ULTRASONIC MONITORING OF MATERIAL PROCESSING USING CLAD BUFFER ROD SENSORS

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Abstract

Ultrasonic sensors and techniques are developed for in-line monitoring of polymer extrusion, cleanliness of molten metals and liquid flow speed at elevated temperature. Pulse-echo mode is used for the first two processes, while the through-transmission mode is applied in the third one. The ultrasonic probe consists of high performance clad buffer rods with different dimensions to thermally isolate the commercial ultrasonic transducer from materials at high temperature. The clad buffer rods are made of steel, polymer and ceramic.

Steel clad buffer rods are introduced for in-line monitoring of polymer extrusion processes. Owing to its superior performance in pulse-echo mode, for the first time such a probe is installed and performs ultrasonic monitoring in the die of a co-extrusion machine and in the barrel section of a twin-screw extruder. It can reveal a variety of information relevant to process parameters, such as polymer layer thickness, interface location and adhesion quality, stability, or polymer composition change. For the ultrasonic monitoring of polymer processes, probes with acoustic impedance that matches that of the processed polymer may offer certain advantages such as quantitative viscoelastic evaluation; thus high temperature polymer clad buffer rods, in particular PEEK, are developed. It is demonstrated that this new probe exhibits unique advantages for in-line monitoring of the cure of epoxies and polymer extrusion process.

Long steel clad buffer rods with a spherical focus lens machined at the probing end are proposed for cleanliness evaluation of molten metals. The potential of this focusing probe is demonstrated by means of high-resolution imaging and particles detection in molten zinc at temperatures higher than 600°C, using a single probe operated at pulse-echo mode.

A contrapropagating ultrasonic flowmeter employing steel clad buffer rods is devised to operate at high temperature. It is demonstrated that these rods guide ultrasonic signals whose velocity is dependent on the average temperature of the flow. Thus, a novel

technique to significantly reduce the temperature effects of ultrasonic flowmeters is successfully developed and tested in motor oil flow at 130°C.

Résumé

Des capteurs ultrasonores et des techniques sont développés pour le contrôle en ligne de l'extrusion de polymères, de la pureté de métaux fondus et pour la mesure de la vitesse d'écoulement de liquides portés à hautes températures. Le mode en réflexion est utilisé pour les deux premiers procédés, quant au mode en transmission est appliqué pour le troisième. La sonde ultrasonore est constituée d'une ligne à retard pouvant avoir des dimensions différentes et ayant subi un revêtement. Ce dernier a pour but d'isoler thermiquement les transducteurs ultrasonores commerciaux des matériaux à hautes températures. Ces lignes à retard revêtues peuvent être soit en acier, soit en polymère ou en céramique.

Les lignes à retard en acier sont employées pour le contrôle en ligne des procédés d'extrusion de polymères. A cause de sa haute performance en mode réflexion, pour la première fois, une telle sonde est installée dans le moule d'une machine à co-extrusion et au niveau d'une section transversale d'une double visse sans fin à extruder. Elle peut révéler diverses informations importantes relatives aux paramètres du procédé, tels que l'épaisseur des différentes couches polymères, la position des interfaces, la qualité des adhésions ainsi que la stabilité ou le changement de la composition des polymères. Concernant le contrôle des procédés de mise en forme des polymères, les sondes ayant une impédance acoustique assortie avec celle du polymère sous analyse peuvent donner certains avantages comme l'évaluation quantitative des propriétés viscoélastiques ; Ainsi, des lignes à retard fabriquées en polymère résistant à haute température, notamment du PEEK, ont été développées. Il est prouvé que cette nouvelle sonde offre des avantages uniques pour le contrôle en ligne de la cuisson des résines époxy et l'extrusion des polymères.

Afin d'évaluer la pureté des métaux fondus, nous utilisons de longues lignes à retard en acier munies à l'extrémité sensible d'une lentille de focalisation sphérique. Le potentiel de cette sonde focalisée est essentiellement dû à sa haute résolution spatiale et à

sa capacité de détecter des particules en suspension dans du zinc liquide, et ce malgré des températures dépassant 600°C.

Par ailleurs, un débitmètre ultrasonore à propagation en contre sens et utilisant une ligne à retard en acier est conçu pour opérer à haute température. Il est démontré que ces lignes à retard guident les ondes ultrasonores dont la vitesse est dépendante de la température moyenne du fluide en écoulement. Ainsi, une nouvelle technique visant à réduire de manière significative l'influence de la température sur les débitmètres ultrasonores est développée. Cette nouvelle procédure est validée et testée avec succès sur l'écoulement d'une huile à moteur portée à une température de l'ordre de 130°C.

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Chapter 1

Introduction

1.1-Background

As new materials are developed, process monitoring and control becomes increasingly complex and significant [1]. The technical specifications of products made from the latest hi-tech materials are usually advanced and precise. Moreover, mass-produced items, even those made from conventional materials, need continuous monitoring and process control to ensure production efficiency [2]. Very often, process monitoring and control requires complex instrumentation during the manufacturing process itself, which can provide precise feedback on process parameters and materials properties during production [2-7]. Ultrasonic techniques [2-9] are often used for such purposes because they are simple, fast and economical, and have the capability to probe the interior of opaque materials. Such techniques are preferred to have ultrasonic transducers (UT) of large bandwidth and high efficiency.

In several situations, in-line monitoring measurements of industrial processes must be performed at elevated temperatures. For instance, in polymer extrusion the processing temperature is between 200 and 300°C, whereas in metal processing, it may be higher than 1000°C. Non-contact ultrasonic methods such as laser ultrasound [10,11] and electromagnetic acoustic transducers (EMAT) techniques [12,13] are possible approaches. Laser ultrasonic systems can provide advantages such as fast scanning and probing materials of complex shapes, but are generally bulky and costly. Laser safety is also a concern. The signals produced by EMATs have much poorer qualities in frequency bandwidth and signal to electronic noise ratio (SNR) than those generated by

piezoelectric UTs. Thus there is a strong need to find broadband ultrasonic probes of high efficiency for measurements at elevated temperatures.

Various efforts have been devoted to the development of high temperature (HT) piezoelectric UTs [14-21]. Commercially, HTUTs are supplied by several companies, such as Etalon (Lizton, IN), Panametrics (Waltham, MA), Krautkramer GmbH (Cologne, Germany), RTD (Rotterdam, the Netherlands), Ultran (Boalsburg, PA), Ishikawajima Inspection and Instrumentation Co. Ltd (IIICL, Tokyo, Japan) [18], *etc.* However, not only is the price of these UTs very high, but at present it seems that there is no good solution for the ultrasonic couplant, for instance, employed between the UTs and the steel walls of the mould and die operated at elevated temperatures. In general, the ultrasonic couplant is a type of grease, for which the ultrasonic coupling coefficient changes significantly as the temperature varies. Also, at high temperatures the grease may be slowly evaporated and/or dried.

Except for the UTs from IIICL, for which the details of UT design appear in [18], the broad bandwidth of the above UTs is provided by a method employing an epoxy backing [19] that is not suitable for thermal cycling. Since the thermal expansion coefficient of the electrode material deposited on the piezoelectric crystal of the UT is quite different from that of the epoxy, a major problem that occurs during repeated thermal cycling in industrial processing is disbonding between the electrode and the epoxy. When this occurs the bandwidth is no longer sufficient for ultrasonic monitoring. HTUTs from IIICL have a reasonable bandwidth, but they suffer from low SNR (< 30 dB). Also, they require a high temperature couplant. Therefore, the classical approach employing conventional UTs with buffer rods is preferable for process monitoring at elevated temperatures [2,3,22-32]. In this approach, the probing end of the buffer rod is in contact with hot material, whereas its other end can be cooled by water or air so that the conventional ambient temperature high performance piezoelectric UTs and couplants can be readily used.

Two common modes of operating UTs with buffer rods are through-transmission and pulse-echo. In the through-transmission mode, one probe serves as transmitter and the other as receiver, whereas in the pulse-echo mode the same probe acts as both transmitter and receiver. For monitoring of industrial processes, the through-transmission mode exhibits disadvantages such as alignment and access to two opposite faces of the sample. In this regard, the pulse-echo mode is often preferable because alignment is no longer a concern any more, and installation of the probe requires access to only one face of the sample, provided that good quality echoes can be obtained.

The well-known problem in using long buffer rods is the presence of spurious echoes due to mode conversion, wave reverberation and diffraction within rods of finite diameter [33,34]. These echoes are unwanted because of their possible interference with the desired signals from the measured sample. One method for eliminating these parasitic echoes is to use buffer rods of large diameter and short length. However, large diameter and short length may make the cooling system impractical and bulky. Also, short buffer rods suffer from the so-called "blind zones", which are caused by echoes reflected at two ends of the rod. To attach buffer rods to machinery or equipment, often discontinuities such as threads and flanges have to be used, with the possibility of introducing additional noises, particularly strong in the case of shear wave in pulse-echo mode. Because shear and longitudinal waves are complementary in terms of material properties they can sense [35], a preferable buffer rod for process monitoring should be capable of propagating both waves with good signal quality such as high signal strength and low noise.

In order to improve buffer rod performance, design and fabrication of clad silica [33], tin alloy [34] and aluminium alloy [36] rods consisting of a core and a cladding have been reported previously. These clad rods have demonstrated ultrasonic performance superior to non-clad rods for longitudinal wave propagation. One attractive feature of clad rods is the ability to maintain good ultrasonic guidance in the core even if the cladding is machined, *e.g.*, threaded for attachment.

Clad silica buffer rods have been made by chemical vapour deposition, and clad tin and aluminium alloy rods are fabricated by the Ohno continuous casting (OCC) process [36]. The clad silica rods are fragile and not easily machined, but it should be noted that shear wave is well guided in the core. On the other hand, the OCC process produces clad tin and aluminium alloy rods with elongated grain structures. These long needle-like grains enhance mechanical strength and corrosion resistance [34,36]. Such rods are also easily machined, but the needle grains deteriorate shear wave propagation significantly [34,36]. In addition, it would be difficult to extend the use of this process to clad rods with high melting temperature materials such as stainless steel. Clad buffer rods (CBRs) made by thermal spray techniques [37,38] overcome all these difficulties of fabrication and give superior performance for both longitudinal and shear waves. They can be machined and made of metals with high melting temperature. Numerous materials can be used for the fabrication of the cladding over a length in excess of one meter. The core materials used are metals with low ultrasonic attenuation and high melting temperature, such as zirconium, titanium and steels. For specific applications, core and cladding materials should be properly selected, for instance, for long immersion in liquid metals [39]. Owing to all these attractions, in this thesis we make extensive use of metal, in particular, steel CBRs for monitoring of industrial processes at high temperature, in situations where other probes have failed or suffer from limitations.

Although metal buffer rods have met widespread use in industry, they are not always the best solution. This is particularly true for monitoring of polymer processes. For metal buffer rods, because the acoustic impedance[1] of metals is much greater than that of molten polymers, the ultrasonic reflection coefficient at the buffer rod/polymer interface, denoted as Γ, is rather large (about 0.9). If the density of the molten polymer, denoted as ρ, is measured through the measurement of Γ, the relative error in ρ caused by that in Γ can be very large, with a ratio of $\Delta\rho/\rho$ to $\Delta\Gamma/\Gamma$ being up to several hundred percent [35]. This large error in ρ could lead to a high uncertainty in the measurement of Young's modulus, shear modulus and viscosity of the molten polymers. In another

[1] The acoustic impedance, Z, is the product of the density, ρ, of a medium and the velocity, V, of a given ultrasonic wave propagating in it: $Z=\rho V$.

aspect, due to the high thermal conductivity of metals, the metal buffer rod must be sufficiently long in order that the cooling at the UT end does not adversely reduce the temperature at the probing end and alter the material processing. However, the great advantages of metal buffer rods, in particular, steel buffer rods, are their robustness and high melting temperature, which are important requirements for many practical applications.

If a polymer buffer rod is used, because of the small difference in acoustic impedance between the buffer rod and polymer sample, the relative error $\Delta\rho/\rho$ caused by $\Delta\Gamma/\Gamma$ is significantly reduced [35]. In addition, because of the small impedance mismatch, a large amount of ultrasonic energy guided by the polymer buffer rod can be transmitted into the molten polymer, leading to improved SNR, particularly for thin samples in reflection configuration. Also, because of its poor thermal conductivity, the polymer buffer rod may be short without letting the cooling at the UT end adversely affect the temperature at the probing end. This shortening of the buffer rod reduces the ultrasonic propagation loss in the buffer rod.

It is well known that polymers have high ultrasonic loss, and relatively low melting temperature and mechanical strength. Thus, it is also the goal of this thesis to search for and study high performance polymer buffer rod materials to be used as robust sensors in the monitoring of polymer processes. In addition, they should not react with molten polymers under extrusion. If produced, these buffer rods might open new routes for monitoring of industrial processes of other low acoustic impedance materials, *e.g.*, epoxies.

1.2-Industrial materials processes and their monitoring

Several industrial materials processes of importance to modern and competitive industries are under exhaustive research and development at the Industrial Materials Institute (IMI) of the National Research Council of Canada (NRC), where this thesis

research has been performed. Ultrasonic techniques based on the use of CBRs are promising for in-line monitoring of some of these processes. Here, we introduce the processes considered for the thesis research, followed by the state of the art on their monitoring. Emphasis is put on the performance of the CBRs, ultrasonic techniques, their merits and limitations.

1.2.1-Polymer extrusion

Extrusion is one of the most commonly used processes in the plastics industry. The standard set-up for the extrusion process includes an extruder and a die for shaping purposes, and auxiliary equipment for sizing, taking-up and cooling. The polymer is fed into the extruder through a hopper in the form of pellets, transported along the barrel through different zones (solid conveying, melting and metering) where it is compacted, melted and mixed, and finally flows through a die where it takes the shape of the product. During the process, the material undergoes a very complex thermo-mechanical history, which affects the qualities of the final product.

As industrial requirements have become more and more stringent in recent years, the need has increased for products with properties tailored for specific applications. One approach is to develop new materials with a wide range of properties through combining various polymers. This can be accomplished by either blending two or more materials in a twin-screw extruder, or combining polymer melt streams from several extruders in the die to form a multi-layer extrudate. In the latter case, the location and stability of the interface are essential to the overall performance of the process, and the final quality of the product. In the case of polymer blends, the physical properties and the performance of the end products depend strongly on the morphology of the material, which in turn is linked to the degree of mixing and other process-based considerations, such as melting and shearing effects of the screw. Twin-screw extruders are also more and more routinely used as continuous chemical reactors, with the polymer species being chemically modified to fulfil specific tasks. In all cases, control of the process is crucial for

productivity, and conventional monitoring methods, including off-line characterisation, often do not provide sufficient and timely information for that purpose.

As a response to the deficiencies encountered in off-line inspection, in-line ultrasonic monitoring of polymer extrusion processes has been investigated [3,40-42]. Advantages of this approach are: its simplicity, reduced cost, non-destructiveness and non-intrusiveness. For instance, Piché, *et al.* [41] demonstrated that ultrasound constitutes a useful probe for monitoring changes in viscoelastic properties, structure and composition of flowing polymer melts during processing. Likewise, Gendron, *et al.* [40] discussed the application of ultrasound to discriminating blend morphology compounded in twin-screw extruders. All of these works utilised ultrasonic sensors consisting of non-clad steel buffer rods operating in through-transmission. A major limitation of this approach is that all the monitoring has to be carried out at the extruder exit (die), since this is possibly the only location where probes can operate in through-transmission. However, it is of practical interest as well to evaluate the composition of polymer blends [41], residence time distribution [40], and flow of the polymer melt in different sections of the barrel where the mixing screw is located. In order to obtain localised information on polymers in the barrel section of the extruder, the monitoring has to be performed off-the-screw in pulse-echo mode [43].

Five major factors which cause considerable difficulty in off-the-screw ultrasonic measurements are: (1) irregular shapes of the screw (narrow and non-flat surface at the top of the flight); (2) near field effects due to the close distance between the screw and the probing end of the ultrasound sensor; (3) screw rotation; (4) deflection and tilting of the screw in the radial direction of the screw shaft due to the viscosity of the materials being extruded; and (5) shifting of the screw in the axial position of the screw shaft during extrusion. These factors significantly affect the precision of tracking the screw position and measuring the sound speed and attenuation in the material being extruded. Because of the practical need to determine the variation in molecular weight and viscosity of polymers or epoxies, the precision of measuring ultrasonic velocity (or its variation) and attenuation (or signal amplitude variation) should be better than a few percent. Thus, a versatile ultrasonic monitoring of extrusion processes in the barrel

section (off the screw) requires high performance buffer rods operating in the pulse-echo mode. Such buffer rods should exhibit high SNR for ready identification of the relevant signals, and be machined in the same shape as conventional temperature and pressure sensors coming along with extrusion machines. This attractive feature would not alter the extruder machine design for ultrasonic probe installation, but would allow interchanging sensors depending on the desired monitoring purpose. Pulse-echo mode, as opposed to through-transmission, would make it possible to install the probes in the barrel section of the extruder, preferable for off-the-screw measurements.

1.2.2-Cleanliness evaluation for molten metals

In metal processing, quality is frequently associated with the entrainment of non-metallic material within the liquid metal during the primary manufacturing steps. In the case of aluminium (Al), the entrained material can be oxide film together with hard carbide inclusions derived from the original smelting process [44-46]. Al also picks up hydrogen readily, which can, on solidification, diffuse to entrained oxide particles, and during the finishing stages, result in blisters on the sheet material. The hard particles, which may be associated with the oxide films, are detrimental to the forming of thin-wall cans. These particles scratch or deform the draw dies, and part rejection due to such a defect can be costly.

The magnesium (Mg) industry [47,48] has similar problems. There are many sources from which particles can be introduced into liquid metal. Liquid Mg readily oxidises in the atmosphere during transfer operations, leading to the formation of inclusions, which may remain within the melt. In addition other reaction products, such as flux particles, may be present. Particle sizes can vary from less than 1 micron to greater than 150 microns.

Filtration systems developed by the Al industry have been extremely successful as an adjunct to the continuous casting process. However, any handling or movement of

filters can send entrapped oxides or particles down-line into the product. Filtration techniques have been also applied in the Mg and steel industry. Various techniques currently available for evaluating metal quality are normally based on the extraction of a metal sample, followed by analysis in the laboratory. Although this approach is often capable of providing the desired information regarding inclusion content, it also requires considerable sample preparation and analysis time to discover possible liquid metal processing problems. In addition, the information is obtained too late to make rapid adjustments in the casting process. Beyond the inability of the laboratory techniques to provide feedback control of the casting process, they are also not sufficiently rapid to prevent the rolling and treatment of poor quality metallic products. Clearly, while many of the existing sampling and analysis techniques are capable of determining product quality, a more direct method could be highly beneficial.

Ideally, the evaluation of the cleanliness of liquid melts should be conducted on large quantities, which could be rapidly analysed with little or no sample preparation. This may be achieved by analysing the metal, such as Al or Mg, while it is still in the liquid state. When successfully implemented in industry, this type of in-line monitor could potentially provide information regarding liquid metal processing problems, and also prevent further processing of unsatisfactory slabs, billets, ingots and castings.

For Al, a commercial on-line monitoring device is available to measure cleanliness: the Liquid Metal Cleanliness Analyser (LiMCA) [45]. Such devices are commonly used in the aluminium industry. Its basic operating principle is that when non-conducting inclusions (particles) pass between two electrodes inside a capillary, the electrical impedance between these two electrodes varies. The magnitude and width of the variation in electrical impedance determines the size of the non-conducting particles. LiMCA is convenient, but still suffers several limitations. One limitation is that this device uses a capillary with a diameter of 500 microns or less, through which the liquid Al is pumped for particle size evaluation and counting. If an inclusion has a diameter greater than that of the capillary, the capillary is blocked and must be replaced. Another constraint of the LiMCA is that the volume of liquid Al being on-line analysed for

cleanliness is limited due to sampling through the capillary. The third limitation of the device is that large inclusions (> 100 μm diameter) are normally carried at the bottom of the transportation channel, due to their weight; it is very difficult for the capillary to collect such large particulates. At the present time, many researchers are attempting to develop a modified version of LiMCA to characterise inclusions in liquid Mg. However, no such device has been established yet.

In parallel to LiMCA development, ultrasonic techniques [49-53] have also been reported as in-line methods to monitor liquid metal properties. The merits of ultrasound are: 1) it can propagate in liquid metals without much attenuation; and 2) when inclusions are present in liquid metals, the ultrasonic properties such as velocity and attenuation of the liquid may change. This means that the variation in velocity and attenuation of the ultrasound propagating in the liquid metal may be used to characterise inclusions, such as their population density. However, the probes used in earlier studies with ultrasonic techniques [49-53] were non-clad buffer rods with no focusing lens. Due to the finite dimensions of the rod and wave diffraction, the SNR of the transmitted ultrasonic signal in such non-clad buffer rods is poor. Because of the poor ultrasonic guidance in such non-clad buffer rods, two buffer rods have commonly been used in a pitch-catch configuration. In this approach, one rod is used for transmitting the ultrasonic energy, and the other for receiving it. The disadvantages of the two buffer rod system are: 1) the alignment of the two rods with a reflector located inside the molten metal is critical and inconvenient; and 2) two rods together with the associated cooling system make the system bulky and expensive. In addition, once the non-clad buffer rod is immersed into the molten metal, some ultrasonic energy will leak from the periphery of the buffer rod into the surrounding molten metal. This results in a further reduction in the SNR

The reported ultrasonic techniques [49-53] also do not use an acoustic lens to focus the ultrasonic energy. The concern is that aligning two focused probes is impractical in liquid metal environments. However, in at least one study [53] an additional reflector was used to focus the ultrasound; this configuration which uses two probes is difficult for alignment and inconvenient for practical use.

Therefore, there is still an industrial need to develop novel and convenient ultrasonic sensors to quantify inclusions in liquid metals.

1.2.3-High temperature flow speed measurement

Flow measurement is an important parameter for engineers and scientists in general engaged in the development of systems for process monitoring and control in industries [2,54]. Presently, the main types of industrial flow meter technology are ultrasonic, magnetic, coriolis mass and vortex flowmeters. Each type exhibits specific advantages depending on the particular application [55].

In contrast to others, ultrasonic techniques are attractive because they are non-intrusive, applicable to fluids regardless of their conductivity and opacity, robust and inexpensive [2]. Therefore, fluid speed measurements at room temperature by ultrasound means are a well-established technology. Indeed, publications and practical implementations of flowmeter devices date back to the 1950's [54-56]. Applications of ultrasonic flowmeters include: assessing gases and various other fluids (including poisonous and corrosive ones) in petrochemical industries and nuclear plants [56-62]; determining water flow in large conduits and measuring ocean currents [54]; and blood flow measurement and imaging in diagnostic tests [54,63].

An ultrasonic flowmeter system should be accurate over a large range of flow speeds and duct sizes, quick to respond for effective control of the process and correction of eventual irregularities, independent of different shapes of flow profiles (laminar, transitional or turbulent), and able to sustain high temperatures (*e.g.*, 200°C), without deterioration of its performance. The latest developments in instrumentation techniques, measurement principles and microprocessor technology have contributed significantly to improving accuracy (±0.3% of flow) and time response (< 1ms) [2,54,55]. Vontz, *et al.* [64] report an accurate measurement technique independent of flow profiles, based on the

use of a helical sound path for interrogating the fluid. This approach provides optimal integration over different flow speeds in the duct. Nevertheless, due to its intrinsic difficulty, few results have been published on high temperature ultrasonic measurements of fluid speed [65]. In polymer injection moulding and metal die casting, for instance, the speed of the injected molten material at the gate or runner can affect considerably the quality of the final part [66]. Obviously, because of the involvement of molten materials, in-line ultrasonic techniques suitable for liquid flow measurement at elevated temperatures are of interest.

Two common concerns limiting the high temperature performance of conventional ultrasonic systems for flow measurement are transducers and couplants, as mentioned before. It is well known that most so-called *off-the-shelf* broadband UTs are designed for operations up to 50°C, a limit imposed by the softening and melting temperatures of the damping material, *e.g.*, epoxy impregnated with tungsten powder. A suitable approach to overcoming this drawback is to insert a thermal isolating buffer rod with good ultrasonic performance (*e.g.*, high SNR) [67,68]. This requirement is important because, *a priori*, the noises generated in the buffer rod may bury the desired signals, so that no meaningful information is extracted. Besides protecting the ultrasonic transducer from overheating in applications such as high temperature flow measurements, buffer rods are also a solution for the couplant between the probe and tested sample, since their probing end can be directly wetted by fluids [66].

Proper selection of buffer rod materials and understanding of wave propagation mechanisms in finite bounded media have culminated in buffer rod designs with properties tailored to flow measurement at high temperature [31,67]. These two previous studies utilised two different non-clad buffer rod geometries to propagate a single mode, either longitudinal or shear. For longitudinal wave guidance, the buffer rod has a conventional cylindrical shape, but for shear wave guidance, it resembles a thin *hockey stick*. This latter geometry was chosen to improve the shear wave guidance itself in the buffer rod, and effectively generate longitudinal waves in fluid by mode conversion.

However, no efforts are reported in these works to eliminate temperature effects in flow measurement.

For industrial environments, where melt flow may undergo quick temperature variation, CBR probes are proposed here as an alternative for high temperature flowmeters, immune to temperature variation.

1.3-Thesis Content

Since sensors and techniques are the main tools for process monitoring and control, this thesis focuses on the development of ultrasonic sensors and techniques exploring both metal and polymer CBRs for process monitoring of common industrial materials, namely polymers, resins and metal alloys.

In Chapter 2, we introduce different kinds of steel CBRs utilised in the thesis research. Details regarding the design of the rod core and fabrication of the cladding are addressed. These CBRs have either a focusing or a flat probing end, appropriate for a variety of ultrasonic monitoring purposes. The ultrasonic field distribution generated by the probe consisting of CBRs and commercial UTs is assessed through reflection over a small target. We evaluate pulse duration, centre frequency, and the -3 dB-bandwidth achieved by different combinations of CBRs and UTs. Identifying these features is important in order to select the best probe for a given application. In particular, for the focusing probes, we evaluate their true focal length and spatial resolution. It will be seen that the high spatial resolution attained by such probes is promising for small particle detection in liquids, *e.g.*, inclusions in molten metals. With respect to the CBR with a flat probing end, we show that the ultrasonic field distribution at the output of a long probe has a far field radiation pattern, appropriate for ultrasonic monitoring in the off-the-screw region of extruder machines. In addition to experimental evaluation of CBRs, numerical simulation is also performed. We comment on similarities and discrepancies between the experimental and numerical approaches.

Innovative in-line ultrasonic monitoring of polymer co-extrusion and twin-screw extrusion using steel CBRs is presented in Chapter 3. These newly developed CBRs, machined as conventional temperature and pressure sensors and operating in pulse-echo mode, are installed in actual machines without disturbing material processing at temperatures higher than 200°C. First, co-extrusion of HDPE and a thermoplastic elastomer based on polypropylene-EPDM (ethylene-propylene-diene monomer) is investigated. We monitor extrusion stability and layer thickness *via* ultrasonic detection of the interface between the polymers. Ultrasound is also used to study adhesion qualities of a plastic bottle produced by co-extrusion blow moulding of HDPE and Santoprene. Digital signal processing for improvement of ultrasonic signals is investigated. For the twin-extrusion process, ultrasonic sensors operating in pulse-echo mode are used for the first time in the barrel section of the extruder, to demonstrate the potentiality of the new probe for off-screw measurements. Experiments are performed using PS (polystyrene) and PE (polyethylene) polymers, to reveal the sensitivity of ultrasonic signals to the properties of these polymers and their mixtures; this technique can be used to track changes in polymer properties, as is been clearly demonstrated in [5], where ultrasonic monitoring is carried out at the exit of the extruder.

Because quantitative ultrasonic measurement of viscoelastic properties of polymers and thermoset resins needs low acoustic impedance buffer rods, Chapter 4 is concerned with material selection, fabrication and performance evaluation of these probes. For the first time, polymer buffer rods made of PEEK (polyetheretherketone) are used as ultrasonic probes. It is shown that PEEK rods exhibit low ultrasonic loss at low MHz frequencies for longitudinal and shear waves, when compared to other reported polymer buffer rods, namely those made of HDPE, and Vespel (polymide). PEEK also has relatively a high melting temperature (~350°C), which makes it an interesting candidate for monitoring industrial processes. To this end, low acoustic impedance and heat resistant cladding materials are investigated to further improve ultrasonic guidance in the PEEK core. To demonstrate the capability of the newly developed probe to monitor viscoelastic changes of materials, two sensors composed of longitudinal and shear UTs

and PEEK CBRs are employed together for a detailed characterisation of polyester curing. Based on this experiment, we demonstrate the simultaneous generation of shear and longitudinal waves during the cure using a single UT and the PEEK CBR. This makes ultrasonic inspection simpler and more economical. Applications of PEEK CBRs for in-line ultrasonic monitoring of polymer extrusion in the barrel section are presented as well. Merits and drawbacks of the new probe are identified and compared to those of steel CBRs employed in Chapter 3.

Chapter 5 is an account of our effort to demonstrate that steel CBRs may be used as ultrasonic probes to identify and quantify inclusion particles in molten metals. This ability is important for cleanliness evaluation in metal processing industries. In order to achieve the necessary high spatial resolution, a point focus lens is machined in the probing end of the buffer rod. Thus, ultrasonic images of a small object are acquired by the common C-scanning technique, to provide quantitative imaging resolution of the probe. Such images are obtained from amplitude and time variations of the reflected signals. Two propagation media are chosen for this demonstration: water at room temperature, and molten zinc at approximately 600°C. It is shown that the high SNR of the focus lens leads, for the first time in our knowledge, to clear ultrasonic images in molten metal. Thus, taking advantage of the high spatial resolution and SNR of the developed ultrasonic probe, we attempt to detect impurity particles present in molten zinc. First, however, a simulation is carried out in a solution of water and PVC particles, to study time and frequency domain characteristics of the ultrasonic signals scattered from particles at the focal region of the lens.

Chapter 6 addresses the important issue of flow speed determination by ultrasound at high temperatures, which may be used to monitor the melt flow in polymer and metal processing. A brief description of the theory underlying ultrasonic contrapropagating flowmeters is presented. We analyse different configurations, and select the optimal one for flow measurement at high temperature without disturbing the process. Details regarding the most effective way to generate and detect ultrasonic energy in the contrapropagating approach are introduced as well. Before attempting liquid flow

measurement at high temperature, the performance of steel CBRs as part of contrapropagating ultrasonic flowmeters is first verified in water at constant room temperature. Here, we demonstrate that CBRs also exhibit higher SNR than the reported non-clad rods even in through-transmission mode, a standard way to operate contrapropagating flowmeters. This signal superiority can lead to improved measurement accuracy. Ultrasonic wave radiation patterns of the chamfered probing end are assessed, to determine the spatial limits of signal detection provided by CBRs. The effect of water flow speeds on the difference between upstream and downstream ultrasonic transit times are measured and compared with theoretical expectations. Finally, a flowmeter exploring two steel CBRs is designed and installed in a heater machine to measure the flow speed of motor oil at temperatures near 130°C. We discuss and introduce a novel technique for compensating for temperature variation during flow speed measurement. This feature extends the applicability of the designed flowmeter as a potential candidate for industrial environments, where fast temperature fluctuation exists.

Finally, Chapter 7 presents the summary and claims of originality of the thesis.

Chapter 2

Ultrasonic Evaluation of Steel Clad Buffer Rods

2.1-Introduction

As mentioned in Chapter 1, metal CBRs have evolved over the last several years, thanks to efforts of Jen, *et al.* [33,34,38,39,68,69]. In these rods, the cladding is fabricated by the thermal spray technique. This results in ruggedness, endurance and superior guidance for both longitudinal and shear waves. Our focus in this chapter is on the experimental investigation of the ultrasonic field distribution at the exit of the probing end and pulse-echo response generated by probes consisting of commercial UTs and steel CBRs with different lengths for specific applications, to be explained in Chapters 3, 5 and 6. The CBRs used in this thesis have, in general, the diameter and length much smaller and longer in wavelengths, respectively, than those of the buffer rods of acoustic microscopes, which have already been well characterised in previous publications [70-72]. Therefore, there is a need to characterise the radiation fields of the non-focusing and focusing CBRs. It is not the scope of the present work to introduce detailed analyses of the ultrasonic guiding mechanism in such CBRs. For the purpose of completeness, design considerations and fabrication process of CBRs, which have been reported in [39], are present in this chapter.

Because the porous cladding material of the CBRs considered here has ultrasonic velocities and impedances much lower than those of the core [73], the propagation constants for the guided modes are complex numbers, leading to complicated theoretical analyses [74], which is not the objective of this research. Furthermore, it seems that there

is no available software that can provide such analyses, neither analytically nor numerically.

In order to show that the cladding does affect the propagation characteristics of CBRs, such as radiation pattern, signal strength and SNR, the longitudinal and shear velocities of the cladding are assumed to be 10, 20 and 40% higher than the corresponding ones in the core. In this case, the propagation constants of the guided modes in the core are real, and commercially available softwares can be used. It is noted here that the simulation is merely used to observe the trend of the change of radiation pattern, signal strength and SNR imposed by the cladding.

2.2-Clad steel buffer rods with a double-taper shape

2.2.1-Design

In previous studies, silica and metallic CBRs made by the OCC process were designed according to the theoretical modelling of, for example, Thurston [75] and Jen, *et al.* [76,77]. In these cases, the bulk longitudinal and shear wave velocities of the core were less than those of the cladding. In the case of the CBRs made by thermal spraying, the velocities in the cladding are much lower than those in the core. Thus, the propagation characteristics of the predominant longitudinal and shear guided modes, for which the experimental data are shown below, cannot be explained by the theories given in [75-77]. Although Simmons, *et al.* [74] describes leaky modes in clad rods, in which the velocities of the core are higher than those of the cladding, these leaky modes are radial-axial types, and do not correspond to the guided longitudinal and shear waves reported here. Based on analogies between the ultrasonic and optical modes in a clad waveguide [77], the guidance principle of leaky optical guided modes in a clad waveguide in which the velocity of the core is higher than the cladding [78,79] was used for the preliminary design of the metallic CBRs used in this thesis research. From

experience, the general rules are the following: 1) the velocity of the core should be different from that of the cladding, and this difference can be even higher than 20%; 2) the acoustic impedance of the core should be close to that of the cladding. It is also noted that reference [78] indicates that not only may the leaky modes be strongly guided in the core, but also the loss in the cladding does not significantly affect the attenuation of the guided core modes.

In a previous work [68] it was also found that the spurious signal in a non-clad tapered buffer rod is much smaller than that in a non-clad rod with a uniform diameter. For the in-line ultrasonic monitoring of interest in our research, the reduction of spurious signals is vital. Therefore, CBRs with a steel core and a double-taper shape as shown in Fig.2.1 are used. In Fig.2.1, $2l$ denotes the length of the rod, d_L and d_S refer to its diameter at the large and small ends, respectively, and θ is the taper angle. In reference [68], it is also experimentally demonstrated that $\theta > 1°$ should be preferred for an aluminium tapered rod. For the rods used here, the cladding was directly deposited on top of the tapered core by the thermal spray technique.

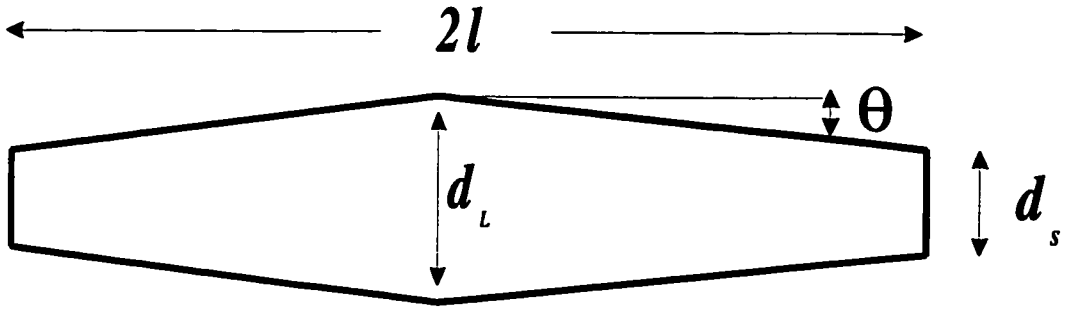


Fig.2.1: Schematic view of a CBR with a double-taper shape.

Since the length requirement for an ultrasonic CBR is only hundreds of wavelengths, orders of magnitude less than that used in optical fibre waveguides, the tolerance of the design is much less critical in ultrasonic CBRs than in optical fibres. In fact, preliminary trials of reference [38] paid a lot of attention to the interfacial conditions

between the core and the cladding during the fabrication, and less to the velocity requirements. For example, the surface of the core rod was prepared in a proprietary manner before the deposition of the cladding.

2.2.2-Fabrication of cladding

Several thermal spray techniques, such as arc spray, plasma spray and high velocity oxygen fuel, may be used to fabricate the cladding. Electric arc thermal spray was used to produce the claddings of the CBRs utilised in our work. This process uses the thermal energy provided by an electric arc formed between two consumable wires to produce molten droplets from them. The molten material is atomised and propelled onto a substrate by a gas stream, usually air. In fabricating CBRs, the substrate is the core of the rod, which can be of uniform diameter or taper shape, as shown in Fig.2.1. It is not the purpose of this thesis to describe in detail the fabrication procedure; this point has been addressed elsewhere [80]. The arc-spray process allows a high deposition rate, up to 100 kg per hour in certain cases, and wide compositional ranges, but it also permits the fabrication of thick metallic cladding (> 5 mm) [80]. Since the coating is formed by the incremental flattening and solidification of molten droplets, it is a line of sight process that gives rise to a heterogeneous and anisotropic microstructure. The splats form an elongated structure, while the exposition of molten metal to air forms an oxide layer surrounding the splats. Due to the piling process and contraction at solidification, thermally sprayed coatings contain many pores. By controlling the operating parameters of the arc-spray gun, it is possible to tailor the microstructure of the coating, and thus its ability to form a thick adherent coating with the desired ultrasonic properties. For instance, the droplet size and their flattening can be controlled. Also, the porosity level varies between 4 and 10%, while variations in the oxide content between 9 and 25% are achieved. All these microstructural characteristics affect wave propagation, and are briefly described in work [80]. For the steel CBRs considered here, the porosity and oxide contents were measured using back-scattered electron images and analysed using

image analysis software from NIH. In general, the results show that the void contents are less than 7.5%, and the oxide less than 17.5% by volume [39].

2.2.3-Fabrication of lenses

As mentioned in Chapter 1, one of the objectives of this thesis research is to develop ultrasonic sensors and techniques for particle detection in molten metals. This monitoring requires ultrasonic probes with high spatial resolution. For a given frequency, improved spatial resolution is achieved by generating and detecting focused ultrasonic waves in the medium under inspection, as it is done in acoustic microscopy [70-72]. In acoustic microscopy, the lens is made at the end of buffer rods which have, in general, a diameter of about 100 wavelengths or more and a length of about 150 wavelengths or less. In our approach, focused ultrasonic waves interrogate the molten metal through a focused CBR operated with a commercial UT. The diameter of the core of the CBR is about 10 wavelengths, and the length can be more than 300 wavelengths. Here, similar to acoustic microscopy, we use spherical focus lens because they are relatively simple to be ground and polished, and sufficient spatial resolution can be achieved.

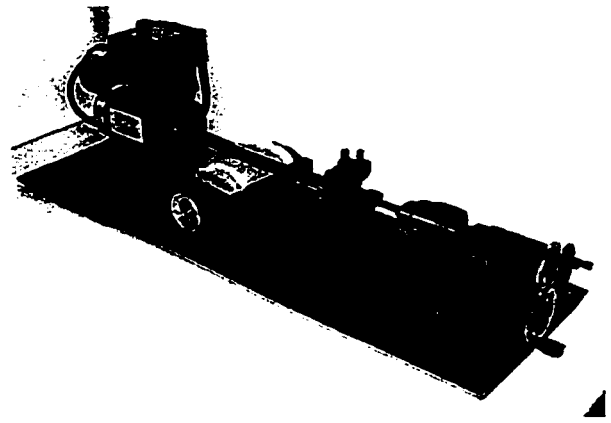
For CBRs made of rugged materials, such as steel, spherical lenses can be directly machined on the probing end by conventional workshop techniques. Fine polishing is required in order to improve the lens performance. In certain situations, however, other materials than steel may be selected for the ultrasonic probe fabrication, in particular ceramics, as it will be discussed in Chapter 5. It is well known that ceramics are fragile during machining and micro-cracks may appear. Therefore, we devised a technique to safely machine a spherical lens at the probing end of ceramic CBRs. This technique is also applied to polish the lenses of the steel CBRs used in our study.

The setup is shown in Fig.2.2. It consists of a miniature lathe (model 4000, Sherline Products Inc., San Marcos, CA) and a steel ball with a desired radius to simultaneously grind and polish the probing end of the CBR. Normally the diameter of

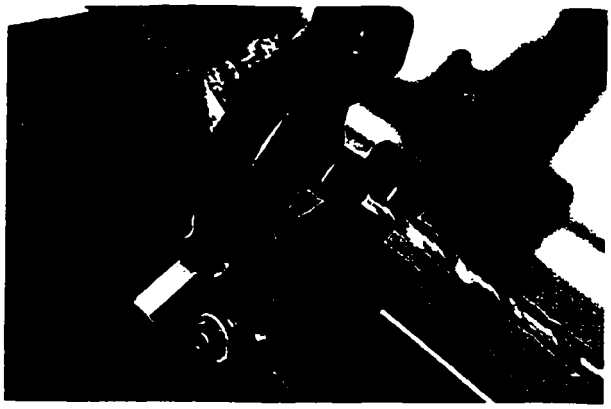
the ball is larger than the diameter of the probing end, depending on the desired focusing properties. Diamond polishing paste of different grain sizes is used. The procedure is summarised in the following steps: 1) the CBR is fixed in the rotating motor of the lathe, for centring and initial grinding, as shown in Fig.2.2(a); 2) next, a small indentation hole is drilled in the probing end of the CBR for centring and accommodating the steel ball. This is shown in Fig.2.2(b); 3) the lens is then fabricated by the continuous rotation of the CBR around the steel ball (Fig.2.2(c)). In order to guarantee a steady pressure on the probing end of the CBR, the steel ball is pushed by a spring. The ball will be changed after a short while of polishing due to erosion by the diamond paste.

2.3-Experimental evaluation of ultrasonic fields of clad steel buffer rods

Steel CBRs have been demonstrated to be effective for conveying ultrasonic waves with low attenuation in situations rather prohibitive for conventional UTs [39]. For our investigation of ultrasonic monitoring of industrial material processes, the probe consists of conventional UT and CBR. Knowledge about its transmit-receive spatial and temporal acoustic field distribution is, therefore, of importance for gaining insight into the physical properties governing CBRs and interpreting the ultrasonic signals interacting with materials. Several techniques for predicting the ultrasonic field distribution generated by single UTs have already been reported [81-85]. In our study, we apply the techniques based on both a thin wire [85] and a small ball bearing [84] immersed in water, as ultrasonic targets in the pulse-echo mode. These techniques are well accepted because 1) they take into account the transmitter and receiver operations of the ultrasonic probe and electronics; 2) they can be used for a broad range of frequencies; 3) they provide good spatial and temporal resolutions; and 4) two scan directions (axial and lateral) are required for a spatial field projection, as long as an axial-symmetry field distribution can be assumed [84,85].



(a)



(b)

(c)

Fig.2.2: Fabrication of a lens at the probing end of ceramic CBRs. (a) Centring and grinding; (b) Drilling an indentation hole; (c) Grinding and polishing.

For the steel CBRs used in this investigation, two different types of probing ends are considered, with a focus lens and with a flat surface. Assessment of the ultrasonic field distributions for the former type is done by the thin wire technique, while for the latter, it is carried out with a small ball bearing. The reason for different ultrasonic targets is solely based on the satisfactory SNR of the detected signals.

Four different steel CBRs with focusing lenses machined on one extremity, and a steel CBR with flat probing end, as shown in Fig.2.3, were characterised. Dimensions of the rods are given in Table 2.1. They are mainly chosen for the reason of different lengths for our investigation with roughly the same taper angle, which ranges from 1° to 3°. For simplicity, from now on in this chapter we refer to the focusing CBRs as rod#1, rod#2, rod#3 and rod#4, from the shortest to the longest, respectively. The probe with the flat end is designated as rod#0. All these rods have a double-taper shape, which is to enhance the SNR, as reported in reference [39]. The small probing end is required so that it can be machined into the shape of conventional temperature and pressure probes (*e.g.*, the rod#0), as it will be explained in the next chapter; the small UT end is used so that, at high temperature applications, less cooling is demanded due to the small thermal mass. The ultrasonic targets were a 25 μm diameter tungsten wire (Johnson Matthey Inc., Seabrook, New Hampshire) and a 1.5 mm diameter stainless steel ball bearing. Evaluation of the ultrasonic field distribution was carried out in an immersion water tank equipped with micropositioners for scanning, and controlled by a PC computer through a GPIB board. The computer was programmed *via* Labview® (National Instruments Inc., Austin, Texas) to acquire and process ultrasonic signals. The A/D board was a CS2125 card (Gage Applied Science, Montreal) with 8-bit resolution and 256 K sample on-board memory. Both 5 and 10 MHz longitudinal UTs (6.35 mm element diameter) were selected to constitute the ultrasonic probes, together with the CBRs[1]. The UTs were driven by a PR35 pulser/receiver (JSR Ultrasonics Inc., Pittsford, NY) in the pulse-echo

[1] If not mentioned otherwise, all longitudinal and shear UTs utilised in this thesis research have 6.35 mm element diameter, and are produced by Panametrics, Inc.

mode. Signals were acquired at 100 MHz sampling rate, and averaged 10 times. The experimental setup is shown in Fig.2.4.

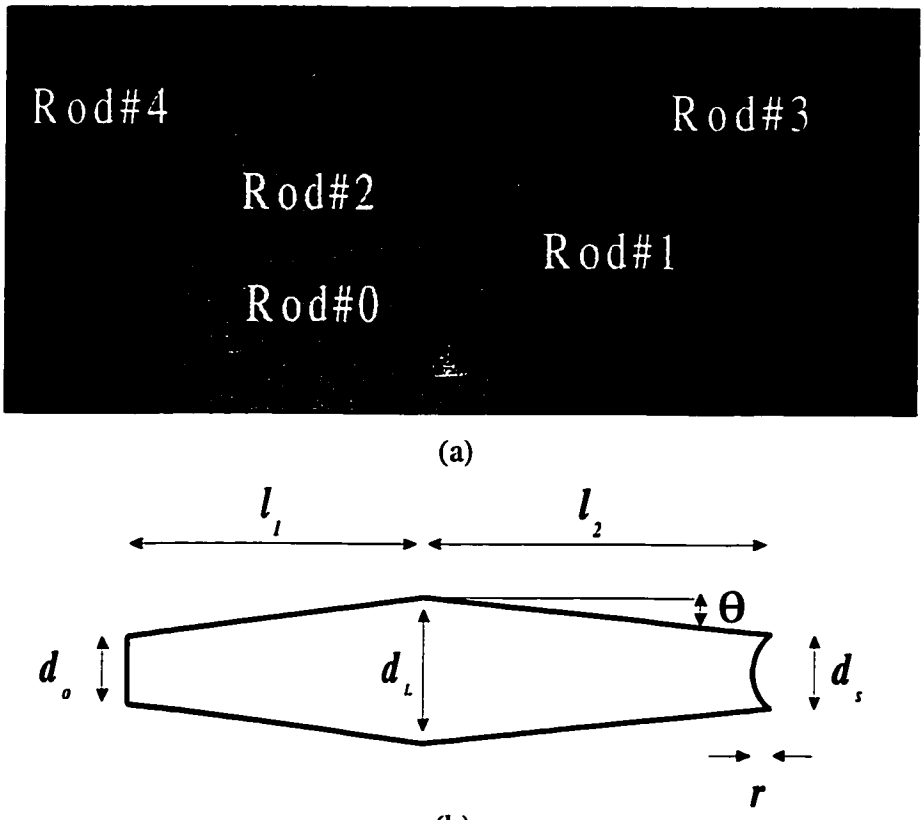


Fig.2.3: Steel CBRs. (a) Picture showing different rods: rod#0 has a flat probing end; rod#1, rod#2, rod#3 and rod#4 have a focusing lens machined on the probing end. (b) Schematic view.

Table 2.1: Dimensions (in mm) and taper angle, θ (in degrees), of the steel CBRs shown in Fig.2.3.

	d_o	l_1	l_2	d_L	d_S	r	θ
rod#0	5.25	76	76	10.6	5.25	-	2.0
rod#1	11.39	35.08	38.78	13.56	11.21	1.57	1.7
rod#2	14.05	115.7	48.30	21.74	16.75	3.92	3.0
rod#3	13.25	144.0	141.0	23.44	12.65	5.70	2.2
rod#4	12.70	500.0	384.0	31.80	17.50	3.22	1.1

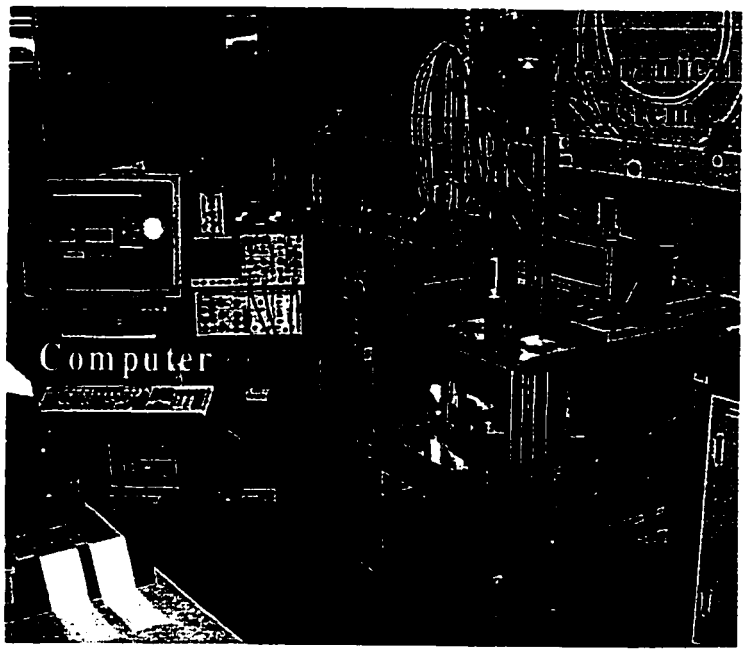


Fig.2.4: Experimental setup used for ultrasonic characterisation of different CBRs.

In order to determine the pulse-echo response for each focusing probe operating at 5 or 10 MHz, the pulse duration, centre frequency and -3 dB-bandwidth were obtained from the focal point (location of maximum axial intensity) using the thin wire. The pulse duration here is defined as the duration between the times when the pulse amplitude is at -20 dB of its maximum values[2]. A typical ultrasonic echo reflected from the wire target is given in Fig.2.5. In these figures, $\mathbf{L^1}$ and $\mathbf{L^2}$ are the first and second echoes reflected from the end of the focusing CBR (rod#1), respectively, and $\mathbf{L_{Wire,F}}$ is the echo reflected from the thin wire at the focal point. Although the diameter of the wire target is much less than the wavelength, the SNR of the desired echo is adequate. For the small ball target, the pulse-echo response of rod#0 (with a flat probing end) was evaluated at 5 MHz, because the SNR at 5 MHz is higher than that at 10 MHz. This evaluation was performed at a location of maximum detected amplitude apart from the flat probing end. For comparison purposes, the time and frequency domain characteristics of a commercial 5 MHz UT were also assessed by the same technique. The results are presented in Figs.2.6 (for 5

[2] Alternatively, the time duration corresponds to the interval in which the pulse amplitude is at 10% of its maximum values.

MHz, V110 Panametrics) and 2.7 (for 10 MHz, V112 Panametrics), and then summarised in Table 2.2. Several interesting features should be noted: 1) steel CBRs behave like high-pass filters, attenuating frequency components less than 6 MHz; 2) the focusing CBRs operating at 5 and 10 MHz do not alter significantly the time duration of the UT; 3) due to the dispersion, the longer the rods, the larger the pulse duration. Because of the poor SNR of the signal reflected from the target, rod#3 at 5 MHz, rod#0 and rod#4 at 10 MHz are not presented.

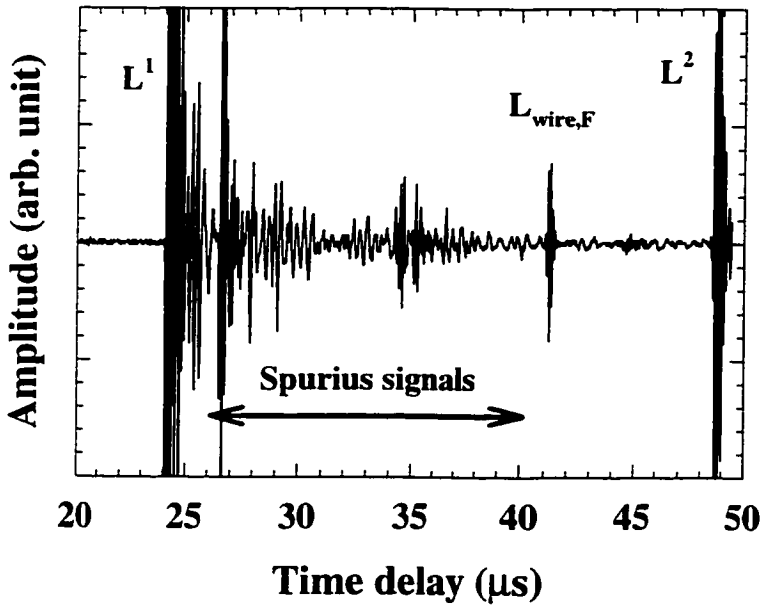
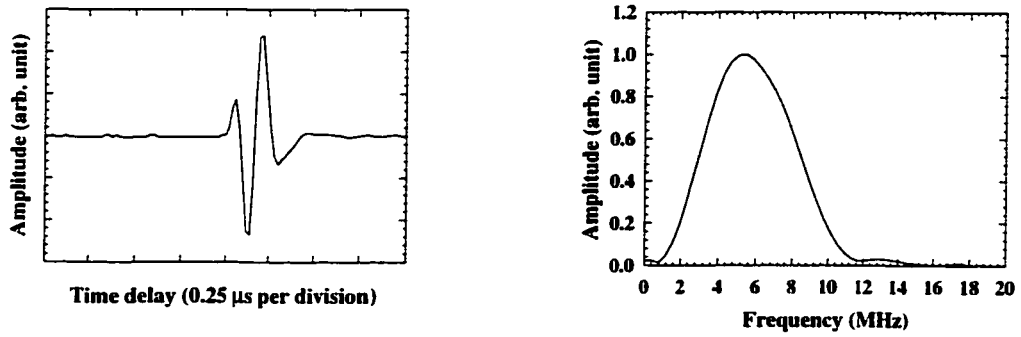


Fig.2.5: Typical ultrasonic echo reflected from the wire target.



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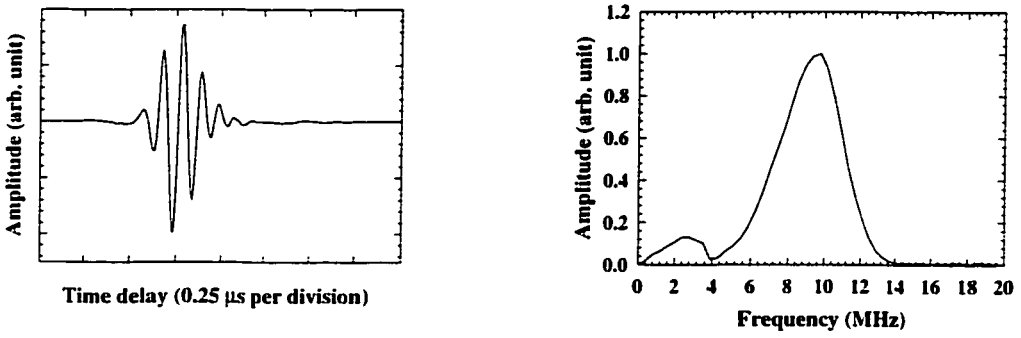

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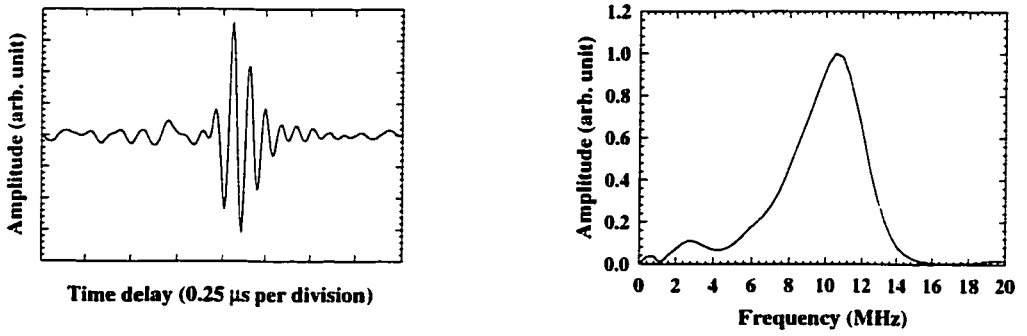
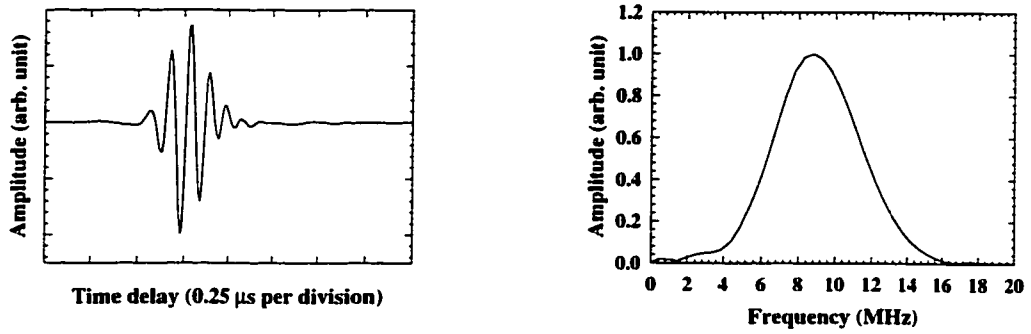
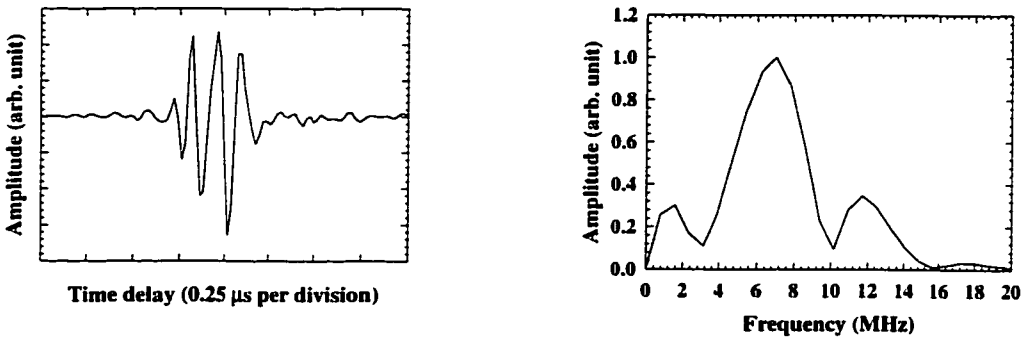


(c)

Fig.2.6: Time and frequency domain characteristics of the V110 longitudinal wave UT (5MHz centre frequency) and V110 UT with CBRs:
(a) V110 UT using a small ball bearing as reflector; (b) V110 UT with rod#1 using a thin wire; (c) V110 UT with rod#2 using a thin wire.

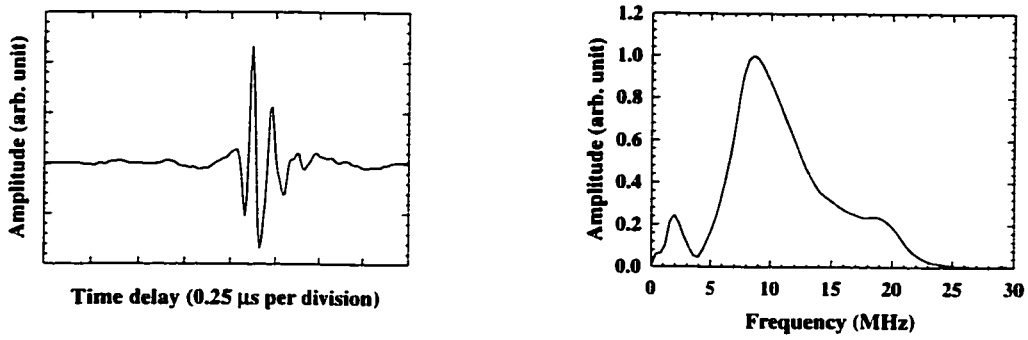


(d)

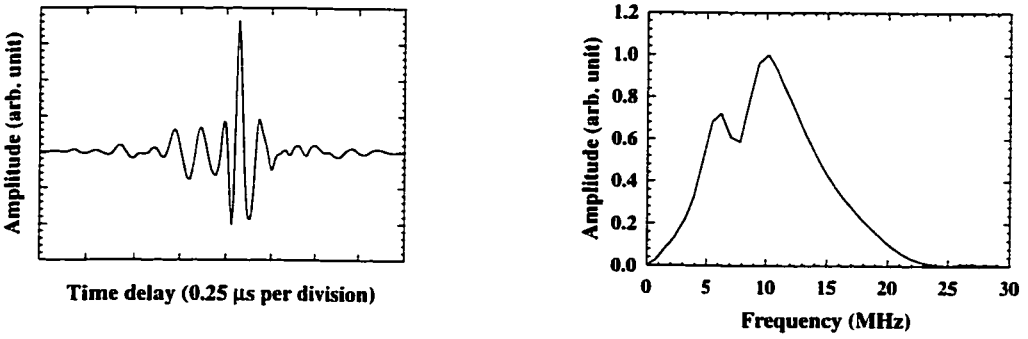


(e)

Fig.2.6: Time and frequency domain characteristics of the V110 longitudinal wave UT (5MHz centre frequency) and V110 UT with CBRs:
(d) V110 UT with rod#4 using a thin wire; (e) V110 UT with rod#0 using a small ball bearing.



(a)



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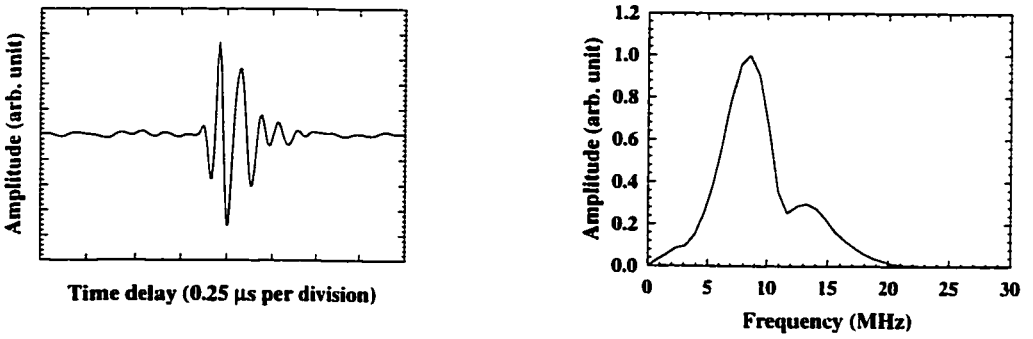


(c)

Fig.2.7: Time and frequency domain characteristics of CBRs operating with V112 longitudinal wave UT (10 MHz centre frequency):
(a) V112 UT with rod#1 using a thin wire as reflector; (b) V112 UT with rod#2 using a thin wire; (c) V112 UT with rod#3 using a thin wire.

Table 2.2: Pulse-echo responses of ultrasonic probes consisting of commercial UTs (5 MHz V110 and 10 MHz V112) with steel CBRs showed in Fig.2.3. For comparison purposes, the pulse-echo response of the commercial 5 MHz V110 UT was also measured. A PR35 pulser/receiver (JSR Ultrasonics Inc.) was used for the measurements.

5 MHz centre frequency (using Panametrics V110 Longitudinal UT, 6.35 mm diameter)			
	Pulse duration (ns)	**Centre frequency (MHz)**	**-3dB-bandwidth (MHz)**
Panametrics V110	350	5.2	4.2
rod#0	500	7.0	3.0
rod#1	320	9.6	2.8
rod#2	350	10.4	2.8
rod#4	300	8.8	4.0
10 MHz centre frequency (using Panametrics V112 Longitudinal UT, 6.35 mm diameter)			
	Pulse duration (ns)	**Centre frequency (MHz)**	**-3dB bandwidth (MHz)**
rod#1	275	8.5	4.0
rod#2	550	10.0	4.0
rod#3	510	8.5	3.5

B-scanning images of ultrasonic field distributions were obtained by the amplitude variation of the received signals scattered from the thin wire or small ball targets mentioned above. The scanning was carried out along lateral and axial directions, defining thus the yz-plane of Fig.2.8. For the thin wire target, the grid size spacings in the lateral and axial directions varied from 10 to 25 μm and from 10 to 50 μm, respectively, and for the ball bearing target, these values were 100 and 600 μm, respectively. The scanning required careful alignment of the probes with respect to the targets, in order to assure minimum deviation of the reflected ultrasonic beam. The results for commercial focusing UTs and ultrasonic probes consisting of commercial non-focusing UTs with focusing CBRs (rod#1,..., rod#4) are shown in Fig.2.9. Compared with commercial focusing UTs, the insertion of CBRs did not affect significantly the pattern of ultrasonic field distribution. However, the use of CBRs provides different spatial resolutions at the same frequency: it suffices to modify the lens shape in order to meet the resolution

required. For points of maximum ultrasonic energy, corresponding to Fig.2.10, the spatial (lateral and axial) resolution was also determined from the –3 dB amplitude range. The measured values are presented in Table2.3, along with the focal length. As expected, Table 2.3 indicates that higher spatial resolution is attained at higher frequencies [72]. All these parameters are taken into account when selecting probes for applications. For instance, ultrasonic imaging or detection of small particles should be carried out with probes exhibiting high spatial resolution together with sufficient SNR and signal strength.

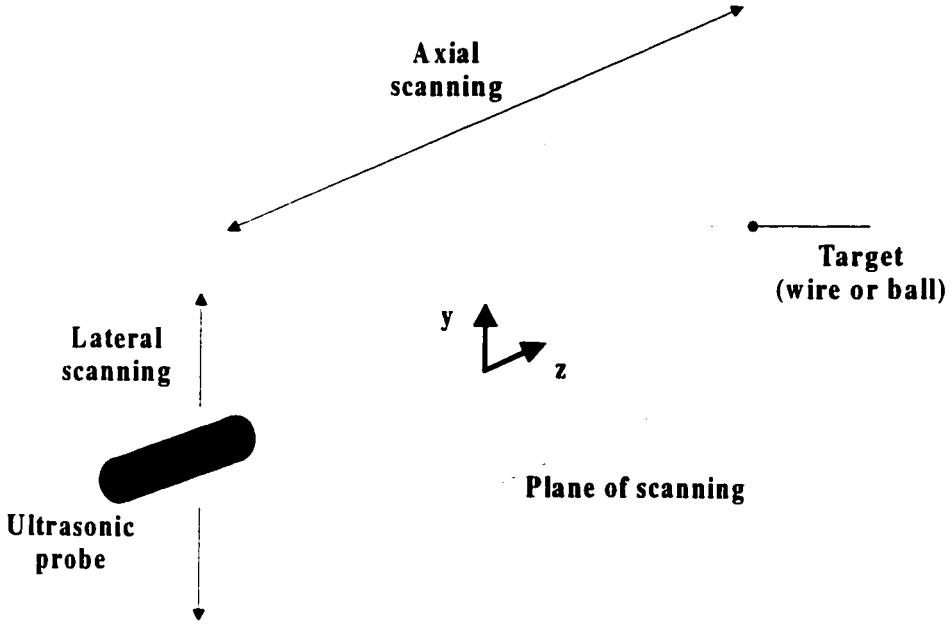
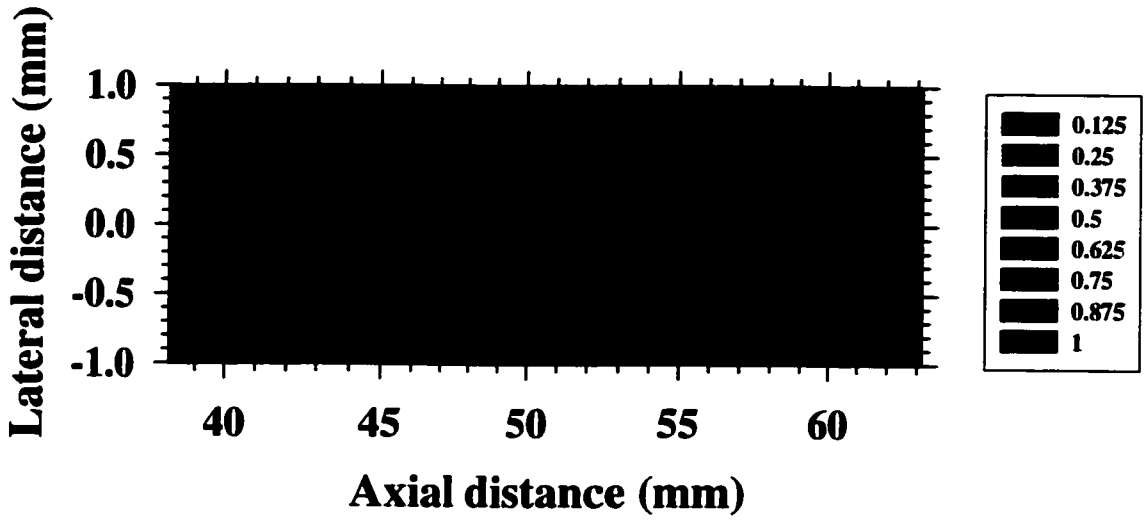
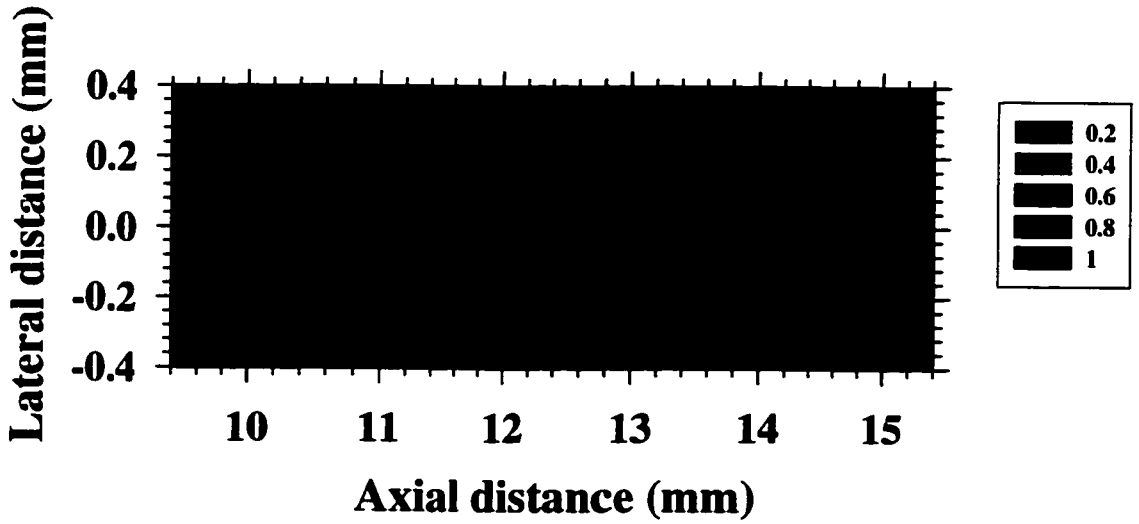


Fig.2.8: Schematic showing the plane on which B-scanning images of the ultrasonic field distribution were obtained.

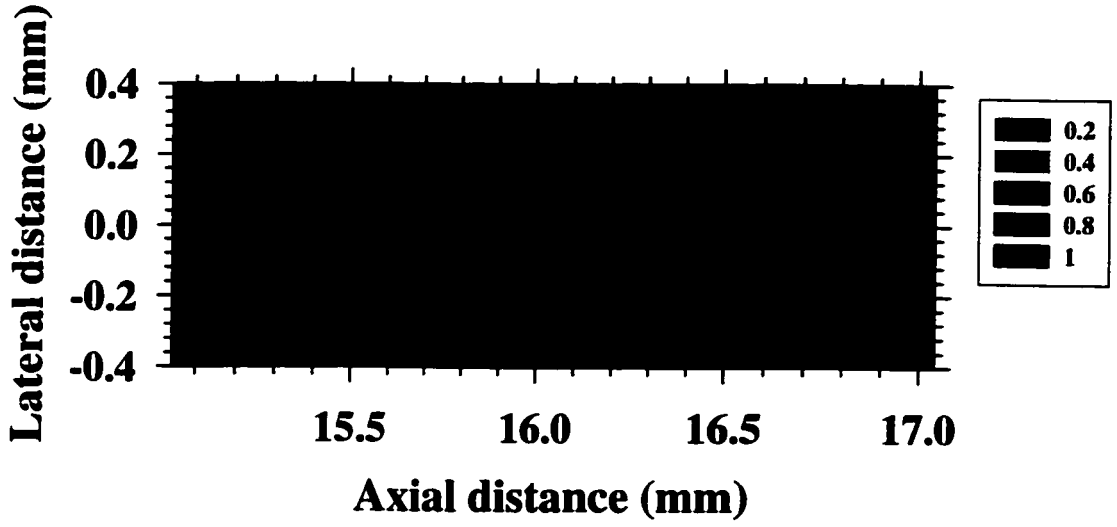


(a)

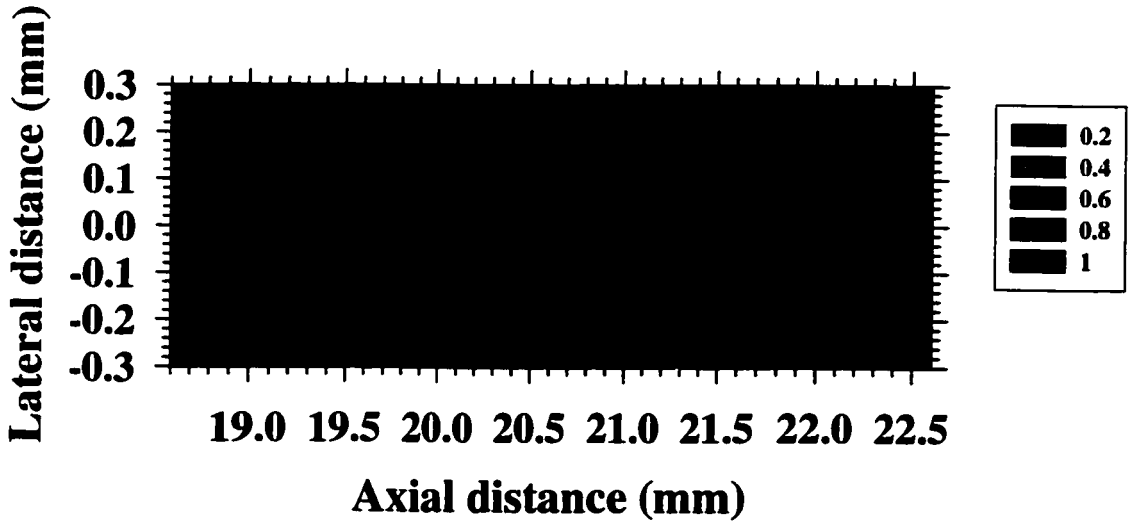


(b)

Fig.2.9: Contour plots of the ultrasonic field distributions of commercial focusing UTs and ultrasonic probes consisting of commercial non-focusing UTs with focusing CBRs. All images were generated with a thin wire as ultrasonic target.
(a) Focusing V307 UT (5 MHz centre frequency); (b) Non-focusing V110 UT (5 MHz centre frequency) with rod#1.

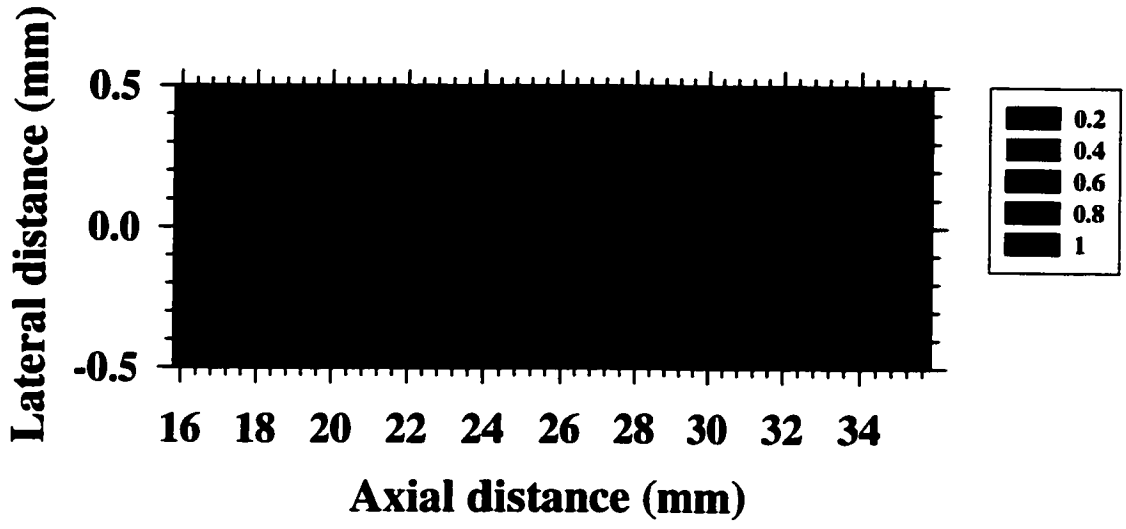


(c)

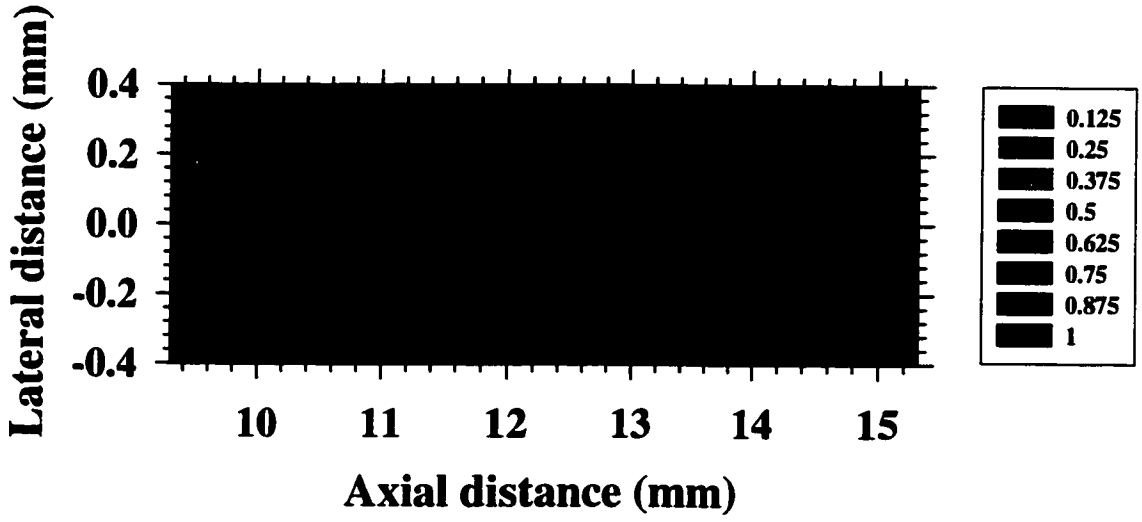


(d)

Fig.2.9: Contour plots of the ultrasonic field distributions of commercial focusing UTs and ultrasonic probes consisting of commercial non-focusing UTs with focusing CBRs. All images were generated with a thin wire as ultrasonic target.
(c) V110 UT with rod#2; (d) V110 UT with rod#4.

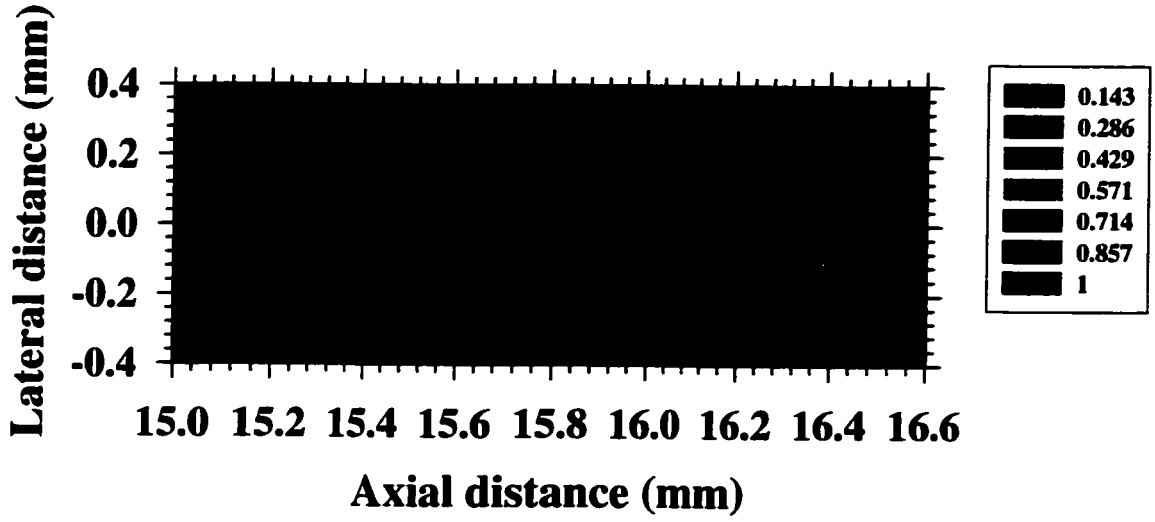


(e)

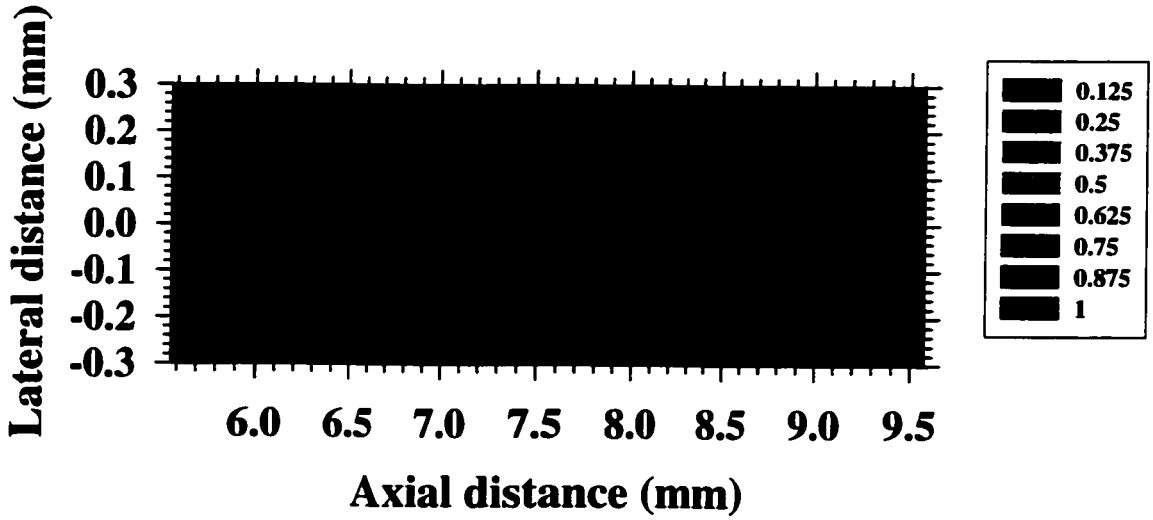


(f)

Fig.2.9: Contour plots of the ultrasonic field distributions of commercial focusing UTs and ultrasonic probes consisting of commercial non-focusing UTs with focusing CBRs. All images were generated with a thin wire as ultrasonic target.
(e) Focusing A315S UT (10 MHz centre frequency); (f) Non-focusing V112 UT (10 MHz centre frequency) with rod#1.

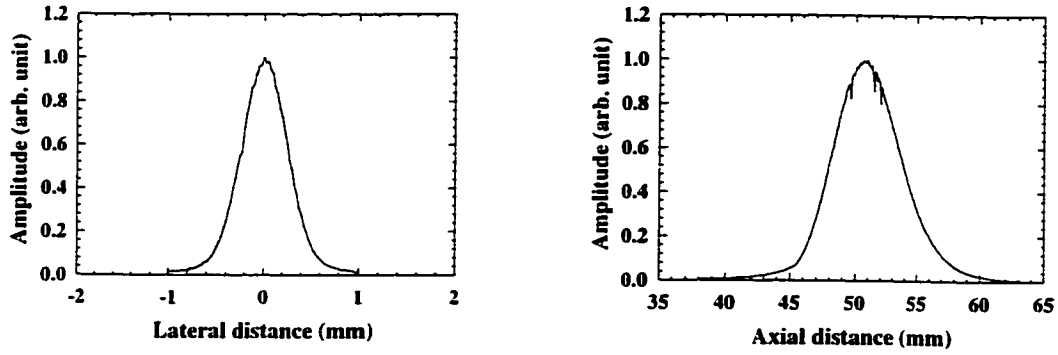


(g)

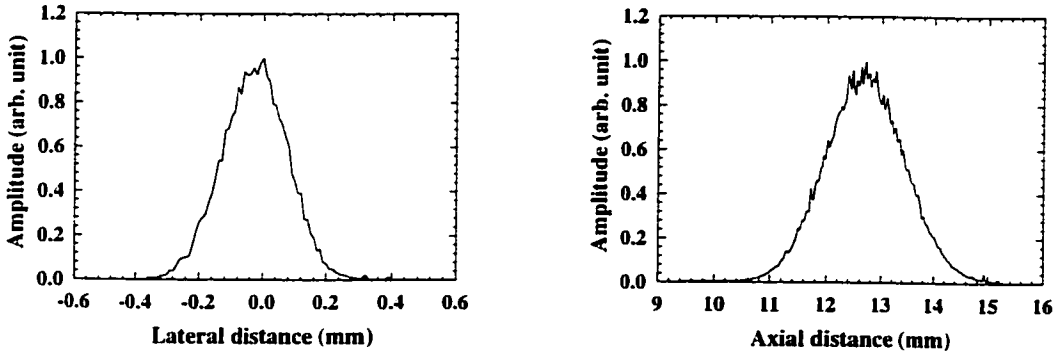


(h)

Fig.2.9: Contour plots of the ultrasonic field distributions of commercial focusing UTs and ultrasonic probes consisting of commercial non-focusing UTs with focusing CBRs. All images were generated with a thin wire as ultrasonic target. (g) V112 UT with rod#2; (h) V112 UT with rod#3.



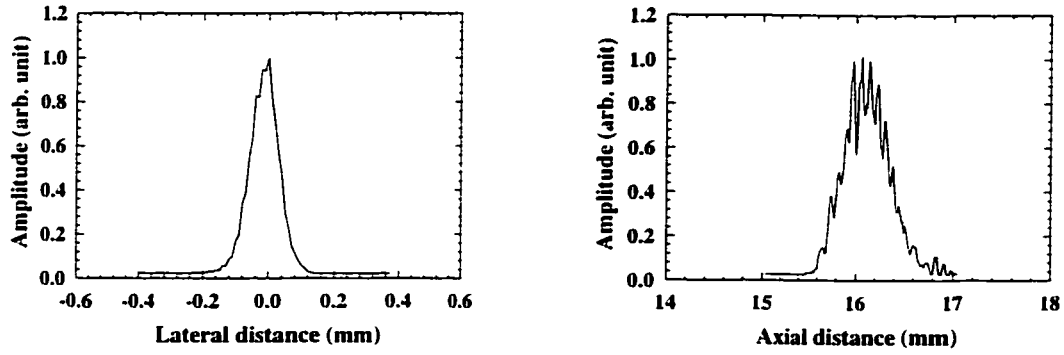
(a)



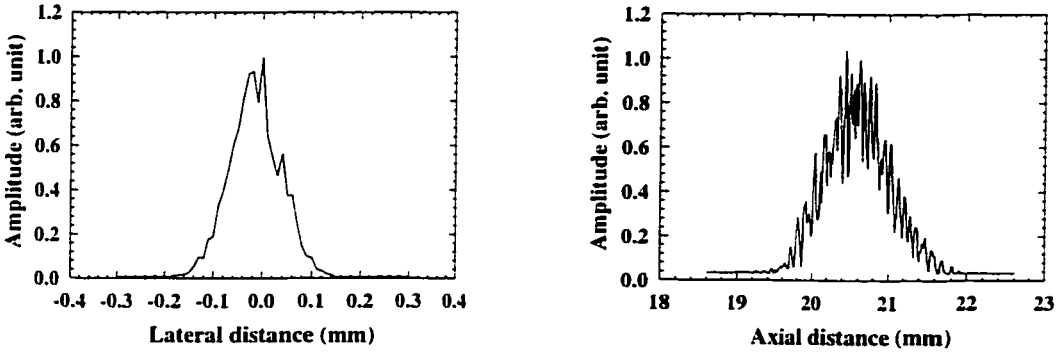
(b)

Fig.2.10: Lateral and axial resolution of commercial focusing UTs and ultrasonic probes consisting of commercial non-focusing UTs with focusing CBRs. All curves were generated at points of maximum ultrasonic energy reflected from a thin wire.

(a) focusing V307 UT (5 MHz centre frequency); (b) non-focusing V110 UT (5 MHz centre frequency) with rod#1.



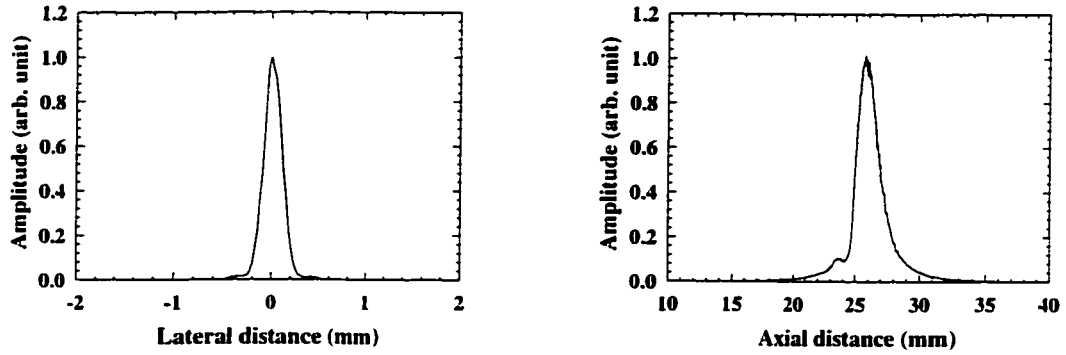
(c)



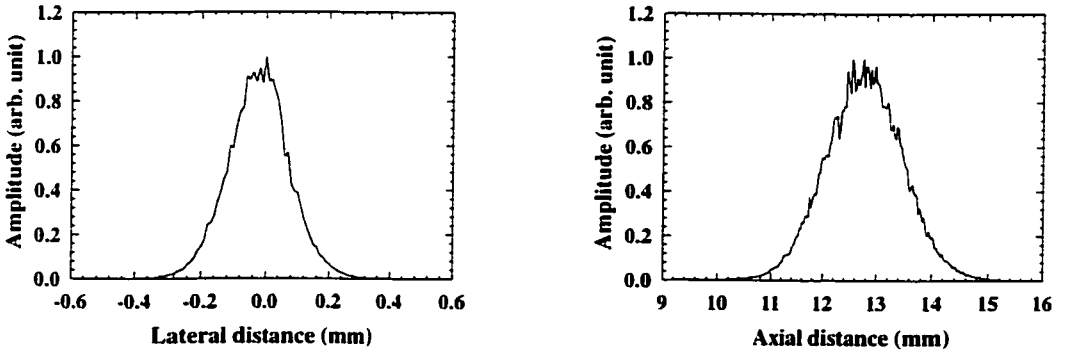
(d)

Fig.2.10: Lateral and axial resolution of commercial focusing UTs and ultrasonic probes consisting of commercial non-focusing UTs with focusing CBRs. All curves were generated at points of maximum ultrasonic energy reflected from a thin wire.

(c) V110 UT with rod#2; (d) V110 UT with rod#4.



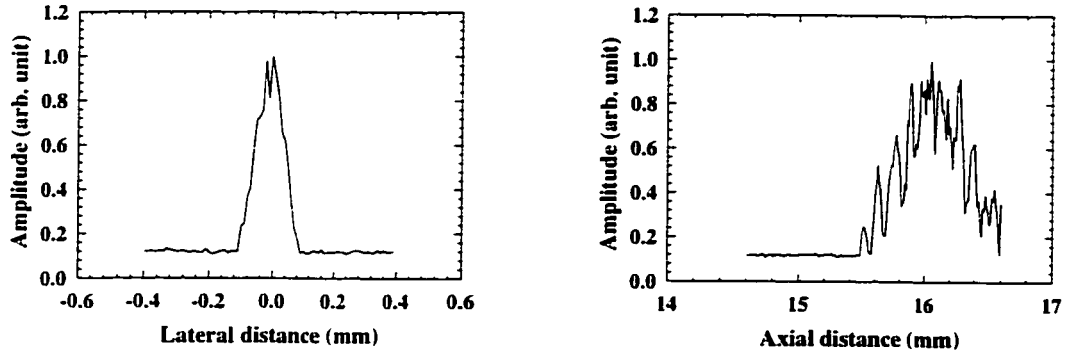
(e)



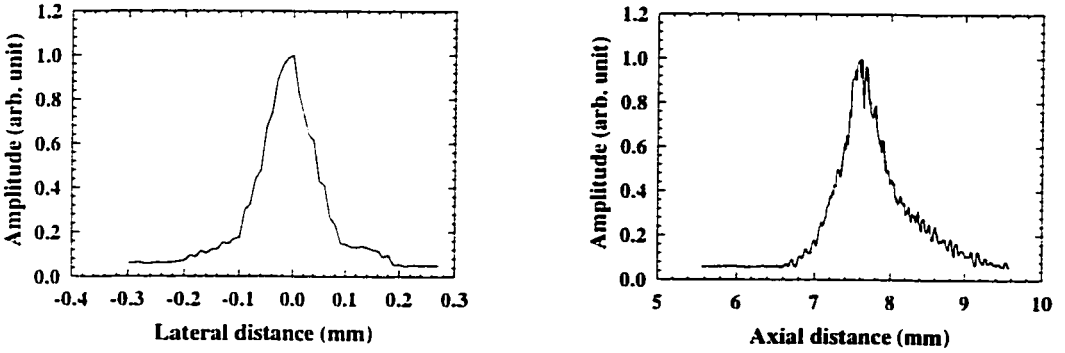
(f)

Fig.2.10: Lateral and axial resolution of commercial focusing UTs and ultrasonic probes consisting of commercial non-focusing UTs with focusing CBRs. All curves were generated at points of maximum ultrasonic energy reflected from a thin wire.

(e) focusing A315S UT (10 MHz centre frequency); (f) non-focusing V112 UT (10 MHz centre frequency) with rod#1.



(g)



(h)

Fig.2.10: Lateral and axial resolution of commercial focusing UTs and ultrasonic probes consisting of commercial non-focusing UTs with focusing CBRs. All curves were generated at points of maximum ultrasonic energy reflected from a thin wire.

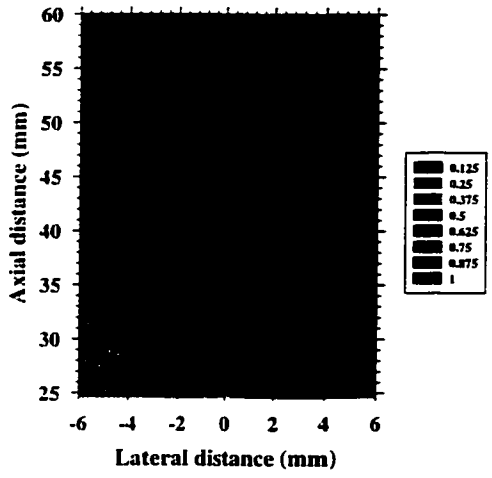
(g) V112 UT with rod#2; (h) V112 UT with rod#3.

Table 2.3: Spatial resolution of ultrasonic probes consisting of commercial UTs (5 MHz V110 and 10 MHz V112) and focusing steel CBRs shown in Fig.2.3. For comparison purposes, the spatial resolution of the commercial focusing UTs (5 MHz V307 and 10 MHz A315S) were also measured. A PR35 pulser/receiver (JSR Ultrasonics Inc.) was used for the measurements.

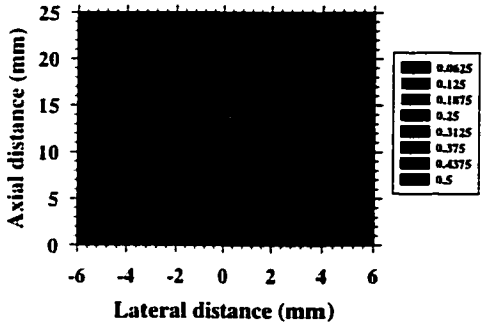
5 MHz centre frequency (using Panametrics V110 Longitudinal UT, 6.35 mm diameter)			
	Focal length (mm)	**Lateral resolution (μm)**	**Axial resolution (μm)**
Panametrics V307	50.5	400	4000
rod#1	12.7	160	1200
rod#2	16.1	100	500
rod#4	20.6	800	800
10 MHz centre frequency (using Panametrics V112 Longitudinal UT, 6.35 mm diameter)			
	Focal length (mm)	**Lateral resolution (μm)**	**Axial resolution (μm)**
Panametrics A315S	25.2	200	1500
rod#1	12.8	140	1100
rod#2	16.0	90	450
rod#3	7.6	80	500

The images of the ultrasonic field distributions for the commercial 5 MHz UT (V110) and the ultrasonic probe consisting of V110 with rod#0 (with a flat probing end) are shown in Figs.2.11 and 2.12, respectively. The amplitude variation along axial and lateral directions are presented in Figs.2.13 and 2.14, respectively. These figures indicate that the ultrasonic field distribution generated by rod#0 follows a pattern similar to the far field region (Fig.2.11(a)), *i.e.*, the ultrasonic echo amplitude decays gradually in both lateral and axial directions. Thus, unlike commercial UTs, the near field region (Fig.2.11(b)) is not generated at the exit of the rod#0 probe. The near field is the region right in front of the commercial UT, where the echo amplitude varies rapidly within very small distances both in lateral and axial directions, going through a series of maxima and minima due to diffraction effects [72]. Hence, the near field region should be avoided for ultrasonic quantitative measurements based on amplitude techniques. Naturally, this

cannot be achieved if the sample under inspection is thinner than the near field region of the UT. From our experimental observation, the use of CBRs with a suitable length overcomes this problem, and provides an effective approach for ultrasonic probing of thin samples, *e.g.*, the off-the-screw section of extruders, as it will be described in the next chapter.



(a)



(b)

Fig.2.11: Contour plot of the ultrasonic field distributions of the commercial V110 UT (5MHz centre frequency). All images were generated with a small ball bearing as ultrasonic target. (a) Far-field region; (b) Near-field region.

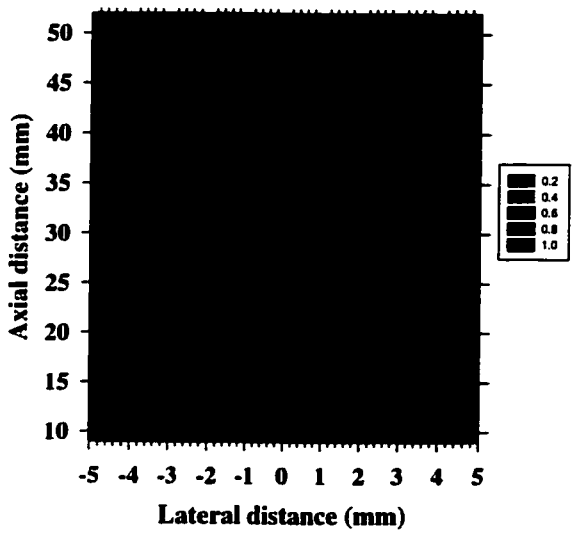


Fig.2.12: Contour plot of the ultrasonic field distribution of the probe consisting of the commercial V110 UT (5MHz centre frequency) with rod#0. The image was generated with a small ball bearing as ultrasonic target.

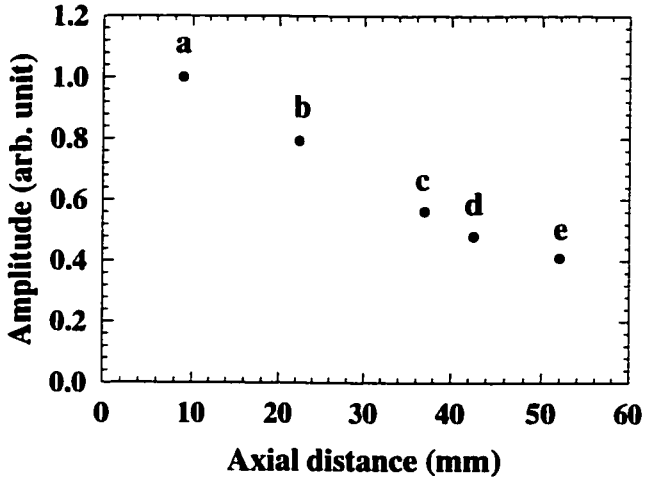


Fig.2.13: Amplitude variation of the ultrasonic field along axial direction for the probe consisting of the commercial V110 UT (5MHz centre frequency) with rod#0. Five given points apart from the probing end were selected. These points are indicated by the lowercase letters a, b, c, d and e.

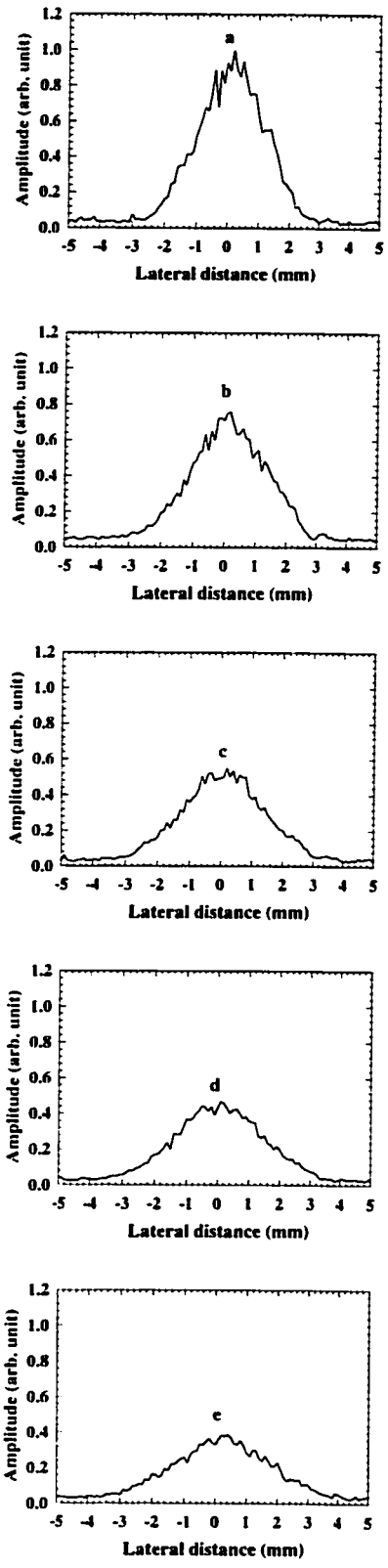


Fig.2.14: Amplitude variation of the ultrasonic field along lateral direction for the five axial distances given in Fig.2.13.

2.4-Numerical evaluation of clad steel buffer rods

Experiments have proven that the high performance of CBRs depends on selection of both proper materials and taper angle [39]. It seems to be difficult to analytically predict the pulse-echo response of buffer rods having a taper shape and thermal sprayed cladding. It would be highly desirable to use numerical simulations as a first guideline for buffer rod design. Here, we intend to verify whether the experimental pulse-echo response obtained for rod#0 (with flat probing end), operating at 5MHz, for example, can be simulated by existing software for acoustic wave propagation in bounded media. For this purpose, a program from CyberLogic Inc. (New York, NY) was chosen. This software is based on a finite difference method, and uses a two-dimensional isotropic model made by a discrete grid. The governing equations are the two coupled wave equations for displacements [86]:

$$\rho\frac{\partial^2 u_y}{\partial t^2}=(\lambda+2\mu)\frac{\partial^2 u_y}{\partial y^2}+\mu\frac{\partial^2 u_y}{\partial z^2}+(\lambda+\mu)\frac{\partial^2 u_z}{\partial y\partial z}$$

$$\rho\frac{\partial^2 u_z}{\partial t^2}=(\lambda+2\mu)\frac{\partial^2 u_z}{\partial y^2}+\mu\frac{\partial^2 u_z}{\partial z^2}+(\lambda+\mu)\frac{\partial^2 u_y}{\partial y\partial z}, \qquad (2.1)$$

where u_y and u_z are displacements in y and z directions, t is time, λ and μ are Lamé's constants of the medium, and ρ is the density. For longitudinal waves, the propagation direction is assumed to be along the z direction (the rod axis). A set of coupled finite-difference equations corresponding to Eq.(2.1) was used to compute the displacements u_y and u_z at each spatial grid point at every time step, under boundary conditions at every interface that impose continuity of stresses and displacements. The motion of longitudinal and shear waves in the medium can be obtained at every time step, as long as the stability requirement for the finite difference equations is satisfied. In order to make the stable calculation, the time step was chosen according to the von Neumann stability criterion [87], $\Delta t=\varepsilon/(V_L^2+V_S^2)^{1/2}$, where V_L and V_S are the longitudinal and shear wave

velocities, respectively, and ε is the grid spacing. In order to achieve sufficient accuracy in the calculation, the grid spacing was chosen to be smaller than the shortest wavelength related to the highest frequency in the pulse, and the lowest wave velocity in the medium. In this simulation, the grid points were spaced at intervals of 1/10 of shear wavelength.

For simulation, the materials properties at room temperature of the core and cladding of rod#0, and for water, are presented in Table 2.4. The cladding (1 mm thick) is modelled as having acoustic properties 10% higher than those of the core, which is assumed to be steel. The shear velocity in water is chosen arbitrarily small compared to the longitudinal velocity (10%), due to the requirements of the numerical simulation. The parameters used were: grid size = 6 μm for y and z directions, and time step Δt = 0.8 ns. A Gaussian longitudinal pulse of 5 MHz generated by a 6.35 mm diameter transducer is assumed.

Table 2.4: Materials parameters at room temperature for numerical evaluation of the ultrasonic field distribution generated by steel CBRs in water.

	ρ (kg/m^3)	$V_L = \sqrt{\lambda + 2\mu/\rho}$ (m/s)	$V_S = \sqrt{\mu/\rho}$ (m/s)
Core (mild steel)	7900	5900	3200
Cladding	8690	6490	3520
water	1000	1500	150

For rod#0, Figs.2.15 and 2.16 show the experimental and numerical results of the axial and lateral distribution in five points apart from the flat probing end of the rod#0. The experimental and numerical contour plots of the ultrasonic field distribution are shown in Fig.2.17. The experimental results have already been shown in Figs.2.12, 2.13 and 2.14, but they are repeated here for ready comparison. The experimental ultrasonic field distribution was obtained through variation of the ultrasonic pressure on the UT surface. In the numerical simulation, the ultrasonic field distribution was calculated from the particle displacement caused by the propagation of ultrasonic waves. From Figs.2.15 and 2.16, it can be noted that both experimental and numerical approaches have the same trend: the signal amplitudes gradually reduce along axial and lateral distances. In addition, the lateral amplitude distribution is broadened along the axial direction. This

similarity is expected, since pressure (or stress) is proportional to strain (Hook's law), which is directly related to displacement [72,85]. The peak values of the lateral amplitude distribution are normalised in Fig.2.16. The relative values are shown in Fig.2.15. The numerical result confirms that the ultrasonic field distribution at the exit of the clad buffer rod with a flat probing end behaves like the far field region, as it has been found experimentally.

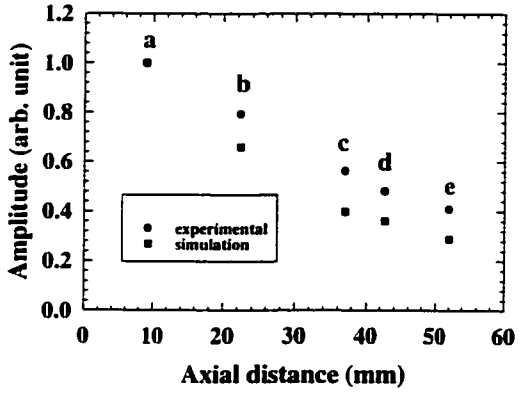


Fig.2.15: Experimental and numerical results of the axial amplitude distribution in five points apart from the flat probing end of rod#0. It is noted that the experimental result is the one shown in Fig.2.13.The centre frequency is 5 MHz.

The effect of the cladding on the SNR and signal strength of the ultrasonic pulse-echo response is shown numerically in Fig.2.18. For this simulation, the cladding of rod#0 is assumed to have three values for the ultrasonic velocities: 10, 20 and 40% higher than those of the core. The first echo corresponds to $\mathbf{L^1}$, *i.e.*, the first longitudinal round-trip echo inside the rod#0. It is clearly observed that the simulating cladding does affect the pulse-echo response of the buffer rod. The best SNR and signal strength is obtained for the case in which the longitudinal velocity of the cladding is 40% higher than the corresponding one in the core. However, at present, it seems not possible to fabricate CBRs that can have a rugged and high temperature cladding, and whose velocities are higher than those of the core. CBRs produced by the thermal spray technique still exhibit high ultrasonic performance.

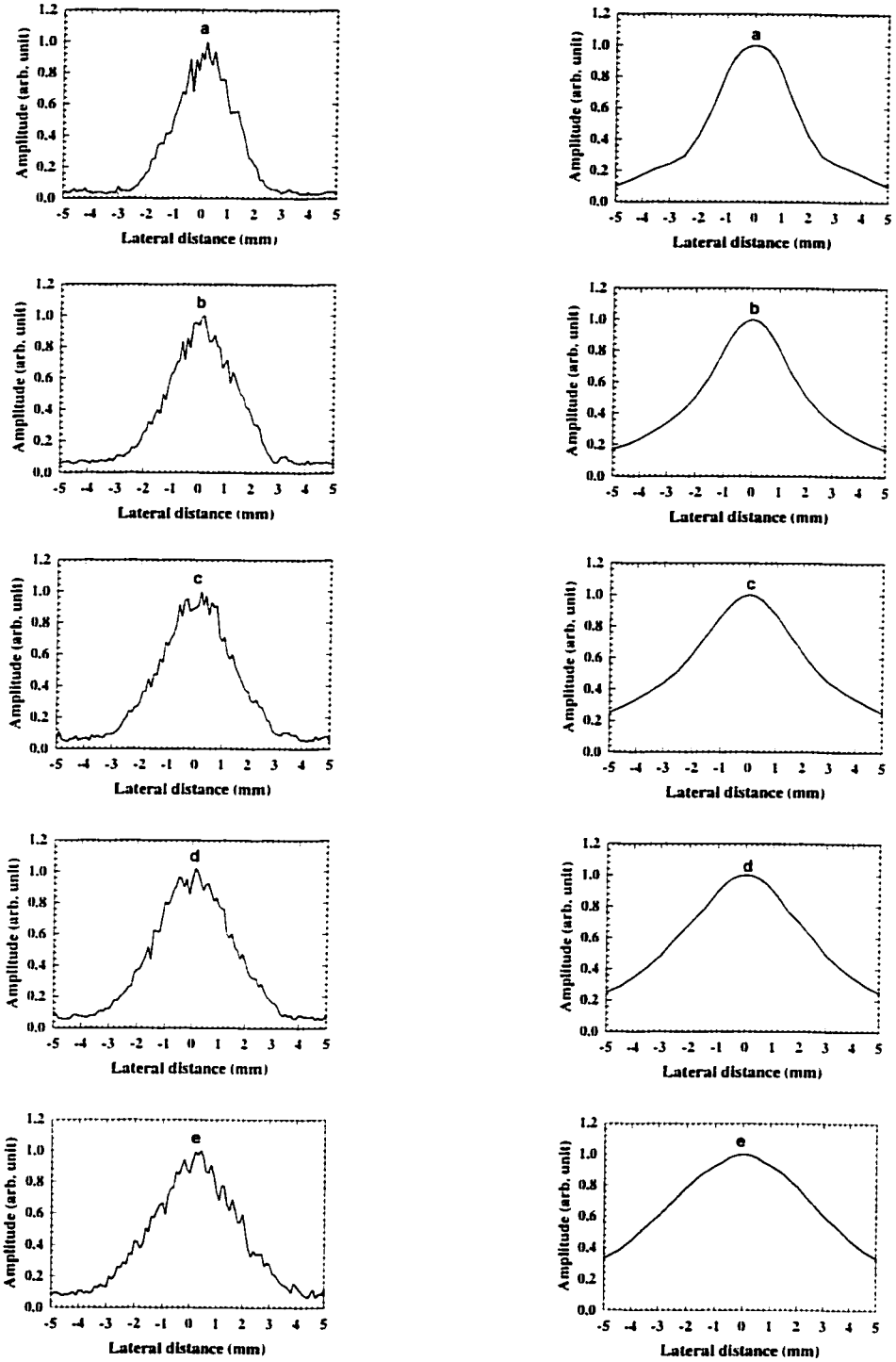
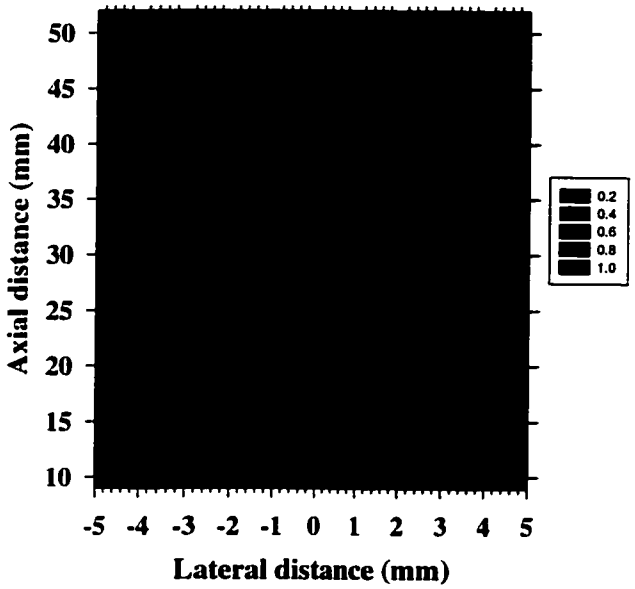
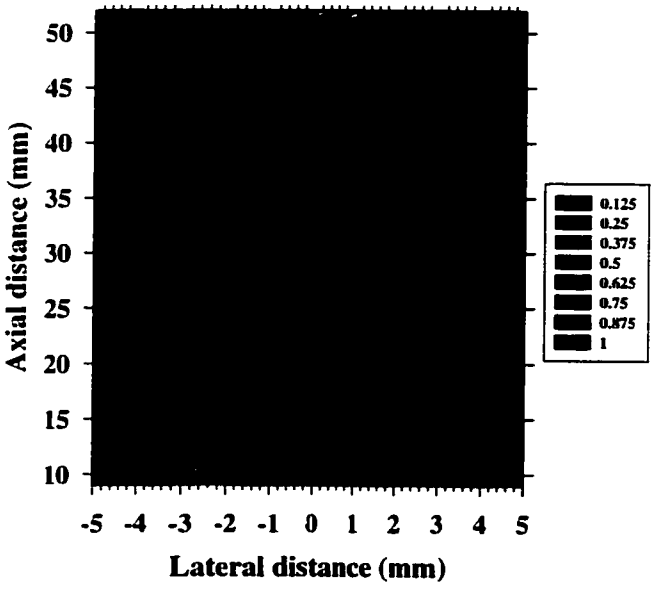


Fig.2.16: Experimental (left) and numerical (right) results of the lateral amplitude distribution in five points apart from the flat probing end of rod#0, as indicated in Fig.2.15. It is noted that the experimental curves are the same shown in Fig.2.14 (not normalised). The centre frequency is 5 MHz.

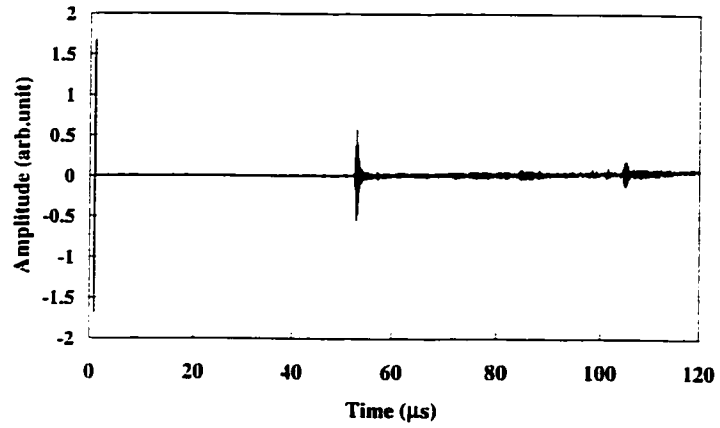


(a)

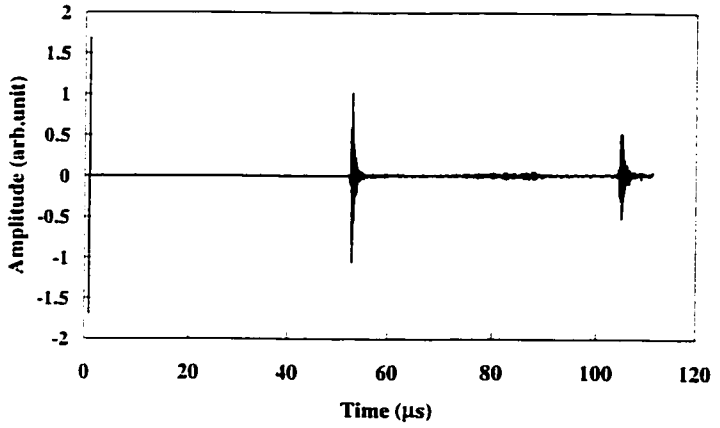


(b)

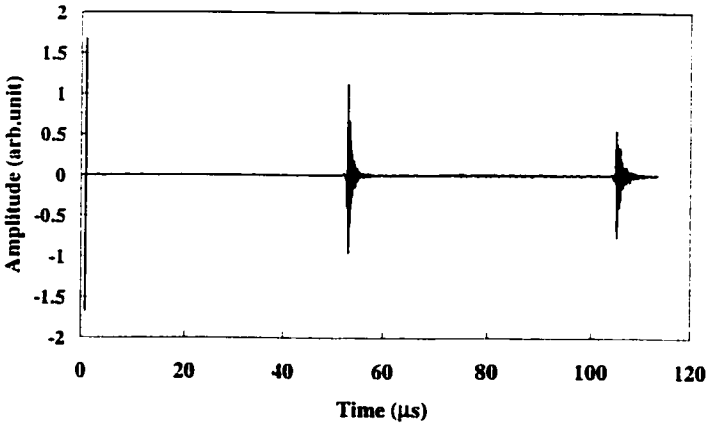
Fig.2.17: Contour plots of the ultrasonic field distribution at the exit of rod#0 (with a flat probing end). (a) Experimental result. (b) Numerical result.



(a)



(b)



(c)

Fig.2.18: Numerical results of the effect of the cladding (1 mm thick) on the SNR and signal strength of the ultrasonic pulse-echo response of the rod#0 (with a flat probing end). Ultrasonic velocities in the cladding are: (a) 10% higher than those of the core;. (b) 20% higher than those of the core; (c) 40% higher than those of the core.

2.5-Summary

The ultrasonic field distribution generated by the probe consisting of short and long CBRs with commercial 5 and 10 MHz UTs operating in pulse-echo mode were evaluated. This evaluation was performed through reflection over a thin tungsten wire or small ball bearing immersed in water. We examined the pulse duration, centre frequency and –3 dB-bandwidth of different combinations of CBRs and UTs. Compared with focusing UTs, the probe consisting of UT and focusing CBR exhibits similar pattern for the ultrasonic field distribution. In addition, CBRs can achieve different spatial resolutions at the same frequency by modifying the lens shape in order to meet the resolution required. With respect to the probe consisting of UT and CBR with a flat probing end, it was learned that at the exit the wave behaves as in the far field region. Thus, ultrasonic quantitative measurements based on amplitude techniques may be performed in thin samples.

For CBRs in which the velocities of the cladding are higher than those of the core, numerical simulation carried out by commercial software on acoustic wave propagation showed that the cladding has effect on the wave propagation. However, CBRs which have a thermal sprayed cladding still display high ultrasonic performance even being the velocities in the cladding less than those of the core.

Chapter 3

In-line Ultrasonic Monitoring of Polymer Extrusion Processes

3.1-Introduction

The ever-increasing need for high quality products puts strong pressure on improving process control systems. This is clearly illustrated by the large number of attempts to monitor polymer properties during processing [3-9]. On-line or side-stream rheometers have been developed to monitor the viscoelastic properties of flowing polymer melts, and to use these properties to control the process. Apart from problems inherent to mechanical devices, there are also a number of disadvantages associated with this test method. For example, only part of the flowing polymer is tested, and the sampled polymer stream may not be representative of the main stream. In addition, the sampled stream may have a different shear history than the main stream [41].

Because ultrasound causes no disruption of the process, and the characteristics of the signals passing through materials are strongly dependent on the properties of these materials, in-line ultrasonic techniques have found numerous uses in widely different fields of application, in particular to characterise polymers in molten states [3-9]. The benefits associated with the application of these techniques can be directly related to improved quality and reduced production costs [42]. For the polymer extrusion process, in-line ultrasonic techniques have been applied successfully in the exit of the extruder [3-5], where the through-transmission configuration using metallic non-clad buffer rods can be installed. However, due to the high spurious noises in such probes during pulse-echo mode, no monitoring has been carried out in the off-the-screw section of the barrel.

Monitoring of this section is important to reveal the transient behaviour (melting, mixing, blending and conveying) of the polymer extrusion process, which may not be assessed in the extruder exit.

To overcome all the above drawbacks, one of the objectives of this thesis research is to provide ultrasonic sensors and techniques for in-line characterisation of polymer melts under processing conditions, suitable for monitoring at different sections of the extruder barrel. The pulse-echo mode is chosen in our applications because it requires access to only one side of the equipment, and is therefore appropriate for off-the-screw inspection. These extrusion processes typically reach temperatures on the order of 200°C. Since high-temperature piezoelectric UTs are generally not reliable, the classic approach using buffer rods is adopted here, due to its simplicity and low cost. The CBRs, composed of a central core and an outer cladding, provide good SNR when used in ultrasonic pulse-echo instruments [27,38,88], as illustrated in the previous chapter. One end of the CBR (the UT end) is air-cooled so that the high performance room temperature UTs can be readily used, while the probing end contacts the polymer melt, but without disturbing the process. A schematic of the CBR used here for in-line monitoring of extrusion processes is shown in Fig.3.1. This CBR was characterised in Chapter 2 (designated as rod#0) in terms of its ultrasonic field distribution and pulse-echo response. It was demonstrated that the ultrasonic field at the probe exit behaves like the far field region, and therefore it may be used for quantitative off-the-screw measurements.

Here, for the first time, in-line ultrasonic techniques using steel CBRs in the pulse-echo mode are developed to monitor: 1) a two-layer polymer extrusion in the co-extrusion blow moulding process; and 2) the ultrasonic properties of the polymers under processing in a twin-screw extruder, at a given screw location in the barrel section. With respect to the co-extrusion blow moulding process, the objective of this study focuses on the type of information that can be obtained by our novel ultrasonic technique during the process, such as the location and degree of adhesion of the interface between polymers and its stability. For the twin-screw extrusion process, the goal is to validate the

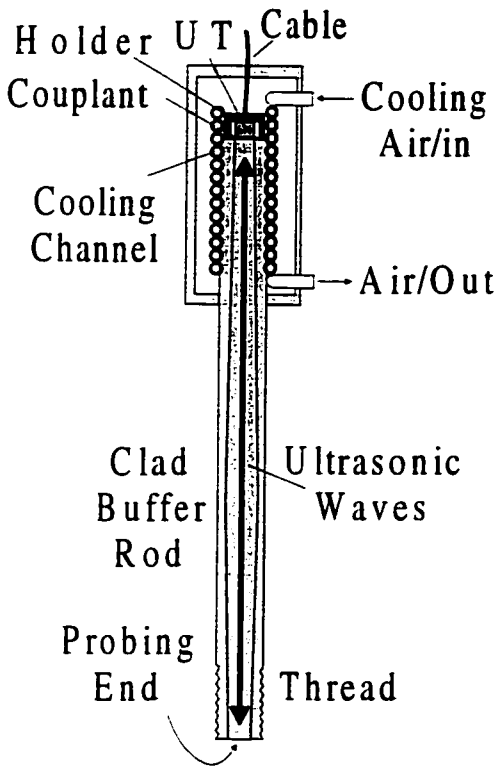


Fig.3.1: Schematic of the steel CBR.

implementation of the ultrasonic technique at the off-the-screw section of the extruder machine, in order to provide information on the transient states or properties of the polymer material being processed.

3.2-Monitoring of the co-extrusion process

3.2.1-Experimental setup

The acquisition system was set up with a Pentium® PC and a 50 MHz double-channel acquisition card (CS-250, Gage Applied Science, Inc.). This card has 8-bit resolution and 8M sample on board memory. The ultrasonic sensors, both 5 and 10 MHz

longitudinal UTs (6.35 mm element diameters), were driven by a 5055PR pulser/receiver (Panametrics, Inc.). Signals were digitised, stored, processed and displayed using Labview® software.

In the co-extrusion blow moulding process, the location of the interface, *i.e.*, the thickness of the individual layer, is dependent on the head and die geometrical parameters, material properties and processing conditions such as relative flow rates, extrusion temperatures and parison programming. In practice, for a given head and die setup and material combination, the desired interface location is achieved following a time-consuming trial and error procedure including off-line measurement [89,90]. To facilitate the optimisation of this process, ultrasonic sensors and techniques are developed to determine in-line the location of the interface and its stability.

The experiments were carried out using a co-extrusion blow moulding machine (Placo model 3xy5.5_7), shown in Fig.3.2. The materials employed in this work were blow moulding grade high-density polyethylene (HDPE DMDF 6200, melt index M.I.= 0.40, from Petromont™), and a polypropylene-EPDM-based thermoplastic elastomer (Santoprene 101-55 from Advanced Elastomer Systems, L.P.). For the sake of brevity, Santoprene will be used for the remainder of this chapter. An annular die having a die gap of 2 mm and a length of 101.6 mm was used. The machine was operated in intermittent mode in cycles of about 45 s.

Since the extrusion temperature was maintained close to 200°C, monitoring was carried out using steel CBRs, which are shown to provide a good SNR [27,38,88]. For this investigation, the CBRs are composed of a steel core, a stainless steel inner cladding and a bronze outer cladding. The stainless steel inner cladding is to ensure the proper ultrasonic guidance in the core, and the bronze outer cladding is solely for machining purposes. The steel core and cladding were chosen to match the thermal characteristics of the extrusion die. This steel CBR has a length of 131 mm and a probing end diameter of 7.8 mm. The UT end of the rod was cooled with air-cooling pipes to keep it below 50°C,

so that high performance room temperature UTs could be utilised. 10 MHz longitudinal broadband UTs were selected for this experiment. The length of the CBR was chosen such that the air cooling would not affect the temperature at the probing end of the CBR. The probing end of the CBR was machined to the same shape as the Dynisco pressure (PT462E-5M) and pressure/temperature (TPT463E-1M) sensors. Besides preserving the UT from high temperature exposure, this shape allows it to be applied in a variety of material processes. It also permits the three types of sensors to be interchanged depending on the desired monitoring purposes. Fig.3.3 shows the installation of the steel CBRs in the extruder die, together with commercial temperature and pressure sensors.

3.2.2-Off-line ultrasonic characterisation of the interface between HDPE and Santoprene layers of a co-extrusion blow moulded part

The major purpose of this experiment is to verify how versatile ultrasonic technique is to assess the adhesion quality between two polymers processed by co-extrusion. The polymer sample under investigation is the one shown in Fig.3.4 (a bottle made by co-extrusion blow moulding of HDPE and Santoprene). A sample of this bottle exhibiting both good and poor adhesion was selected, and immersed in water for ultrasonic testing; the results are displayed in Fig.3.5. In the case of good adhesion, three echoes are generated corresponding to water/HDPE boundary $\mathbf{L_1}$, HDPE/Santoprene interface $\mathbf{L_2}$ (designated as the interface echo) and Santoprene/water boundary $\mathbf{L_3}$, respectively. As expected, the amplitude of the interface echo is rather small, since the acoustic impedance mismatch between HDPE and Santoprene is not sufficient to yield a high reflection coefficient. However, in the case of poor adhesion, the presence of the gap prevents the transmission of ultrasound to the Santoprene, resulting in only two echoes instead of three, and a larger amplitude of the second echo. Even in the case where the interface echo is too weak to be detected, the adhesion quality can still be inferred from the total travel time in the polymers. Such results are quite encouraging, in the sense that ultrasound can be an effective technique in the quality control of polymer adhesion during

Fig.3.2: View of the co-extrusion machine from PLACO.

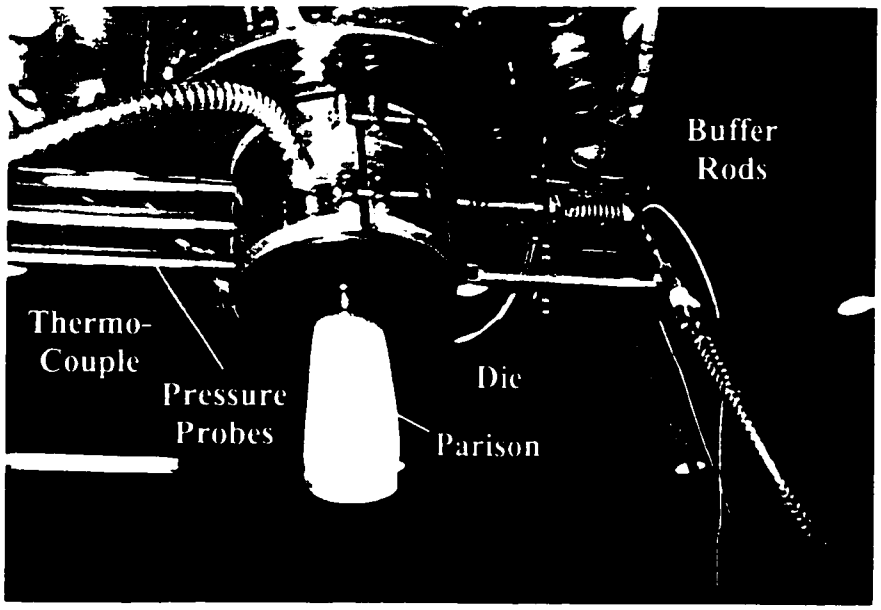


Fig.3.3: CBRs installed in a die of a co-extrusion blow molding machine from PLACO.

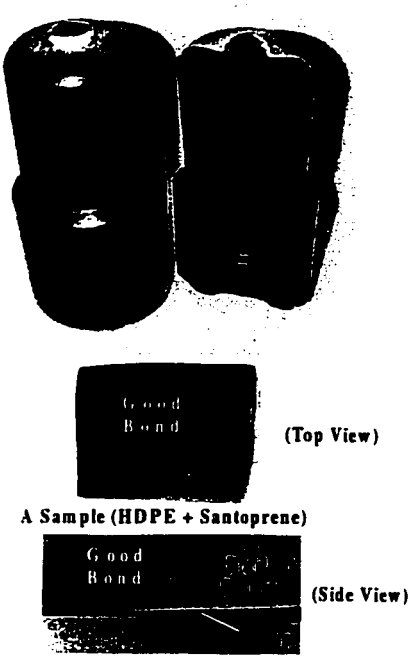


Fig.3.4: Plastic bottle made by co-extrusion of HDPE and Santoprene.

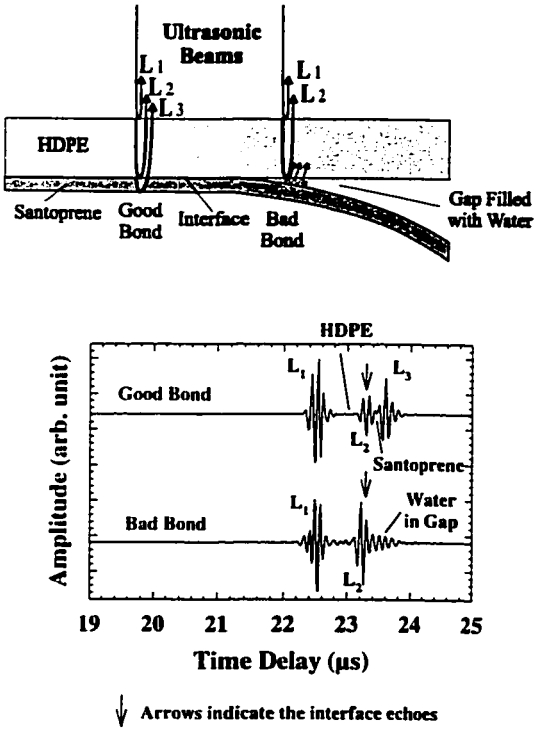


Fig.3.5: Ultrasonic monitoring of the interface between HDPE and Santoprene layers of a co-extrusion blow molded part.

the co-extrusion process, as the amplitude of the interface echo is directly related to the quality of the adhesion.

3.2.3-In-line monitoring of interface position and process stability during co-extrusion

In this section we present the in-line ultrasonic monitoring of the interface position and its stability, during co-extrusion of HDPE and Santoprene in the Placo machine. The reflected echoes from the probing end of the CBR show excellent SNR (Fig.3.6), where $\mathbf{L^1}$ and $\mathbf{L^2}$ are the 1st and 2nd round trip echoes in the CBR, respectively. The upper, middle and lower curves in Fig.3.7 display the typical reflected echoes during the single extrusion of 2 mm thick HDPE, single extrusion of 2 mm thick Santoprene, and co-extrusion of HDPE/Santoprene (~1 mm thick for each layer, HDPE being located on the UT side), respectively. $\mathbf{L_2}$, $\mathbf{L_4}$ and $\mathbf{L_6}$ are the 1st, 2nd and 3rd round trip echoes inside the extruded polymer, 2 mm in total thickness. In Fig.3.7 the longitudinal velocities, V_L, of the molten HDPE and Santoprene under extrusion conditions are 1275 and 1110 m/s, respectively, as computed from the time delay between consecutive echoes and the total travel distance during the single extrusion. The ultrasonic attenuation is higher in Santoprene than in HDPE, as seen from the rapid diminution of the amplitude of the signal from one echo to the other for Santoprene when compared to HDPE.

The stability of the extrusion process can be determined by the absolute time delay of echo $\mathbf{L_2}$ ($\mathbf{L_2}$-$\mathbf{L^1}$) or $\mathbf{L_4}$ ($\mathbf{L_4}$-$\mathbf{L^1}$), because it is related to the properties of the extruded polymers. Since the ultrasonic travel time in $\mathbf{L_4}$ is twice that in $\mathbf{L_2}$, the time delay of $\mathbf{L_4}$ is more sensitive to interface stability, *i.e.*, interface instability can be detected more easily with this echo. Figs.3.8 and 3.9 show the signals from a stable and an unstable run, respectively, for the co-extrusion of HDPE and Santoprene. In both figures, the spurious signals (acoustic noise generated and propagated in the CBR) and the desired signals are interspersed, making the identification of the interface between two polymers

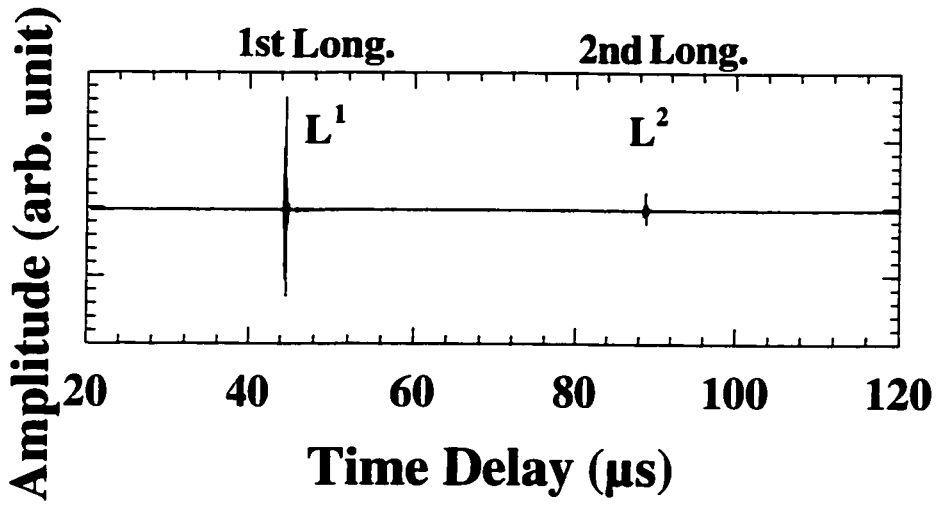


Fig.3.6: Reflected longitudinal wave signals from the end of the CBR..

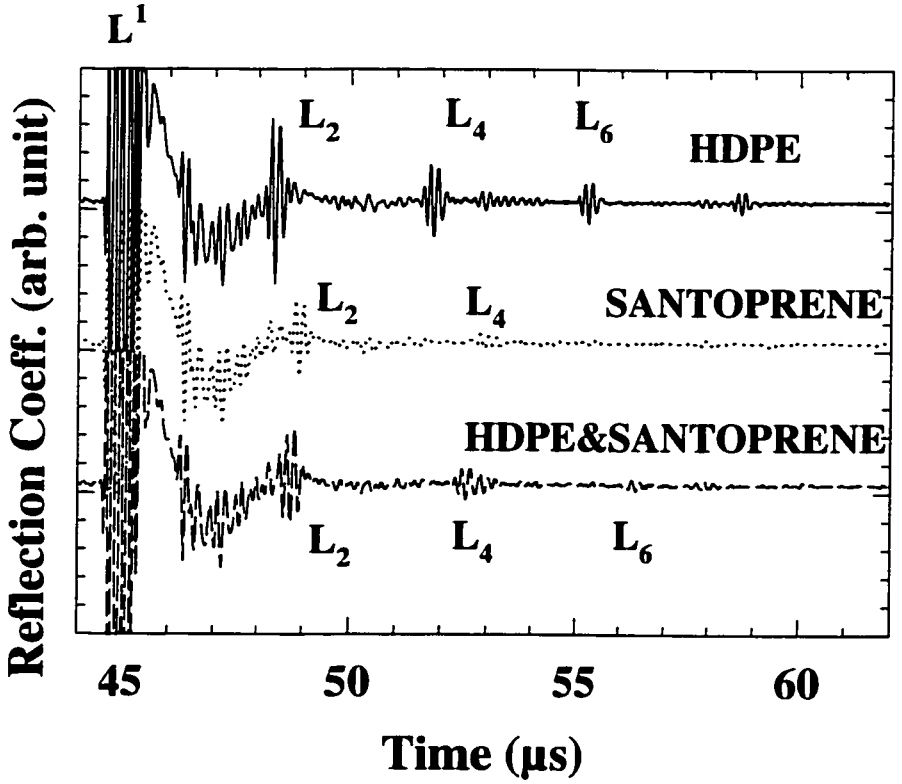


Fig.3.7: Reflected longitudinal wave signals during extrusion.

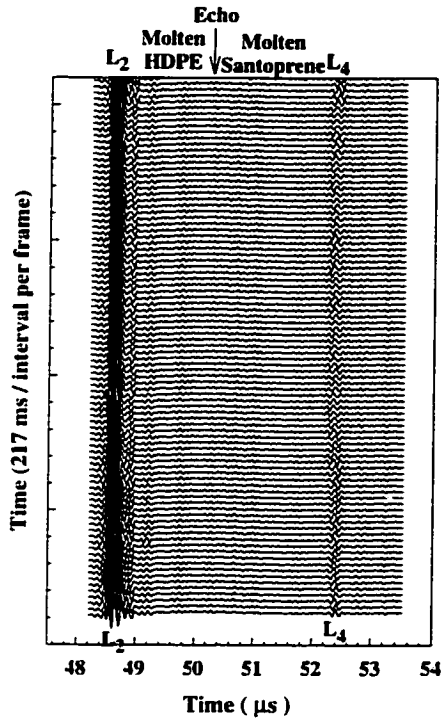


Fig.3.8: Stable run of the co-extrusion of HDPE and Santoprene.

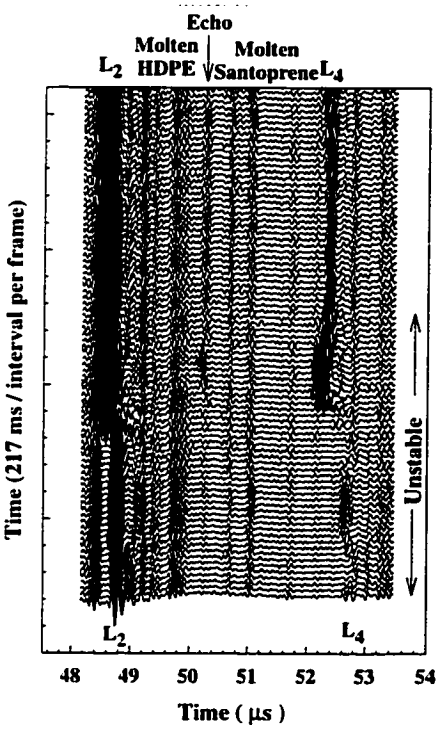


Fig.3.9: Unstable run of the co-extrusion of HDPE and Santoprene.

very difficult. However, the interface echoes are identified using three factors. The first factor is the fact that the time delay of this echo is almost synchronous with respect to those of $\mathbf{L_2}$ or $\mathbf{L_4}$. The second factor is the variation of this signal compared to the noise. Due to the high ultrasonic attenuation in molten polymers, and the large impedance mismatch between the CBR and the extruded polymers, the amplitudes of the signals $\mathbf{L_2}$, $\mathbf{L_4}$ and the interface echo are slightly larger than those of the spurious signals [88]. However, these spurious signals remain essentially unchanged both in amplitude and time delay during the extrusion, while the amplitude of $\mathbf{L_2}$, $\mathbf{L_4}$ and interface echo varies with time. The third factor is the estimated position of the interface echo. From the flow rate controller, it is estimated that the interface exists almost in the middle of the 2 mm gap between the two die walls. Even though the pressure and temperature during the co-extrusion are not identical to the conditions during the single extrusion of HDPE and Santoprene, the V_L of the molten HDPE and Santoprene at the co-extrusion conditions can still be estimated at 1275 and 1110 m/s, respectively. Thus, the time delay of the interface can be roughly calculated. As described in the previous section, it is also speculated that the larger the amplitude of this interface echo, the worse the adhesion between the two polymers.

3.2.4-Improvement of the interface echo by signal processing

Besides information about the stability of the co-extrusion process, the precise identification of the interface echo leads to both the thickness measurement of each polymer layer and the adhesion quality between them. For a given set of temperature and pressure, the ultrasonic velocity and attenuation can be simultaneously determined. This information may be used to in-line monitor the thickness and adhesion quality of the polymer layers. The thickness of each polymer layer is calculated from the measured travel time inside the layer and its corresponding velocity. On the other hand, adhesion quality between polymers is related to the magnitude of the interface echo. The higher its

magnitude, the higher is the reflection coefficient of the interface echo, and the worse is the adhesion between polymers.

As mentioned previously, the spurious signals are mainly generated by the CBR, and as a consequence their frequency components are the same as those of reflected echoes L_2, L_4 and L_6. Therefore, filtering techniques based on frequency selection cannot be used. Similarly, improvements cannot be accomplished by subtraction from a reference signal, since signal phases shift slightly during the co-extrusion process, because of variations in temperature and pressure. To overcome this problem, deconvolution methods were investigated. It was found that Wiener Filtering, a classic approach widely adopted in seismic and non-destructive evaluation signal processing [91-95], provides particular advantages in treating the tiny interface echo in the co-extrusion of HDPE and Santoprene, since a reference signal can be properly chosen. Theoretical aspects of this technique and the relevant equations are discussed in detail in Appendix A.

The Wiener Filter algorithm was programmed, and signal processing was carried out for both stable and unstable runs. Figs.3.10 and 3.11 illustrate the filtered data using 256-point FFT (Fast Fourier Transform) of the stable and unstable runs, respectively, as compared to the raw data shown in Figs.3.8 and 3.9. The interface echo has been significantly improved, and the noise remarkably reduced. This algorithm can be implemented in the Labview® environment to provide simultaneous filtering of the raw signals, and display of the filtered signals during data acquisition.

3.3-Monitoring of polymer processing in a twin-screw extruder

Single and twin-screw extruders have different end uses. The main functions of a single-screw extruder are to melt and pump the polymer through a die that will give the extrudate a particular shape. More advanced applications require the use of a twin-screw extruder. Because it has a modular design, screw and barrel configurations can be altered

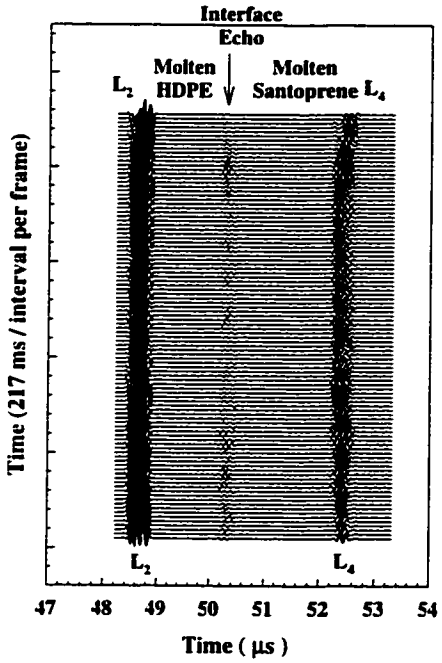


Fig.3.10: Wiener Filter deconvolution applied to stable run of the co-extrusion of HDPE and Santoprene.

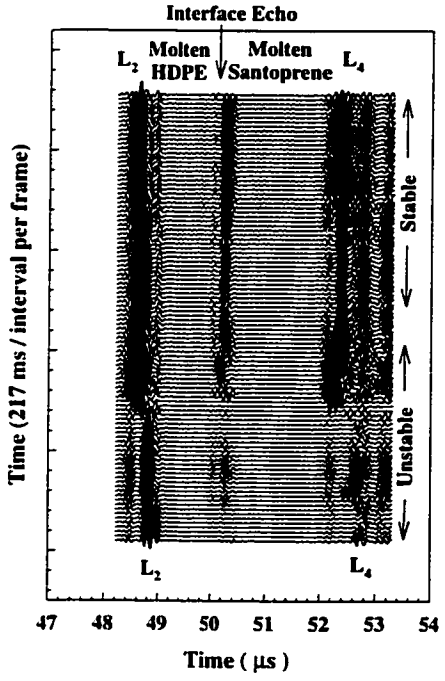


Fig.3.11: Wiener Filter deconvolution applied to unstable run of the co-extrusion of HDPE and Santoprene.

by modifying the sequence of the screw and barrel elements, to match the exact needs for each particular application. Since the flow pattern can be controlled through an adequate selection of the screw elements, one can design a twin-screw extrusion process like a continuous chemical reactor, with temperatures and residence times as part of the process design. Twin-screw extruders also have excellent mixing capability, which makes them the preferred choice for compounding applications. However, even though an accurate control of the properties and performance of the material is extremely important to meet target applications, characterisation of the resins is commonly performed off-line, *i.e.*, in the laboratory on discrete samples taken from the process line. Off-line techniques are usually not adequate for industrial quality control due to long delay time. In-line monitoring, where applicable, allows feedback control and optimisation of the process. Ultrasonic techniques appear to provide several advantages over conventional methods, and have already been explored for compounding applications, through ultrasonic measurements performed at the die level [5]. However, since the material properties are gradually transformed along the extruder from the feed throat up to the die exit, as the material undergoes mixing, chemical changes, or mechanical and thermal degradation, there is a strong interest in having access to these properties at any location throughout the process, and not only at the die level. This approach is explored in the present work. The same ultrasonic sensor described in the previous section is employed.

3.3.1-Experimental setup

The experiments were carried out in a co-rotating, intermeshing twin-screw extruder (Leitstritz, 34 mm diameter, length to diameter ratio L/D = 42) using an extrusion grade high-density polyethylene (HDPE H-1008-A, melt index[1] M.I.=0.25) and polystyrene (PS-115, M.I.=3.5), both from Nova Chemicals Ltd. The twin-screw extruder has a modular design, and allows the screw to be assembled in different configurations depending on the application. In this work, a section of the barrel has been specially

[1] Melt index (M.I.) is the number of grams of polymer extruded in a time period of 10 minutes.

designed to permit installation of different sensors for monitoring purposes. The twin-screw extruder and sensors are shown in Fig.3.12.

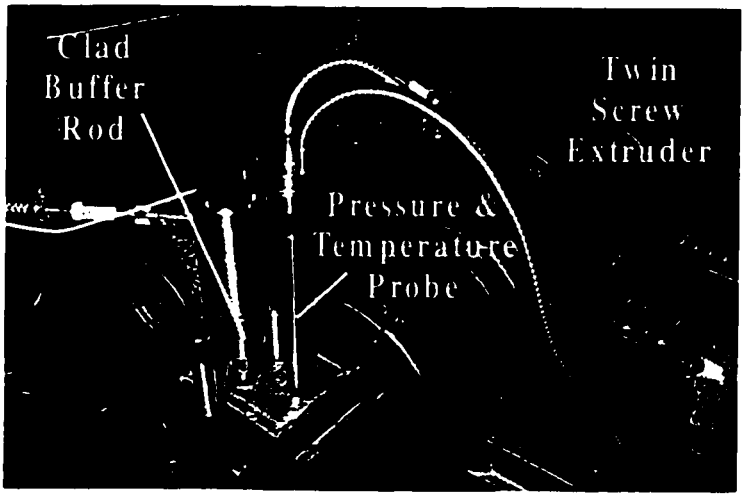


Fig.3.12: The twin-screw extruder from Leitstritz and sensors.

The CBR was installed in such a way that its tip was flush with the barrel inner wall, as shown schematically in Fig.3.13. Twin-screw extruders are typically starve-fed, which implies that some sections may be only partially filled with polymer. The signal in the polymer can only be obtained if the polymer is in contact with the CBR, so a starved screw would lead to the absence of the signal. The screw configuration is then designed with reversed elements at the end of the monitored zone, to ensure that the screw is full of polymer in this section during the measurements.

3.3.2-Ultrasonic responses for different screw elements in water

Before carrying out the in-line monitoring in the twin-screw extruder, it is essential to understand the ultrasonic probe (UT and CBR) response to both rotational and translational deviations from the optimal alignment, where the reflected ultrasonic energy is supposed to be at a maximum. This is due to the curved surface of the screw, which splits the ultrasonic energy emitted upon it, resulting in null reflection on the

transducer. Furthermore, polymers in general are very attenuative to ultrasonic waves [3], and it is necessary to reduce as much as possible the ultrasonic path in such media. To maximise the performance of the ultrasonic probe, the reduction of the detected signal amplitude caused by misalignment while installing the CBR inside the extruder barrel must be accounted for. Since it is not practical to study the sensitivity of the ultrasonic response to translation and rotation of the probe with respect to the reflector in molten polymer, this part of the work was carried out in water. The screw element was immersed in a water tank (the same described in Chapter 2) with a positioner and a turntable controlled by a stepping motor. The turntable is used for rotational scanning, and the positioner for axial scanning of the screw. Four different screw elements, shown in Fig.3.14, were examined.

The first step of the experiment in water consisted of analysing the ultrasonic response (reflected signal magnitude and time delay) for different relative positions between the ultrasonic probe and screws. The screw element (reflector) was fixed at the centre of the turntable, and the ultrasonic probe on the positioner. The movements of both the turntable and the positioner were controlled until the magnitude of the reflected signal was at a maximum. A 5 MHz longitudinal transducer (6.35 element diameter) in pulse-echo mode was used along with an oscilloscope. Next, the turntable and the positioner were set, one at a time, to automatic rotation in steps of 0.1 degree and axial translation in steps of 0.1 mm, respectively. Fig.3.15 illustrates the different screws investigated, and their corresponding ultrasonic responses. Table I provides the descriptions of these screws. With respect to the first screw shown in Fig.3.15(a) (conveying element GFA 2-45-R), a strong signal was detected each time the probe passed by the screw root, which corresponds to each 180° axial rotation. Both the amplitude and time delay (time of flight of the echo round trip from ultrasonic probe to screw) of the reflected signal turned out to be irregular; the reason may be the irregular screw shape itself along axial rotation. Nevertheless, Fig.3.15(b) shows that the screw is almost uniform along axial translation, since the magnitude of the ultrasonic responses is about the same over the screw root. Similar responses were observed for conveying element GFA 2-20-R2, shown in

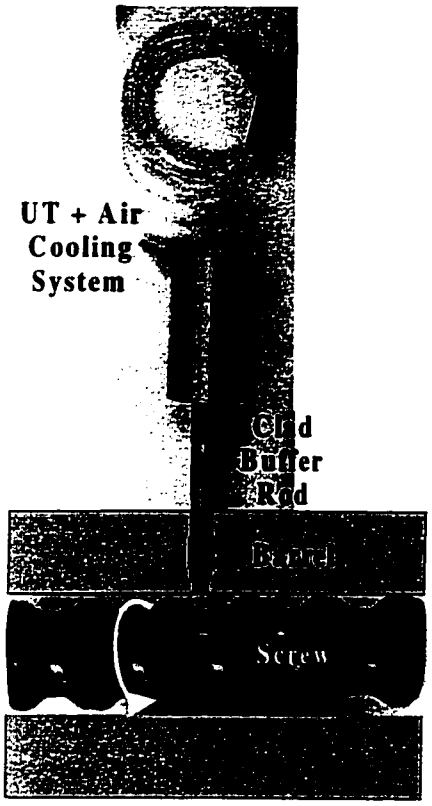


Fig.3.13: View inside the barrel of the twin-screw extruder from Leitstritz.

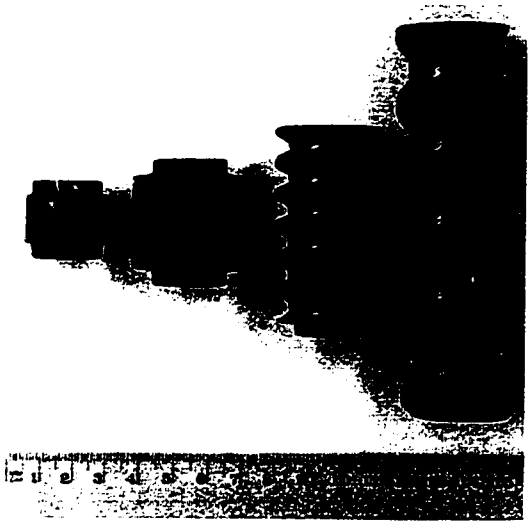
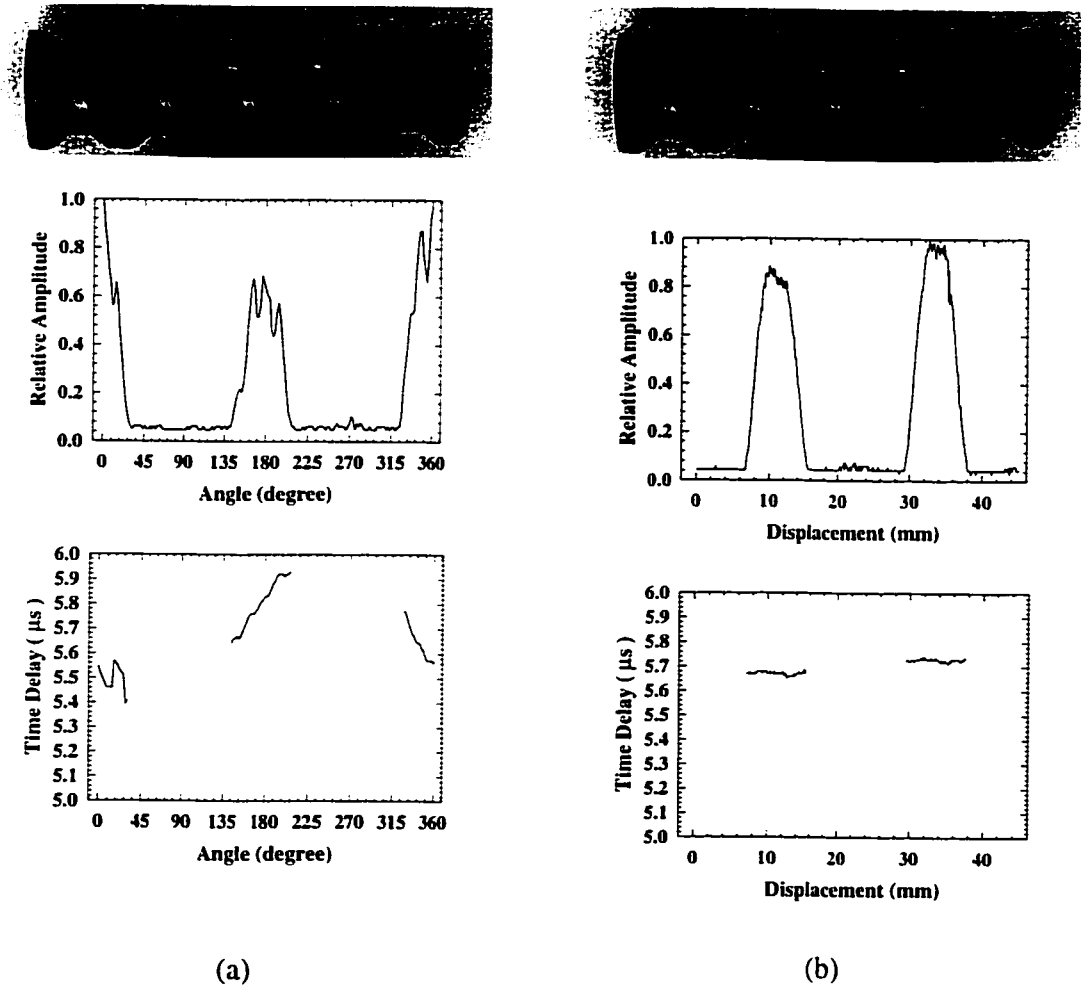
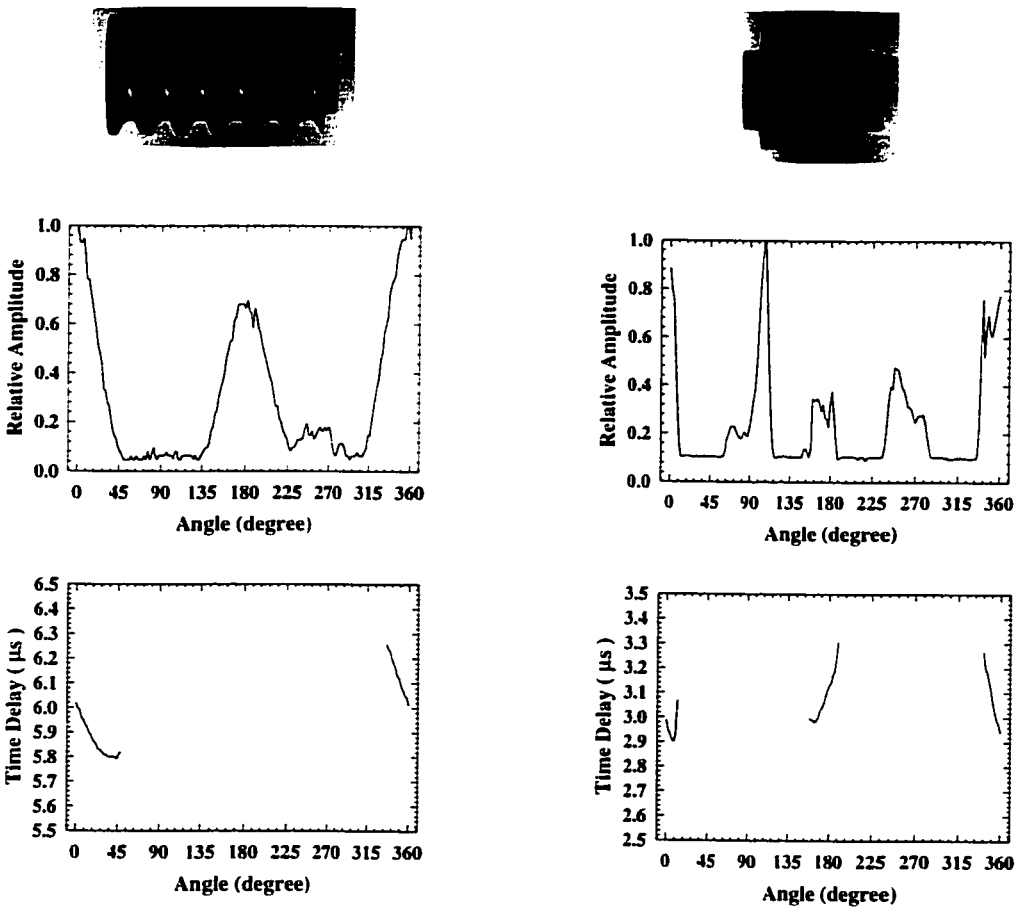


Fig.3.14: Four different screw elements used in the twin-screw extrusion process.



(a) (b)

Fig.3.15: Ultrasonic measurements in reflection
(a) during axial rotation (conveying element GFA 2-45-R); (b) during axial translation (conveying element GFA 2-45-R).



(c) (d)

Fig.3.15: Ultrasonic measurements in reflection
(c) during axial rotation (conveying element GFA 2-20-R2); (d) during axial rotation (kneading block KS 2-2-60-4r).

Fig.3.15(c). But for this element the time delay in the vicinity of 180° was so irregular that no information could be drawn, and so it was not plotted. Finally, the kneading block illustrated in Fig.3. 15(d) was characterised. Due to its ellipsoidal shape, maximum signals were observed each 90° rotation. Again, because of ambiguous time delay responses at 90° and 270°, these data were not considered. To summarise, in Fig.3.15 ((a), (c) and (d)) both the relative amplitude and the time delay of the ultrasonic signal plotted *versus* angle (degrees) are for double flight screws, as shown in Table 3.1. For each different screw pattern, the ultrasonic transducer was aligned with respect to the screw root (where maximum reflection was detected), and then the screw was automatically rotated 360°, thus getting back to the start position. By doing so, the ultrasonic response was revealed, shown to be specific for each screw geometry, as the amplitude and time of flight of the reflected ultrasonic signals depend on the shape of the reflector (screw) and the relative distance from it. As much valuable information may be inferred from the time delay response in the actual process, it is mandatory that its reading be unequivocal. One way to accomplish this is to synchronise screw rotation with respect to ultrasonic pulse generation. Since maximum reflection occurs each time the ultrasonic waves impinge at the root of the screw, a good approach is to generate and receive ultrasonic signals as the screw root faces the ultrasonic transducer. Therefore, the same location on the screw will be measured every complete (360°) rotation, and any of the screw elements discussed will be adequately monitored by ultrasound.

A distance (spacing) element was also chosen to evaluate the ultrasonic alignment sensitivity with regard to translation and rotation (horizontal and vertical) from the optimal detection position. Because of its regular, circular shape, it is particularly suitable for alignment with the ultrasonic probe. The results are presented in Fig.3.16. It is observed that either a translation of 2 mm, a horizontal rotation of 1 degree, or a vertical rotation of 2 degrees reduces the magnitude of the total detected signal by about 80%. This provides the allowed range of uncertainty in installing the CBR in the extruder barrel for which strong signals reflecting from the screw can still be detected.

Table 3.1: Description of the geometry of the screw elements illustrated in Figs.3.15((a)-(d)) 3.16.

Code Name	Figure	Description	Length (mm)	Pitch (mm)	Number of Flights
GFA 2-45-R	15a-b	Conveying element	120	45	2
GFA 2-20-R2	15c	Conveying element	60	20	2
KS 2-2-60-R4r	15d	Kneading block (2 kneading disks staggered clockwise by 60°)	30	90	2
ZD 26-R8	16	Distance element (26 mm diameter)	15	---	none

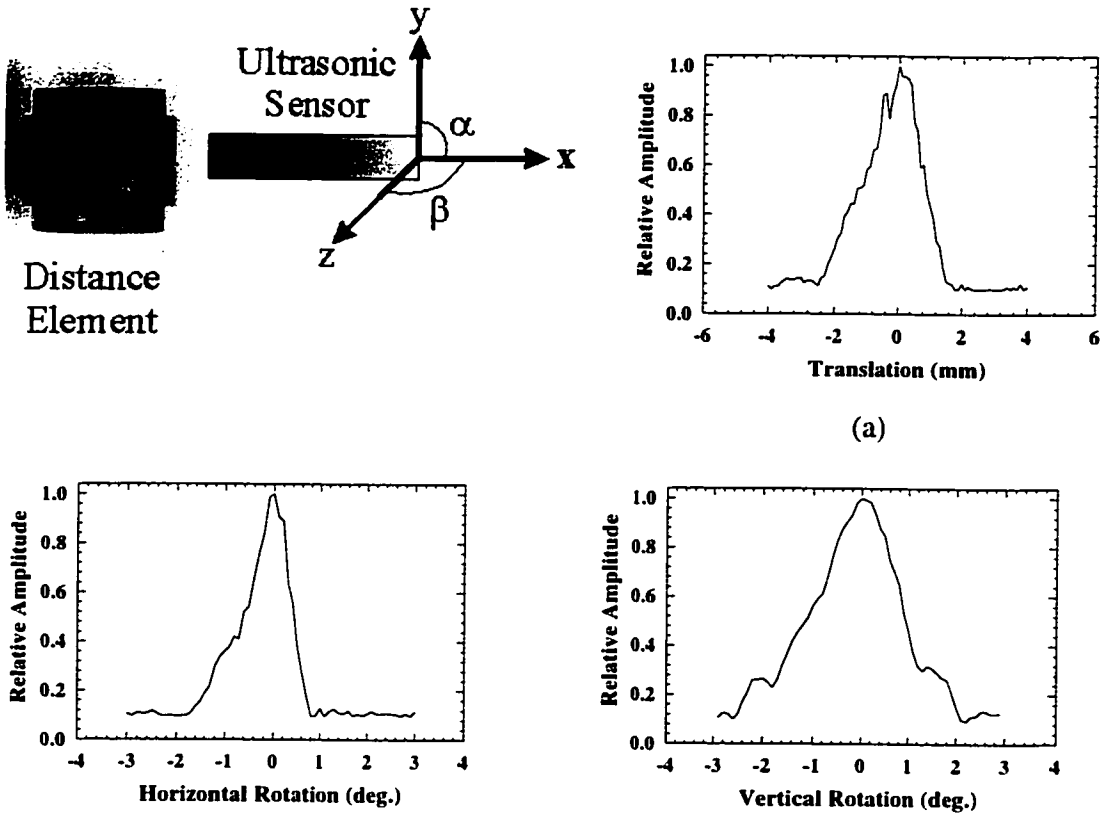


Fig.3.16: Alignment sensitivity evaluation using the distance element (ZD 26-R8). (a) Translation, (b) horizontal rotation and (c)vertical rotation are along **z**, β and α, respectively.

3.3.3-In-line monitoring of twin-screw extrusion of PS and HDPE

The conveying element GFA 2-45-R, illustrated in Fig.3.15(a), was used in the experiment. The experiments began with the extrusion of HDPE. The screw speed was varied from 5 rpm to 200 rpm, and the ultrasonic signals were acquired at predetermined speed. The screw was then purged with PS at 200 rpm until it was completely free of HDPE. The screw speed was again varied, and the ultrasonic signals were acquired for PS. Subsequently, the screw was purged again with HDPE at 200 rpm, and the signals were obtained over time. As time went on, the concentration of HDPE in the monitored zone became higher, until the entire zone section was filled with HDPE. The melt temperature and pressure were measured at about 220°C and 3.7 MPa for HDPE, and about 210°C and 1.6 MPa for PS. In order to obtain ultrasonic signals exhibiting sufficient SNR and signal strength, it was observed that the pressure in the extruder barrel has to be at least 0.7 MPa. Thus, the barrel is completely filled and the ultrasonic signal can propagate from the CBR into the molten polymer.

Fig.3.17 shows the data for pure HDPE at different screw speeds. In this figure, the first echo comes from the end of the CBR, and the second from the root of the screw, as indicated by the arrows in the figure. The difference between these two echoes represents the time delay for a round trip in the polymer between the barrel wall and the root of the screw. Even though the temperature of the barrel is controlled, at higher screw speed and hence higher shear rate, the heat generated by viscous dissipation cannot be removed fast enough, resulting in a higher local melt temperature. Since the velocity of sound decreases with higher temperature, the time-delay of echoes increases with the screw speed. In this range of extruder screw speed, it was observed that the ultrasonic velocity in PE changes about 28%, while in PS just 7% (not shown here). The behaviour of the amplitude of the signals is quite different. For PS, the amplitude of the signals increases significantly with the screw speed, while it decreases slightly for HDPE.

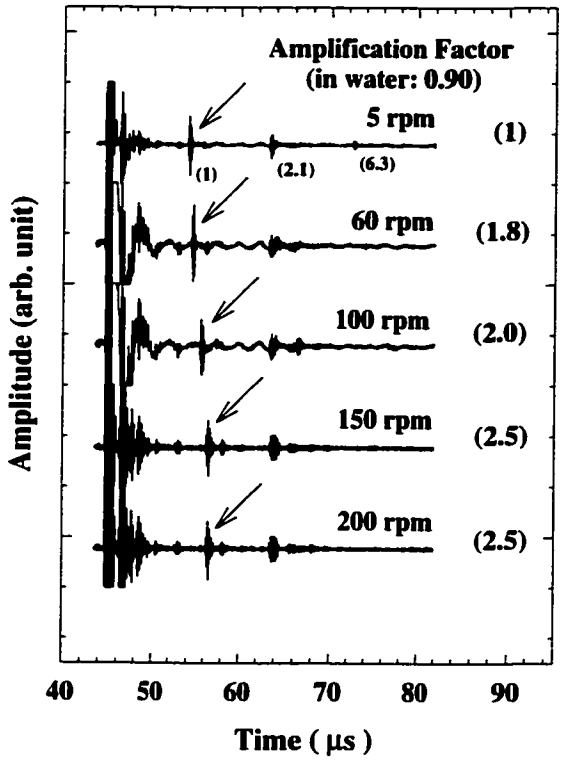


Fig.3.17: Twin-screw extrusion of polyethylene (PE) under different screw angular frequencies (arrows indicate the first echo on the screw).

Fig.3.18 demonstrates the sensitivity of ultrasonic waves to different concentrations of PS and PE, as the feed is being switched from one polymer to the other. The transient conditions may correspond to resulting polymer blends. Even though this part of the experiment is not controlled, and the exact composition of the blend is not known, this approach illustrates clearly that the ultrasonic in-line sensor can monitor changes in properties as the polymer being processed undergoes chemical or structural modifications. For this technique to be fully explored in polymer blend applications, it is however necessary to know precisely the relationship between the velocity of sound as a function of temperature, pressure and blend composition [5]. More studies are required to take further advantage of these results.

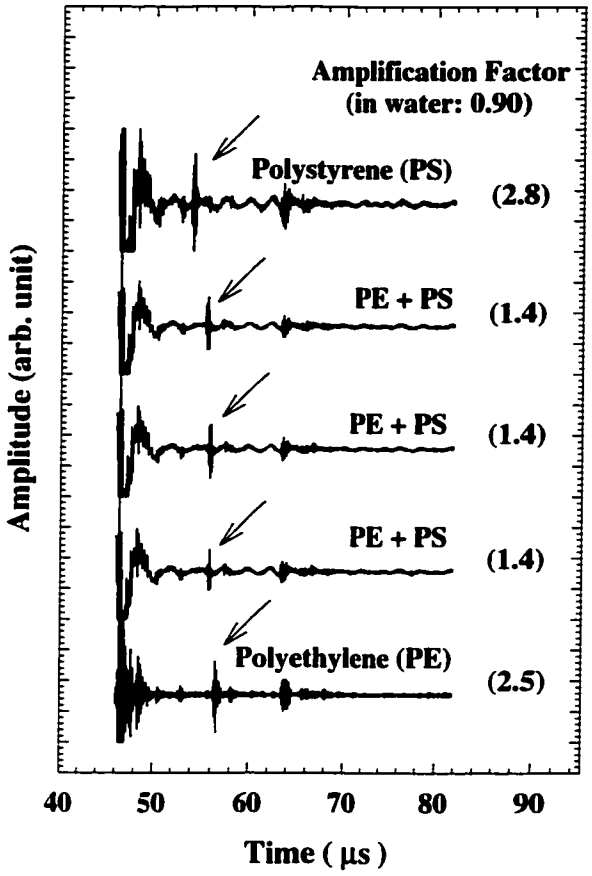


Fig.3.18: Twin-screw extrusion of polystyrene (top) and polyethylene (botton) switch from one polymer to the other at 200 rpm screw speed.

Similarly, the in-line ultrasonic monitoring of low density polyethylene (LDPE) was carried out. The same ultrasonic probe described previously was installed in the barrel section of a Werner & Pfleiderer 30 mm twin-screw extruder. The barrel temperature, pressure and screw rotation speed at the probed location were 200°C, 1.4 MPa and 200 rpm. In the experiment 2 and 4 wt % of $CaCO_3$ were added to the host LDPE during extrusion. Fig.3.19 shows that the changing of ultrasonic amplitude and velocity are sufficiently sensitive to the addition of the small amount of $CaCO_3$.

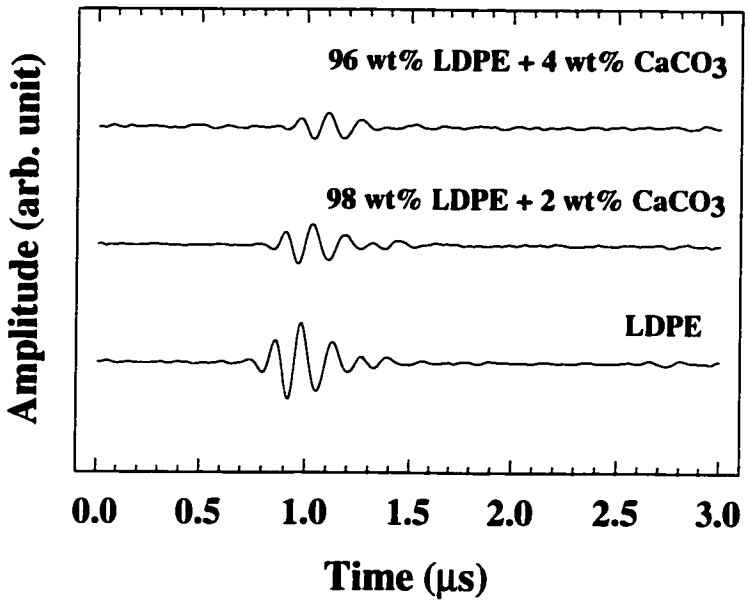


Fig.3.19: Twin-screw extrusion of low density polyethylene (LDPE) with different concentrations of $CaCO_3$.

3.4-Summary

In-line ultrasonic monitoring of multi-layer extrusion and twin-screw extrusion are presented. Steel CBRs providing high SNR in pulse-echo mode were used to isolate the commercial UT from molten polymer. The pulse-echo mode allowed access to just one side of the extruders. The probing end of the CBRs was machined to the same shape as the Dynisco pressure and temperature sensors delivered with the extruders, allowing the three types of sensors to be interchanged depending on the desired monitoring purposes.

During the co-extrusion of HDPE and Santoprene, the interface between the polymers could be detected, and the stability of the extrusion inferred from the signals.

The detection of the interface may provide the thickness of each polymer layer. Ultrasound was also used to study the adhesion quality of a plastic bottle produced by co-extrusion blow moulding of HDPE and Santoprene. As the accurate polymer thickness measurement and adhesion quality between parts rely on the precise identification of the interface echo, signal processing techniques have been investigated to reduce spurious signals and improve the interface echoes. It was found that deconvolution using Wiener Filtering is quite suitable, due to its fast processing time. This algorithm was implemented in the Labview® environment using 256-point FFT (Fast Fourier Transform).

Monitoring of the polymer behaviour in a twin-screw extruder presents several challenges. The difficulties come from not only the complicated geometry of the screw, but also from the transient nature of the process due to the screw rotation. The sensitivity of the signal to rotational and translational movement has been evaluated for several screw elements in an immersion tank, using water as the propagation medium. Such responses reveal the behaviour of the expected echoes for each screw pattern in the actual polymer extrusion. The effect of sensor misalignment is also established. The technique was implemented on a twin-screw extruder, with the ultrasonic sensor located in the extruder barrel and over a transport element. The screw was designed so that the monitored section was kept continuously full of molten polymer. It was observed that in order to obtain high quality ultrasonic signals (*i.e.*, stable and with sufficient SNR and signal strength), the pressure in the extruder barrel has to be at least 0.7 MPa. In this situation, the barrel is completely filled and the ultrasonic signal can well propagate from the CBR into the molten polymer. Experiments were performed using PS, HDPE and LDPE . It is demonstrated that the signal is sensitive to the properties of these polymers, and that the technique can track changes in polymer type, as has been clearly demonstrated in [5], where ultrasonic monitoring was carried out at the exit of the extruder. Here, we emphasise that our technique extends the possibility of performing ultrasonic monitoring in the off-the-screw section of the extruder. These results open routes for the development of various applications, from in-line monitoring of the

chemical transformation of the polymer during reactive extrusion, to in-line measurements of the rheological properties of polymers subjected to reticulation or controlled degradation.

Chapter 4

Development and Evaluation of Polymer Clad Buffer Rods

4.1-Introduction

As mentioned in Chapter 1, for ultrasonic evaluation of the viscoelastic properties of molten polymers the use of metallic CBRs can exhibit drawbacks because of the high impedance mismatch between metal and polymer. The relative error of density ($\Delta\rho/\rho$) *versus* that of the reflection coefficient ($\Delta\Gamma/\Gamma$) at the interface probe/polymer during the measurement is large, and can be up to several hundred percent [35]. This large error in the measurement of density will lead also to a large error in the measurement of viscosity and mechanical moduli. If a polymer buffer rod is used, because of the better impedance matching with the sample, then this relative error may become within acceptable limits [35]. In addition, it has been demonstrated that polymer rods can deliver ultrasonic pulses of lower frequencies [35]. Because of these features, polymer buffer rod sensors may be preferable in situations when high sensitivity is required to monitor changes in polymer properties [96], or when low-frequency interrogative signals are needed to achieve deeper acoustic penetration in the polymer. However, the ultrasonic loss in polymers is commonly very high. Thus, it is our objective in the present study to search for polymer buffer rod materials that offer high ultrasonic performance, such as low ultrasonic attenuation and low spurious noises [35]. Since high density polyethelene (HDPE) used in reference [35] may react to the polyester, a material widely used in industry, it is avoided here as the core material of the polymer buffer rod. For this work, we select polyetheretherketone (PEEK) as the buffer rod core material, because it does not react to polyester. In addition, PEEK has a melting temperature near 350°C, higher than that of

HDPE (~150°C), thus it may also be used to monitor polymer curing or other polymer processes at elevated temperatures (near 200°C). Next, we search for an appropriate cladding material as well, to improve the ultrasonic wave guidance for both longitudinal and shear modes, capable of standing high temperature for use in industrial applications.

The performance of the newly developed PEEK CBR for in-line ultrasonic monitoring is to be evaluated in two contexts: first, the cure of a polyester epoxy; and second, the polymer extrusion process. For cure monitoring, it is demonstrated here that the PEEK CBR can guide simultaneously and at the same location both shear and longitudinal waves, with a single commercial shear UT. The combined use of both shear and longitudinal waves provides an extra edge in material characterisation, because of the simplicity, reduced cost and one-point access to the sample. For the extrusion process, the new-developed PEEK CBR, machined as a conventional temperature and pressure sensor found in extruder machines, is installed in the barrel section of the extruder, to demonstrate its capability of in-line monitoring of polymer extrusion processes at temperatures up to 200°C and pressures up to 1.2 MPa

4.2-Fabrication and characterisation of clad PEEK buffer rod

4.2.1- Fabrication

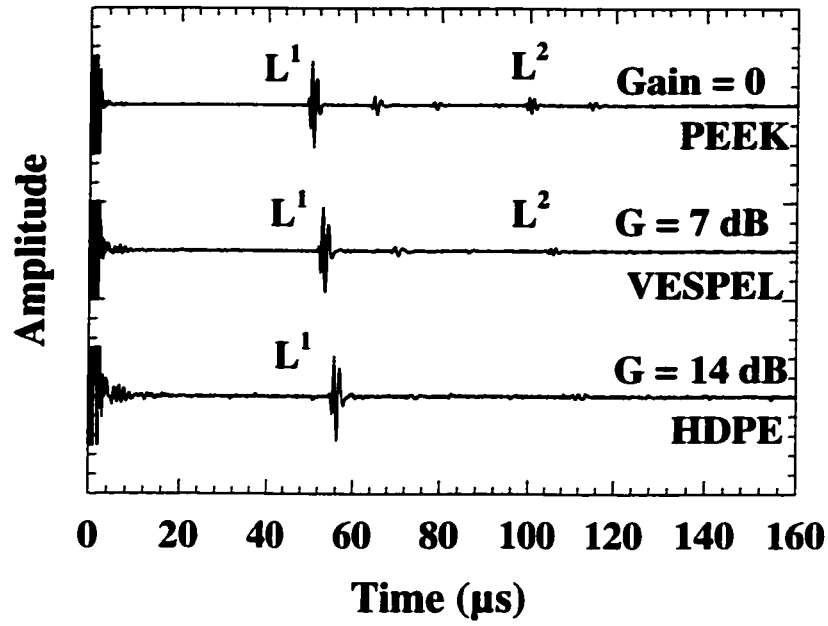
It was discussed in Chapter 2 that steel CBRs, consisting of a core and cladding, significantly outperform those without cladding. However, on the contrary, Legros, *et al.* [35] found that for polymer CBRs consisting of an oriented high-density polyethylene (HDPE) core and an epoxy cladding, the performance of the shear waves deteriorates because of the cladding. In the present study, our purpose is to develop and test polymer CBRs with superior performance for both longitudinal and shear waves. For this purpose, we selected polyetheretherketone (PEEK) as the buffer rod core material, because of its high melting temperature (~350°C) and relatively low ultrasonic loss at low MHz frequencies. A composite made of a heat-resistant epoxy mixed with aluminium powder

(45% by weight) is used as the cladding material. The cured heat-resistance epoxy can be operated at temperatures up to 275°C. Aluminium powder was added into this epoxy to increase the acoustic impedance of the cladding material.

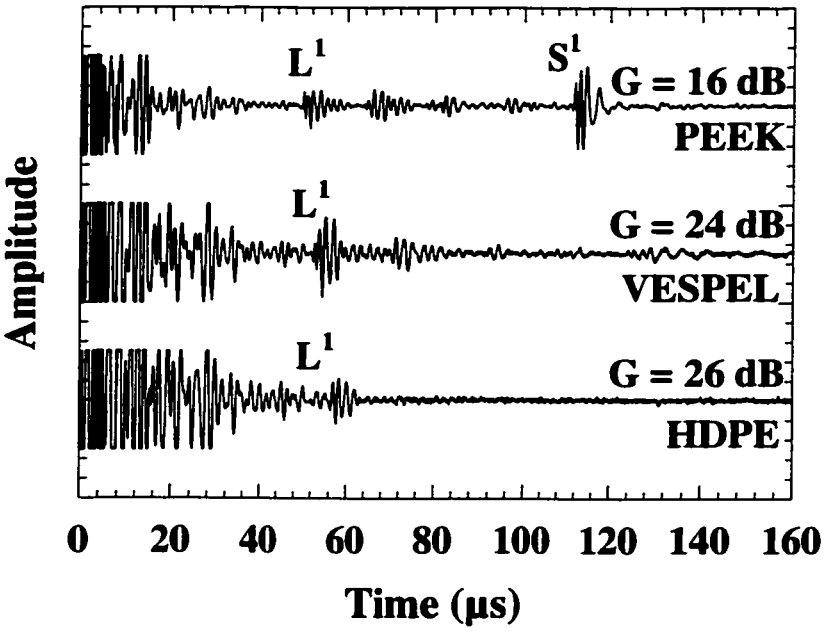
For the fabrication of a PEEK CBR, a PEEK rod was first placed inside a cylindrical plastic tube, both mounted on a holder with their central axes aligned. The inner diameter of the tube was several millimetres larger than the diameter of the PEEK rod. Then the heat-resistant epoxy-aluminium composite was poured into the gap between the tube and the rod, and cured for 48 hours. After curing, the external tube material was removed. The measured longitudinal, V_L, and shear, V_S, wave velocities at ~1 MHz; density, ρ; and longitudinal, Z_L, and shear, Z_S, wave impedances of the PEEK core and composite cladding materials are given in Table 4.1. For comparison purposes, those of polyimide (VESPEL) [97] and HDPE [35] are listed as well.

The ultrasonic characterisation of different polymer rods was carried out using 1 MHz longitudinal and shear UTs (Panametrics V103 and V153, respectively), both with a 12.7 mm element diameter. The UTs were driven by a 5072 PR pulser/receiver (Panametrics, Inc.) in pulse-echo mode. The ultrasonic signals were sampled at a rate of 50 MHz.

Figs.4.1(a) and 4.1(b) show the measured longitudinal and shear wave echoes, respectively, in the non-clad PEEK (upper curve), polyimide (VESPEL, middle curve) and high-density polyethylene (HDPE, lower curve) rods. The diameter and length of the rods are 17.5 mm and 63 mm, respectively. In Figs.4.1(a) and 4.1(b), $\mathbf{L^n}$ and $\mathbf{S^n}$ denote the nth round trip longitudinal and shear wave echoes, respectively, in the rod, and G denotes the relative amplification gain of the receiver electronics. Although the central frequency of the commercial UT used here is 1 MHz, our data (not shown here) indicate that the frequency at the maximum of the spectrum, f_M, of these echoes ranges from 0.3 to 0.9 MHz, because of the high attenuation nature of the polymer rod. Higher attenuation in higher frequency components makes polymer buffer rods act as a mechanical low pass filter.



(a)



(b)

Fig.4.1: Ultrasonic (a) longitudinal and (b) shear wave echoes of non-clad PEEK (upper curve), VESPEL (middle curve) and HDPE (lower curve) rods in air. G represents amplification in gain (dB).

In Fig.4.1(a), we can see that for the longitudinal waves, the signal strength of $\mathbf{L^1}$ in the PEEK rod is 7 dB and 14 dB higher than that of VESPEL or HDPE, respectively. In addition, for the shear waves, as shown in Fig.4.1(b), the signal strength of $\mathbf{S^1}$ in the PEEK rod is more than 20 dB larger than in VESPEL or HDPE, the latter hardly detectable. Therefore, PEEK is chosen for further studies.

4.2.2-Experimental evaluation

Experiments were carried out in order to evaluate several rods in pulse-echo mode. Performance of both non-clad and clad buffer rods (CBRs) will often be compared in this chapter. For simplicity, here we refer to non-clad buffer rods as NCBRs.

4.2.2.1-Clad buffer rod consisting of a uniform diameter PEEK core

Fig.4.2 shows the measured longitudinal wave echoes in a PEEK NCBR (upper curve) and a PEEK CBR (lower curve) in air. The core diameter is 17.5 mm for both NCBR and CBR, and the cladding thickness is 5 mm. The corresponding frequency spectra of the longitudinal wave signals, $\mathbf{L^1}$'s, shown in Fig.4.2, are given in Fig.4.3. Under the same operating conditions, the signal strength in the PEEK CBR is a few dB stronger than that in the NCBR, but with nearly the same bandwidth. In both cases, f_M is about 0.9 MHz. This means that the cladding enhances longitudinal wave guidance in the rod a little. In addition, we notice that the trailing echoes in the PEEK NCBR are eliminated in the PEEK CBR, so that a high SNR is achieved. Here SNR is the ratio of the amplitude of the signal over that of the trailing echoes.

The measured time domain shear wave signals and the corresponding frequency spectra in the NCBR (upper curve) and CBR (lower curve) are given in Figs.4.4 and 4.5, respectively. The signal $\mathbf{S^1}$ in the PEEK CBR is 10 dB stronger than that in the PEEK NCBR. Also the echoes are "cleaner" in the CBR than in the NCBR. Furthermore,

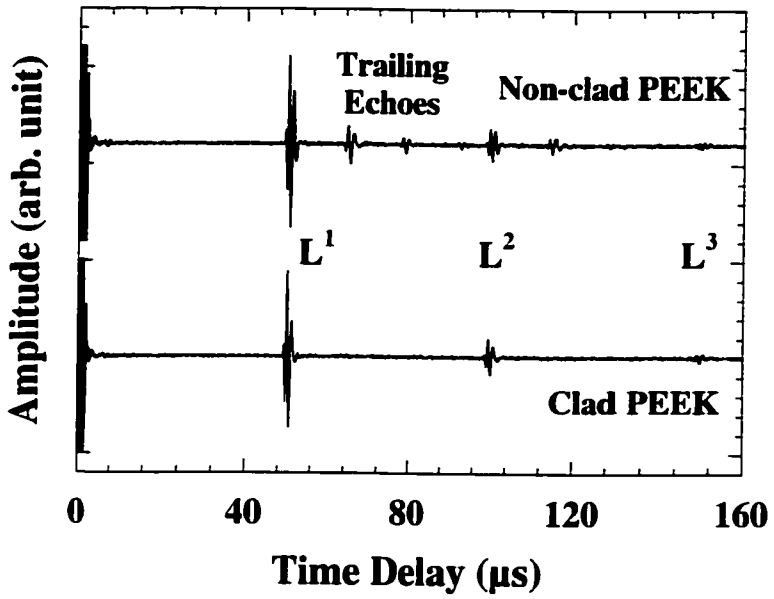


Fig.4.2: Ultrasonic longitudinal wave echoes of non-clad PEEK (upper curve) and clad PEEK (lower curve) rods in air. The transducer was a longitudinal wave UT.

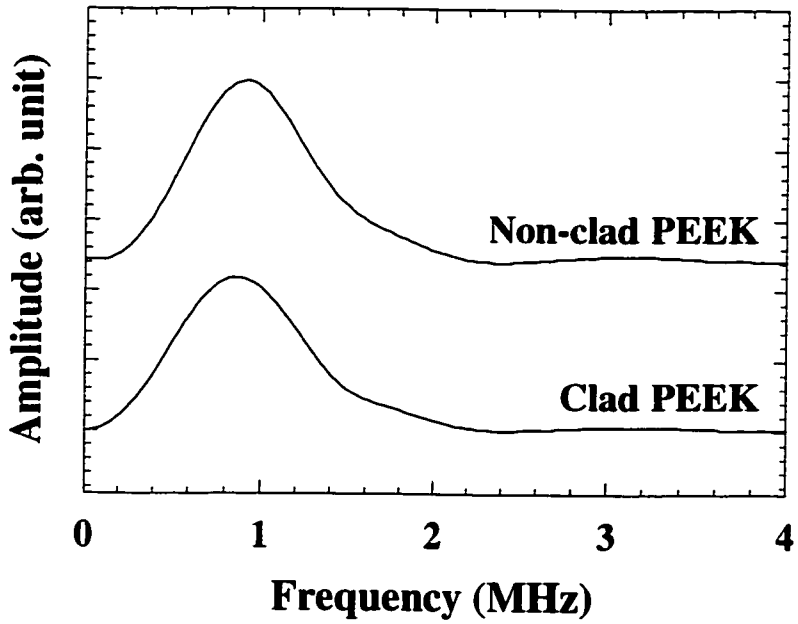


Fig.4.3: Frequency spectra of L^1 echoes in rods shown in Fig.4.2.

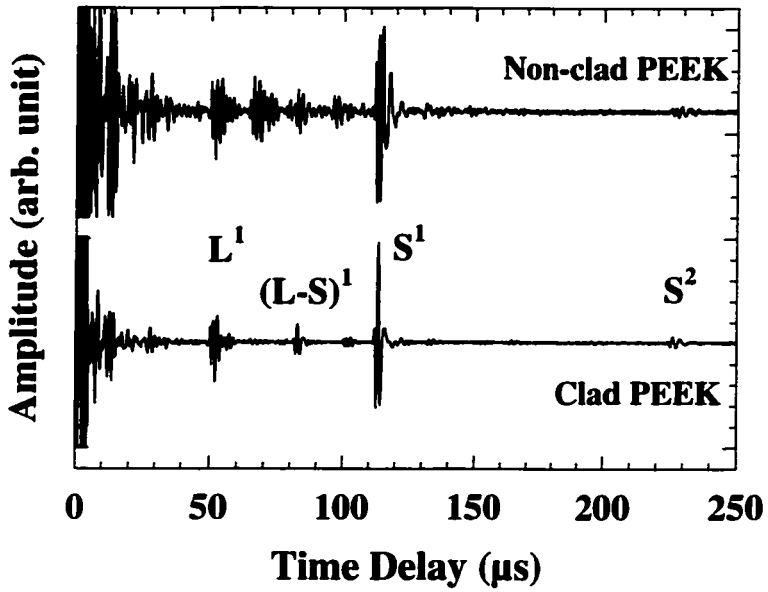


Fig.4.4: Ultrasonic wave echoes of non-clad PEEK (upper curve) and clad PEEK (lower curve) rods in air. The UT can generate and receive both longitudinal and shear waves.

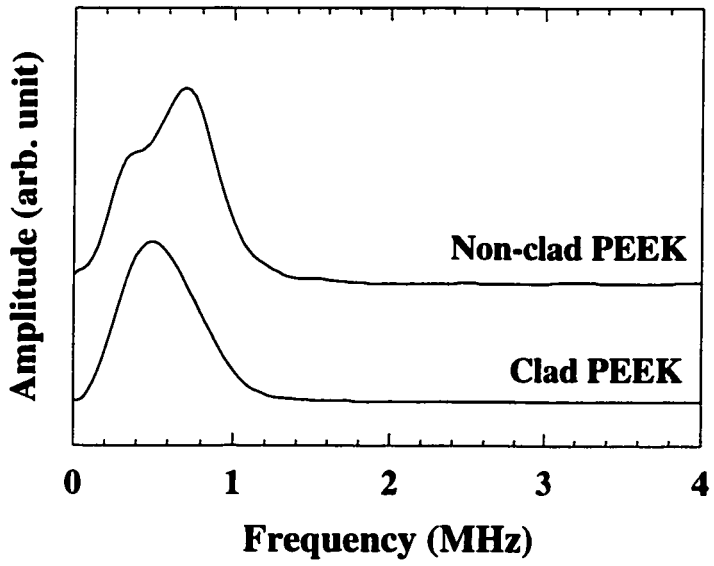


Fig.4.5: Frequency spectra of S^1 echoes in rods shown in Fig.4.4.

although the transducer is a commercially designated "shear wave" UT, not only can the shear wave signals $\mathbf{S^1}$ and $\mathbf{S^2}$ be observed, but also the longitudinal wave signal $\mathbf{L^1}$ and the signal $\mathbf{(L\text{-}S)^1}$, caused by mode conversions (**S→L** and **L→S**), are generated and received in this case. From Fig.4.5 we notice that f_M is around 0.6 MHz, and the cladding did not change significantly the frequency spectra of signals $\mathbf{S^1}$. This means that for shear waves this PEEK CBR also acts as a low pass filter. Since both longitudinal and shear waves can be generated and received by the same UT, this could be advantageous for certain applications [35]. This property will be discussed later in this chapter.

The improved signal strength and SNR in the CBR over the NCBR geometry can be explained by the fact that both ultrasonic velocity and impedance in the epoxy-aluminium cladding are higher than those in the PEEK core (shown in Table 4.1), as explained in Chapter 2. Such clad configuration enhances both longitudinal and shear wave guidance in the core. It also means that the problem of the cladding which deteriorates the shear wave propagation reported in reference [35] has been eliminated.

Table 4.1: Measured properties of buffer rod materials.

Materials		V_L (m/s)	V_S (m/s)	ρ (kg/m^3)	Z_L (10^6 kg/ m^{2}s)	Z_S (10^6 kg/ m^{2}s)
Cladding	Epoxy-Al Composite	2743	1410	1810	4.965	2.552
Core	PEEK	2549	1125	1290	3.288	1.451
	VESPEL	2414	1014	1421	3.430	1.441
	HDPE	2292	922	950	2.178	0.876

4.2.2.2-Clad buffer rod consisting of a tapered PEEK core

Chapter 2 mentioned, based on experimental verification, that a tapered shape is an effective approach to reducing unwanted trailing echoes. Therefore we used the approach described in section 4.2.1, and fabricated tapered PEEK CBR. Its top, bottom and side views are shown in Figs.4.6(a) and 4.6(b), respectively. In Figs.4.6(a) and 4.6(b), d_L, d_S and l, similar to those in Fig.2.3, here also denote the large diameter, small diameter, and length of the tapered rod, respectively. A small diameter at the probing end is necessary for some applications. As mentioned in Chapter 3, for polymer extrusion monitoring, there is a need to make the probing end of the CBR in the same external shape as that of some commercially available pressure and temperature sensors, such as those produced by Dynisco [43], so that the CBRs may be installed wherever conventional Dynisco pressure and temperature probes are installed. Therefore, a tapered PEEK CBR with the Dynisco shape is shown in Fig.4.7. It is specially designed for in-line ultrasonic monitoring of polymer extrusion processes, as is demonstrated later in this chapter.

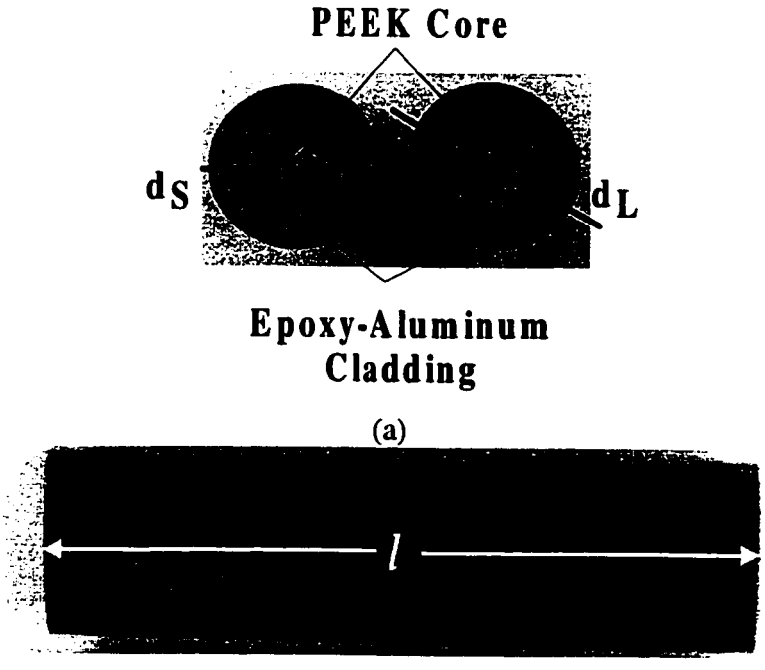


Fig.4.6: (a) Top & bottom and (b) side views of a clad taper PEEK buffer rod.

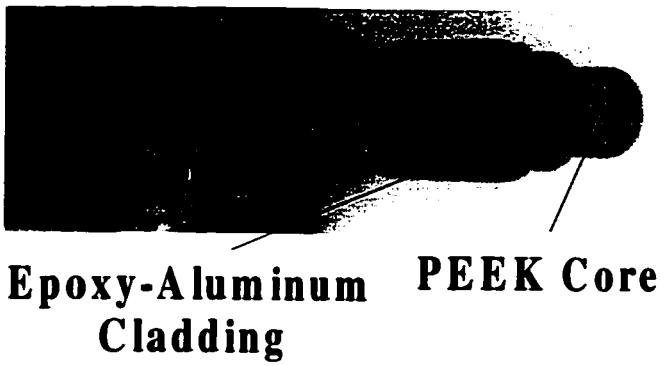


Fig.4.7: A clad tapered PEEK buffer rod having the same external shape as that of a Dynisco pressure and temperature sensor.

Fig.4.8 shows the measured longitudinal wave echoes in the tapered PEEK NCBR (upper curve) and tapered PEEK CBR (lower curve) with the probing end in air. For the tapered PEEK NCBR, d_L is 19 mm, d_S 13.1 mm and l 73.8 mm. For the corresponding CBR, d_L is 19.4 mm, d_S 12.9 mm, and l 74.9 mm. The corresponding frequency spectra of the longitudinal wave signals, $\mathbf{L^1}$, shown in Fig.4.8 are given in Fig.4.9. Similar to the uniform core case, the signal strength in the tapered PEEK CBR is several dB stronger than in the tapered PEEK NCBR. We also notice that the trailing echoes in the uniform PEEK NCBR are reduced in the tapered PEEK NCBR, and finally eliminated in the tapered PEEK CBR. In addition, because of the smaller d_S, the strength of the reflected $\mathbf{L^1}$ in the tapered PEEK CBR is a few dB lower than that in the uniform PEEK CBR. Furthermore, f_M is now at about 0.7 MHz rather than 0.9 MHz for the uniform core case (Fig.4.3).

The measured time domain shear wave signals, and the corresponding frequency spectra in the tapered PEEK NCBR (upper curve) and tapered PEEK CBR (lower curve), are given in Figs.4.10 and 4.11, respectively. The signal strength of $\mathbf{S^1}$ in the tapered PEEK CBR is about 10 dB stronger that in the corresponding NCBR. In this tapered configuration, both longitudinal and shear waves are still generated and received simultaneously by a single shear wave UT. Fig.4.11 indicates that the frequency, f_M, of signals $\mathbf{S^1}$ is now around 0.4 MHz.

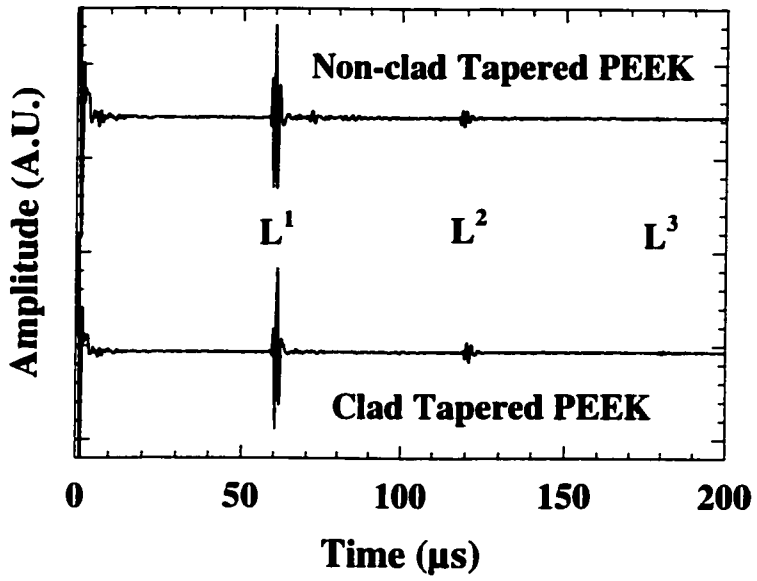


Fig.4.8: Ultrasonic longitudinal wave echoes of non-clad tapered PEEK (upper curve) and clad tapered PEEK (lower curve) rods in air. The transducer was a longitudinal wave UT.

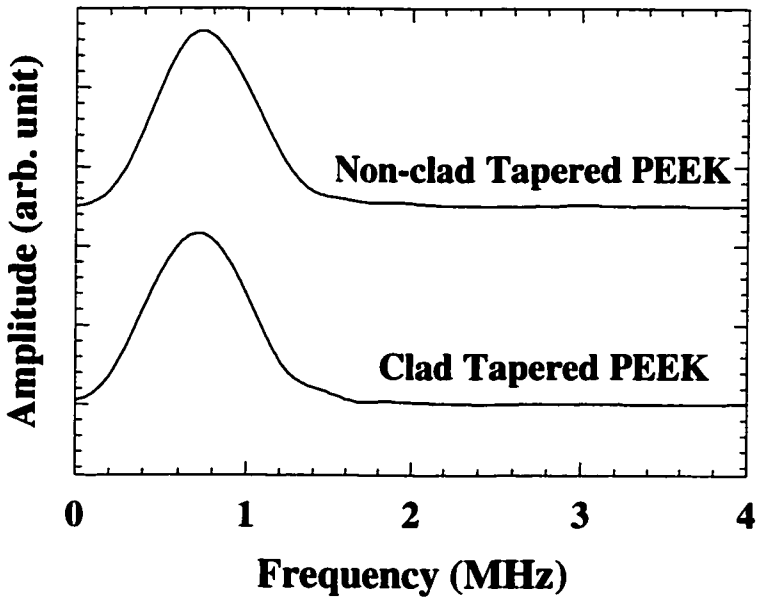


Fig.4.9: Frequency spectra of L^1 echoes in rods shown in Fig.4.8.

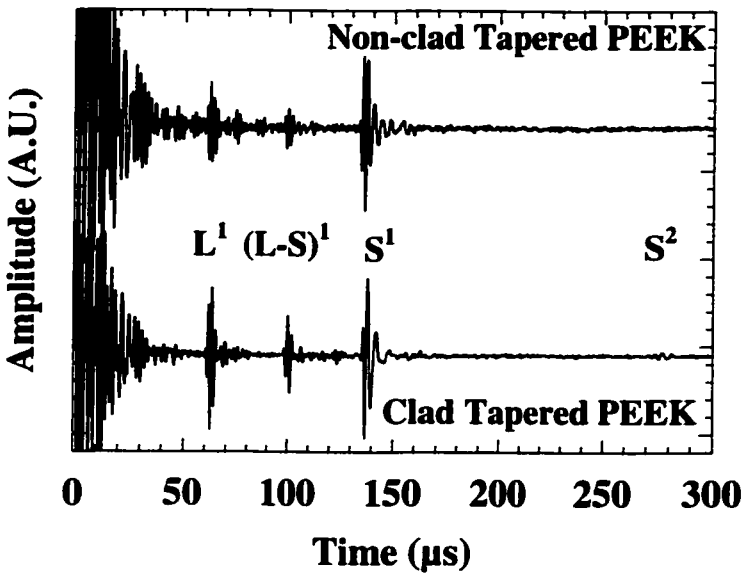


Fig.4.10: Ultrasonic wave echoes of non-clad tapered PEEK (upper curve) and clad tapered PEEK (lower curve) rods in air. The UT can generate and receive both longitudinal and shear waves.

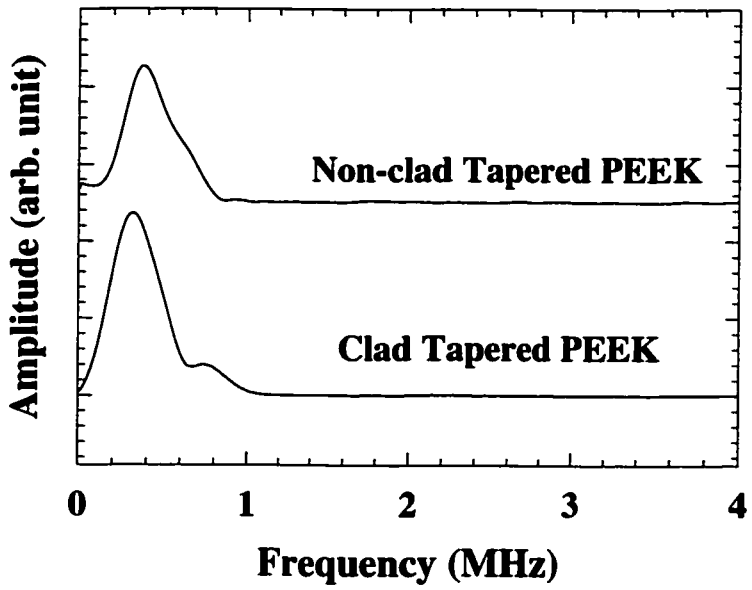


Fig.4.11: Frequency spectra of S^1 echoes in rods shown in Fig.4.10.

4.3-In-line ultrasonic monitoring of epoxy curing

In order to explain the principles involved in the in-line ultrasonic monitoring of epoxy curing, we use the reflection configuration shown in Fig.4.12. Ultrasonic waves generated by the UT (longitudinal or shear) propagate through the CBR and reach the interface with a polymer, which is under monitoring. Part of the energy is reflected, and part is transmitted, at the interface. The transmitted energy is further reflected at the boundary between the other side of the polymer and the container. In Fig.4.12, $\mathbf{L_2}$, $\mathbf{L_4}$ and $\mathbf{L_6}$ represent the 1st, 2nd, and 3rd round trip ultrasonic echoes in a polymer sample with thickness h. The same UT receives all reflected ultrasonic energy reaching it. The reflection coefficient at the PEEK CBR/polymer interface can be given as [72]:

$$\Gamma = (Z_B - Z_P)/(Z_B + Z_P), \tag{4.1}$$

where Z_B and Z_P are the ultrasonic impedance of the PEEK CBR and polymer, respectively. For the longitudinal wave, $Z_{L,B} = \rho_B V_{L,B}$ and $Z_{L,P} = \rho_P V_{L,P}$, and for the shear waves, $Z_{S,B} = \rho_B V_{S,B}$ and $Z_{S,P} = \rho_P V_{S,P}$, where ρ is the material density, and V_L and V_S are the longitudinal and shear wave velocities, respectively.

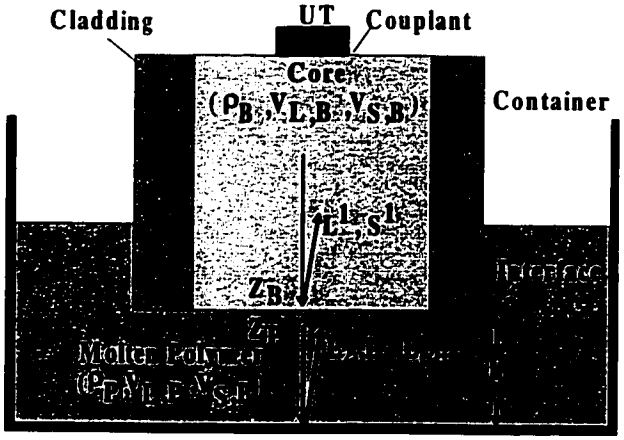


Fig.4.12: Ultrasonic pulse-echo measurement using a polymer CBR in the reflection mode.

For the PEEK CBR used here, the parameters ρ_B, $V_{S,B}$, $V_{L,B}$, $Z_{L,B}$ and $Z_{S,B}$ are known (Table 4.1). Therefore, from Eq.(4.1) and the signals $\mathbf{L^1}$ and $\mathbf{S^1}$ obtained in the longitudinal and shear wave measurements, we can calculate $Z_{L,P} = \rho_P V_{L,P}$ and $Z_{S,P} = \rho_P V_{S,P}$. Later, we will also show that from the time delays $(\mathbf{L_4} - \mathbf{L_2})$ or $(\mathbf{L_6} - \mathbf{L_4})$ *etc.* of longitudinal waves, $(\mathbf{S_2} - \mathbf{S^1})$ of shear waves, and polymer thickness h, $V_{L,P}$ and $V_{S,P}$ can be derived, respectively. Then ρ_P can be evaluated. It is also noted that the Young's, $E = \rho_P V_{S,P}^2 \left(3V_{L,P}^2 - 4V_{S,P}^2\right)/\left(V_{L,P}^2 - V_{S,P}^2\right)$, and shear, $G = \rho_P V_{S,P}^2$, moduli [72] of the polymer sample can be obtained as well.

The viscosity of a viscous fluid such as polyester can be determined from the complex reflection coefficient of shear waves reflected from a solid/fluid interface [3,35,97-99], as shown in Fig.4.12. Assuming that the fluid is perfectly viscous (Newtonian fluid) and the PEEK CBR is purely elastic, the shear impedance for the CBR and fluid are then expressed as $Z_{S,B} = \rho_B V_{S,B}$ and $Z_{S,P}=(i\,\omega\,\rho_P\,\eta)^{1/2}$, respectively, where η is viscosity, i denotes the imaginary unit, and ω is the angular frequency of the ultrasound. If one takes the absolute value of the shear reflection coefficient in Eq.(4.1) and solves this equation for η, the viscosity can be obtained by [35]

$$\eta = \frac{2}{\rho_P \omega} Z_{S,B}^2 \left[\frac{-(|\Gamma|^2 + 1) + \sqrt{-|\Gamma|^4 + 6|\Gamma|^2 - 1}}{2(|\Gamma|^2 - 1)} \right]^2 \qquad (4.2)$$

Here, the absolute shear wave reflection coefficient is experimentally determined by the ratio of the amplitude of $\mathbf{S^1}$ in the fluid over that in air. In this process, the density ρ_P of the fluid must be known. As mentioned earlier, the density can be determined from both the longitudinal wave velocity, $V_{L,P}$, and impedance, $Z_{L,P} = \rho_P V_{L,P}$, which is obtained from the reflection coefficient of the longitudinal waves at the interface.

Another advantage of CBRs over NCBRs is that the capillary effect which affects the reflection coefficient in the NCBRs does not exist in the CBR. With NCBRs, the level

of a liquid sample under monitoring can rise higher than the interface between the end of the rod and the liquid, because of the capillary effect. This additional liquid surrounding the NCBR near the end contacting the liquid can cause a significant effect on the reflection coefficient measurement, and induce measurement errors. In our PEEK CBR, the ultrasound is tightly guided inside the PEEK core. Thus the liquid, which can only surround the cladding of a reasonable thickness near the probing end, does not cause any adverse effect on the reflection coefficient measurement.

We used the PEEK CBRs to perform in-line ultrasonic monitoring of the entire curing process of a polyester sample. The sensors were composed of a longitudinal or shear UT (1 MHz central frequency with a 12.7 mm-element diameter) and uniform PEEK CBRs. The CBR used with the longitudinal UT was 54.2 mm long, with 17.5 mm core diameter. For the one used with the shear UT, these values were 44.4 and 19.2 mm, respectively. The CBRs had the same outer diameter of 31.5 mm. It was observed that the sensor with the longitudinal UT generates a longitudinal wave centred at 0.8 MHz, with 0.5 MHz -3dB bandwidth. The one with the shear UT generated a shear wave centred at 0.2 MHz, with 0.3 MHz – 3dB bandwidth. These probes were placed side by side at a fixed distance of 3.1 mm from the bottom of the container made of stainless steel, as shown in Fig.4.13. This distance was measured by filling the container with water and then measuring the time delay between the probing end of the sensor and the bottom of the container. The polyester resin was cured at room temperature with a curing agent (methyl ethyl ketone peroxide) [100].

The acquisition system was set up with a PC and Labview® program. The A/D board was a Gage CS250. The ultrasonic echoes were acquired at a sampling rate of 50 MHz. The results for longitudinal and shear UTs are shown in Figs.4.14 and 4.15, respectively. Both the shear wave signals $\mathbf{S^1}$ and $\mathbf{S_2}$ and longitudinal wave signals $\mathbf{L^1}$, $\mathbf{L_2}$ and $\mathbf{L_4}$ are simultaneously observed in Fig.4.15, where $\mathbf{L_n}$ and $\mathbf{S_n}$ are the *n*th round trip longitudinal and shear waves, respectively, in the polyester sample, and $\mathbf{(L\text{-}S)^1+(S_2\text{-}S^1)}$ represents the echoes resulting from the mode conversions between $\mathbf{L^1}$ or $\mathbf{S_2}$ and $\mathbf{S^1}$. If we let the reflection coefficients of the longitudinal $\mathbf{L^1}$ and shear waves $\mathbf{S^1}$ equal 1 (*i.e.* total

reflection) for the case in air, as shown in Figs.4.2 and 4.4, respectively, then the absolute (normalised) reflection coefficients, Γ, for the longitudinal and shear waves at the interface between the PEEK CBR and polyester can be obtained by normalising the amplitudes of $\mathbf{L^1}$ and $\mathbf{S^1}$, shown in Fig.4.15 with respect to the amplitudes of the waves shown in Fig.4.4. From this figure, we can also obtain the shear wave velocity, $V_{S,P}$, in the 3.1 mm-thick polyester from the time delay between $\mathbf{S^1}$ and $\mathbf{S_2}$. Therefore, we can obtain $Z_{L,P}$, $Z_{S,P}$, $V_{L,P}$, ρ_P, $V_{S,P}$, E and G simultaneously at the same probing location of a polyester with a single PEEK CBR and shear UT as the probe. However, in the present case, the poor signal to noise ratio of the shear-UT-generated longitudinal waves do not allow us to use the information to accurately measure the acoustic properties of the polymer. Instead, the longitudinal-UT-generated signals, shown in Fig.4.14, are used.

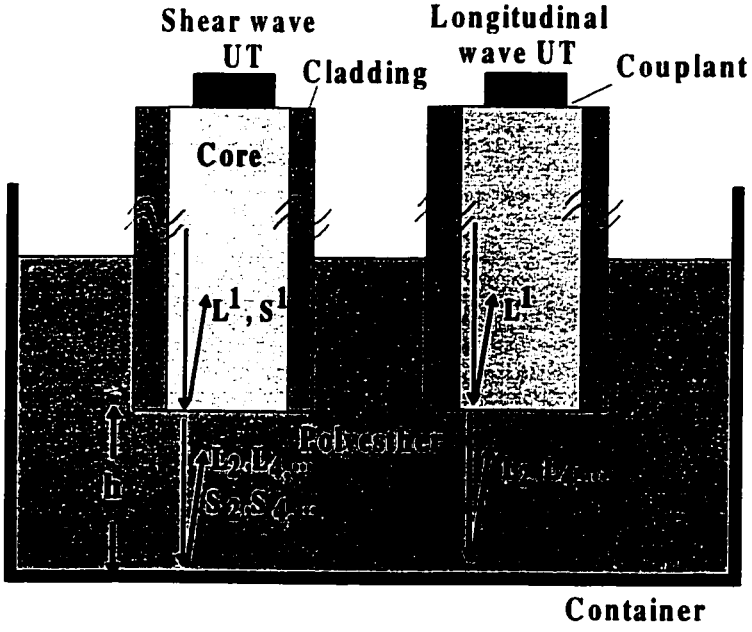


Fig.4.13: Experimental setup for cure monitoring.

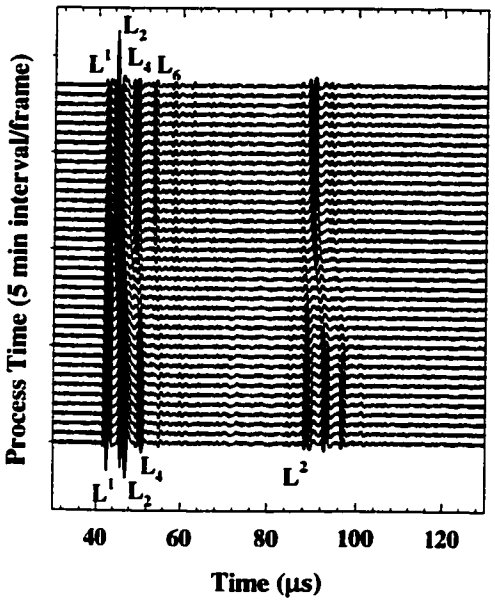


Fig.4.14: Ultrasonic signals in the PEEK CBR during monitoring of the cure process of polyester. The transducer is a longitudinal wave UT.

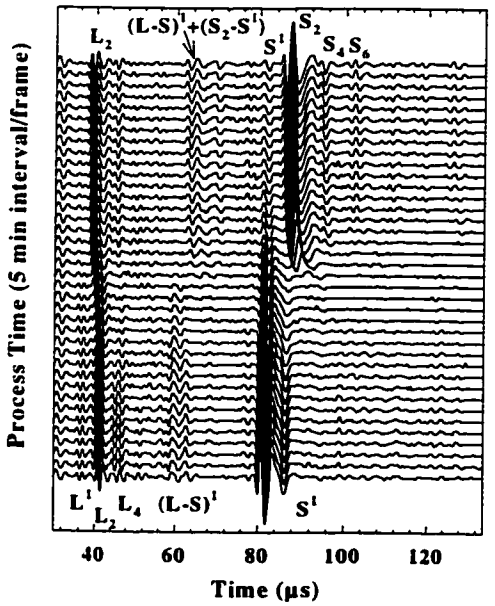


Fig.4.15: Ultrasonic signals in the PEEK CBR during monitoring of the cure process of polyester. The transducer is a shear wave UT.

Figs.4.16 and 4.17 show the measured variations of the ultrasonic velocity and reflection coefficient during the curing process for longitudinal and shear waves, respectively. In Fig.4.16, it should be noted that both the longitudinal wave velocity and reflection coefficient are drastically changed with respect to curing time. Our measurement accuracy of the velocity is about 1m/s and the total change of V_{LP} is 1073 m/s (from 1452 m/s to 2525 m/s). In Fig.4.17, since shear waves have high attenuation in polyester (which has very low viscosity), V_S can be only observed after 60 minutes curing time. However, after this time, the change in V_S is very similar to that in V_L. In the very beginning of the curing process, nearly all the energy associated with the shear wave is reflected at the CBR/polyester interface, because low viscosity liquid polyester does not support shear waves. The normalised shear wave reflection coefficient is 1, as expected. However, due to the small difference between the acoustic impedances of the PEEK CBR and polyester, it can be seen that the reflection coefficient is less than 0.30 for the longitudinal and shear waves, from 60 minutes of curing time throughout the 180 minute curing process. Such low reflection coefficients confirm the desired property of the PEEK CBR, to transmit the majority of the ultrasonic wave energy from the rod to the polyester and *vice versa*. More ultrasonic energy entering the polyester enables the measurement of longitudinal and shear wave velocities of a thicker polyester sample. Using the results shown in Fig.4.16, the density change is obtained and given in Fig.4.18. In addition, with the use of the absolute shear wave reflection coefficient shown in Fig.4.17, the viscosity of the epoxy during curing is also derived from Eq.(4.2), and shown in Fig.4.18 as well. These data may be utilised for quantitatively monitoring and even controlling the curing process.

4.4-In-line ultrasonic monitoring of polymer extrusion

In order to evaluate the performance of PEEK CBRs in pressure and temperature tests and polymer extrusion monitoring, the tapered one shown in Fig.4.7 was screwed into the barrel of a polymer extruder from Werner & Pfleiderer (also used for the

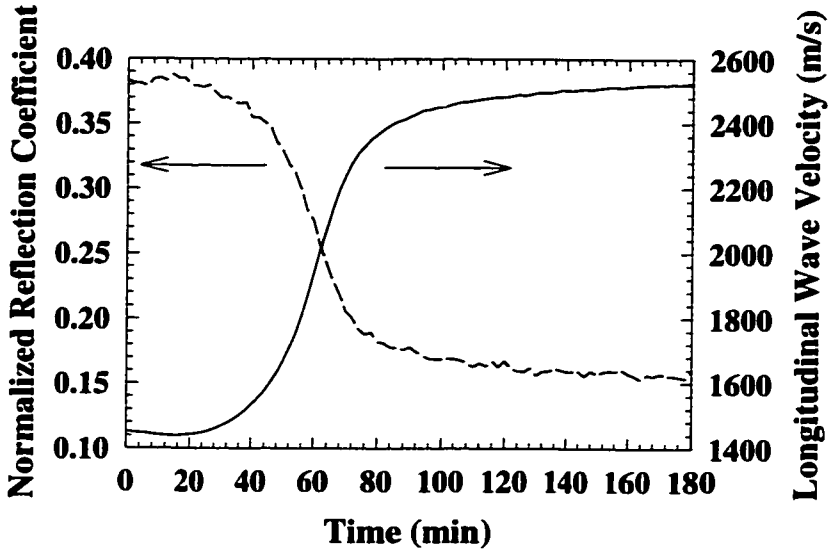


Fig.4.16: Longitudinal wave velocity and reflection coefficient variations during the curing process of a polyester.

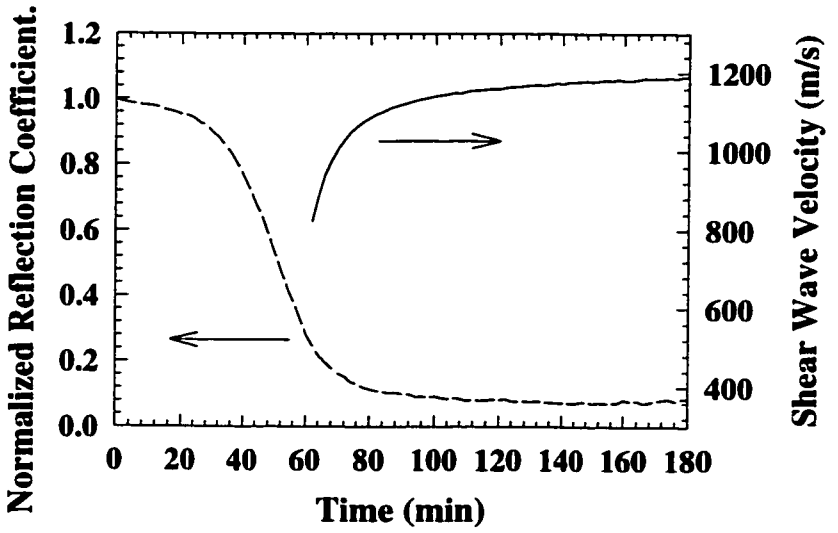


Fig.4.17: Shear wave velocity and reflection coefficient variations during the curing process of a polyester.

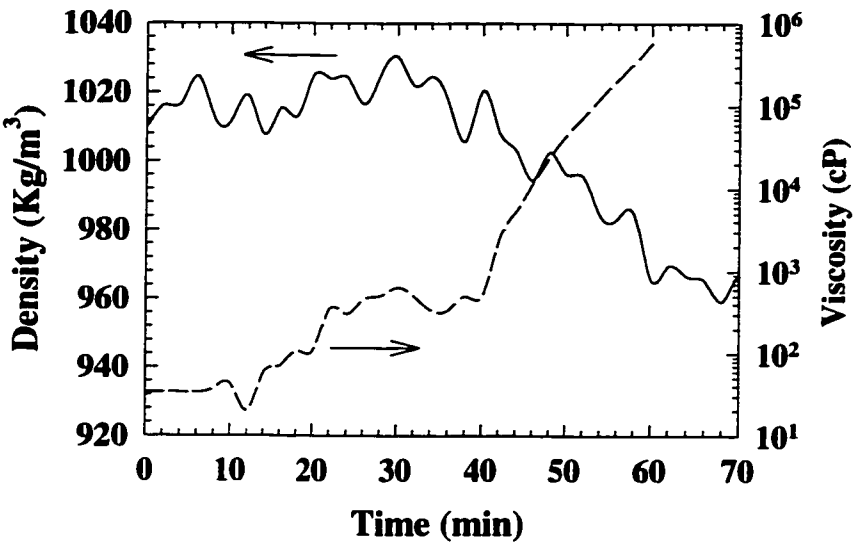


Fig.4.18: Density and viscosity variations during curing process of polyester.

experiment in chapter 3), as shown in Fig.4.19. This time, a 5 MHz longitudinal wave UT (6.35 mm-element diameter) was attached firmly to the d_L side. In this case, d_L, d_S and l were 10.7, 5.7 and 68.8 mm, respectively. This UT is cooled by a cooling fan during monitoring. A schematic figure of the PEEK CBR probe, barrel (wall) and screw is given in Fig.4.20. In order to monitor the pressure and temperature, a Dynisco temperature & pressure probe was screwed into the barrel 2 cm away from the PEEK CBR probe, as shown in Fig.4.19. Here, the acquisition system was set-up with a Pentium® PC and a 50 MHz double-channel acquisition (CS12100, Gage Applied Science, Inc.), with 12-bit resolution and 8M-sample on board memory. The 5 MHz longitudinal UT was used in pulse-echo mode with a PR35 pulser/receiver (JSR Ultrasonics, Inc.). Signals were digitised, stored, processed and displayed using Labview®.

Fig.4.21 shows the amplitude in dB of the $\mathbf{L}^1$ echo *versus* the temperature of the barrel measured by the Dynisco sensor. The cavity was empty; thus there was no pressure exerted on the rod. It is to be noted that only half (34 mm long) of the PEEK CBR was screwed into the heated barrel. At a barrel temperature of about 160°C, the signal strength of $\mathbf{L}^1$ decreases about 12 dB.

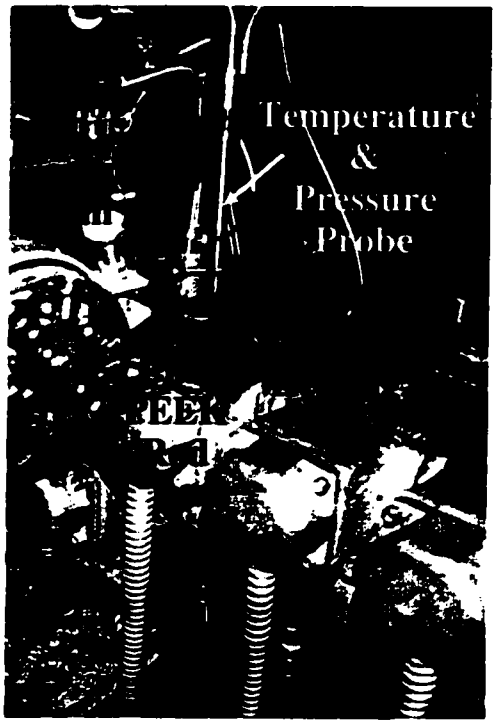


Fig.4.19: The tapered PEEK CBR shown in Fig.4.7 is inserted into the barrel region of an extruder for high temperature and pressure tests, and polymer extrusion monitoring.

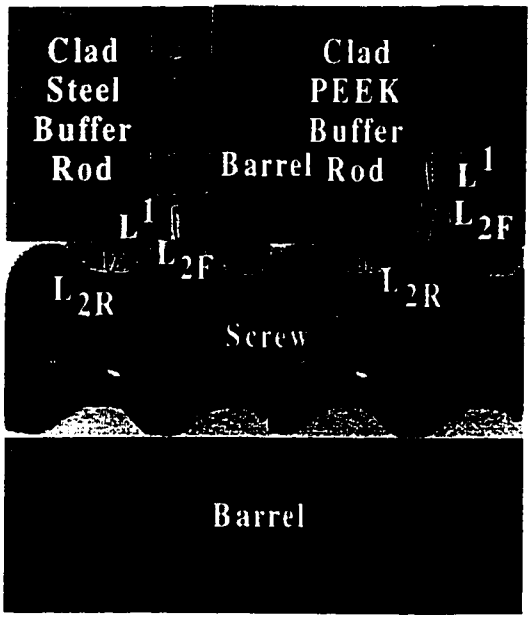


Fig.4.20: Measurement configuration in the barrel section for pressure and temperature tests, and polymer extrusion monitoring.

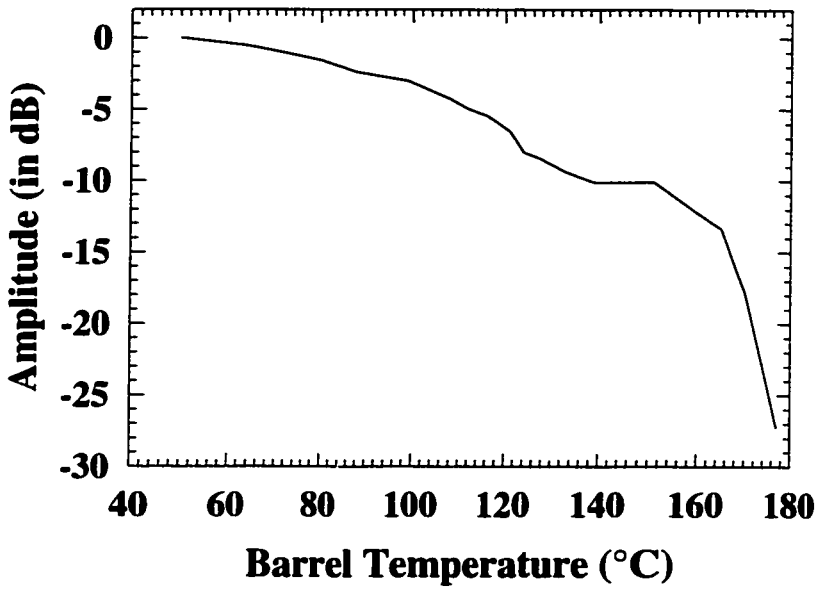


Fig.4.21: The amplitude of L^1 in the tapered PEEK CBR shown in Fig.4.7 versus the barrel temperature. There is no polymer inside the barrel.

Using the PEEK CBR and the above-described experimental configuration, we monitored polymer extrusion. For proprietary reasons, the name of the polymer is not released here. Ultrasonic signals having passed through this molten polymer and reflected from the top of the screw flight, L_{2F}, (upper two curves) and screw root, L_{2R}, (lower two curves) are presented in Fig.4.22, where subscript "2" denotes the round trip distance between the end of the probe and the screw position. The screw was a 20/20 conveying element (pitch and element length are both 20 mm), and the distance between the flight and root was about 4.5 mm. The temperature of the barrel, the temperature and pressure of the molten polymer, and the screw rotation speed were 147°C, 107°C, 1.1 MPa, and 100 rpm, respectively. The original recorded signal is marked as raw, and the one having undergone digital signal processing is denoted as processed [101]

For the case in which the temperature of the barrel, the temperature and pressure of the molten polymer, and the screw rotation speed were 200°C, 130°C, 1.2 MPa, and 100 rpm, respectively, the ultrasonically monitored results are shown in Fig.4.23. We can

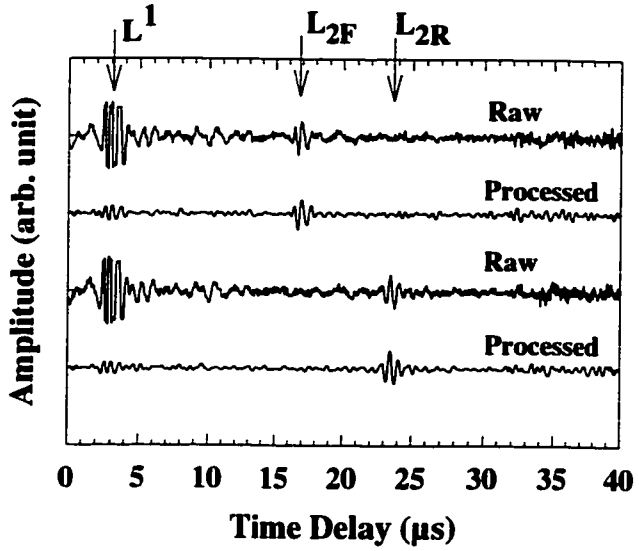


Fig.4.22: Ultrasonic monitoring results of the PEEK CBR shown in Fig.4.7 at the flight (upper two curves) and root regions (lower two curves). The temperature of the barrel, the temperature and pressure of the molten polymer and the screw rotation speed are 147°C, 107°C, 1.1 MPa and 100 rpm, respectively.

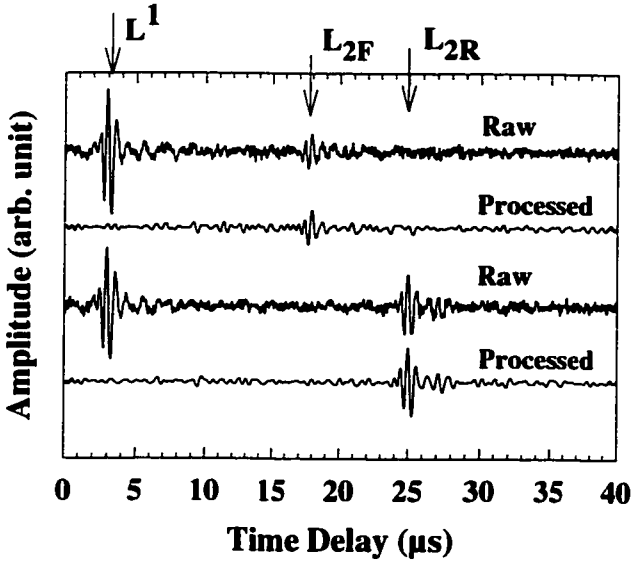


Fig.4.23: Ultrasonic monitoring results of the PEEK clad buffer rod shown in Fig.4.7 at the flight (upper two curves) and root regions (lower two curves). The temperature of the barrel, the temperature and pressure of the molten polymer and the screw rotation speed are 200°C, 130°C, 1.2 MPa and 100 rpm, respectively.

see that in spite of the increase of acoustic attenuation inside the CBR with temperature, shown in Fig.4.21, the strength of the signal $\mathbf{L_{2F}}$ is about the same at the higher temperature and pressure (Fig.4.23) as at the lower temperature and pressure (Fig.4.22), and the signal strength of $\mathbf{L_{2R}}$ is even higher in Fig.4.23 than that in Fig.4.22. This is because the ultrasonic attenuation (loss) in the extruded molten polymer is reduced at the higher temperature and pressure. This amount of loss reduction in the extruded polymer is larger than the increased ultrasonic loss with temperature in the PEEK CBR (Fig.4.21). The f_M of echoes $\mathbf{L_{2F}}$ and $\mathbf{L_{2R}}$ is about 1.7 MHz.

We also observe that the time delay between $\mathbf{L^1}$ (the probing end) and $\mathbf{L_{2F}}$ is twice that between $\mathbf{L_{2F}}$ and $\mathbf{L_{2R}}$. This means that the distance between the probing end and the top of the screw flight is about 9 mm. Because the cladding thread made of the epoxy-aluminium composite is not strong, we did not exert too much force to screw the buffer rod further into the barrel, for fear of damaging the thread. To eliminate this concern, a metal casing will be used in a future work. The casing will embrace the entire periphery except the UT and probing ends of the PEEK CBR, and threads will be cut on this metal casing. Such a probe then can be screwed fully into the barrel in such a way that the probing end is flush with the inner cavity wall of the extruder.

Furthermore, it is of interest to compare the ultrasonic performance of the PEEK CBR with that of the steel CBR, reported in Chapter 3, for ultrasonic monitoring of polymer extrusion. Thus, the steel CBR with the Dynisco probe shape was screwed into the barrel together with the PEEK CBR (88 mm apart, as shown in Fig.4.20) and a Dynisco temperature and pressure sensor. The probing end of the steel CBR was flush with the inner cavity wall of the extruder. A UT (5 MHz, 6.35 mm diameter) identical to that attached to the PEEK CBR was mounted on the UT end of the steel CBR. It was observed that the frequency f_M of $\mathbf{L^1}$ in the steel CBR is around 7.2 MHz. This up-shift of frequency from 5 MHz to 7.2 MHz is caused by the special shape and material combination of the core, and the cladding of the steel CBR [43] used here. With regard to operating frequency, the PEEK CBR operating at f_M of 1.7 MHz has some advantages

over the steel CBR operating at f_M around 7.2 MHz, because the ultrasonic attenuation in the molten polymer within the temperature range mentioned earlier is approximately proportional to the square of the operation frequency [3].

Fig.4.24 shows the monitored results when the barrel temperature, the temperature and pressure of the molten polymer, and the screw rotation speed were 147°C, 107°C, 1.1 MPa, and 100 rpm, respectively. For the case in which the temperature of the barrel, the temperature and pressure of the molten polymer, and the screw rotation speed were 200°C, 130°C, 1.2 MPa, and 100 rpm, respectively, the monitored results are given in Fig.4.25. In Fig.4.24, because of the short distance between the probing end (flush with the inner cavity wall) and the top of the flight, multiple echoes such as $\mathbf{L_{2F}}$, $\mathbf{L_{4F}}$, $\mathbf{L_{6F}}$ *etc.* traversed back and forth. Due to the large acoustic impedance mismatch between the steel CBR and the molten polymer, only a small amount of the ultrasonic energy was transmitted into the molten polymer. As a consequence, $\mathbf{L_{2R}}$, the signal reflected from the screw root, was not detected. However, once the pressure and the temperature increase, $\mathbf{L_{2R}}$ is clearly seen, as shown in Fig.4.25. This is due to the fact that the attenuation (loss) in the extruded molten polymer reduces at higher temperature and pressure.

The above comparison suggests that, within a certain pressure and temperature range, PEEK CBRs have advantages over steel CBRs for polymer extrusion monitoring. Certainly, steel is much stronger and more rugged than polymer. In a future work, we will improve the mechanical strength and ruggedness of the PEEK CBR and explore further its advantages, in particular, for shear wave reflection measurements [35,97] for high accuracy in-line probing of the viscosity of molten polymers.

In Figs.4.22-4.25, ultrasonic signals $\mathbf{L^1}$ and $\mathbf{L_{2F}}$ have good SNR. This means that an accurate distance between the probe end and the top of the screw flight can be obtained if the ultrasonic velocity of the molten polymer is known.

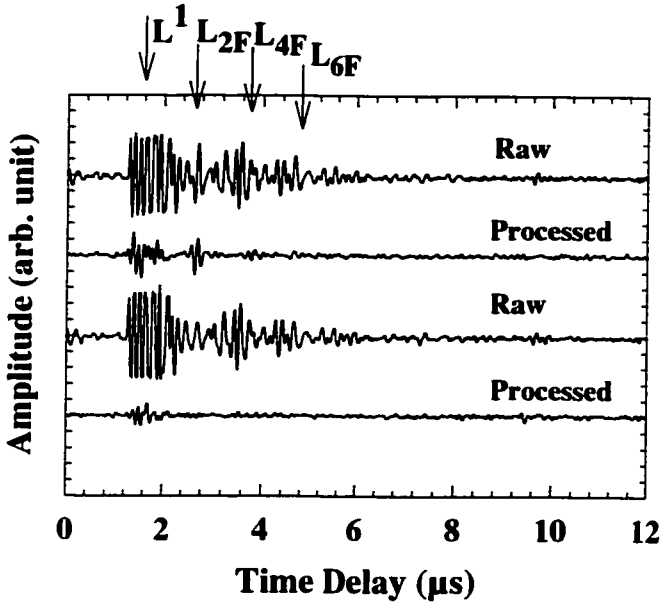


Fig.4.24: Ultrasonic monitoring results of a steel CBR (Fig.3.3) at the flight (upper two curves) and root regions (lower two curves). The temperature of the barrel, the temperature and pressure of the molten polymer and the screw rotation speed are 147°C, 107°C, 1.1 MPa and 100 rpm, respectively.

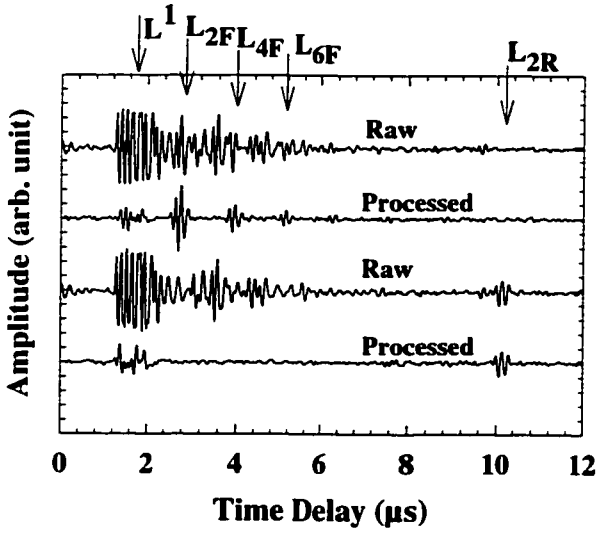


Fig.4.25: Ultrasonic monitoring results of a steel CBR (Fig.3.3) at the flight (upper two curves) and root regions (lower two curves). The temperature of the barrel, the temperature and pressure of the molten polymer and the screw rotation speed are 200°C, 130°C, 1.2 MPa and 100 rpm, respectively.

4.5-Summary

The fabrication and performance of polymer CBRs consisting of a polyetheretherketone (PEEK) core and a cladding made of a heat resistance epoxy-aluminium composite are presented. The core can have a uniform diameter or a tapered shape. Comparisons between PEEK, polyimide (VESPEL) and HDPE rods are given. For longitudinal waves, the signal strength of the $\mathbf{L^1}$ echo in the PEEK rod is 7 dB and 14 dB higher than that of VESPEL and HDPE, respectively. And for shear waves, the signal strength of the $\mathbf{S^1}$ echo in the PEEK rod is more than 20 dB larger than that in VESPEL or HDPE. Thus PEEK was chosen for our core material.

Ultrasonic measurement results indicate that the ultrasonic signal strength and SNR of the PEEK CBR with either a uniform or a tapered core shape are better than those of the corresponding PEEK NCBRs, for both longitudinal and shear waves. For this core-cladding combination, the acoustic impedance of the epoxy-aluminium composite cladding is higher than that of the core, and also improves ultrasonic wave guidance in the core itself [75,76].

Ultrasonic monitoring of the curing process of a 3.1 mm polyester sample at ambient temperature using the developed PEEK CBRs was performed. During the curing process, it was demonstrated that the PEEK CBR together with a single shear wave UT operating in pulse-echo mode could generate, simultaneously and at the same location, both shear and longitudinal waves. An additional advantage of PEEK CBRs over the non-clad ones is that the problem caused by the capillary effect in reflection coefficient measurement is eliminated. Ultrasonic velocities and reflection coefficients of longitudinal and shear waves were then measured in order to determine the density and viscosity change of polyester during the cure.

Applications of tapered PEEK CBRs for ultrasonic monitoring of polymer extrusion were carried out as well. The barrel temperature and the temperature and

pressure of the molten polymer ranged from 147 to 200°C, 107 to 130°C, and 1.1 to 1.2 MPa, respectively. The rotation speed of the screws was 100 rpm. The monitoring results also reveal that within certain operating temperature and pressure ranges, polymer CBRs operating at around 1.7 MHz show specific advantages over steel CBRs operating at around 7.2 MHz.

Chapter 5

In-line Ultrasonic Monitoring in Molten Metals

5.1-Introduction

Current ultrasonic techniques for evaluating the cleanliness of molten metals are based on the use of non-clad metal buffer rods. Due to the poor ultrasonic wave guidance provided by such rods, the pitch-catch configuration, consisting of a transmitting and a receiving rod, is usually used. However, this system has the disadvantages of alignment and bulkiness, being of reduced practicality in the harsh industrial environment.

In this chapter, our purpose is to demonstrate the potentiality of steel CBRs for ultrasonic inspection of molten metals, including cleanliness assessment. As CBRs have shown excellent ultrasonic guidance in pulse-echo mode at high temperature [22,39], just one probe is required for monitoring molten metal processes, thus overcoming the problem of alignment. In the present study, we perform ultrasonic imaging and particle detection in molten zinc, with the requirement of both high SNR and high spatial resolution at temperatures near 600°C, which is higher than the melting point of zinc (420°C). In fact, temperatures higher than 700°C have been reached in our experiments in order to improve the ultrasonic coupling (good wetting) to the molten zinc and simulate the melting temperature of aluminium (660°C) and magnesium (650°C), common industrial materials. Our technique follows a standard approach of assembling a UT on the cooled end of a CBR. High spatial resolution is then achieved by making a spherical acoustic lens at the probing end of the CBR. It will be shown that, at 600°C, the ultrasonic velocity in steel is much faster than in molten zinc; thus the spatial resolution

of the focus lens is improved. An additional advantage of using CBR is that the cladding material protects the core from the reactive molten metal.

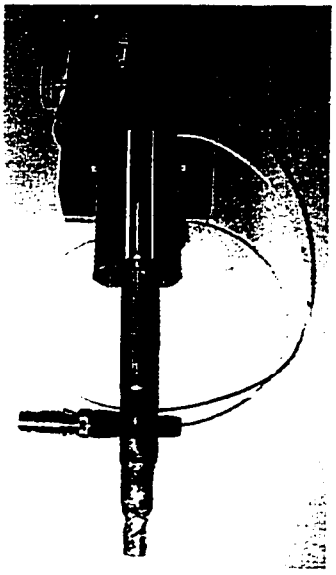
Images exhibiting high resolution in molten zinc are generated using the common C-scan imaging technique. Following that, we detect small particles present in molten zinc, as they pass by the focal zone of the focusing CBR. To demonstrate the high resolution imaging and particle detection capabilities achieved by the CBR in molten zinc, similar experiments will be carried out in water at room temperature.

It is well known that ceramics such as alumina has higher corrosion resistance in molten metals (*e.g.*, zinc) than steel does. Here, we present a focusing alumina CBR. It can sustain high temperatures (>1000°C), but attention should be paid to its poor thermal shock resistance. Ultrasonic images produced by this focusing alumina CBR of a small object in water at room temperature will be presented. These images are intended to demonstrate that high spatial resolution can be achieved by such a probe due to the high ultrasonic velocity in alumina, leading to a reduction of the lens spherical aberration [72].

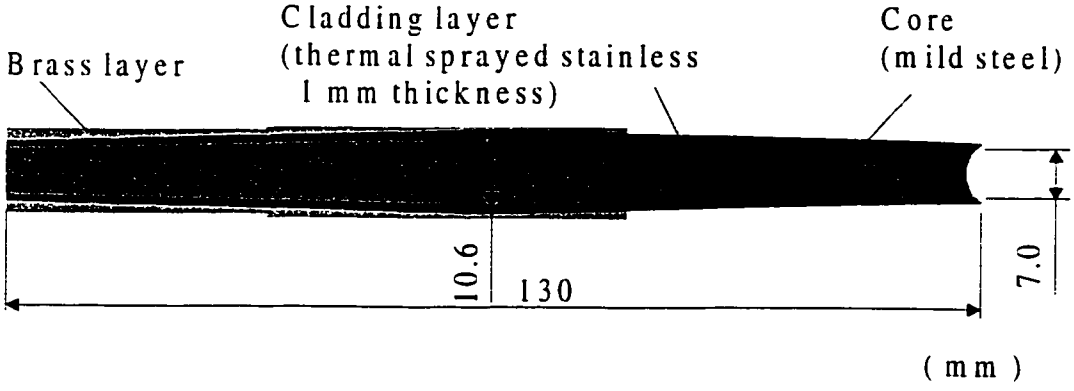
5.2-Ultrasonic probes and experimental setup

The CBR used in this part of the thesis research consists of a mild fine grain steel core with a double-taper shape, a thermal-spray stainless steel inner cladding, and a bronze outer cladding, but only for cooling section near the UT end. Fig.5.1(a) shows the CBR, and its schematic view with dimensions is depicted in Fig.5.1(b). The thermal-spray stainless steel cladding provides sufficient thermal shock resistance when the CBR is immersed in molten metal. Furthermore, it protects the steel core against interaction with molten zinc, and ensures the proper ultrasonic guidance in the core, resulting in signals with good SNR [22,86]. The bronze outer cladding is merely used for machining efficient cooling channels in the vicinity of the UT, and holding the CBR itself. This combination of materials for the core and cladding sustains the CBR against the

interaction with molten zinc during long time exposure [22], while providing ultrasonic signals with high quality [22,39,86].



(a)



(b)

Fig.5.1: Steel CBR used for ultrasonic monitoring of molten metal. (a) external view; (b) schematic diagram.

The experimental setup is shown in Fig.5.2. The C-scanning system introduced in Chapter 2 is used here for imaging generation. The ultrasonic probe, consisting of CBRs and 5 or 10 MHz broadband longitudinal UT (6.35 element diameter), is fixed in a mechanical arm equipped with micropositioners, with the UT extremity air-cooled. The ultrasonic probe is driven by a 5072 PR pulser/receiver (Panametrics, Inc.) in the pulse-echo mode. Signals are acquired at 100 MHz sampling rate, and averaged 10 times. A furnace (Lucifer Furnaces, Inc.) reaching temperatures near 2000°C is employed for melting zinc.

The ultrasonic longitudinal velocity of the molten zinc here investigated is not a known parameter. Knowledge about its value at a given temperature is important for estimating the spatial resolution of focusing steel CBRs in molten zinc, for example. Therefore, the classic approach based on the measurement of the time-of-flight of ultrasonic waves reflected from a target in pulse echo mode is utilised for determining the longitudinal velocity in molten zinc. For this end, we chose a CBR operating with a 5 MHz longitudinal UT, similar to the one designated as rod#0 in Chapter 2. We chose a small piece of alumina (Al_2O_3) plate to be the reflector of ultrasonic pulses in molten zinc. The alumina plate was chosen because of its high acoustic impedance, which leads to a high reflection coefficient at the liquid zinc/alumina interface. The CBR fixed in the mechanical arm was then immersed in the molten zinc bath, and aligned with respect to the alumina plate. The distance between the CBR probing end and the alumina reflector was adjusted to 14 mm. Fig.5.3 shows a typical ultrasonic signal obtained at 650°C; signals were also acquired at 600°C. The time difference between echoes $\mathbf{L_2}$ and $\mathbf{L_4}$ (the first and second round-trip echoes over the alumina plate) leads to the ultrasonic longitudinal velocity, V_L=2800 m/s, in molten zinc at this temperature. In addition, the longitudinal velocity in the steel CBR can be obtained from echo $\mathbf{L^1}$ (the first round-trip echo in the CBR), being about V_L=5500 m/s at 600°C.

In order to provide high spatial resolution, a spherical concave surface [70-72] was machined at the end of the CBR, as shown in Fig.5.4. It is used as a point-focus

acoustic lens for generating and receiving focused ultrasonic waves at a point. This lens has a radius, R, of 4.75 mm and an aperture diameter, D, of 7.0 mm. The lateral

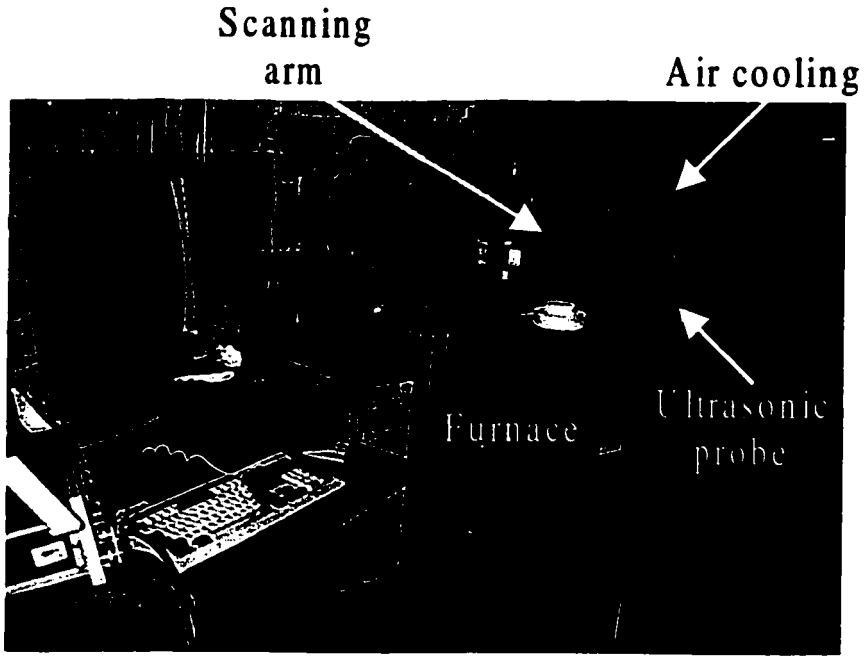


Fig.5.2: Experimental setup used for ultrasonic monitoring of molten metal.

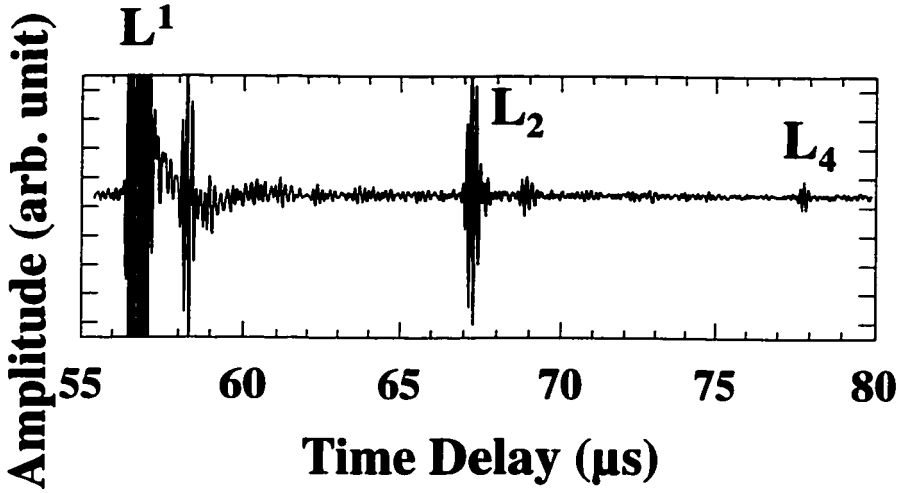


Fig.5.3: Ultrasonic signal reflected from an alumina plate immersed in molten zinc at 650°C.

resolution, dr, and the focusing depth, dz, of the acoustic lens can be roughly estimated from well-known formulae [72]: $dr = Kr\ \lambda\ F/D$ and $dz = 4\ Kz\ \lambda\ (F/D)^2$, where λ is the wavelength, F is the focal length, and Kr and Kz are constants close to unity, respectively. F can be determined from $R / (1 - V_{L2} / V_{L1})$ [70], where V_{L1} and V_{L2} are the longitudinal velocities in the steel CBR and in liquid, respectively. At 600°C, $V_{L1} = 5500$ m/s for steel, and $V_{L2} = 2800$ m/s for molten zinc. At room temperature, these values are $V_{L1} = 5980$ m/s for steel and $V_{L2} = 1497$ m/s for water [102]. Therefore, we obtain $F = 9.7$ mm for molten zinc and $F = 6.4$ mm for water. If $Kr = Kz = 1$, dr and dz at 10MHz are derived to be about 388 and 2150 μm in molten zinc, and 136 and 498 μm in water, respectively. This means that the spatial resolution in molten zinc is worse than in water. In this work, two kinds of experiments are performed using focusing CBRs: C-scan imaging and impurity detection. The probes utilised in these experiments use the focusing CBR shown in Fig.5.1, and another much longer focusing CBR, to be described later.

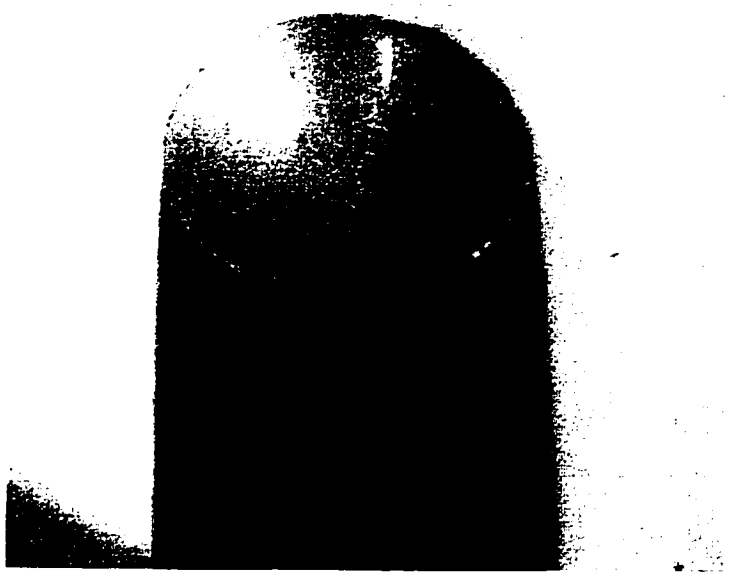


Fig.5.4: Picture of a point-focus acoustic lens fabricated at the end of the steel CBR.

In order to investigate the focusing ability of the acoustic lens shown in Fig.5.4, the lateral resolution was evaluated by using a thin stainless wire (0.38 mm in diameter)

as a target immersed in both molten zinc and water, as described in Chapter 2. But this time the wire material and thickness were selected to last long enough in the hostile molten zinc. The wire was laterally scanned across the focal point in the lens focal plane. A typical back-scattered echo from the wire in molten zinc at 650°C is shown in Fig.5.5. In this figure, L^1 denotes the first round-trip echo in the CBR, as mentioned before, and $L_{Wire,F}$ is the echo reflected from the wire at the focal distance. It is noted that such a thin wire is clearly detectable. For comparison purposes, Fig.5.6 shows the variation in echo amplitude with respect to the lateral scanning of the focusing CBR in molten zinc and water. Thus, the ultrasonic wave can be focused onto a small spot of about one wavelength to provide high lateral resolution, even in molten zinc. Since molten zinc is corrosive, it may affect the performance of the acoustic lens by distorting the spherical concave shape, if the lens is immersed in molten zinc for a long period of time. We observed no significant deterioration of the lens during an immersion time of about two hours. Based on this result, an attempt was then made to perform ultrasonic imaging in molten zinc.

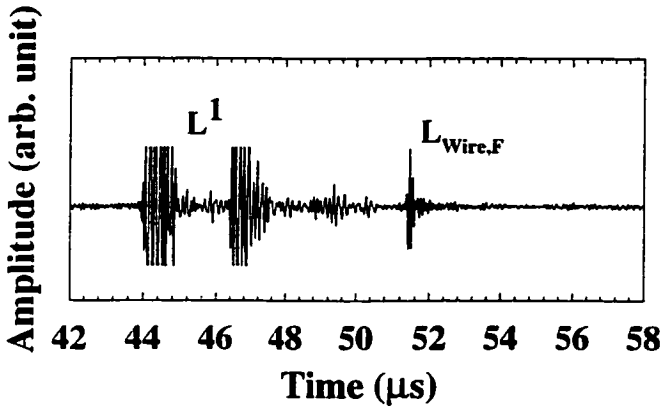


Fig.5.5: Reflected echo from a thin stainless wire located in the focal plane of the acoustic lens in molten zinc at 650°C.

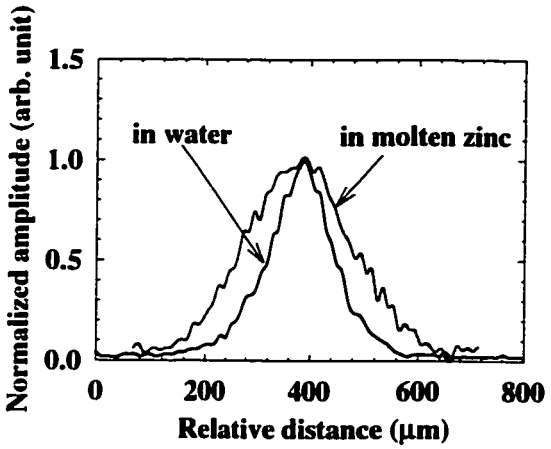


Fig.5.6: Lateral variation of the reflected echo shown in Fig.5.5. The data for water and molten zinc were normalised to the same peak amplitude.

5.3-Ultrasonic imaging in molten zinc

Ultrasonic images may provide a fair quantitative evaluation of the spatial resolution achieved by the probe, before attempting particle detection. For the demonstration, a small steel object with the three letters NRC engraved on its surface was immersed in both water and molten zinc with the grooves upwards, facing the lens. Fig.5.7 is a photograph of the steel object. The width and depth of the grooves are 1.2 mm and 0.4 mm, respectively. The CBR operated with a 10 MHz longitudinal UT was lowered into the liquid bath so that the surface of the steel object was brought into the focus of the acoustic lens, with the incident ultrasonic waves normal to the surface. A spherical ball was welded to a 25 cm L-shape wire at its end, and it was used to remove the air bubble trapped in the spherical concave lens. The scanning was carried out with a displacement step size of 200 μm. Fig.5.8 shows typical reflected echoes from water/steel and molten zinc/steel interfaces. The reflected echo from the molten zinc/steel interface has an SNR of 35 dB, which is almost the same as from the water/steel interface, and sufficient for performing ultrasonic imaging. It is noted in Fig.5.8 that the amplitude of

the reflected echo from the molten zinc/steel interface is larger than the one from the probing end of the CBR, which is denoted as L^1. This is explained by the small ultrasonic

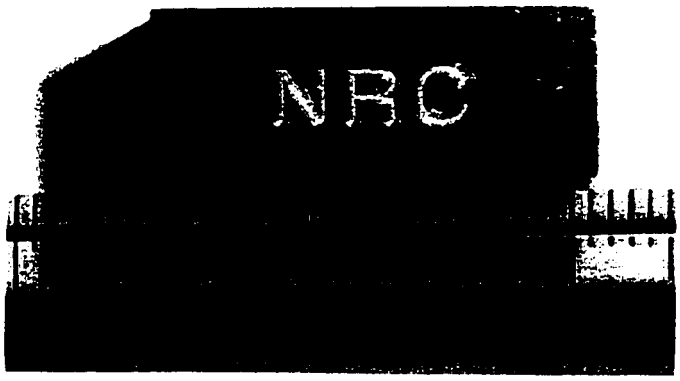


Fig.5.7: Photograph of the steel object showing the three letters NRC engraved on a surface.

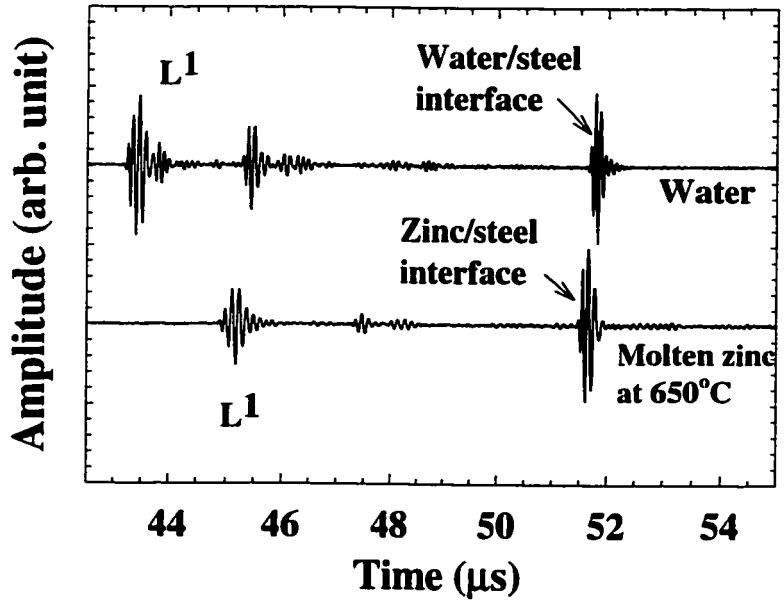
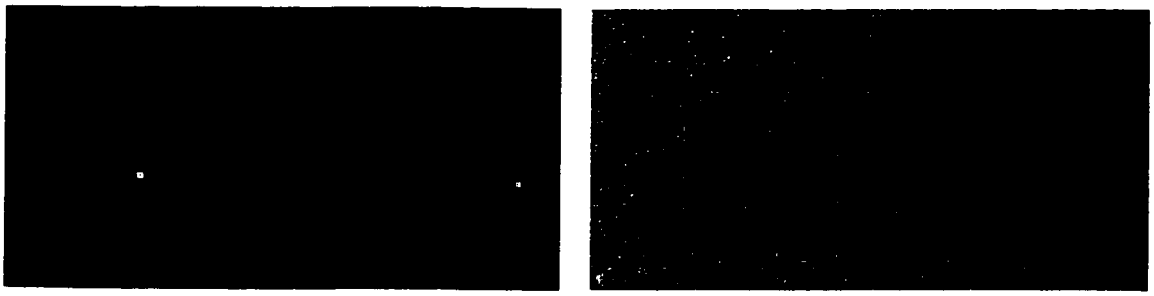


Fig.5.8: Reflected echoes from water/steel and molten zinc/steel interfaces.

impedance mismatch between the CBR and molten zinc, and good wetting (*i.e.*, ultrasonic coupling) between them, enabling a large amount of ultrasonic energy to be transmitted from the CBR into molten zinc. There was no significant difference in the frequency spectra for both echoes at the liquid/steel interfaces. In Fig.5.8 we can observe that the echo from the rod end in molten zinc is delayed compared to that in water. The reason for this time shift is due to a significant increase in temperature of the CBR immersed in molten zinc, and then the velocity in the CBR reduces.

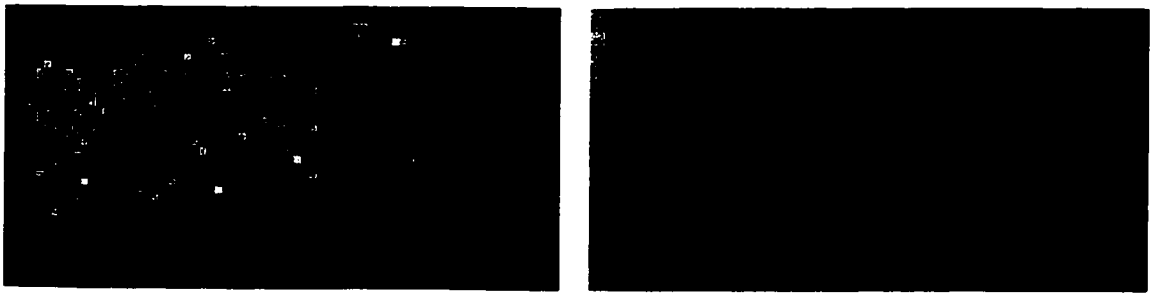
Ultrasonic images in water and in molten zinc at 600°C are shown in Figs.5.9 and 5.10, respectively. Each of these images took about 30 minutes to complete. Here the raw data are presented, but image quality could be improved by applying signal processing techniques. The variations in peak amplitude, or the time delay of the reflected echo coming from the focal zone, mainly caused by irregularities in the sample surface, were used for imaging. Higher intensity in the time-delay or amplitude images corresponds to a longer time delay or greater amplitude, respectively. In the amplitude plots shown in Figs.5.9(b) and 5.10(b), the dark zone delimiting the letters NRC is caused by a weaker echo at locations where the engraved surface is inclined and curved. It is noted that, since the wave velocity in molten zinc at 600°C is about twice as fast as in water, the variation of time delay in water is about twice as large as in molten zinc. Also, as expected, the images obtained in water have a better spatial resolution than those obtained in molten zinc because of the shorter wavelength. This is in agreement with Fig.5.6. The dirty spots observed in Fig.5.10 may be caused by chemical compound layers, such as oxide films, formed on the steel sample surface due to chemical reactions at high temperature, or by deterioration of the acoustic signal because of the ultrasonic scattering induced by impurities floating in the molten zinc. Although there is a steep temperature gradient in the CBR due to a large temperature difference between the immersed and air-cooled parts, no marked influence on the images of Fig.5.10 is observed, provided the temperature gradient is kept nearly constant. As both images shown in Figs.5.10(a) and 5.10(b) have sufficient quality to recognise the letters NRC, the results of this experiment

reveal that the use of CBRs with acoustic lens is appropriate for ultrasonic imaging in molten metals.



(a) (b)

Fig.5.9: Ultrasonic images of "NRC" in water at room temperature: obtained (a) by plotting the time delay of the echo; (b) by plotting the echo amplitude.



(a) (b)

Fig.5.10: Ultrasonic images of "NRC" in molten zinc at 600°C: obtained (a) by plotting the time delay of the echo; (b) by plotting the echo amplitude.

5.4-Detection of particles

One of the aims of this thesis research is to develop ultrasonic sensors and techniques based on CBRs for quantitatively estimating the cleanliness of molten metals by directly detecting the presence of impurities and inclusions. Usually, the inclusions found in aluminium ingots are alumina. In order to simulate detection of such particles in the laboratory, the detection of PVC particles suspended in water was carried out using a long CBR at room temperature, the one designated as buffer#4 in Chapter 2. It was chosen for this demonstration because it exhibits both good spatial resolution and SNR in pulse-echo mode. Furthermore, this CBR is almost one meter long, offering operational safety and isolation between the molten metal and the UT, and thus well fitted for in-line monitoring in the aluminium industry. The particle size was distributed between 15 and 50 μm, with an average size of 30 μm. Their concentration in water was about 30 ppm. The experiment with the PVC particles in water may be appropriate for simulating detection of alumina particles in molten aluminium, since the impedance ratio of PVC to water is almost equal to that of alumina to molten aluminium. It is also known that PVC particles can be well dispersed in water. Thus, clusters are not likely to be formed.

Fig.5.11 shows typical results of back-scattered signals from PVC particles near the focal area. A 10 MHz longitudinal UT was selected for this experiment. Although the particle sizes were smaller than the lateral resolution of the acoustic lens, clear signals from the particles were observed with high SNR (about 20 dB) due to the low noise in the CBR. The signal denoted by F in Fig.5.11 was saturated because the amplitude was too large. It should be noted that not only the amplitudes but also the shapes of the signals differ from each other. Fig.5.12 shows the frequency spectra corresponding to signals A to E in Fig.5.11. It can be seen that each signal has different frequency characteristics. It is believed that this difference in spectrum may probably reveal a variety of particle characteristics, such as the size, shape and clustering related to the particle volume fraction.

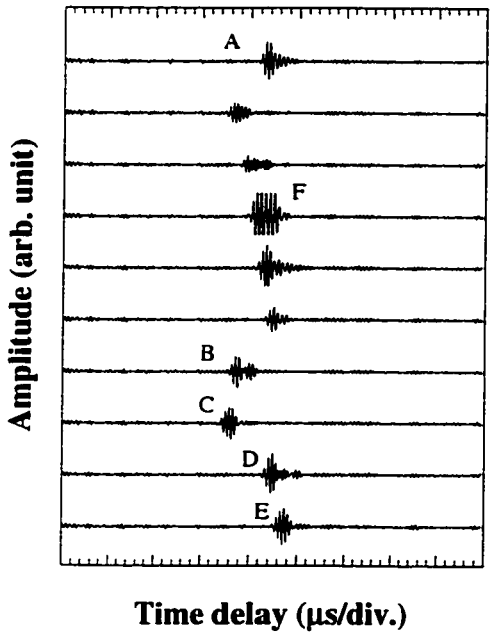


Fig.5.11: Back-scattered signals from PVC particles suspended in water at 10 MHz.

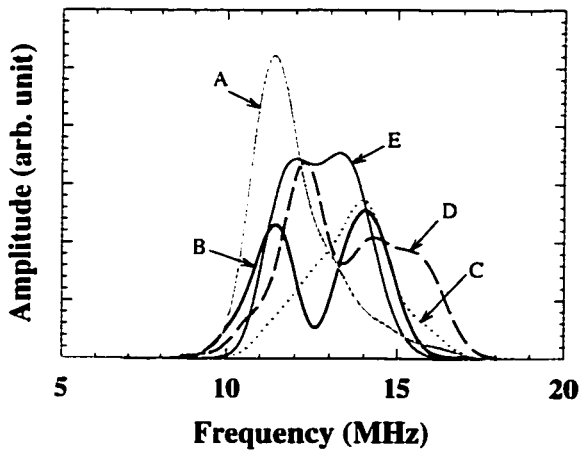


Fig.5.12: Frequency spectra of signals A to E in Fig.5.11.

In addition, we attempted particle detection in molten metal using the same focusing CBR, which provided high spatial resolution measurements in molten zinc. In the beginning of the experiment, the molten zinc at 650°C was at rest, and no significant echo was observed, as shown in the top trace of Fig.5.13. However, once the molten zinc was manually stirred, many echoes were observed near the focusing zone, as shown in the other traces of Fig.5.13. It is reasonable to consider that these echoes are scattered from particles in molten zinc passing through the focusing zone. Furthermore, it is observed that the number of echoes detected and their amplitudes decrease gradually in time after stirring, as shown in Fig.5.14. This means that many particles, including relatively large ones, are actively floating at the beginning of the measurement, and then, due to precipitation, the movement of the particles slows down as time goes on. Although details about these particles are not known at this moment, it is expected that focusing CBRs may be used for impurity particle detection in molten metals, *e.g.*, oxides or intermetallic particles suspended in molten zinc.

Figs.5.13 and 5.14 indicates significant differences in the amplitudes of the echoes scattered from these particles. However, small differences in the shape of the corresponding frequency spectra were observed. More investigation is needed. As was just mentioned, these differences may reflect a variety of particle characteristics.

5.5-Ceramic clad buffer rod

It was observed that focusing steel CBRs are promising tools for imaging and particle detection in molten metals. At 10 MHz operating frequency, these probes can attain simultaneously high spatial resolution and sufficient SNR and signal strength. In addition, these probes exhibit high thermal shock resistance and can operate at temperatures near 1000°C. However, for applications in corrosive environments, such as molten zinc, aluminium and magnesium, it is well known that steel CBRs will be chemically attacked [51]. As a result, after several hours of exposure, this probe should be replaced or the focus lens machined again. It is then desirable to develop probes chemically resistant to the severe molten metal environment. Ceramic materials, which

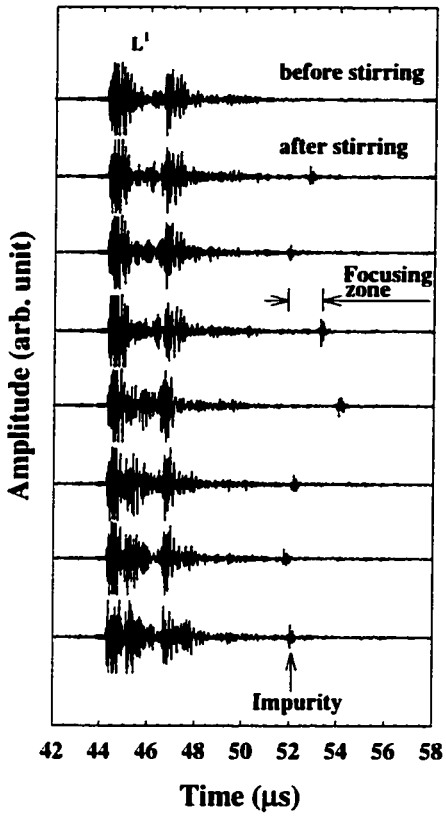


Fig.5.13: Back-scattered echoes from particles suspended in molten zinc at 650°C.

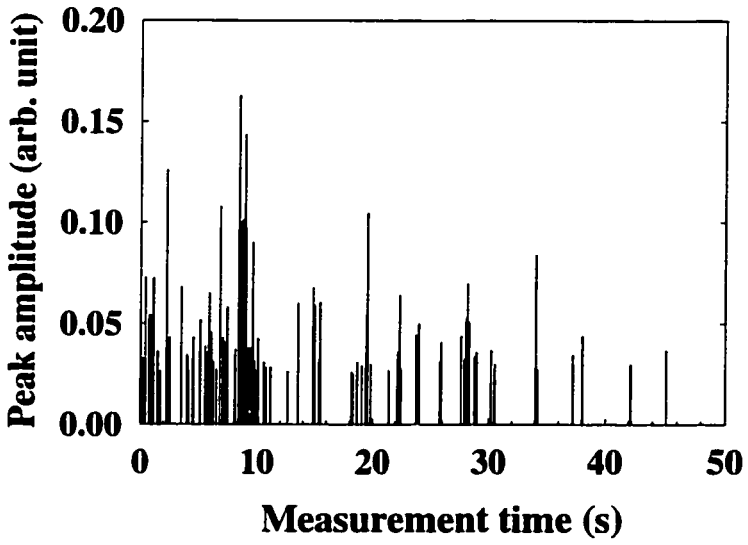


Fig.5.14: Change in the peak amplitude of the observed ultrasonic ehoes after stirring the molten zinc.

have high resistance to corrosion and also can sustain high temperatures, are interesting candidates for making CBRs for industrial applications. Here, our intention is to demonstrate that ceramic CBRs with a lens can be made and offer high spatial resolution.

For the demonstration, we select the alumina (Al_2O_3) CBR, shown in Fig.5.15, for performing ultrasonic imaging in water at room temperature. The Al_2O_3 cladding was fabricated by the thermal spray technique described in Chapter 2. The lens at the probing end was machined according to the procedure also described in Chapter 2. It was reported in reference [103] that these ultrasonic rods with a flat probing end could achieve sufficient SNR and signal strength in a pulse-echo mode. The experimental setup was the same used previously with the focusing steel CBR to produce the ultrasonic images of the small steel object containing the letters NRC, shown in Fig.5.7. Basically, the steel object immersed in water at room temperature was brought into the focal point of the probe for C-scanning. The step size used here was also 200 μm. The results for time delay and amplitude variations at 10 MHz are shown in Fig.5.16(a) and 5.16(b).

For Figs.5.9(a) and 5.9(b), obtained with the steel CBR, and Figs.5.16(a) and 5.16(b), obtained with the alumina CBR, the images were produced by different lenses; thus a precise comparison is not possible. However, it is clearly observable that the imaging quality achieved by the alumina CBR is superior. This may be attributed to the inferior spherical aberration provided by the alumina CBR, whose ultrasonic longitudinal velocity (10800 m/s) is roughly twice as the one in steel CBRs (5880 m/s) at room temperature. The spherical aberration is the difference between the geometrical focal length of the lens and its true focal length, which is caused by diffraction effects. A smaller spherical aberration leads to a better spatial resolution [72]. Therefore, focusing alumina CBRs may be an appropriate candidate for molten metal inspection. Special attention has to be paid to its poor thermal shock resistance. From our experience, it is needed to gradually heat the ceramic CBR before immersing it into the molten metal.

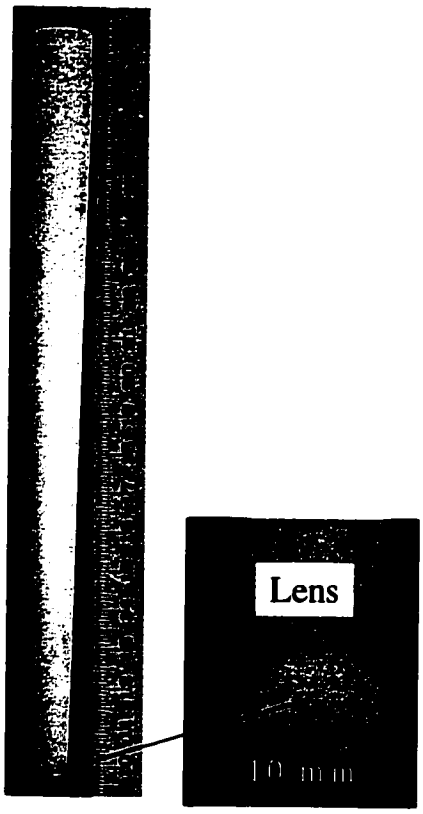
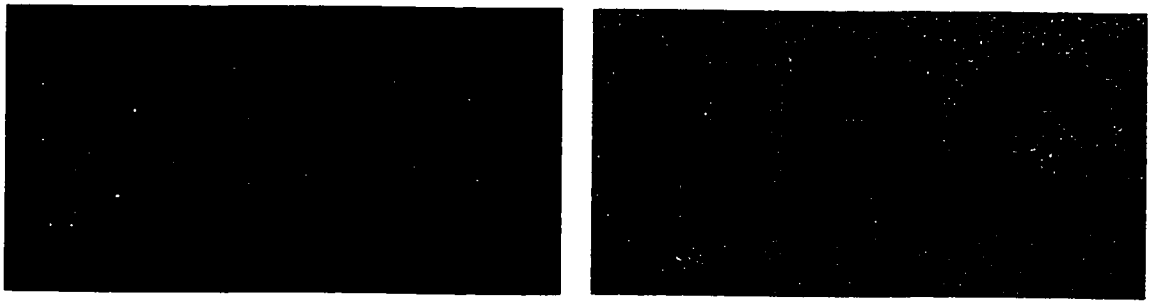


Fig.5.15: Photograph of the alumina CBR with a focus lens.



(a) (b)

Fig.5.16: Ultrasonic images of "NRC" in water at room temperature: obtained with the alumina CBR shown in Fig. 5.15: (a) by plotting the time delay of the echo; (b) by plotting the echo amplitude.

5.6-Summary

Ultrasonic imaging and particle detection were performed using focused steel CBRs in water at room temperature, and in molten zinc at temperatures higher than 600°C. In the experiments, molten zinc at temperatures higher than 600°C was chosen because it has a slow ultrasonic velocity (2800 m/s) compared to steel (5500 m/s), thus improving the spatial resolution of the focusing lens. In addition, it "wets" properly the focus lens of the steel CBR, allowing ultrasonic coupling to the molten metal, and simulates the melting point of aluminium (660°C) and magnesium (650°C), which are common used industrial materials. The focused ultrasonic waves were generated by a spherical acoustic lens fabricated at the end of the steel CBRs.

In order to evaluate the focusing ability of the steel CBRs, several experiments were carried out at 10 MHz in a pulse-echo mode. The lateral resolution at the focus of the spherical acoustic lens in molten zinc was quantitatively examined, and compared with that in water, using a thin stainless wire with a diameter of 380 μm as the ultrasonic target. High-resolution ultrasonic imaging of a small steel sample immersed in water or molten zinc was carried out by the common C-scan technique. The SNR of the reflected signals from the flat sample surface at the focus was better than 35 dB. These ultrasonic images were obtained from the amplitude and time delay variations of the reflected signals.

Taking advantage of the good spatial resolution demonstrated by our ultrasonic probes at 10 MHz, an attempt was also made to detect PVC particles of 30 μm average size suspended in water at room temperature, and oxides in molten zinc at 650°C. Back-scattered signals from particles were clearly visible at the focal region of the lens. The results reveal that the amplitude and shape of the frequency spectra of the ultrasonic signals may be sensitive to a variety of particle characteristics, such as the size, shape and clustering related to the particle volume fraction.

Because ceramic materials can sustain high temperature and have a higher corrosion resistance in molten metals than steel does, a focusing alumina CBR was fabricated and investigated. Such a probe produces ultrasonic images in water at room temperature exhibiting high spatial resolution. It is believed that the high ultrasonic velocity in alumina leads to a reduction of the lens spherical aberration, thus enhancing the spatial resolution of the probe.

Chapter 6

Contrapropagating Ultrasonic Flowmeter Using Clad Buffer Rods

6.1-Introduction

The determination of the liquid flow speed is important for process optimisation and control [104]. Among all available techniques for liquid flow evaluation, ultrasound is advantageous owing to non-intrusiveness, applicability to a large class of materials, robustness and low cost. In fact, ultrasonic inspection of liquid flow at room temperature is a well-established technique. However, several industrial processes, such as the polymer extrusion, polymer injection moulding and metal die casting, demand high temperature flowmeter systems for in-line monitoring and control of the melt flow speed. The difficulties encountered by ultrasonic measurement of flow speed and other processes at elevated temperatures are, as mentioned in Chapter 1, related to the Curie point of the UT piezoelement, disbonding between the electrode and the epoxy backing material and ultrasonic couplant [31]. These drawbacks may be overcome if a suitable buffer rod is interposed between the UT and the process liquid. The typical problem of using buffer rods is the appearance of spurious signals due to dispersion, multipaths, mode conversion and beam spread (diffraction loss) [68]. Thus, new materials and buffer rod configurations have been developed recently in order to reduce the spurious signals and allow flow speed measurements at high temperatures [31]. In these works, an ultrasonic contrapropagating flowmeter was designed employing a *hockey stick* buffer rod with superior waveguidance just for shear waves. This novel configuration could then successfully generate longitudinal waves for sensing the liquid flow. However, no attempts have been reported to cancel out the effect of temperature variation in the flow

measurement. Due to the rapid temperature variation that the melt flow of industrial processes may undergo, there is an urgent need of developing sensors and techniques immune to temperature variation.

The concept of CBRs for ultrasonic investigation of materials and processes have been used throughout this dissertation, and owing to certain advantages (superior wave guidance, robustness, machinability and capability to stand high temperature and pressure) [39], their performance for flow speed measurements at high temperature is investigated here. At this point, we emphasise that it is not the purpose of this work to provide a complete solution to the flowmeter problem, but rather to add contributions in the aspect of high temperature measurement for industrial applications.

In the present study, we intend to demonstrate that in the standard ultrasonic contrapropagating flowmeters, these CBRs exhibit superior SNR compared to the reported non-clad rods in through-transmission mode. A high SNR is desirable for more accurate evaluation of flow speeds [105]. In addition, specific ultrasonic signals in CBRs can be used for temperature calibration of flowmeters. Such a feature, which is explored for the first time, affords temperature variation while measuring accurately flow velocities.

6.2-Basic ultrasonic flowmeters

Most ultrasonic methods developed for flow measurement are categorised into three groups [2,57]: i) contrapropagation (travel time difference), ii) beam deflection, and iii) Doppler shift. The first two methods are based on the principle that the resultant velocity of acoustic waves travelling in a flowing fluid is the vector summation of the fluid speed and the acoustic velocity in the fluid at rest. In terms of measurement techniques, the contrapropagation method measures the difference in transit time between the acoustic waves transmitted downstream (with the flow) and upstream (against the flow) across the moving fluid. The beam deflection method, as suggested by the name

itself, measures the drift caused by the flow on the normal transmission of the acoustic waves. The Doppler shift method, on the other hand, is based on the well-known Doppler effect, and measures the shift in frequency in the reflected acoustic waves from scatterers in the fluid. Both contrapropagation and beam deflection methods are suited for homogeneous fluids, while the Doppler shift method depends on solid particles and gas bubbles in the liquid flow to scatter acoustic waves.

The large amount of work done on beam deflection and Doppler shift flowmeters testifies to their importance. Their unique features make them the obvious choice for some specific tasks [57]. In particular, the Doppler shift method has widespread use for measuring blood flow from outside the body, since it can sense a remote point employing just a single transducer [63]. However, such methods are sensitive to ultrasonic velocity in fluids, and compensation techniques must be implemented to account for velocity variation with temperature. In the industrial domain, where the process temperature of the liquid flow may vary quite often, the favoured method would be the one insensitive to ultrasonic velocity. As the contrapropagation method may attend to such a requirement [54], it is selected in this dissertation. The subsequent sections provide theoretical considerations underlying its flow sensing principle as well as its applicability for high temperature measurements.

6.2.1-Contrapropagating ultrasonic flowmeter

In contrapropagating ultrasonic flowmeters, the flow speed is obtained by measuring, over the same path, the time difference between two ultrasonic waves travelling downstream and upstream. The acoustic waves can be transmitted simultaneously, but typically they are alternated in order to avoid interference between them [58]. In terms of flowmeter realisation, ultrasonic sensors are placed either on opposite facing surfaces or on the same side, as illustrated in Fig.6.1. Here, we focus on the latter configuration (Fig.6.1(b)), for two basic reasons: it can be mounted on duct

surfaces with limited access, and it also enlarges the acoustic travel time difference inside the flow, which enhances resolution in time-domain measurements.

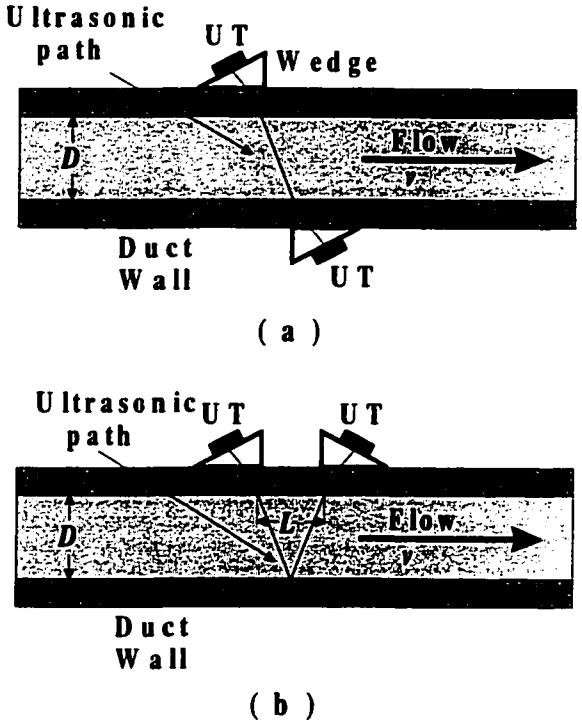


Fig.6.1: Basic configurations of contrapropagating ultrasonic flowmeters. (a) Ultrasonic probes assembled on the same side. (b) Ultrasonic probes assembled on opposite sides.

Pertaining to the generic duct of Fig.6.1, the downstream and upstream travel times are found by vector operation using either of the following two extreme assumptions on the ultrasonic source: it is infinitely wide (the wave front always travels parallel to itself) or it is a point source [59]:

$$t_d = \frac{L^2 + 4D^2}{V\sqrt{L^2 + 4D^2} + vL} \tag{6.1}$$

$$t_u = \frac{L^2 + 4D^2}{V\sqrt{L^2 + 4D^2} - vL}, \tag{6.2}$$

where L is the axial interaction length between ultrasonic beams, D is the distance perpendicular to the axis between ultrasonic beams, V is the ultrasonic velocity in the fluid and v is the average flow speed along the path. Indeed, if the ultrasonic velocity V were known accurately enough or remained constant, the average flow velocity v could be determined by transmission in one direction only (using either Eq.(6.1) or (6.2)), similar to what is normally done by the beam deflection method. For most flowing liquids, however, V is usually 300 to 700 times larger than v. Therefore if the fractional uncertainty in V is not exceedingly small, it is not possible to measure v to 1% or better unless transmission in both directions (*i.e.*, contrapropagation) is used to cancel V out [54]. Such an important feature of the contrapropagation approach is better appreciated by assuming that V is invariant during measurements of t_d and t_u. Hence V can be eliminated from Eqs.(6.1) and (6.2) to yield:

$$v = \frac{L^2 + 4D^2}{2L}\left(\frac{1}{t_d} - \frac{1}{t_u}\right). \tag{6.3}$$

Eq.(6.3) indicates that the average flow speed is a function of downstream and upstream acoustic travel times and duct geometry. Furthermore, it is no longer dependent upon ultrasonic velocity, V, justifying why the contrapropagation method is by far the main flowmeter technique applied in industrial environments.

It is always desirable that during flow measurement the sensor does not disturb the interrogated flow at all. In the contrapropagation method, this can be achieved by means of different installations of buffer rods and UTs over the duct, the weld-on, weld-in and clamped-on configurations being the most popular [54]. For industrial fluids subjected to severe conditions of temperature and pressure, those techniques are safe and effective. Whenever allowed, the weld-in configuration is the simplest one to analyse, for acoustic wave propagation is limited to the buffer rod and fluid only. Weld-on and clamped-on versions are more complex because the duct is encompassed by the buffer rod and fluid (Fig.6.1), requiring knowledge about material properties and thickness of

the duct wall for proper flowmeter design. Besides, the wall surface may also complicate the acoustic coupling to the fluid. These reasons suggest the weld-in type as the one to be analysed and implemented in this dissertation. Such an approach, shown in Fig.6.2, eliminates the coupling problem by setting the buffer rod probing end flush to the inner surface of a rectangular duct wall.

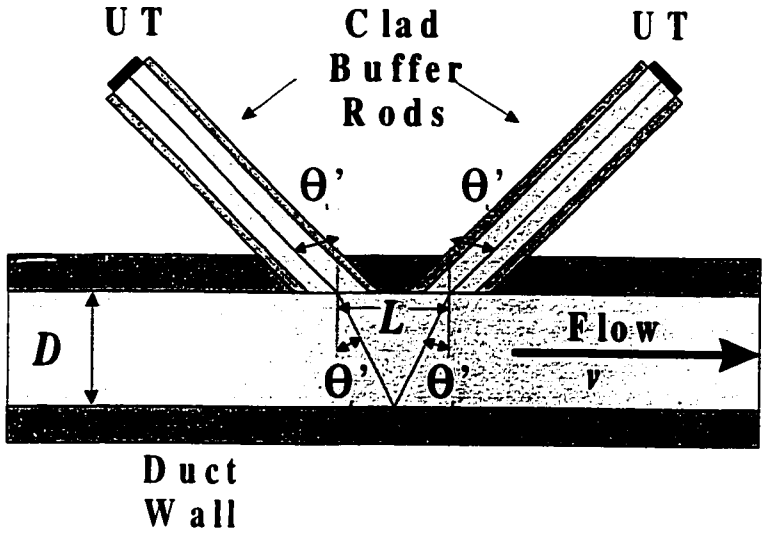


Fig.6.2: Weld-in contrapropagating ultrasonic flowmeter. The probing ends of the buffer rods are flush with the inner surface of the wall.

In the measurement of liquid flow by the weld-in contrapropagation method, it is desirable to launch the acoustic beam at a large angle to the duct, 40-60° being a usual angle of incidence [54]. Snell's Law and energy partitioning of acoustic modes dictate this criteria. These issues will be addressed in more detail later in this chapter. To introduce a few key concepts, let us estimate the angle at which the ultrasonic waves propagating in a chamfered buffer rod enter the liquid. Although two distinct sources, longitudinal and shear wave UTs, can be used in connection with chamfered buffer rods to generate the sensing signals, only longitudinal waves are transmitted through the liquid, since fluids in general do not support shear motion. The reason shear waves, under certain conditions such as polarisation and incidence angle, can generate longitudinal modes at the interface of two different media is explained by the mode conversion

property of the oblique incidence [102]. For steel CBRs, the longitudinal wave velocity in the core is about 6000 m/s at 25°C, and the shear wave velocity is about 3300 m/s. At high temperature, *e.g.* 200°C, these values are reduced a few percent (less than 5%). For simplicity, the velocity variation will be neglected in this first calculation. In hot water at 200°C, the longitudinal wave velocity is further reduced, to about 1000 m/s [2]. Assuming 40° incidence angle from steel to water, at 200°C, the angle of refraction is calculated according to Snell's Law [102]:

$$\frac{1}{V_i}\sin\theta_i' = \frac{1}{V_r'}\sin\theta_r' \tag{6.4}$$

where, regarding Fig.6.2, V_i is the incident ultrasonic velocity (longitudinal or shear) in steel clad buffer rods, θ_i is the angle of incidence to the normal of the surface, *i.e.* 40°, V_r' is the refracted longitudinal velocity in water, and θ_r' is the angle of refraction to the normal of the surface. If the longitudinal mode is transmitted, the angle of refraction in water is found to be $\theta_r' = \sin^{-1}[(V_r'/V_i)\sin\theta_i] \approx 6.1°$. Instead, if the shear mode is chosen, the refracted angle becomes $\theta_r' = \sin^{-1}[(V_r'/V_i)\sin\theta_i] \approx 11.2°$. Thus, for the same angle of incidence and propagation media, shear mode incidence results in a larger angle of refraction than longitudinal. Consequently, the propagation path in water and so the transit time difference between upstream and downstream transmissions are also enlarged. To put it in a quantitative way, we subtract Eq.(6.2) from (6.1) and obtain:

$$\Delta t = t_{up} - t_{down} \approx 2(v/V^2)L = 4(v/V^2)D\tan\theta_r', \tag{6.5}$$

assuming $V >> v$. In this example, therefore, the transit time difference measured by employing shear UTs is $\tan 11.2°/\tan 6.1° \approx 1.8$ times that measured by longitudinal UTs. Clearly, shear UTs are preferable over longitudinal ones for the weld-in contrapropagating flowmeter proposed herein: the resolution in time difference to be measured is increased about 80%, as well as the axial interaction length $L = 2D\tan\theta_r'$

between transmitter and receiver probes. Since UTs cannot be scaled down at will, the latter feature may be an extra advantage. Finally, the ultrasonic flowmeter to be implemented and tested in this work is the one illustrated in Fig.6.3, with shear transducers playing the role of both transmitter and receiver.

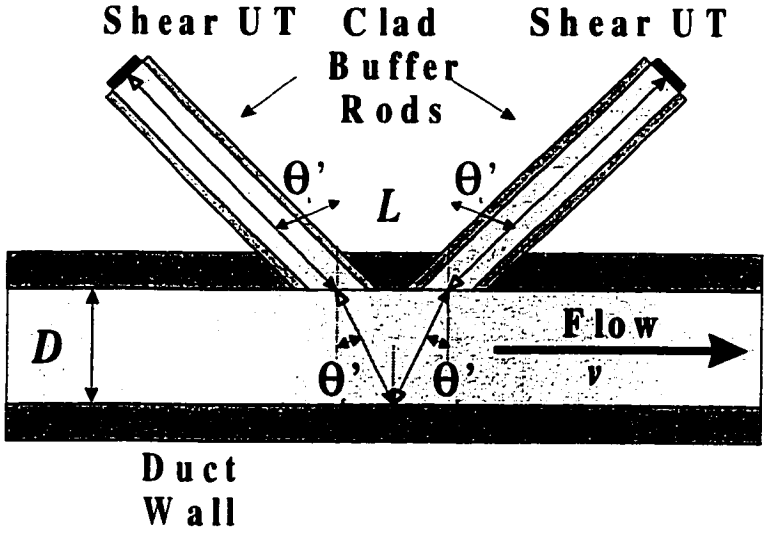


Fig.6.3: Weld-in contrapropagating ultrasonic flowmeter. Shear wave UTs are used to transmit and receive ultrasonic pulses.

6.3-Scattering theory for shear and longitudinal acoustic waves in isotropic media

In the contrapropagating flowmeter proposed in this chapter, shear wave UTs launch shear waves in the buffer rod, which are converted into longitudinal waves in fluids through mode conversion at the chamfered probing end of the buffer rod. The symmetric configuration provides that, after interacting with the flowing liquid, longitudinal waves are converted back into shear waves and detected by the receiving shear wave UT. Physical insight into the wave propagation phenomenon governing the flowmeter can be obtained from scattering theory of acoustic plane waves at the interface

of two media. These two media are treated here as isotropics, leading to simplification of the analyses.

When acoustic plane waves propagate along a given direction in the interior of isotropic or anisotropic solid materials, three mutually orthogonal solutions (or polarisation modes) exist [102]. In the specific case of isotropic solid materials, one wave solution is a pure longitudinal mode, and the other two degenerated pure shear (or transverse) modes. In the longitudinal mode particle displacement is parallel to the wave propagation direction; accordingly, in the shear mode particle displacement is perpendicular to the wave propagation direction. In fluids, only the longitudinal mode is present. It is worthwhile to mention that mode conversion at the interface of isotropic materials does not exist in the case of normal incidence of acoustic waves, but exists in the case of oblique incidence, except for horizontally polarised shear SH wave[1] [102]. In this case, the waves reflected back and transmitted through maintain the original polarisation. Nevertheless, for an oblique incidence of both vertically polarised shear SV and longitudinal L waves, mode conversion generally occurs[2]. However, it does not happen at certain incidence angles, called critical angles, at which either the reflected or transmitted converted mode completely disappears. In principle, one may obtain a fair idea of the oblique incidence characteristics by considering merely the slowness surfaces of the media involved in the scattering problem. Slowness surface is a plot of the inverse of the wave phase velocity *versus* the wave vector direction [102]. Although this tool is quite convenient for obtaining the directions of reflected and transmitted waves, it is limited in the sense that it does not address the energy partitioning among scattered acoustic waves. It reveals, at most, the range of angles of incidence at which certain scattered modes may exist. However, in the design of flowmeters, it is desirable to know at which angle of incidence a generic acoustic wave should impinge an interface in order to generate a specific mode with the highest efficiency (*i.e.*, highest energy). This is analytically achieved by solving the stress and particle velocity wave equations at both sides of the interface, and combining these solutions by means of boundary conditions

[1] SH wave corresponds to particle displacement of the medium parallel to the interface.

[2] For SV wave propagation, there is a component of the particle displacement orthogonal to the interface.

[102]. The resultant equations, known as acoustic Fresnel's equations, turn out to be suitable for calculating reflection and transmission coefficients for all scattered modes. These coefficients are defined, respectively, as the ratio of reflected and transmitted particle velocity wave amplitudes to the incident amplitude. The usefulness of such coefficients is that they can be related to the energy coupled with each scattered mode.

In this section, we are concerned with the scattering of both SV and L waves, the modes present in the contrapropagating flowmeter under investigation [54]. Basically, for either incident SV or L acoustic wave, the angle of incidence and materials properties are related to scattered angles and relative amplitudes and energies of all generated modes [106]. These results serve as a theoretical guide for designing a contrapropagating ultrasonic flowmeter with high efficiency for generating and detecting ultrasonic energy.

For the contrapropagating flowmeter configuration of Fig.6.3, ultrasonic waves generated by the transmitting shear UT are intended to propagate in the CBR, and be mode converted into longitudinal waves in the solid/liquid interface. After sensing the liquid, longitudinal waves must be converted back into shear waves in the liquid/solid interface, and finally be detected by the receiving shear UT. For an effective contrapropagating flowmeter design, it is necessary to maximise the fraction of energy reaching the receiving probe. Thus, three scattering coefficients should be determined: $(\Gamma_T)_{SL}$ (shear incidence to longitudinal transmission coefficient from solid to liquid), $(\Gamma_R)_{LL}$ (longitudinal incidence to longitudinal reflection coefficient from liquid to solid), and $(\Gamma_T)_{LS}$ (longitudinal incidence to shear transmission coefficient from liquid to solid). All these coefficients are functions of scattering angles as well as material properties of the propagation media. They can be found in most classical books on acoustics [106].

For the flowmeter considered here, assuming that the total ultrasonic power delivered by the transmitting probe is a unity $P_T = 1$, and neglecting losses in the media due to attenuation, the total ultrasonic energy conveyed to the receiving probe is

proportional to the product of the squared magnitudes of the scattering coefficients $(\Gamma_T)_{SL}$, $(\Gamma_R)_{LL}$ and $(\Gamma_T)_{LS}$, according to the following relation [2]:

$$E_R \propto [(\Gamma_T)_{SL}]^2 \cdot [(\Gamma_R)_{LL}]^2 \cdot [(\Gamma_T)_{LS}]^2 \qquad (6.6)$$

Thus, for a given combination of solid and liquid media, its magnitude is determined by the angle of incidence of shear waves in the solid/liquid interface.

6.4-Design of a contrapropagating ultrasonic flowmeter with clad buffer rods

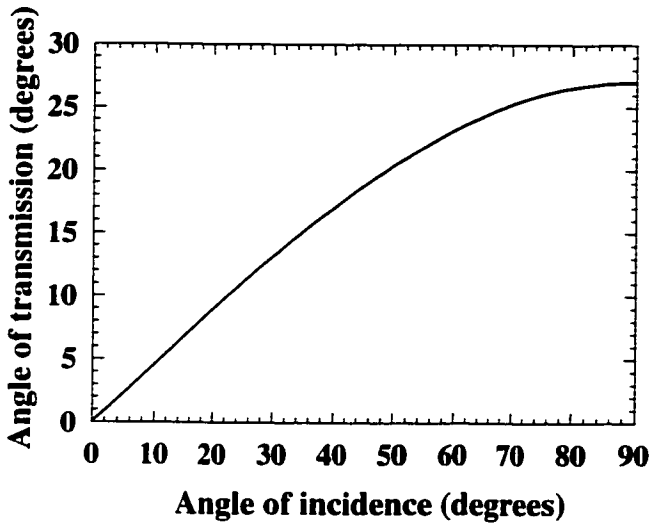
Before attempting liquid flow measurements at high temperature, the performance of CBRs as part of a contrapropagating flowmeter should be assessed. For this purpose, water flow at constant room temperature, the simplest alternative, is selected for initial tests. If the temperature is kept constant or varies slightly over the period in which ultrasonic signals are acquired, the contrapropagating approach is convenient for measuring flow speeds, because it is not necessary to know the ultrasonic velocity, V, in the liquid medium. The velocity, V, is cancelled out when combining upstream and downstream time measurements, according to Eq.(6.3). Alternatively, in this work we will make use of Eq.(6.5) because the ultrasonic velocity in water is a well-known parameter [2,54]. Its advantage is that one needs to measure only the difference in transit times, rather than their absolute values. Therefore, characterisation of the CBRs is carried out in an immersible water tank, which exhibits high thermal capacity.

As the first design criterion for the flowmeter configuration of Fig.6.3, a proper angle for the CBRs must be decided. The energy partitioning equation (Eq.(6.6)) is used for this purpose. This equation depends on the density, ρ, longitudinal velocity, V_L, and shear velocity, V_S, of the materials involved. At room temperature, these values for steel are: 7800 Kg/m^3, 5980 m/s and 3297 m/s. For water, the density and longitudinal velocity

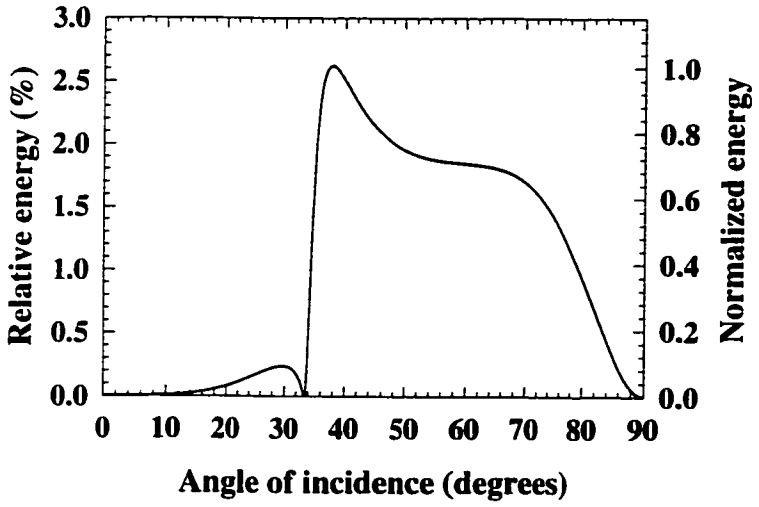
are: 1000 Kg/m^3 and 1497 m/s [2]. Substituting these values in Eq.(6.4) (Snell's Law) and finally in Eq.(6.6) (energy partitioning), we obtain in Fig.6.4 the angle of refraction in water and the relative detected ultrasonic energy in the flowmeter receiver, as a function of the angle of incidence of shear waves. The following interesting features are readily realised: 1) as the angle of incidence of shear waves in the steel/water interface varies from 0 to 90°, the angle of transmission of longitudinal waves in water varies from 0 to 27°; 2) angles of incidence near 34° must be avoided, as at this particular value shear waves are not converted into longitudinal waves; 3) also very important to be noted is that at around 37° incidence, maximum power is coupled from the transmitter shear probe to the receiver shear probe. Therefore, for effective detection of ultrasonic energy, an angle of incidence close to 37° should be selected. We decided for 40° incidence, corresponding theoretically to approximately 94% of the maximum coupled energy between probes. In addition, the energy curve is less sensitive to angle variation around 40° than at 37° incidence.

6.4.1-Comparison between clad steel buffer rods and non-clad buffer rods for contrapropagating ultrasonic flowmeter design

Cross-correlation is a popular technique for measuring downstream and upstream transit times in contrapropagating ultrasonic flowmeters [105]. Accurate transit time measurements require that spurious noises in the buffer rod do not interfere with the desired ultrasonic signals. It has been reported that steel CBRs are superior to non-clad counterparts for wave guidance in pulse-echo mode [39], leading to high SNR, *e.g.*, SNR $\geq$ 35 dB. Here, our aim is to verify, in through-transmission mode, the SNR superiority of CBRs over non-clad rods for use in the contrapropagating flowmeter.



(a)



(b)

Fig.6.4: Design parameters for the flowmeter shown in Fig.6.3. Liquid medium is water at room temperature. (a) Angle of transmission of longitudinal waves in water *vs.* angle of incidence of shear waves in steel.(b) Relative detected ultrasonic energy *vs.* the angle of incidence of shear waves in steel.

Fig.6.5 shows steel buffer rods selected for this comparison. One pair (at the left) are clad by the thermal spray technique (mentioned in Chapter 2), and the another pair are non-clad rods. The cladding material is stainless steel. They have approximately the same length, and each of their probing ends is cut at the same angle of 40°. Commercial 5 MHz shear UTs (6.35 mm element diameter) attached to the flat end of the buffer rods and operated in through-transmission mode were used for signal generation and detection. Each pair of steel CBRs and non-clad buffer rods were aligned with respect to a steel plate reflector immersed in water, lying 1 cm from the probing end surfaces, as illustrated in Fig.6.6. The signals acquired for each configuration are shown in Fig.6.7. It is noted that the signal amplitude for the case of CBRs is 6 dB stronger than that obtained from non-clad rods. In addition, detection of $\mathbf{S^1\text{-}S^1}$ echo is possible only with CBRs. The explanation and application of $\mathbf{S^1\text{-}S^1}$ are given later in this chapter. Here, it can be said that the $\mathbf{S^1\text{-}L_1\text{-}S^1}$ echo is used to sense flows in contrapropagating flowmeters.

Fig.6.5: Pairs of steel clad (left) and non-clad (right) buffer rods selected for performance comparison in through-transmission mode.

Motivated by these results, in this work we propose to use CBRs in our contrapropagating flowmeter. Their superior SNR compared to the reported non-clad rods is expected to improve precision in transit time measurements by the cross-correlation technique, implying more accurate flow speed determination. In the next section, we assess the ultrasonic wave radiation patterns for the 40°-chamfered steel CBR, in order to determine the spatial limits of signal detection provided by this probe.

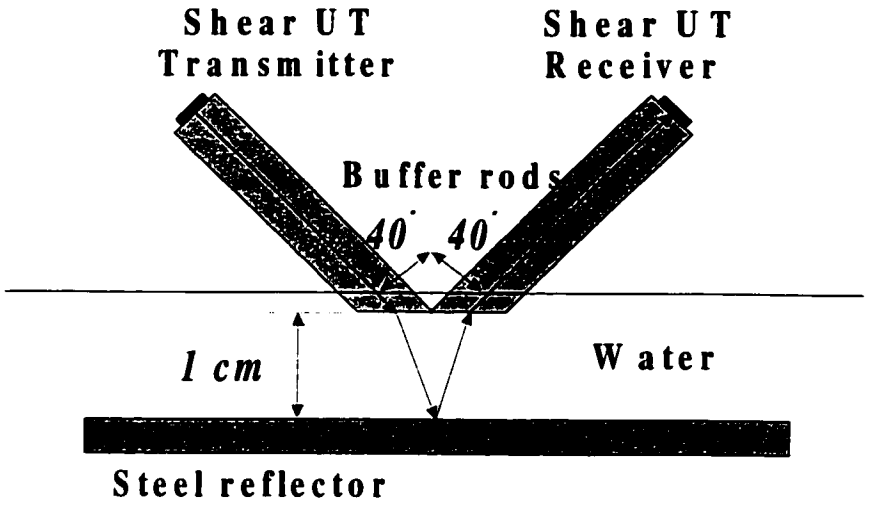
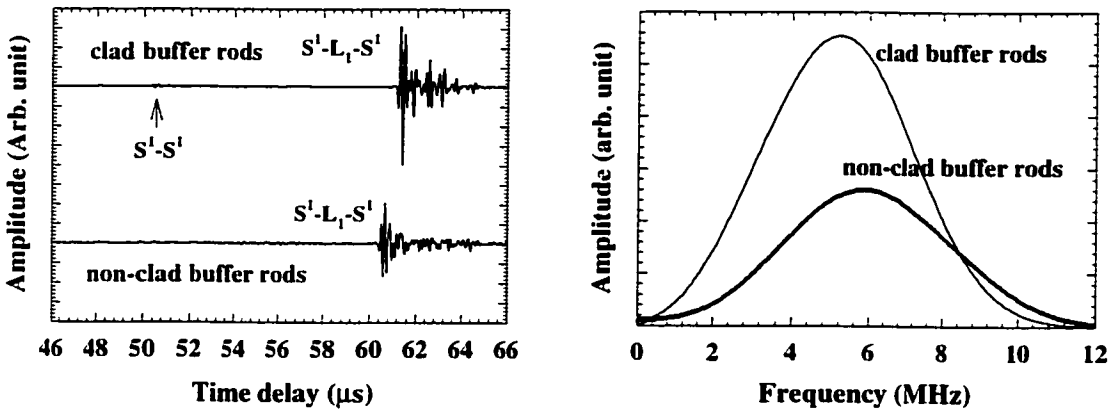


Fig.6.6: Experimental configuration for evaluating buffer rod performance in through-transmission mode.



(a) (b)

Fig.6.7: Experimental results obtained for the configuration shown in Fig.6.6. (a) Time domain signals. (b) Frequency spectra of the S^1-L_1-S^1 echoes shown in Fig.6.7(a).

6.4.2-Experimental evaluation of the 40°-chamfered steel clad buffer rod in water at room temperature

In addition to the theoretical model and calculations for the flowmeter, experimental verification is also needed. In particular, the angle of refraction in liquid is important, since it determines the separation of the probes. Applying wave scattering theory to find the angle of refraction from steel buffer rod to water is entirely based on the assumption of plane wave propagation. However, buffer rods are bounded media, and deviation from theoretical simplifications should be expected due to diffraction and other physical phenomena. To verify the validity of our assumption, we compare the theoretical angle of refraction obtained by Snell's Law ($\theta_S = \sin^{-1}[(1497/3297)\sin 40°] = 16.97°$) with the actual measured value. If good agreement were achieved, we would expect that the calculated ultrasonic energy partitioned in the flowmeter is a satisfactory approximation.

This experiment was carried out in an immersion water tank. As described in Chapter 2, it consists of positioners and a turntable controlled by stepping motors and driven by a PC equipped with a 125 MHz double-channel acquisition card (Gage Applied Science, Inc.), and the Labview® software program, which allows signal acquisition, processing and display. The temperature in the water tank was kept at 25 ± 0.5 °C. The 40°–chamfered CBR was operated at 5 MHz by shear UT. Additionally, a 5 MHz immersible longitudinal ultrasonic transducer (ILUT) was installed in the positioner in order to detect signals emitted by the CBR. A 5072 PR pulser/receiver (Panametrics, Inc.) was used to generate and detect ultrasonic pulses. The basic experimental setup is shown in Fig.6.8. The positioner could be displaced along the x, y and z-axes, and also rotated 360° in the yz-plane and 180° in the xy-plane, offering flexibility in aligning ultrasonic probes. The probing end of the CBR was fixed in the turntable in order to lie in the xz-plane passing by the centre of the turntable, which can be rotated 360° either clockwise or counterclockwise. To determine experimentally the angle of refraction in water, the surface of the CBR probing end was aligned with respect to the ILUT. At this position,

the turntable reference angle was set to 0°. The pulser/receiver was used in through-transmission mode, with the CBR probe serving as transmitter and the ILUT as the receiver. From the 0° reference angle, the turntable was rotated at steps of 0.5° clockwise, and the signal received by the immersible transducer was recorded in the PC. The results are shown in Fig.6.9. The maximum signal amplitude was detected at 17°, corresponding to the refracted angle in water. This value exhibits a remarkable agreement with the theoretical expectation of 16.97°, which is based on plane wave propagation.

It is of interest to evaluate the radiation pattern of the 40°–chamfered CBR, in order to determine its spatial limits of detection. Here, the evaluation with respect to rotation and translation of the probe from its optimal (aligned) position is discussed.

6.4.3-Radiation pattern for the 40°-chamfered clad buffer rod – rotation and translation tests

Our first objective is to verify the sensitivity of the CBR with respect to angle misalignment. In ultrasonic flowmeters, angle misalignment may originate from several factors, such as imprecise installation of the CBRs in the duct, especially curved ones. Our approach was to reproduce a contrapropagating flowmeter configuration (Fig.6.3) using the alignment facilities of the water tank. The probe, consisting of a CBR with a 5 MHz shear UT, was operated as transmitter, a stainless steel plate reflector served as echo-target to direct back ultrasonic waves, and the ILUT fixed in the positioner assessed the alignment sensitivity of the configuration. This is illustrated in Fig.6.10. From the aligned position (0° reference angle), the ILUT was rotated in both clockwise and counterclockwise directions, in steps of 0.2°. Relative amplitudes of the received signals are plotted in Fig.6.11. The –3dB amplitude range extended from about –1.3° to 1.3°.

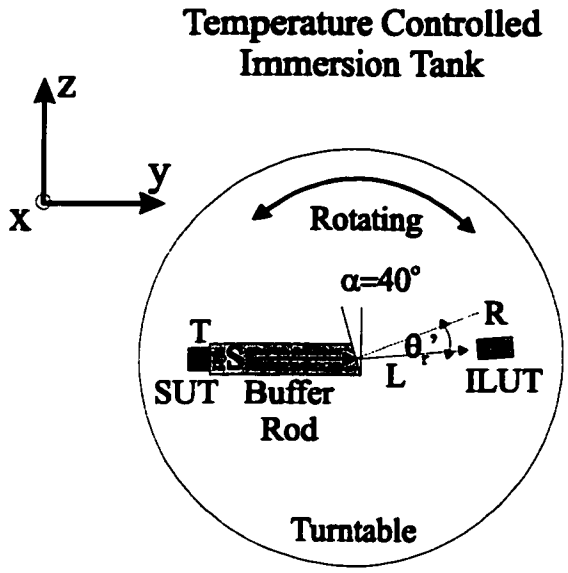


Fig.6.8: Experimental configuration for evaluating the angle of transmission of longitudinal waves in water from mode conversion of shear waves in the steel CBR.

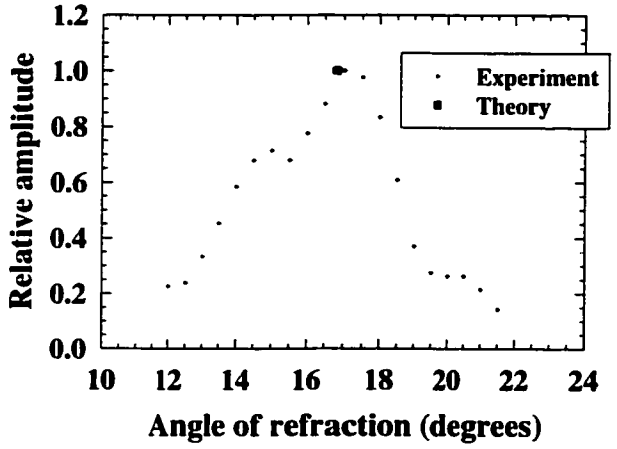


Fig.6.9: Results obtained with the experimental configuration of Fig.6.8.

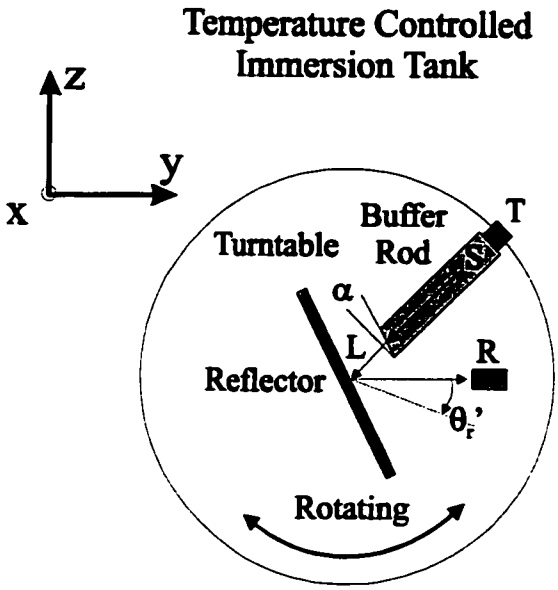


Fig.6.10: Experimental configuration for the rotation test of the steel CBR.

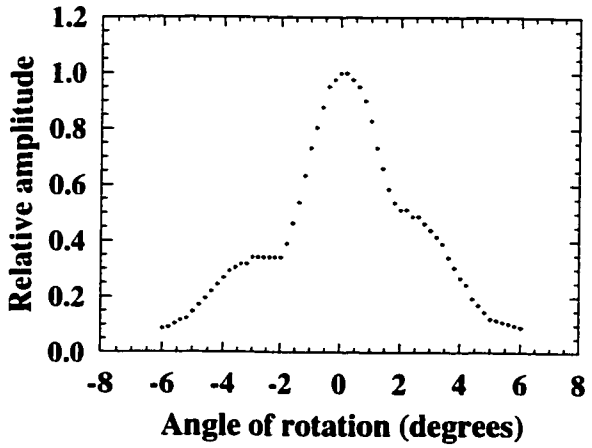


Fig.6.11: Results obtained with the experimental configuration of Fig.6.10.

We also assessed the sensitivity with respect to the distance separation between probes. The alignment steps followed the procedure described before. From the starting point (0 reference displacement), the ILUT was translated in both directions perpendicular to the reflected ultrasonic beam, as depicted in Fig.6.12. Relative signal

amplitudes detected in this experiment are plotted in Fig.6.13. The –3dB amplitude range extended from about –2 mm to 2 mm.

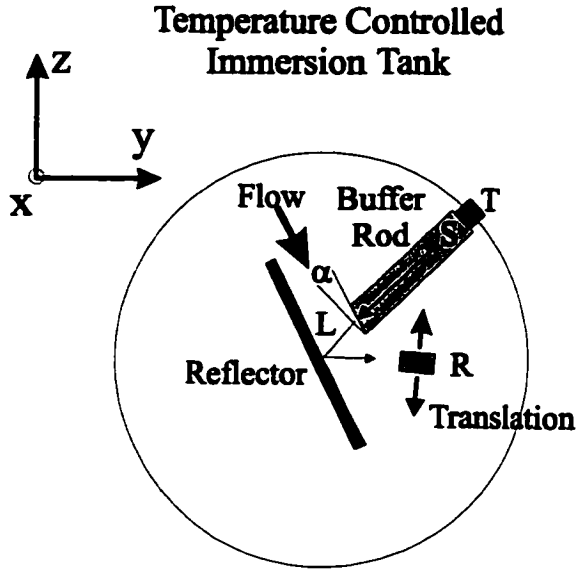


Fig.6.12: Experimental configuration for the translation test of the steel CBR.

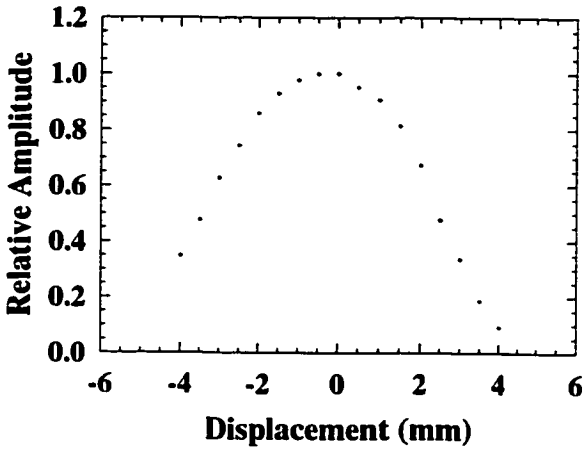


Fig.6.13: Results obtained with the experimental configuration of Fig.6.12.

6.5-Building and testing the ultrasonic contrapropagating flowmeter with clad buffer rods in water flow at room temperature

For a contrapropagating ultrasonic flowmeter operated with water flow, the incidence angle was chosen to be 40°, exhibiting high effectiveness for converting shear waves in steel CBR into longitudinal waves in water and *vice versa*. The actual refracted angle in water (corresponding to 17°) was experimentally verified, showing a remarkable approximation with the theoretical expected value (16.97°). This agreement suggests that the plane wave model can be applied for analysing acoustic wave scattering in flowmeters. Finally, the –3dB amplitude range of detection of ultrasonic signals was assessed in two different situations, arising from misalignment in angle and distance of the flowmeter transmitter and receiver probes from the optimal designed position. It was verified that these limits are within 1.3° and 2 mm, in either direction.

Before evaluating the contrapropagating flowmeter with CBRs at high temperatures, we started by building a prototype to be tested in water at constant room temperature. This test is intended mainly to ascertain the accuracy of flow speed measurements. In order to minimise errors due to temperature variation and flow profiles, two considerations are taken into account. First, flow velocity measurements will be carried out in a water tank with high thermal capacity. Since the temperature is constant, the average flow speed is proportional to the difference between downstream and upstream transit time measurements. Second, this proportionality holds if the ultrasonic beam interrogates the entire cross-sectional area of the duct; otherwise, integration techniques, based on additional ultrasonic probes along the duct sectional periphery or numerical correction factors, have to be used to average the flow speed measurements [107]. Therefore, we selected a stainless steel duct with square sectional area whose dimensions are roughly the diameter of the buffer rod core (5 mm). Thus, ultrasonic beams interact with a large section of the flow profile.

The duct in which the contrapropagating flowmeter was assembled is 151 mm long, with internal edge measurement D = 10 mm. In order to install the chamfered CBRs

flush with the duct inner surface, a small rectangular hole was made in the duct top wall to accommodate the CBR probing ends. For the materials involved, *i.e.*, steel and water at 25°C, Snell's Law determines the separation L between the centres of the probing ends to be $L = 2D\tan\theta_r' = 6.11$ mm, where $\theta_r' = 17°$ is the angle of refraction in water. 5 MHz shear UT's operating in through-transmission mode were clamped on the CBRs, and sealed to prevent their electrical terminals from being attacked by water. Due to the 1.5 mm thick cladding, the precise separation L of 6.11 mm could not be met. Instead, the probing ends were situated 12 mm apart, which is still within the –3 dB amplitude range. Finally, the ultrasonic probes were aligned with respect to the duct walls. The resulting flowmeter prototype was then fixed in the centre of the water tank turntable, as shown in Fig.6.14. One extremity of the duct was connected *via* a hose to the water input terminal of the tank. The tank is also equipped with a regulator valve to control the flow rate. It was verified that the water flow input could be up to 2 m/s.

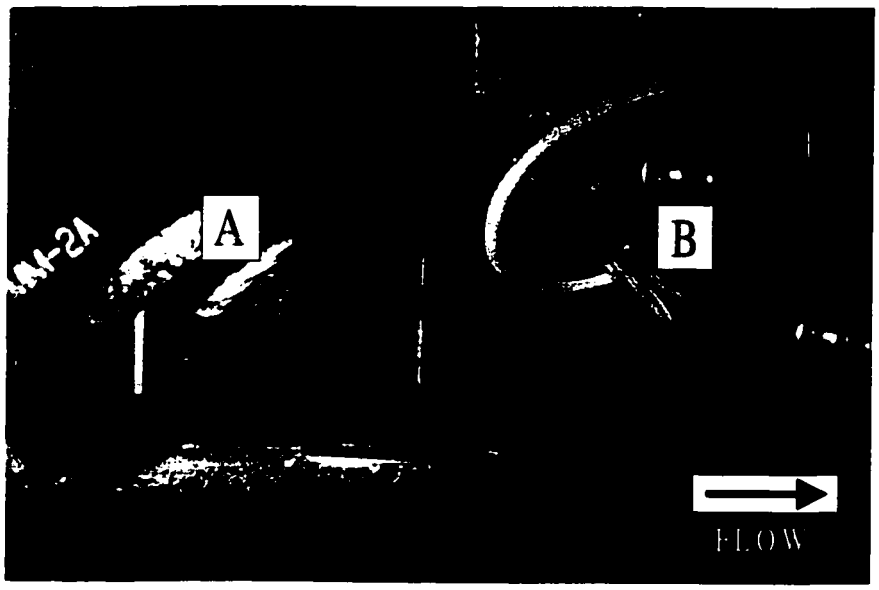


Fig.6.14: A contrapropagating ultrasonic flowmeter prototype for water flow measurements at room temperature. For downstream transit time measurements, probe B is the receiver; for upstream transit time measurements, probe A is the receiver.

The use of the designed flowmeter requires calibration at the operating temperature (25°C), to account for unexpected differences between downstream and upstream transit times at zero flow speed. This difference may be attributed to the fact that the ultrasonic propagation paths are not exactly reciprocal. The signals recorded for downstream (probe B as receiver) and upstream (probe A as receiver) are shown in Fig.6.15. These signals were acquired at 125 MHz sampling rate and averaged 50 times. Cross-correlation time delay measurements revealed a difference of (downstream minus upstream times) = (6.112 ± 0.7795) ns. This is the calibration factor used for measurement corrections.

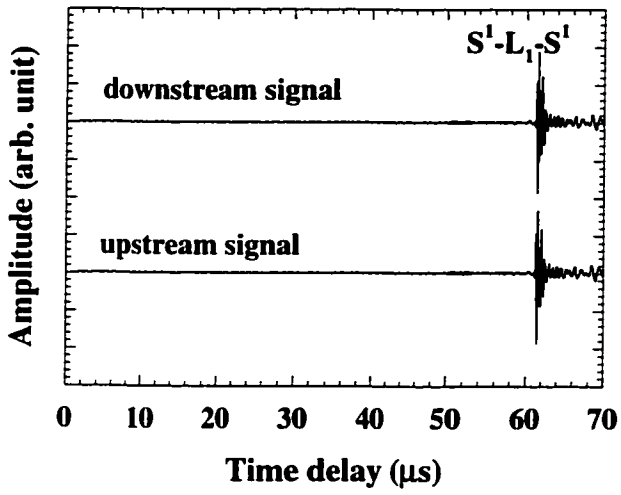


Fig.6.15: Typical ultrasonic signals obtained with the flowmeter prototype of Fig.6.14.

Excluding the valve at closed position, four other apertures were selected to control the flow rate. The corresponding flow velocities were determined by measuring the volume rate, *VR*, provided in the output of the square duct through the well-known equation $VR=F/A$, where F is the flow (volume/time) and A is the cross-sectional area of the square duct. Measurement results were averaged 10 times, and the maximum ratio of standard deviation to average flow speed was about 1%. Owing to this repeatability, these values were utilised as flow speed standards to be compared with ultrasonic

measurements through Eq.(6.5). Using the same procedure adopted for the flowmeter calibration, upstream and downstream signals were acquired 10 times, compensated by the calibration factor and finally substituted into Eq.(6.5). The results are shown in Fig.6.16. It is observed that ultrasonic measurements were repeatable, and the error bars intersected with the theoretical curves (upstream minus downstream times). Accuracy of the order of 1 ns has been reached.

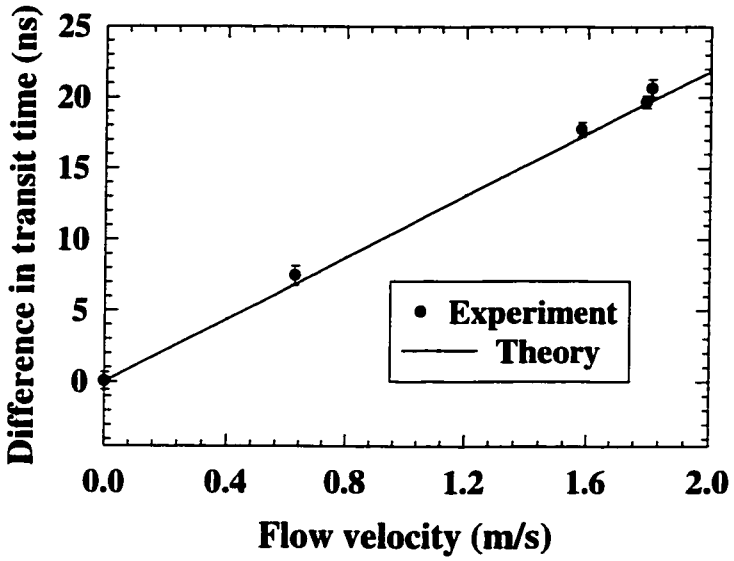


Fig.6.16: Experimental and theoretical results for the contrapropagating ultrasonic flowmeter shown in Fig.6.14.

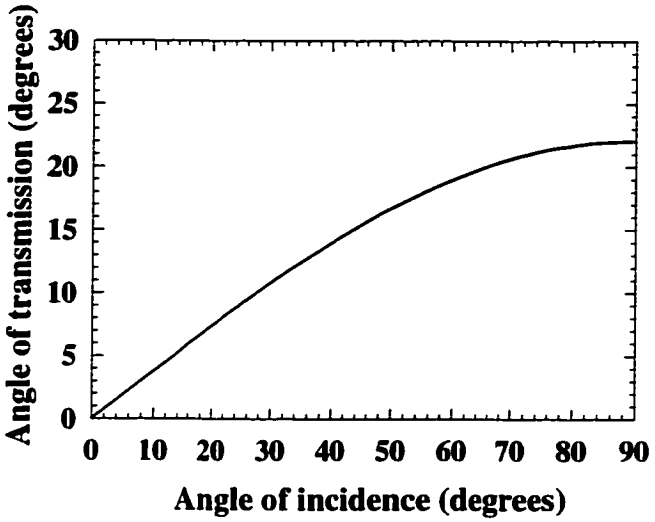
6.6-Building and testing the ultrasonic contrapropagating flowmeter with clad buffer rods in oil flow at elevated temperature

At the Industrial Materials Institute (IMI), a Thermocast™ heater machine, used in connection with polymer and metal processing machines, provides flow of Thermia-C oil (Shell, Inc.) at temperatures near 130°C. This machine is used to demonstrate the high temperature flow speed measurement by the flowmeter. Because of the different

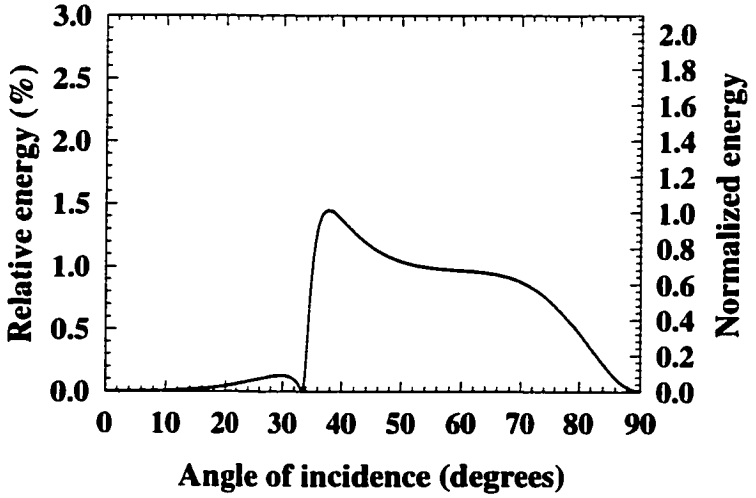
operating temperature and liquid media considered now, buffer rod angles and separation L between probes have to be re-evaluated. We turn again to Snell's Law and Eq.(6.6). At 130°C, the density, longitudinal velocity and shear velocity for steel are taken to be: 7800 Kg/m^3, 5880 m/s and 3242 m/s, respectively. For Thermia-C oil, at this temperature, the density and longitudinal velocity are: 860 Kg/m^3 and 1220 m/s, respectively[3]. The results of numerical calculations are presented in Fig.6.17. Due to high impedance mismatch between steel and Thermia-C oil at 130°C, there is a decrease of the ultrasonic energy generated and detected by the flowmeter. A 40° incidence angle is still an effective design value. However, the angle of refraction in hot oil is reduced to 14°, which yields L = 4.99 mm. Thus, the cladding close to the probing end was partially removed, resulting in a minimum separation L = 8 mm. A flowmeter was then constructed. The CBRs were welded into the duct wall, flush with the duct inner surface. Figs.6.18 and 6.19 illustrate the setup for high temperature measurement and details on the flowmeter installation, respectively. In the former figure are shown a PR35 pulser/receiver (JSR Ultrasonics, Inc.); a SR630 16-channel thermocouple monitor (Stanford Research Systems, Inc.); an oscilloscope (model 7854 Tektronix, Inc.); a PC with Labview® program and 50 MHz double-channel acquisition card (Gage Applied Science, Inc.); the Thermocast™ machine and the flowmeter. In the latter figure, appear the fans to cool down the CBR UT extremities and 3 type-K commercial thermocouples to sense the temperature of the flowmeter in the vicinities of the CBRs. The temperature data are sent over a GPIB interface to the PC, and the Labview® program acquires temperature and ultrasonic signals simultaneously.

Proper functionality of the flowmeter system at high temperature, such as generation and detection of ultrasonic signals, proper cooling of the UTs and absence of hot oil linkage, have been verified. Typical ultrasonic signals acquired for downstream and upstream operations are shown in Fig.6.20. It was demonstrated earlier in this chapter that the superior SNR of the CBRs over the non-clad rods leads to detection of two

[3] The values of densities were taken at room temperature (25°C). The longitudinal velocity in oil was measured by the pulse-echo technique. The longitudinal and shear velocities in steel were determined by the laser-interferometry technique at IMI.



(a)



(b)

Fig.6.17: Design parameters for the flowmeter shown in Fig.6.3. Liquid medium is Thermia-C oil at 130°C. (a) Angle of transmission of longitudinal waves in Thermia-C oil *vs.* angle of incidence of shear waves in steel.(b) Relative detected ultrasonic energy *vs.* the angle of incidence of shear waves in steel.

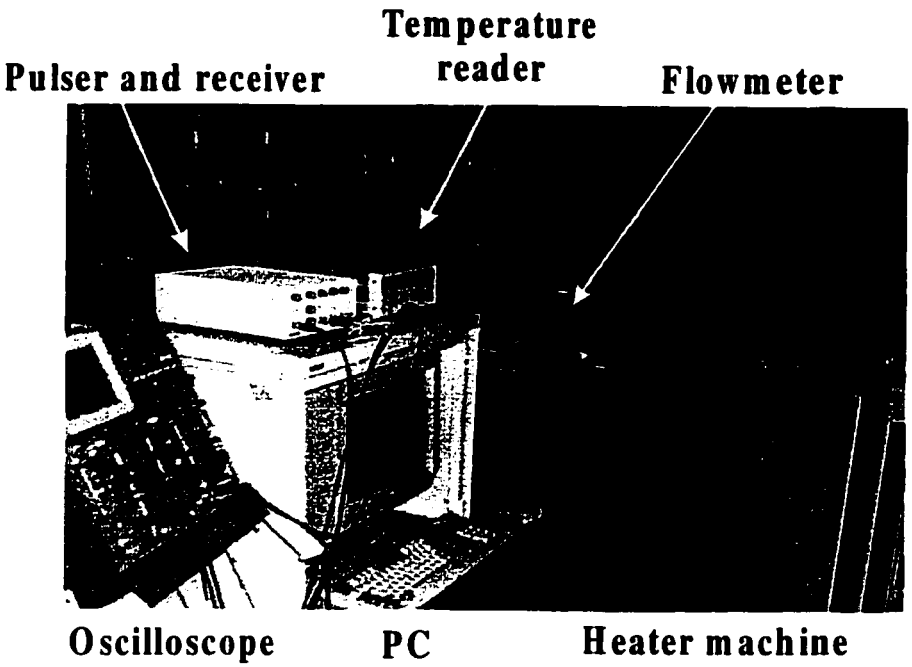


Fig.6.18: Experimental set-up for carrying out flow speed measurements of Thermia-C oil at high temperatures.

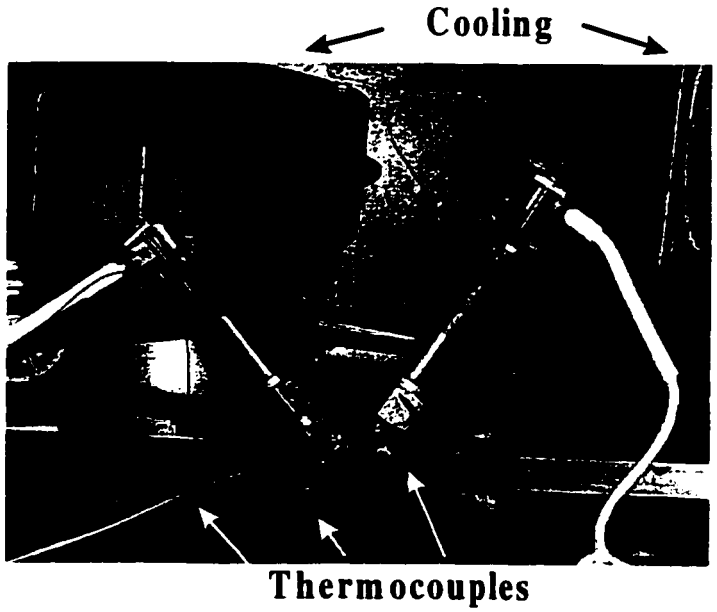


Fig.6.19: Details of the contrapropagating ultrasonic flowmeter designed for operation at high temperatures.

echoes, namely $\mathbf{S^1\text{-}S^1}$ and $\mathbf{S^1\text{-}L_1\text{-}S^1}$. Fig.6.21, intended to illustrate the ultrasonic beam paths in the designed flowmeter, explains the origin of such echoes. The $\mathbf{S^1\text{-}S^1}$ echo comes from the cross-talk between probes. It is originated by shear waves propagating along paths **1,2,3** and **3,2,1** for downstream and upstream operations, respectively. Its transit time is t_{Td} or t_{Tu}, shown in Fig.6.20, where d and u subscripts stand for downstream and upstream respectively. The $\mathbf{S^1\text{-}L_1\text{-}S^1}$ echo propagates as shear waves in the transmitter CBR, and is mode converted into longitudinal waves in the solid/liquid interface. After sensing the liquid, longitudinal waves are converted back into shear waves in the liquid/solid interface, and are finally detected by the receiving shear UT. For downstream and upstream measurements, $\mathbf{S^1\text{-}L_1\text{-}S^1}$ propagates along paths **1,4,5,3** and **3,5,4,1**, respectively. Accordingly, the transit times associated with these paths are t_d or t_u. Thus, downstream signals are characterised by a pair of transit times (t_{Td}, t_d), while upstream signals are characterised by (t_{Tu}, t_u).

It has been observed that downstream and upstream transit times t_{Td} and t_{Tu} of the $\mathbf{S^1\text{-}S^1}$ echo are linearly related with the average temperature measured by commercial thermocouples simultaneously with the acquisition of the ultrasonic signals. For instance, at zero flow rate in the output of the Thermocast™ machine, this linear relationship is shown in Fig.6.22. In this figure, the temperature is the average value of three thermocouples. Therefore, measurement of transit times t_{Td} and t_{Tu} offers a means to calibrate the flowmeter with respect to temperature. In other words, t_{Td} and t_{Tu} may be used as temperature reference to ascertain that downstream t_d and upstream t_u transit times have been taken at the same temperature. This is the essence of the temperature compensation here introduced in order to reduce measurement errors in the contrapropagating flowmeter. It is possible because the SNR of CBRs is high enough to reveal the existence of the $\mathbf{S^1\text{-}S^1}$ echo, in contrast to the reported non-clad rods.

In order to verify the validity of the temperature compensation technique thus devised, downstream and upstream signals were acquired non-simultaneously for three different flow situations: maximum flow rate provided by the Thermocast™ machine, 50% of the maximum flow rate and zero flow rate. In all cases, acquisition started at

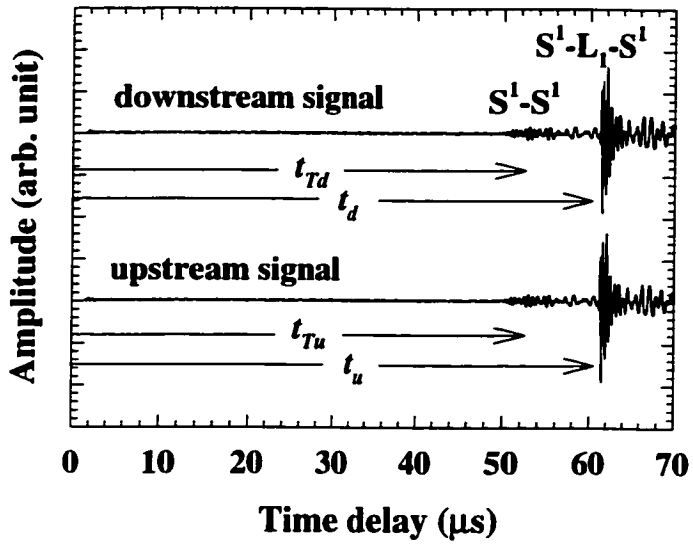


Fig.6.20: Typical ultrasonic signals obtained with the contrapropagating ultrasonic flowmeter shown in Fig.19.

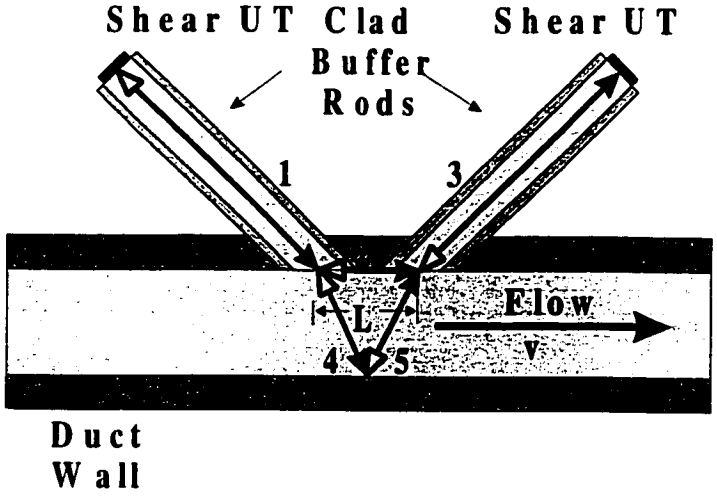


Fig.6.21: Ultrasonic beam paths in the contrapropagating ultrasonic flowmeter.

temperatures around 134°C, prolonging till about 130°C. Signals were acquired at 50 MHz sampling rate, and averaged 50 times. Acquisitions were estimated to take 1 averaged signal per second. For each flow rate, the downstream and upstream pairs (T, t_T) (thermocouple temperature and S^1-S^1 transit time) and (t_T, t_F) (S^1-S^1 and S^1-L_1-S^1 transit times, respectively) were stored in the PC. The common temperature T for downstream and upstream operation was then found, resulting in the pairs (t_{Td}, t_d) and (t_{Tu}, t_u). At this point, Eq.(6.5) holds to determine flow speed from measurements of downstream and upstream times at the same temperature. Equivalent to the pairs (t_{Td}, t_d) and (t_{Tu}, t_u) are the new pairs (t_{Td}, $t_d - t_{Td}$) and (t_{Tu}, $t_u - t_{Tu}$). The latter takes into account propagation of S^1-L_1-S^1 in liquid paths **4,5** and **5,4**, respectively, rather than in solid paths **1,3** and **3,1**. Figs.6.23((a), (b), (c)), show, for each flow rate situation, the relation between times t_T and ($t_T - t_F$).

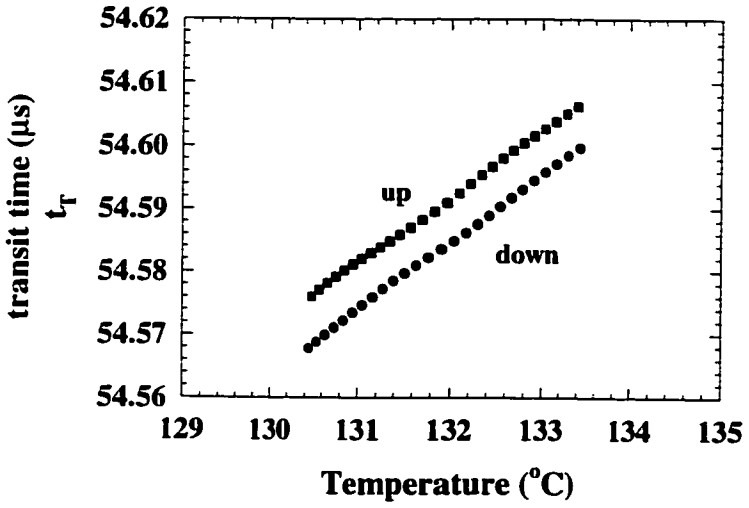
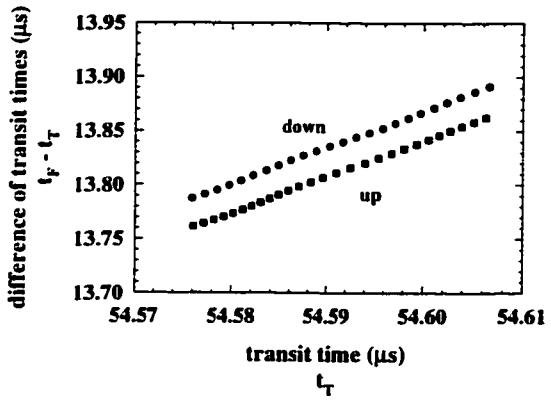
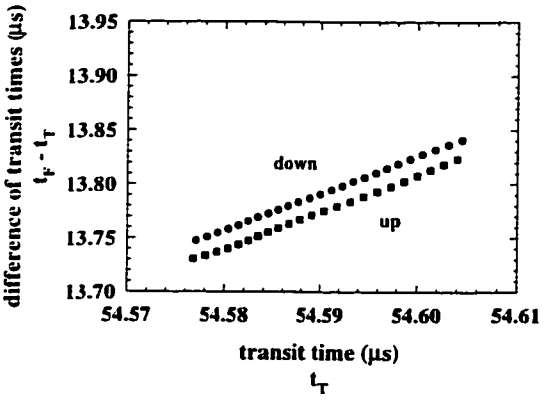


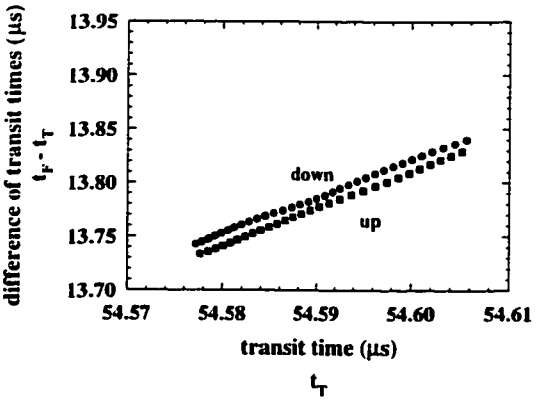
Fig.6.22: Linear relation between the average temperature in the liquid flow and the ultrasonic transit time (upstream and downstream) for the flowmeter shown in Fig.6.19.



(a)



(b)



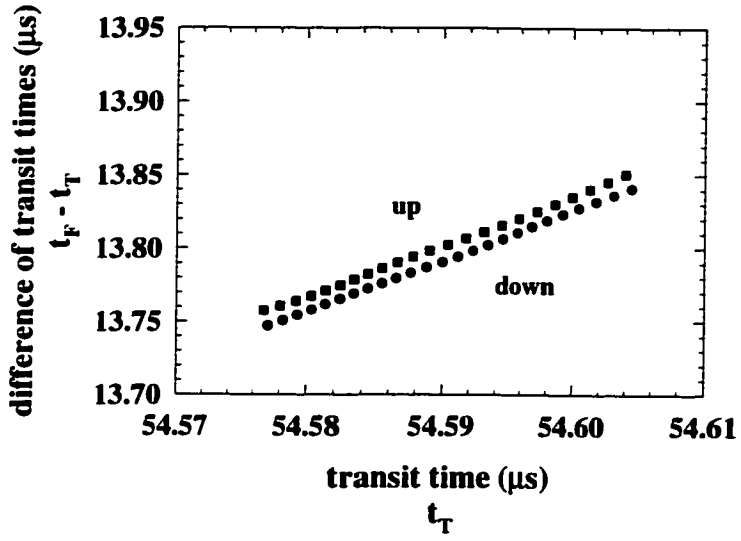
(c)

Fig.6.23: Downstream and upstream transit time measurements. No calibration factor applied for correcting differences of downstream and upstream transit times. (a) Zero flow speed.(b) 50% of maximum flow speed.(c) 100% of maximum flow speed.

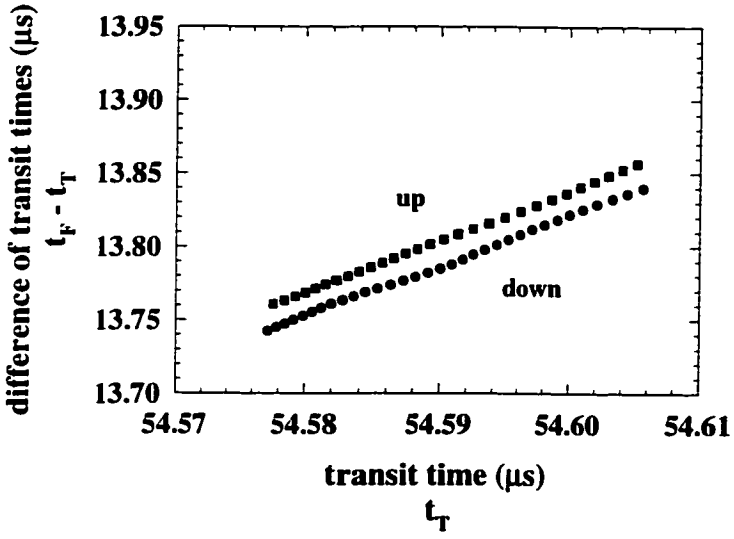
At zero flow rate (Fig.6.23(a)), upstream and downstream curves should coincide, as any difference between them might be caused solely by the flow speed. This means that a calibration factor must be applied to the flowmeter system in order to account for anti-symmetries, which induce non-reciprocity in the time delay measurement. The calibration factor used here is given by a straight line over the measurement range, and results from subtraction of the linear regressions of the curves in Fig.6.23(a). Measurements are then corrected by lifting the upstream transit time curves with the calibration factor curve, as shown in Figs.6.24(a) and 6.24(b). The difference between upstream and downstream transit times can be readily obtained from the latter plots. But in order to highlight the temperature variation during measurements, we transformed the transit time t_T axis into temperature through the linear relation found between them, using either downstream or upstream t_T curve. For this demonstration, we chose the downstream t_T curve. Additionally, the difference between upstream and downstream transit time $(t_u - t_d)$ was defined by linear regression rather than with the experimental points, allowing continuous subtraction over the measurement interval. The result is shown in Fig.6.25. In this figure, intermediate flow refers to 50% of the maximum flow. It is noted that for the 3° temperature range over which measurements were taken, the difference between upstream and downstream times $(t_u - t_d)$, proportional to the flow speed, is kept almost constant, revealing that temperature variation has been tracked by the $\mathbf{S^1}$-$\mathbf{S^1}$ signals generated by the CBRs. For such a temperature range, an error of 1 ns for the difference in transit times has been estimated, which is within the accuracy obtained for the experiment in water at constant temperature.

6.7-Summary

A contrapropagating ultrasonic flowmeter employing steel CBRs was designed for operation at temperatures higher than 100°C, being immune to temperature variation. This ultrasonic flowmeter is driven by shear wave UTs, which generate longitudinal waves to sense the flow speed through mode conversion at the chamfered probing end of the CBRs. It was shown that, compared with longitudinal wave UTs, this configuration



(a)



(b)

Fig.6.24: Downstream and upstream transit time measurements, after applying the calibration factor for correcting differences of downstream and upstream transit times at zero flow speed. (a) 50% of maximum flow speed. (b) 100% of maximum flow speed.

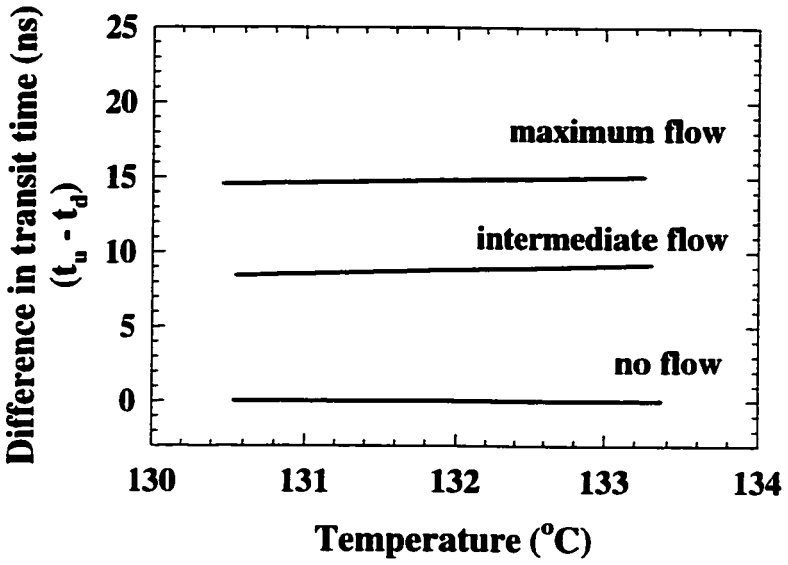


Fig.6.25: Experimental results demonstrating the reduction of the temperature variation effect from the flow speed measurement.

has the advantages of increasing the separation between ultrasonic probes and resolution in time domain measurements, leading to more accurate flow speed measurements.

Theoretical aspects of acoustic plane wave scattering at a solid/liquid interface were taken into account in order to choose the most effective angle for shear-to-longitudinal wave conversion and vice-versa. For the materials of interest in this investigation, it was found that an angle of 40° is effective. One pair of steel CBRs and another pair of non-clad buffer rods, having both the same lengths and diameters, were assembled in a contrapropagating configuration in water, being the probing ends cut at 40°. It was then demonstrated that the SNR of CBRs are superior than that of non-clad buffer rods even in the through-transmission mode, which is a standard way to operate contrapropagating flowmeters. Radiation patterns were assessed in order to reveal the spatial limits of signal detection provided by the steel CBR.

Thus, a contrapropagating flowmeter prototype with steel CBRs was designed for operation with water flow at room temperature. Effects of water flow speeds on the difference between upstream and downstream ultrasonic transit times were measured and compared to theoretical expectations. Good repeatability of measurements and agreement with theory were achieved. An accuracy of 1 ns for the difference between upstream and downstream ultrasonic transit times was reached. Motivated by such results, a contrapropagating flowmeter was then designed and installed in a heater machine for flow speed measurements of oil at temperatures near 130°C. It was shown that these CBRs could generate specific ultrasonic signals to be used in temperature calibration of flowmeters, allowing temperature variation while measuring accurately the flow speed. For a 3°C temperature range, the difference between upstream and downstream ultrasonic transit times was measured within 1 ns accuracy, which is nearly the one obtained in the water flow measurement at constant temperature.

Chapter 7

Conclusion

7.1-Thesis Summary

This thesis is the result of the increasing need for research and development of sensors for in-line monitoring and control of industrial processes. In order to provide precise feedback on process parameters and material properties during production in the harsh industrial environment, these sensors are required to be robust, fast, reliable, and preferably non-intrusive and cost effective. Ultrasound, owing to its ability to interrogate non-invasively, non-destructively and rapidly the internal regions of material objects, has been widely regarded for applications in modern process monitoring and control. It was identified that the major concern to the direct application of ultrasound to industrial processes is related to the limited temperature that conventional ultrasonic transducers (UTs) can withstand. Our approach, which is a classical one, consisted in the use of buffer rods to isolate the material under inspection, usually at elevated temperatures, from the UT. These buffer rods, however, differ from the reported ones in the sense that they are cladded. This feature results in superior wave guidance in the clad buffer rod (CBR) core, making it possible for the use of the pulse-echo technique in situations where the common through-transmission or pitch-catch configuration failed, exhibited partial success or could not even be applied.

All experiments of the thesis research were performed at Industrial Materials Institute (IMI) of the National Research Council of Canada (NRC), where access to a variety of industrial processes, machines and materials was made possible. The industrial

processes investigated were polymer extrusion, liquid metals processing and liquid flow at elevated temperatures.

In order to understand the improvement on the ultrasonic wave guidance caused by the cladding of CBRs and select the most convenient rod for a given application, the ultrasonic field distribution generated by the probe consisting of short and long CBRs with commercial 5 and 10 MHz UTs operating in pulse-echo mode were evaluated in Chapter 2. This evaluation was performed through reflection over a thin steel wire or small ball bearing immersed in water. We examined the pulse duration, centre frequency and –3 dB-bandwidth of different combinations of CBRs and UTs. It was found that all CBRs exhibited sufficient signal strength and SNR for the investigation carried out in this thesis. Compared with focusing UTs, the probe consisting of UT and focusing CBR exhibits similar focusing pattern for the ultrasonic field distribution. In addition, CBRs can achieve different spatial resolutions at the same frequency by modifying the lens shape in order to reach the resolution required. With respect to the probe consisting of UT and CBR with a flat probing end, it was learned that at the exit the wave behaves as in the far field region. These features allow the application of CBRs for quantitative ultrasonic monitoring of industrial processes.

Numerical simulation confirmed that the cladding does have an effect on the ultrasonic wave propagation in CBRs, and suggested that further improvement of the guidance is possible if the velocities of the cladding are higher than those of the core. However, CBRs that have a thermal sprayed cladding still display high ultrasonic performance even being the velocities in the cladding less than those of the core. In addition, the numerical results supported the experimental verification that the ultrasonic field pattern at the exit of the flat probing end of CBRs is similar to the far field region.

In Chapter 3, we demonstrated the potentiality of steel CBRs for industrial applications by in-line monitoring two important polymer extrusion processes. In the first part, co-extrusion of high density polyethylene (HDPE) and a thermoplastic elastomer based on polypropylene-EPDM (ethylene-propylene-diene monomer) was investigated

by ultrasonic sensors consisting of commercial UTs and steel CBRs operating in pulse-echo mode. One extremity of the CBR (probing end) was installed flush with the die surface so as not to disturb the material flow. The other end was air cooled in order to protect the transducer from excessive heating. This approach was demonstrated to be convenient for monitoring industrial material processes: first, it could work at temperatures up to 960°C; second, the probing end of the CBR could be machined to the same shape as those of commercial temperature and pressure sensors commonly used in the extrusion process. Therefore, no modifications were required for installation in the processing equipment. The information obtained included the position of the interface between polymers and the stability of the extrusion process. However, it was realised that due to the large impedance mismatch between the steel CBR and polymer, just a small percentage of the ultrasonic energy penetrated the polymer. Thus, it was necessary to resort to signal processing techniques based on deconvolution in order to reveal the amplitude, which is related to the quality of adhesion between two layers, and location of the interface echoes.

In the second part, the same ultrasonic probe was also installed in the barrel section of a twin-screw extruder for off-the-screw measurements. For our study, polyethylene and polystyrene were arbitrarily chosen as extrusion materials. Before attempting in-line monitoring, the ultrasonic response (reflected signal magnitude and time delay) for different screw geometries (conveying element, kneading block, distance element *etc*) commonly used in extruders was assessed in a water tank. Experiments revealed that the ultrasonic response is specific for each screw geometry. This is an important feature as many informations may be inferred from the time delay response in the actual process. Thanks to the satisfactory SNR achieved by steel CBRs in pulse-echo mode, the ultrasonic sensor could be for the first time successfully operated along the extruder screw, giving access to material properties and blend composition while polymers were being processed. It means that clad buffer rod probes open an important polymer extrusion page which provides possibilities to "see" the material properties of polymers in different zones (melting, mixing *etc*) of the barrel section of extruders.

It was observed that in order to obtain high quality ultrasonic signals (*i.e.*, stable and with sufficient SNR and signal strength), the pressure in the extruder barrel has to be at least 0.7 MPa. In this situation, the barrel is completely filled and the ultrasonic signal can well propagate from the CBR into the molten polymer. It is concluded that our technique could be explored to in-line monitor and control the characteristics of the polymer being transformed in operations typically performed on the barrel section of twin-screw extruders, such as compounding or reactive extrusion. This technique can be equally used to monitor the extrusion performed by single-screw extruders [96]. It is also believed that the monitoring of changes of viscoelastic properties, structure and composition of flowing polymer melts performed in the die exit by two ultrasonic probes in through-transmission, as reported in [5], could be equally achieved in the barrel section, using our CBR probe operated in pulse-echo operation.

It was learned in Chapter 4 that in comparison with metal CBR probes, polymer CBRs have better acoustic impedance matching with processed molten polymers and can deliver ultrasound pulses of lower frequencies. These combined features suggested that polymer CBRs could be preferable for certain quantitative viscoelastic measurement of polymers. A better acoustic impedance matching between ultrasonic probe and polymer sample leads to a higher sensitivity to polymer properties change, being appropriate for density and viscosity measurements. With low frequency interrogative signals, a deeper ultrasonic penetration in polymer is achieved. Thus, even thick polymer samples, which can have very attenuative nature to ultrasonic waves, may be investigated. Therefore, Chapter 4 focused on development of polymer rods with low ultrasonic losses (for both longitudinal and shear modes) and able to sustain temperatures near 200°C for in-line monitoring of extrusion processes. It was found that polymer CBRs consisting of a polyetheretherketone (PEEK) core and a cladding made of a heat resistance epoxy-aluminium composite met such requirements. Measurement results indicated that the ultrasonic signal strength and SNR of these PEEK CBRs are better than those of the PEEK non-clad buffer rods for both longitudinal and shear waves because of the improved ultrasonic wave guidance in the core. This is especially true if a taper geometry

is used rather than a uniform one, as it has been mentioned in chapter 2 for the case of steel CBRs.

Comparisons of PEEK CBRs with those made of polyamide (VESPEL) and high-density polyethylene (HDPE), which have been used as ultrasonic probes, proved the superior waveguidance attained by the former. For longitudinal waves, the signal strength of the $\mathbf{L^1}$ echo in the PEEK rod is 7 dB and 14 dB higher than that of VESPEL and HDPE, respectively. And for shear waves, the signal strength of the $\mathbf{S^1}$ echo in the PEEK rod is more than 20 dB larger than that in VESPEL or HDPE. Practical evaluation of the new-developed PEEK CBRs operating in pulse-echo mode was performed by means of in-line monitoring of polyester cure and polymer extrusion processes. It was demonstrated that the variation of ultrasonic velocities, reflection coefficients, density and viscosity revealed the degree of the curing process. In addition, the use of a single shear wave UT with PEEK CBR could generate simultaneously and at the same location shear and longitudinal waves. The advantage of such an approach is its simplicity and one-location access to achieve a complete ultrasonic history of the cure process. With respect to the polymer extrusion, an objective was to verify the temperature resistance and mechanical strength achieved by the PEEK CBRs during industrial conditions. It was observed that signals with satisfactory SNR were acquired at temperatures up to 200°C and pressures up to 1.2 MPa. Also, within certain temperature and pressure ranges, PEEK CBRs showed advantages over steel CBRs. For instance, the low frequency signals generated by PEEK CBRs have more penetration in polymers, leading to satisfactory SNR in pulse-echo mode. Steel CBRs, however, have more strength and ruggedness.

It was established in Chapter 5 that the pulse-echo technique using focusing CBRs are promising for cleanliness evaluation of molten metals. This approach offers sufficient spatial resolution for small particles detection and also eliminates the need of probe alignment, such as the two-probe configuration used in previous work [53], which is cumbersome under industrial conditions. Because CBRs are mechanical filters that modify the UT-generated pulses, it is necessary to consider the SNR and signal strength when selecting the operation frequency. It was found that steel CBRs operating

at 10 MHz, as shown in Fig.5.1a, exhibited satisfactory spatial resolution, SNR and signal strength in water at room temperature and molten zinc at temperatures higher than 600°C. In the experiments, molten zinc at temperatures higher than 600°C was chosen because it has a slow ultrasonic velocity (2800 m/s) compared to steel (5500 m/s), thus improving the spatial resolution of the focusing lens. In addition, it "wets" properly the focus lens of the steel CBR, allowing ultrasonic coupling to the molten metal, and simulates the melting point of aluminium (660°C) and magnesium (650°C), which are common used industrial materials. The spatial resolution and SNR achieved by focusing steel CBRs were assessed through lateral scanning of a thin stainless steel wire (380 μm-diameter) and C-scanning imaging of a small steel object. Signals with SNR better than 35 dB led to clear ultrasonic imaging in molten zinc, being obtained from both the amplitude and time delay variations of the reflected signals. It is believed that the imaging ability of the new developed probe could be used to perform quantitative and microscopic materials characterisation in molten metals, such as the determination of phase velocity and attenuation, in a way similar to those performed by means of an acoustic microscope at room temperature.

Concerning to the cleanliness evaluation of molten metals by the proposed ultrasonic probe, an attempt was made to detect particles resulting from the interaction between molten zinc and the thin steel wire. Back-scattered signals from particles were clearly visible at the focal region of the lens. The results revealed that the amplitude and shape of the frequency spectra of the ultrasonic signals may be sensitive to a variety of particle characteristics, such as size, shape and clustering that are related to the particle volume fraction.

Because ceramic materials have a higher resistance to corrosion in the hostile molten metal environment than steel does, a focusing alumina CBR was fabricated and investigated. Ultrasonic images produced by this probe in water at room temperature demonstrated that high resolution could be achieved. It is demonstrated in water that the high ultrasonic velocity in alumina leads to a reduction of the lens spherical aberration, thus enhancing the spatial resolution of the probe.

We started Chapter 6 by presenting theoretical aspects underlying the design of contrapropagating ultrasonic flowmeters with CBRs for industrial applications. It was shown that the configuration using shear wave UTs is preferable than the one using longitudinal wave UTs. The former increases the separation between ultrasonic probes and resolution in time domain measurements, thus improving the accuracy in flow speed measurements. In this approach, the liquid flow is sensed by longitudinal waves generated through mode conversion at the chamfered probing end of the buffer rod. For the materials of interest in our research, it was found that 40°-incidence from solid to liquid (and vice-versa) is effective for achieving mode conversion. It was identified that a concern for the accurate flow speed measurement in industrial processes, *e.g.*, the polymer extrusion and metal die-casting was the rapid temperature variation that the melt flow may undergo. Although several flowmeter systems have been proposed recently for high temperature measurement [31], it seems that no efforts have been reported to eliminate the effect of temperature variation from flow measurements. We then introduced a contrapropagating ultrasonic flowmeter to operate at elevated temperatures with significant reduction of the temperature effect. This ultrasonic flowmeter utilises steel CBRs able to sustain temperatures near 1000°C.

The performance of our flowmeter was first evaluated in water flow at constant temperature. It was demonstrated that the superior SNR of the steel CBRs compared to the non-clad ones improved precision in ultrasonic transit time measurements by the cross-correlation technique. Effects of water flow speeds on the difference between upstream and downstream ultrasonic transit times were measured and compared to theoretical expectations. An accuracy of 1 ns for the difference between upstream and downstream ultrasonic transit times was reached. A contrapropagating flowmeter was then designed and installed in a heater machine for flow speed measurements of motor oil at temperatures near 130°C. It was shown that these CBRs could guide specific ultrasonic signals having a linear dependence with the average flow temperature. These signals were successfully applied to the temperature calibration of the flowmeter. For a 3°C temperature range, the difference between upstream and downstream ultrasonic transit

times was measured within 1 ns accuracy, which is nearly the one obtained in the water flow measurement at constant temperature.

7.2-Claims of originality

Here, we summarise the original contributions demonstrated in this study:

In Chapter 2,

- A system based on a miniature lathe and a steel ball was devised to fabricate spherical lenses on the probing end of CBRs;
- The ultrasonic field distributions and pulse-echo responses of different probes consisting of commercial UTs and steel CBRs with and without a focus lens were experimentally evaluated. The focusing patterns of the ultrasonic field distributions for focusing probes were demonstrated to be similar to those of commercial focusing UTs. The ultrasonic field distribution at the exit of the flat probing end of CBRs was experimentally and numerically demonstrated to behave as in the far field region;

The numerical calculations were carried out in collaboration with Prof. Ikuo Ihara at Nagaoka University of Technology, Nagaoka, Niigata, Japan.

In Chapter 3,

- Steel CBRs were machined into the shape of conventional Dynisco temperature and probe sensors and installed in co-extrusion (Placo) and twin-screw extrusion (Leitstritz and Werner & Pfleiderer) machines. This opens an important polymer extrusion page, providing possibilities to "see" the material properties in the different zones (melting, mixing *etc*) of the barrel section of single and twin-screw extruders;

- In-line ultrasonic monitoring at the die of the above co-extrusion (Placo) machine was carried out in pulse-echo mode, revealing information such as polymer layer thickness, interface location and adhesion quality, and stability of the process;
- Digital signal processing based on deconvolution (Wiener filtering) was applied to the ultrasonic signals obtained during polymer co-extrusion process in order to improve the identification of the interface echo between polymers;
- Ultrasonic characterisation of different screws commonly used in single and twin-screw extruders based on amplitude and time delay of echoes was carried out. It is concluded that ultrasonic monitoring can be performed off all screws studied in the barrel section;
- In-line ultrasonic monitoring using pulse-echo mode at the barrel section (off-the-screw) of twin-screw extruders (Leitstritz and Werner & Pfleiderer) was carried out. The change of ultrasonic velocity was demonstrated to be sensitive to polymer properties change and polymer blend composition.

In Chapter 4,

- The materials selection, fabrication and performance evaluation of a polymer CBR for use in in-line ultrasonic monitoring were carried out;
- A technique for in-line generate and detect simultaneously both shear and longitudinal waves at the same location using a single shear UT and a PEEK CBR was introduced for the cure monitoring of epoxies;
- A PEEK CBR having the shape of Dynisco sensors was fabricated. Its temperature resistance and mechanical strength during industrial conditions were evaluated in an extrusion machine (Werner & Pfleiderer);
- In-line ultrasonic monitoring using the developed PEEK CBRs was carried out in the above-mentioned twin-screw extruder.

In Chapter 5,

- The fabrication of focused steel CBRs operating in pulse-echo mode for in-line monitoring of liquid metal processes was carried out;
- High resolution ultrasonic images of a small steel objet immersed in molten zinc at 600°C was performed and compared with the result in water at room temperature;
- Ultrasonic detection of PVC particles (30 μm average size) in water at room temperature was performed using a focusing steel CBR attached to a 10 MHz UT;
- Ultrasonic detection of oxide particles in molten zinc at 650°C was performed;
- The fabrication and evaluation of a spherical lens in alumina CBR was carried out. The lens performance was evaluated through ultrasonic imaging in water at room temperature;

The research and development in this chapter was in collaboration with Prof. Ikuo Ihara at the Industrial Materials Institute, National Research Council, Boucherville, Quebec, Canada.

In Chapter 6,

- The theoretical analysis, design, implementation and test of a contrapropagating flowmeter employing steel CBRs for high temperature industrial applications were carried out;
- The ultrasonic radiation pattern of the chamfered steel CBRs was carried out in water in order to assess the spatial limit of detection provided by the CBRs;
- A contrapropagating ultrasonic flowmeter prototype was implemented and tested in water flow at room temperature. It was demonstrated that the measured results of the difference between ultrasonic upstream and downstream transit times are in good agreement with the theoretical expectations;
- A temperature calibration technique employing specific signals guided by steel CBRs was devised in order to compensate the effect of temperature variation in the flow speed measurement;

- A contrapropagating ultrasonic flowmeter employing steel CBRs was designed and installed in a Thermocast™ heater machine for flow speed measurement of oil at 130°C. It was demonstrated its capability to measure accurately the flow speed in spite of temperature variation.

The work presented in this dissertation resulted in five published refereed journal papers [22,43,100,109,111], one published letter [110], nine published conference papers [66,68,88,101,114-118], and two accepted conference papers [112,113].

APPENDIX A

The general problem of filtering can be described as follows: given an input signal $x(t) = s(t) + w(t)$, where $s(t)$ is the desired signal and $w(t)$ is the noise, how can the noise $w(t)$ be removed while preserving the characteristics of the desired signal $s(t)$? The suitability of deconvolution techniques to handle this specific problem have been demonstrated [91-95]. Although several deconvolution algorithms are available, Wiener Filtering is of particular interest, due to its rapidity and therefore applicability in real time processes [93].

To develop an algorithm to solve the problem computationally, the continuous time t is substituted by kT, where k is an integer representing the time in the discrete domain, and T is the sampling rate of the acquisition system. Furthermore, the filter is constrained to be linear, with impulse response $h(k)$ designed so that its output approximates some specified signal sequence $r(k)$ (reference signal). Fig.A.1 depicts the problem of filtering [108].

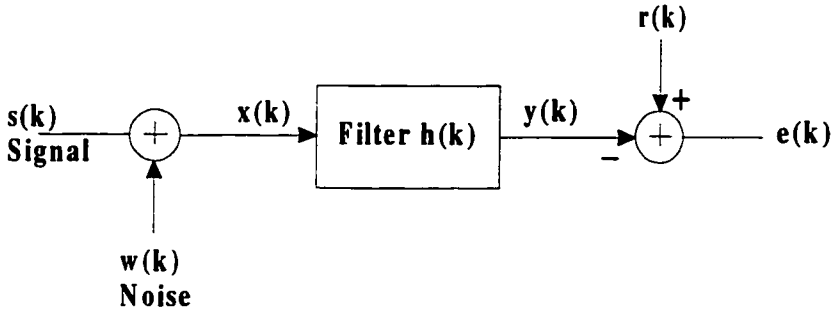


Fig.A.1: Model for filtering problem.

The input sequence to the filter is $x(k)=s(k) + w(k)$, and its output sequence is $y(k)$. The difference between the desired signal and the filter output is the error sequence $e(k)=r(k) - y(k)$. The criterion selected (for its simplicity and mathematical tractability) for optimising filter impulse response $h(k)$ is the minimisation of the mean-square error.

Obviously, the reference signal *r(k)* is chosen to be close to the desired signal *s(k)*. In [91], it is described the procedure to select *r(k)* in such a way that it approaches *s(k)*.

In order to understand the basic concept of deconvolution, it is convenient to start by writing the convolution model for discrete-time signals free of noise:

$$y(k)=s(k)*h(k), \tag{A.1}$$

where the symbol * denotes the mathematical operation of convolution. On the other hand, if noise is present in the system, the previous equation may be written as

$$y(k)=x(k)*h(k)=[s(k)+w(k)]*h(k)=s(k)*h(k)+n(k) \tag{A.2}$$

In Eq.(A.1), if one knows the output signal *y(k)* and the input signal *s(k)*, *h(k)* can be determined by deconvolving both signals *y(k)* and *s(k)*. Nevertheless, the same cannot be applied to Eq.(A.2), since output signal *y(k)* is corrupted by noise *n(k)*. Deconvolution based on Wiener Filtering offers a way to determine an equivalent impulse response for Eq.(A.2), called optimal impulse response $\hat{h}(k)$, by filtering out the noise *n(k)*. Under above considerations, Eq.(A.2) is now expressed as

$$\hat{y}(k)=r(k)*\hat{h}(k) \tag{A.3}$$

The solution to $\hat{h}(k)$ is found first in the frequency domain, by means of the minimization of the mean square error *e(k)* [91]:

$$\hat{H}(w)=\frac{Y(w).R^{*}(w)}{|R(w)|^{2}+S_{n}(w)/S_{h}(w)} \tag{A.4}$$

where $\hat{H}(w)$, $R(w)$ and $Y(w)$ indicate the Fourier Transforms of the optimum estimates of the filter impulse response $\hat{h}(k)$, reference signal $r(k)$ and output signal $y(k)$, respectively, and $R^*(w)$ is the complex conjugate of $R(w)$. $S_n(w)$ and $S_h(w)$ are the power spectral densities of $n(k)$ and $h(k)$.

In practice, to avoid the estimation of the power spectral densities $S_n(w)$ and $S_h(w)$, one may simply replace the ratio $S_n(w)/S_h(w)$ by a positive real constant q (the "noise-desensitizing factor") to be decided [91,92]. Hence, Eq.(A.4) becomes

$$\hat{H}(w) = \frac{Y(w).R^*(w)}{|R(w)|^2 + q} \tag{A.5}$$

Once $\hat{H}(w)$ is determined, the Inverse Fourier Transform gives the optimal estimate of the filter impulse response $\hat{h}(k)$.

Finally, if one applies Eqs.(A.5) and (A.3) to one of the original HDPE and Santoprene co-extrusion data discussed in the section 3.2.3 of Chapter 3 (Fig.A.2), the processed signal, shown in Fig.A.3, turns out to be more suitable for measuring time delays due to the unequivocal identification of echoes.

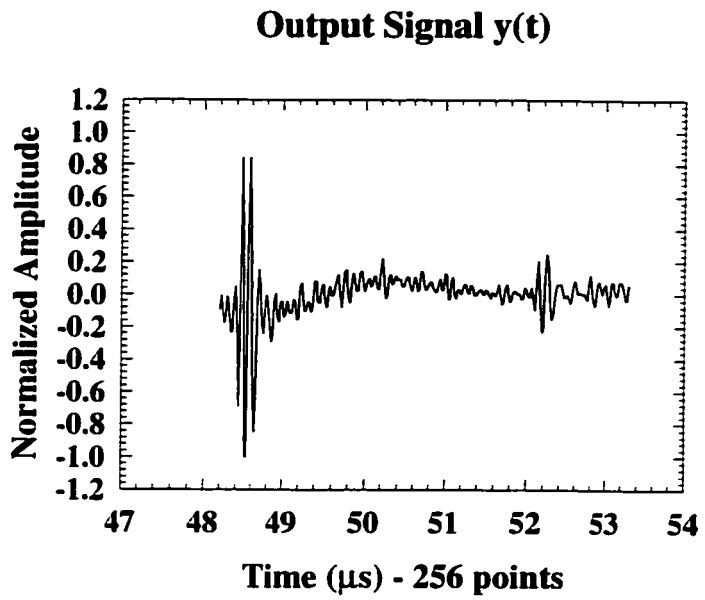


Fig.A.2: Simple trace of HDPE and Santoprene co-extrusion before Wiener filtering.

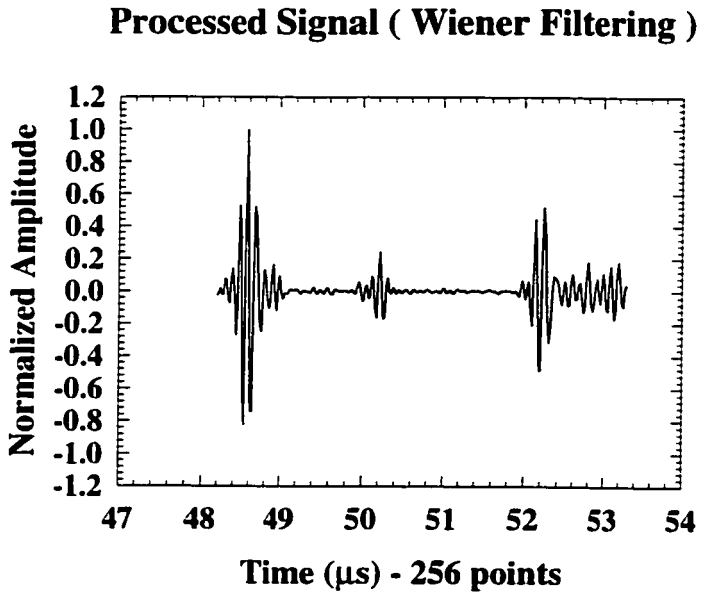


Fig.A.3: Simple trace of HDPE and Santoprene co-extrusion after Wiener filtering.

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Roles of HIV-1 Nucleocapsid Protein (NCp7) in Reverse Transcription Initiation and Viral Genomic RNA Packaging

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ABSTRACT

This work is aimed at understanding the mechanisms that underlie the functions of human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein (NCp7) during reverse transcription initiation as well as during specific viral genomic RNA encapsidation. To pursue the first goal, we developed an *in vitro* reverse transcription reaction system, in which synthetic NCp7 was employed to place natural $tRNA^{Lys,3}$ onto an HIV-1 RNA template containing the primer binding site (PBS) and its flanking sequences. After placement, NCp7 was removed by Proteinase K digestion and phenol:chloroform extraction, so that the function of NCp7 in annealing could be separated from that in subsequent reverse transcription. Low concentrations of dNTPs were used in these reactions in order to detect very early pausing events during the initiation stage of reverse transcription. The results showed that initiation of HIV-1 (-) strand DNA synthesis was characterized by early pausing events at the +1 and +3 nt positions in the cell-free reverse transcription system. The +1 pausing was associated with the use of an RNA primer, while the +3 pausing event was attributable to the existence of a stem-loop structure upstream of the PBS. A mutant NCp7 molecule, devoid of Zn fingers, was able to place $tRNA^{Lys,3}$ onto vRNA as efficiently as wt NCp7; however, the $tRNA^{Lys,3}$:vRNA complex formed with the assistance of wt NCp7 was far more efficient than that formed by the mutant NCp7 in both initiation and the subsequent transition from initiation to elongation during the synthesis of (-) strand DNA.

To study the role of NCp7 in viral RNA incorporation, we deleted the *cis*-acting packaging signals SL1 and SL3, which are located up- and down-stream of the 5′ major splice donor (SD) site, respectively. These deletions led to defected viral replication kinetics. Long-term culture of these mutated viruses gave rise to second-site mutations in the p2 and NC domains of the Gag protein, that were able to rescue the aforementioned deletions. We further deliberately replaced the

corresponding residues within p2 or NC by each of 19 other amino acids in the context of the SL1 deletion, and found that only hydrophobic amino acids with long side chains (including V, L, I, and M) were able to rescue this deletion. In addition, it was observed that compensation of the SL1 and SL3 deletions involved changes at different residue positions in the p2 and NC proteins, indicating that RNA packaging signals up- or down-stream of the 5′ SD site bind to Gag protein in different ways during viral RNA packaging.

RÉSUMÉ

Ce travail a pour but d'essayer de comprendre le méchanisme de fonctionnement de la nucléocapside (NCp7) du virus de l'immunodéficience humaine de type 1 (VIH-1) pendant l'initiation de la transcription inverse ainsi que pendant l'enveloppement spécific de l'ARN génomic viral. Pour atteindre le premier but, nous avons développé *in vitro*, un système de réaction de la tanscritption inverse dans lequel une NCp7 synthétique était utilisée pour placer l'ARN de transfert naturel avec la lysine en position 3 ($tRNA^{Lys.3}$) sur une matrice d'ARN viral contenant le site d'attachement de l'amorce (PBS) et les séquences adjacentes. Après le placement, la NCp7 était enlevée par digestion par la protéinase K et l'extraction avec le phénol chloroforme, de sorte que sa fonction dans la fixation peut être séparée de ses fonctions subséquentes dans la transcription inverse. De faibles concentrations de dNTPs étaient utilisées dans les réactions pour détecter des poses précoces au niveau du stade d'initiation de la transcription inverse. Les résultats ont montré que l'initiation de la synthèse du brin négatif du VIH-1 était caractérisée par des poses précoces aux positions +1 et +3 des nucléotides dans un système de transcription inverse hors cellule. La pose en position +1 est associée à l'utilisation de l'amorce d'ARN, tandis que la pose en +3 est attribuable à l'existence de la structure en queue de loop en amont du PBS. Le mutant NCp7 sans les doigts de Zinc (Zn) peut placer le $tRNA^{Lys.3}$ sur l'ARN viral (vRNA) aussi efficacement que le NCp7 du type sauvage; cependant, le complex $tRNA^{Lys.3}$:vRNA formé avec l'assistance du NCp7 sauvage est beaucoup plus efficace que celui formé avec le NCp7 mutant, tant pendant l'initiation que pendant la transition de l'initiation à l'élongation lors de la synthèse du brin négatif de l'ADN.

Pour étudier le rôle de la NCp7 dans le processus d'incorporation de l'ARN du VIH-1, nous avons effacé les signaux d'enveloppement agissant en cis, SL1 et SL3, qui sont respectivement situé en amont et

en aval du site du donneur principal de nucléotides (SD). L'effacement de ces nucléotides a conduit à un ralentissement de la réplication du virus. Une culture cellulaire prolongée de ces virus mutants a donné naissance à une seconde génération de mutants avec des mutations dans les domaines des protéines p2 ou NC de la protéine majeure Gag, mutations qui peuvent compenser l'enlèvement des nucléotides, mentionés plus haut. Nous avons remplacé les résidus de p2 ou NC correspondants à chacun des 19 autres acides aminés, dans le context des deletions de SL1, et trouvé que seuls les acides aminés hydrophobiques avec de longues chaines sur le côté (incluant V, L, I, et M) étaient capables de compenser les délétions. De plus, il a été aussi observé que la compensation des délétions de SL1 et SL3 entraînait des changements au niveau de différent résidus des protéines p2 et NC, indiquant que les signaux d'enveloppement de l'ARN en amont ou en aval du site SD 5', se fixaient à la protéines majeure Gag, de différentes manières, pendant l'enveloppement de l'ARN viral.

PREFACE

This Ph.D. thesis was written in accordance to the Guidelines Concerning Thesis Preparation from the Department of Graduate Studies and Research at McGill University. The experimental part of this thesis is presented in the form of original papers. This option reads in the following states:

> "The candidate has the option, subject to the approval of the department, of including, as part of their thesis, copies of the text of a paper(s)... If this option is chosen, connecting texts, providing logical bridges between the different papers, are mandatory... The thesis must include, as separate chapters or sections: (1) a Table of Contents, (2) a general abstract in English and French, (3) an introduction which clearly states the rationale and objectives of the study, (4) a comprehensive general review of the background literature to the subject of the thesis, when this review is appropriate, and (5) a final overall conclusion and/or summary.
>
> In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent; ... since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of the different authors of co-authored paper."

The contribution of co-authors to published articles, as well as the information from published articles appear in this thesis on the title page of each chapter. The author's "contribution to original knowledge" and references cited appear at the end of the thesis.

Other manuscripts not included in this thesis, but with which the candidate was involved, are as follows:

1. **Kameoka M., L. Rong, M. Götte, C. Liang, R. S. Russell, and M. A. Wainberg.** 2001. A role for the Tat protein of HIV-1 in suppression of viral reverse transcriptase activity during late stages of viral replication. J Virol. **75**:2675-2683.
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LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
DDDP	DNA-dependent DNA polymerase
ddNC	nucleocapsid protein with both of the Zn fingers replaced by Glycine-Glycine linkage
DIS	dimerization initiation site
dNTP	2'-deoxynucleotide triphosphate
H^{23}C NC	nucleocapsid protein with a point mutation at the 23rd position
HIV	human immunodeficiency virus
LTR	long terminal repeat
mAb	monoclonal antibody
NC	nucleocapsid protein
nt	nucleotide
ODN	oligodeoxyribonucleotide
PBS	primer binding site
R	repeat region
RDDP	RNA-dependent DNA polymerase
RNase H	DNA/RNA-dependent ribonuclease
RT	reverse transcriptase
SD	splice donor site
SL	stem-loop
tRNA	transfer RNA
tRNA:vRNA	an RNA complex involving both tRNA primer and viral RNA template
U3	3' unique region
U5	5' unique region
utRNA	unmodified tRNA/synthetic tRNA
vRNA	viral RNA
Ψ	packaging signal
(-)ssDNA	minus-strand strong-stop DNA

Chapter 1

Literature Review

1.1 GENERAL INTRODUCTION

1.1.1 Retroviruses

Retroviruses comprise a diverse enveloped RNA virus family defined by their structure, composition, and replicative strategy. The uniqueness of this family lies in its replication cycle, which includes, as essential steps, reverse transcription of the viral RNA into proviral DNA and subsequent integration of this DNA into host genome (Nermut and Hockley 1996; Vogt 1997).

According to their genome organization and gene expression patterns, retroviruses can be divided into two categories broadly: simple and complex (Cullen 1992; Weiss 1996; Vogt 1997). Both simple and complex retroviruses contain three major genes: namely *gag*, *pol*, and *env*, which encode internal structural proteins, essential enzymes (*i.e.*, protease, reverse transcriptase and integrase), and viral envelope proteins, respectively. In addition to this elementary information, complex retroviruses also code for additional regulatory proteins derived from multiply spliced mRNAs. As a result, their gene expression can be divided into two temporal phases, an early, regulatory phase and a late, structural phase. Such a gene expression pattern has not been observed in simple retroviruses.

On the basis of sequence homology, retroviruses are further subdivided into seven genera, *i.e.* Avian sarcoma and leukosis viral group; Mammalian B-type viral group; Murine leukemia-related viral group; Human T-cell leukemia-bovine leukemia viral group (HTLV-BLV); D-type viral group; Lentiviruses; and Spumaviruses. The first five groups represent oncogenic retroviruses that have been identified in all classes of vertebrates, many of which contain oncogenes and induce malignancies in host. All oncogenic members except the HTLV-BLV genus belong to simple category, whereas HTLV-BLV, lentiviruses and spumaviruses are complex retroviruses.

There are now six known human retroviral species: human T-cell leukemia virus type I (HTLV-I), type II (HTLV-II), human immuno-

deficiency virus type 1 (HIV-1), type 2 (HIV-2), human foamy virus, and human retrovirus-5 (HRSV-5). HTLV-I and -II are classified as HTLV-BLV group, and are shown to cause T-cell leukemia (Wong-Staal and Gallo 1985). HIV-1, which was originally termed as HTLV-III, belongs to the lentiviruses genus. HIV-1 is the major causative agent of acquired immunodeficiency syndrome (AIDS) (Barré-Sinoussi et al. 1983; Popovic et al. 1984). Compared with HIV-1, HIV-2 also causes AIDS, but produces a lower virus load, is less easily transmitted perinatally, and takes a longer incubation period for the development of the disease (Clavel et al. 1986; Markovitz 1993; Pepin et al. 1991; Weiss 1996). The human foamy virus belongs to the spumaviruses group, which causes no known disease (Weiss 1988). The newly identified HRSV-5 is related to B- and D-type retroviruses, and is detected as an exogenous genome in association with arthritis and systemic lupus erythematosus (Griffiths et al. 1997).

1.1.2 Origin of HIV-1

On the basis of sequence analysis in *pol*, five discrete groups of primate lentiviruses have so far been identified, including HIV-1/SIVcpz, HIV-2/ SIVsmm, SIVagm, SIVmnd, and SIVsyk (Vanden Haesevelde et al. 1996). Cpz, smm, agm, mnd, and syk represent chimpanzees, sooty mangabey monkeys, African green monkeys, mandrill monkeys, and sykes monkeys, respectively.

It is well accepted that some monkey species have been natural hosts for their cognate SIV strains, *e.g.*, SIVcpz, SIVsmm, and SIVagm, without causing any disease (Nathanson et al. 1993). This is expected if a virus and host had sufficient time to adapt to each other. The SIVagm isolates can even be sub-grouped according to the subspecies of monkey from which they were derived (M.C. Müller et al. 1993; Sharp et al. 1995). Similarly, SIVcpz is also found in an ancient infection of chimpanzees, and co-evolved with its host during sub-speciation (Gao et al. 1999).

Through hunting or husbandry, inter-species transmission (zoonosis) of primate retroviruses to humans occurs, though on relatively rare occasions (Voevodin et al. 1997; Mahieux et al. 1998; Heneine et al. 1998). Increasing prevalence and mortality would then be consequences of infection of the new host to which the virus was not fully adapted in some cases. It has been shown that several strains of HIV-2 in West Africa were independently derived from SIVsmm (Gao et al. 1992; Chen et al. 1997).

The origin of HIV-1 remained uncertain until recent times. It has been reported that a species of chimpanzee, *P. t. troglodytes*, which harbors a related SIVcpz, is the primary reservoir for HIV-1 and has been the source of at least three independent zoonotic transfers from chimpanzees to humans (Gao et al. 1999; Hahn et al. 2000).

Through multiple phylogenetic analyses of HIV-1 sequences from an African plasma sample, originally taken in 1959, it was estimated that all major group of HIV-1 viruses may have evolved from a single introduction into the African population not long before 1959, and that diversification of all the major groups occurred in very recent times, *e.g.*, the past 50 years (Zhu et al. 1998).

1.1.3 HIV-1 Viral Dynamics and Immuno-pathogenic Mechanisms of HIV Infection

Infection with HIV-1 is a highly dynamic process associated with progressive immunodeficiency in the patient. During primary infection, there is an initial burst of viremia, which then diminishes due to an early immune response. Following this seroconversion, and the initial clearance of HIV-1 loads by the immune response, is a long clinically latent stage of up to 10 years. Throughout this long asymptomatic phase, a steady decline in the numbers of $CD4^+$ T lymphocytes is observed, which is accompanied by a continuous de novo viral infection and replication in susceptible cells, and rapid cell turnover (Ho et al. 1995; Wei et al. 1995). As the CD4 cell counts drop to around half

of the normal level, affected patients become susceptible to a variety of opportunistic infections (OIs), which becomes the basis of AIDS diagnosis.

Vigorous HIV-1 replication can be observed throughout all stages, including the asymptomatic phase, during which virus production is balanced by virus clearance. Perturbance of this steady state by highly active anti-retroviral therapy (HAART) in HIV patients results in declines of circulating virus and infected cells. These plasma virus decay curves have three phases, each with a different decline rate, implying the presence of at least three classes of HIV-infected cells with different half-life ($t_{1/2}$) spans. The first class of cells turns out to be $CD4^+$ T lymphoblasts, which show a rapid turnover rate ($t_{1/2}$= 1-2 days). They produce virus at high levels, and make a major contribution to plasma viremia. This high level of virus production can also explain the rapid appearance of drug resistance mutations in mono-therapy treated patients. The second class of cells are chronically infected cells with slower turnover rates ($t_{1/2}$= 2 weeks), such as macrophages (Cavert et al. 1997; Perelson et al. 1996, 1997a,b). The resting memory $CD4^+$ T cells with integrated provirus may be the candidate of the third class of cells ($t_{1/2}$= 120 days or more). Instead of contributing to the pool of free virus, they are an extremely stable reservoir for HIV-1, and represent a major barrier for anti-retroviral therapy in regard to eradication of virus (Chun et al. 1995, 1997a,b; Finzi et al. 1997; Wong et al. 1997).

HIV-1 populations in newly infected individuals have been shown to be relatively homogeneous (McNearney et al. 1992; Zhu et al. 1993), while in the subsequent clinically latent period, viral populations undergo a continuous intra-host evolution due to the high degree of genetic variability. Each round of HIV-1 replication produces quasispecies, *i.e.*, a complex mixture of genetically (*e.g.* V3 region variability of envelope glycoprotein gp120) and phenotypically (growth kinetics in cell cultures, tropism, syncytium formation, and/or usage of co-receptor for entry) different viruses (de Jong et al. 1992a,b;

Fouchier et al. 1992; Lukashov et al. 1995; Hoffman et al. 1998). Both the consensus sequence and the complexity of quasispecies change during the course of individual HIV-1 infection, which is thought to be a result of virus escape from immune surveillance, both humoral (Zwart et al. 1992) and cellular (Borrow et al. 1997; Goulder et al. 1997; McMichael 1998). As explained in a continuous virus adaptation model, the destruction of the immune system during the development of AIDS is a process during which the virus continuously adapts itself to the changeable intra-host environment to produce escape mutations until immune collapse (McMichael and Phillips 1997; Lukashov et al. 1998).

One of the early hallmarks of HIV infection is the impairment of a variety of $CD4^+$ T lymphocyte functions (Clerici et al. 1989; Miedema et al. 1994; Rosenberg et al. 1997; Shearer 1998). Besides some cytotoxic cells, most of the $CD4^+$ T lymphocytes are T helper (Th) cells, which can provide critical help to both B lymphocytes and cytotoxic T lymphocytes (CTLs) by secretion of cytokines important for their growth and maturation. The effects of HIV-1 on $CD4^+$ Th can be broadly divided into two categories: one is to induce Th cytopathic death, which is the consequence of HIV infection of Th directly or via HIV-infected antigen presenting cells (APC); the other effect is immunopathogenesis. Major immunopathogenic models that have been suggested include (1) HIV-specific $CD8^+$ CTL that can kill HIV^+ Th cells (Zinkerngel and Hengartner 1994); (2) autoimmune mechanisms which destroy the uninfected immune cells via antigenic cross-reactivity (Pantaleo and Fauci 1995); (3) selective depletion of HIV-specific $CD4^+$ Th via apoptotic T cell death (ATCD), induced by aberrant antigen presentation and increased expression of Fas and FasL in HIV-infected APC (Finkel et al. 1995; Rosenberg et al. 1997; Herbein et al. 1998; Shearer 1998). Through a similar APC-induced mechanism, $CD4^+$ Th clones specific for other antigens could also be gradually depleted over an extended period of time.

Despite compromised $CD4^+$ Th cell functions, infected patients make very strong $CD8^+$ CTL responses to HIV-1 (McMichael 1998). Analysis

of T cell receptors on $CD8^+$ T cells in the blood of acutely infected patients often shows oligoclonal expansion of HIV-specific CTLs. It is estimated that more than 10% of peripheral blood $CD8^+$ T cells are effector CTLs in acute infection. The CTL responses peak as the viraemia starts to fall, and then the expanded T cell populations fade because of apoptosis (Koup et al. 1994; Pantaleo et al. 1994; McMichael and O'Callaghan 1998; Ogg et al. 1998). CTLs are crucial in the control of HIV-1 replication, killing virus infected cells by both cytolysis and release of chemokines and other cytokines (Rowland-Jones et al. 1997; O. O. Yang et al. 1997a,b; Wagner et al. 1998).

Although CTL responses provoked by HIV are strong and effective, they are not sufficient to control the infection permanently. As HIV disease progresses, anti-HIV CTL activity is compromised. CTL killing requires the recognition of viral epitope presented by MHC I molecules on the cell surface. However, this is impaired both by Nef-mediated down-regulation of MHC I expression and by viral epitope mutation. Because Nef only selectively down-regulates MHC-A2- and -B-restricted CTL clones, the potential NK cell-mediated lysis caused by down-regulation of MHC-C molecules can also be avoided (Collins et al. 1998, 1999; Le Gall et al. 1998; Cohen et al. 1999). Other important CTL evasion mechanisms include (1) virus may be sequestered in sites that CTLs do not access effectively, *e.g.* infected glial cells in the brain, and latent proviruses without gene expression; (2) activated T lymphocytes can be susceptible to apoptosis mediated by expressing Fas and FasL (Wu et al. 1995; Herbein et al. 1998). In addition, exhaustion of responding CTL has been reported in some patients (Pantaleo et al. 1994).

Along with escape from CTL response, is the progressively poor $CD4^+$ Th cell responses. With an early and permanent loss of HIV-specific Th cells, it could be difficult to initiate new primary CTL responses demanded by virus escape mutations, which, in turn, could lead to a further increase in virus load and ultimately the collapse of the immune response.

1.2 HIV-1 GENOME AND VIRAL PROTEINS

1.2.1 The Virion

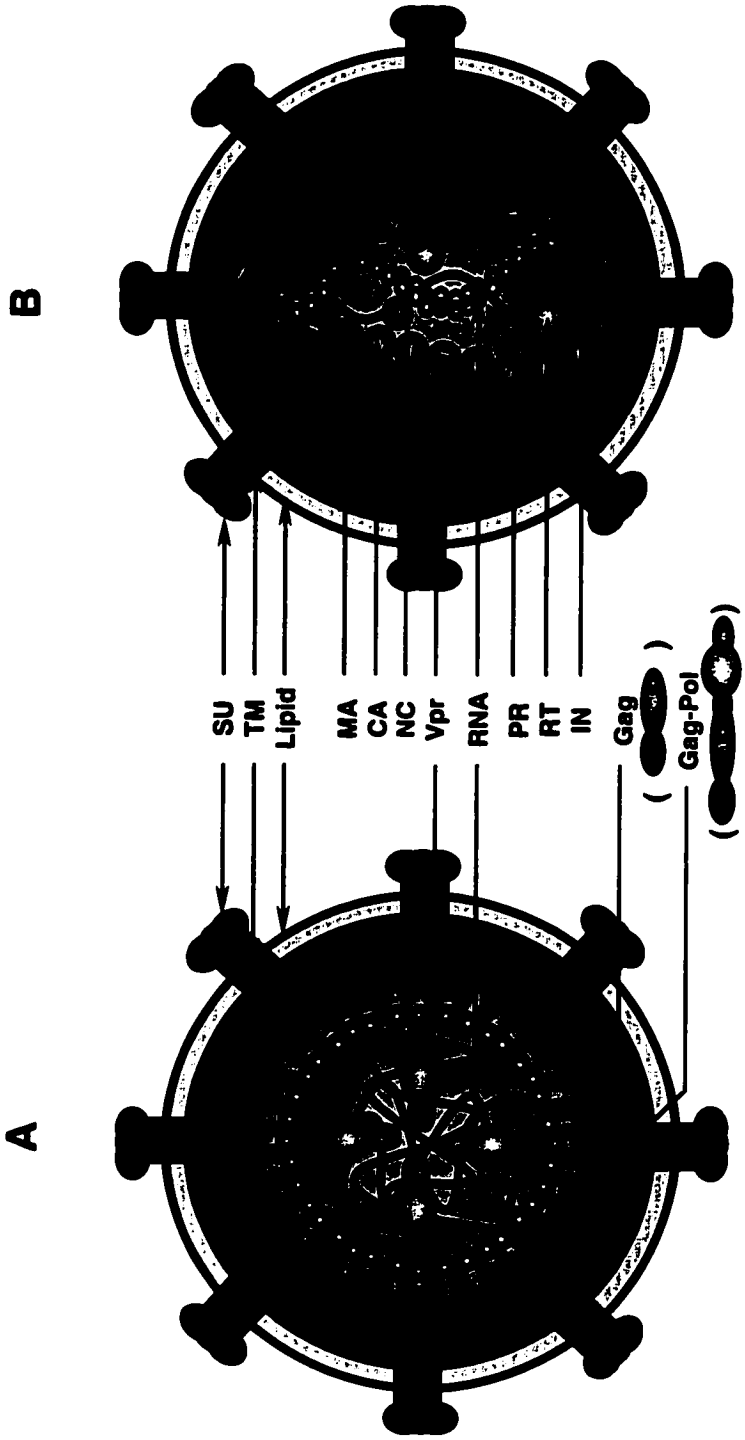
The structure of the HIV-1 virion is represented in models to illustrate what is known about the topographic relationships of components of the virion, as well as other aspects of their structure and function. The newest version of the model is shown in Figure 1-1. The viral envelope is formed by a cell-derived lipid bilayer, into which the viral envelope glycoprotein (Env) complexes are inserted. Each complex consists of the transmembrane (TM, gp41) and the surface (SU, gp120) components, which together form an oligomeric knobbed spike on the surface of the virion.

Within the immature virion, Gag (Pr55) and Gag-Pol (Pr160) polyproteins form a diffuse but relative stable core underneath the envelope (Figure 1-1A). During maturation, the proteolysis of Gag induces a huge transformation in virion structure (Figure 1-1B). The matrix proteins (MA, p17) remain associated with the inner face of the viral membrane, while capsid proteins (CA, p27) condense to form the shell of a conical-shaped core, which is largely detached from the membrane. In the center of the core is the nucleocapsid proteins (NC, p7) complexed with two copies of genomic RNA. Associated with this complex are reverse transcriptase (RT) and integrase (IN), proteolytic products of the Gag-Pol precursor. The location of protease (PR) is unclear.

1.2.2 Genetic Organization

HIV-1 carries two identical genomic RNA molecules that are non-covalently associated at the 5'end to form a dimer. Each of the two genome RNAs is 9.2 kb in length, single-stranded (ss), non-segmented, and of positive polarity (Popovic et al. 1984). For reasons of simplicity, retroviral genome organization is discussed herein in terms

Figure 1-1. Structural models for HIV-1 immature (A) and mature (B) virions.


B
SU
TM
Lipid
MA
CA
NC
Vpr
RNA
PR
RT
IN
Gag
Gag-Pol
A

of proviral DNA. Figure 1-2 is a schematic illustration of HIV-1 genetic organization. The known functions of its gene products are also shown in the figure.

The HIV-1 genome consists of three major genes, *gag*, *pol*, and *env*, as well as six auxiliary genes, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (Cullen 1991). *gag* encodes the internal structural protein of the virion, including MA, CA, and NC. *pol* encodes the enzymes RT, IN, and PR. *env* encodes the surface and transmembrane glycoproteins of the virion, which form a complex that interacts specifically with cellular receptor proteins.

HIV-1 accessory/regulatory genes have different functions. Some regulate viral gene expression, while others have ancillary roles in specific cell lines. These genes are located in a variety of locations downstream of *pol*, partially overlapping portions of *env* and U3, as well as each other.

The HIV-1 genes in the proviral DNA are bracketed by the long terminal repeats (LTRs), identical sequences that are composed of U3, R, and U5 elements. The transcription initiation site and poly(A) addition site separate the R region from the U3 and U5 regions respectively; the initiation sites of plus- and minus-strand DNA synthesis determine the other boundaries of the U3 and U5 regions.

HIV-1 contains a large array of *cis*-acting elements that direct the host cell machinery to function in viral gene expression from its proviral DNA intermediate. Most of the transcription control elements, including the promoter and multiple enhancer sequences, are found within the 5'end U3 region.

In the form of RNA, the 5'end of the genome is a non-coding region that includes the R and U5 regions, the primer binding site (PBS), as well as an exon 1 leader sequence (Figure 1-3A). This region contains multiple essential RNA elements, which form specific secondary structures, and play critical roles for numerous aspects of viral replication (Figure 1-3B). These RNA elements are: (1) Tat protein transactivation region (TAR), which serves as the binding site for the

Figure 1-2. Schematic illustration of HIV-1 genetic organization and known functions of its gene products.

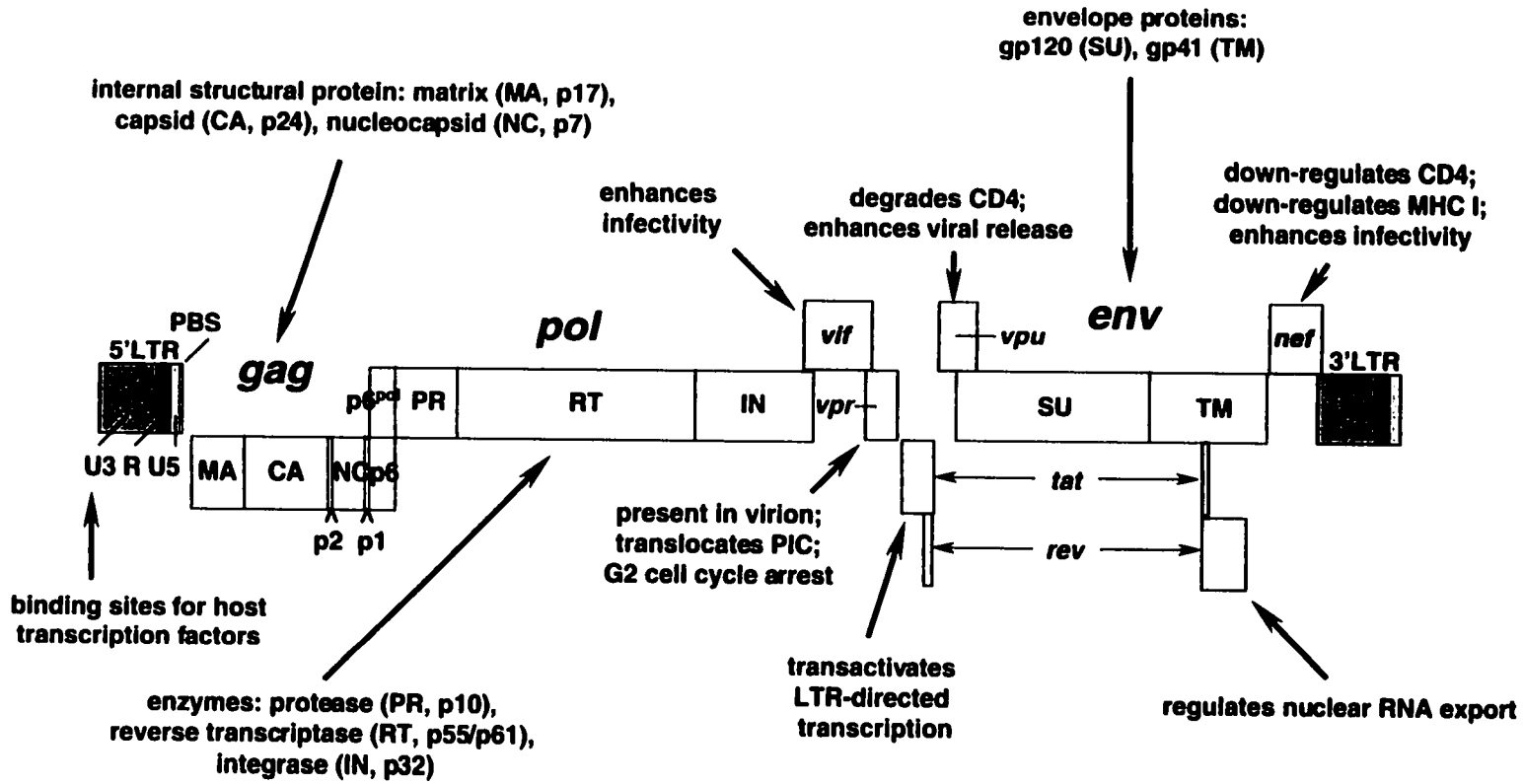
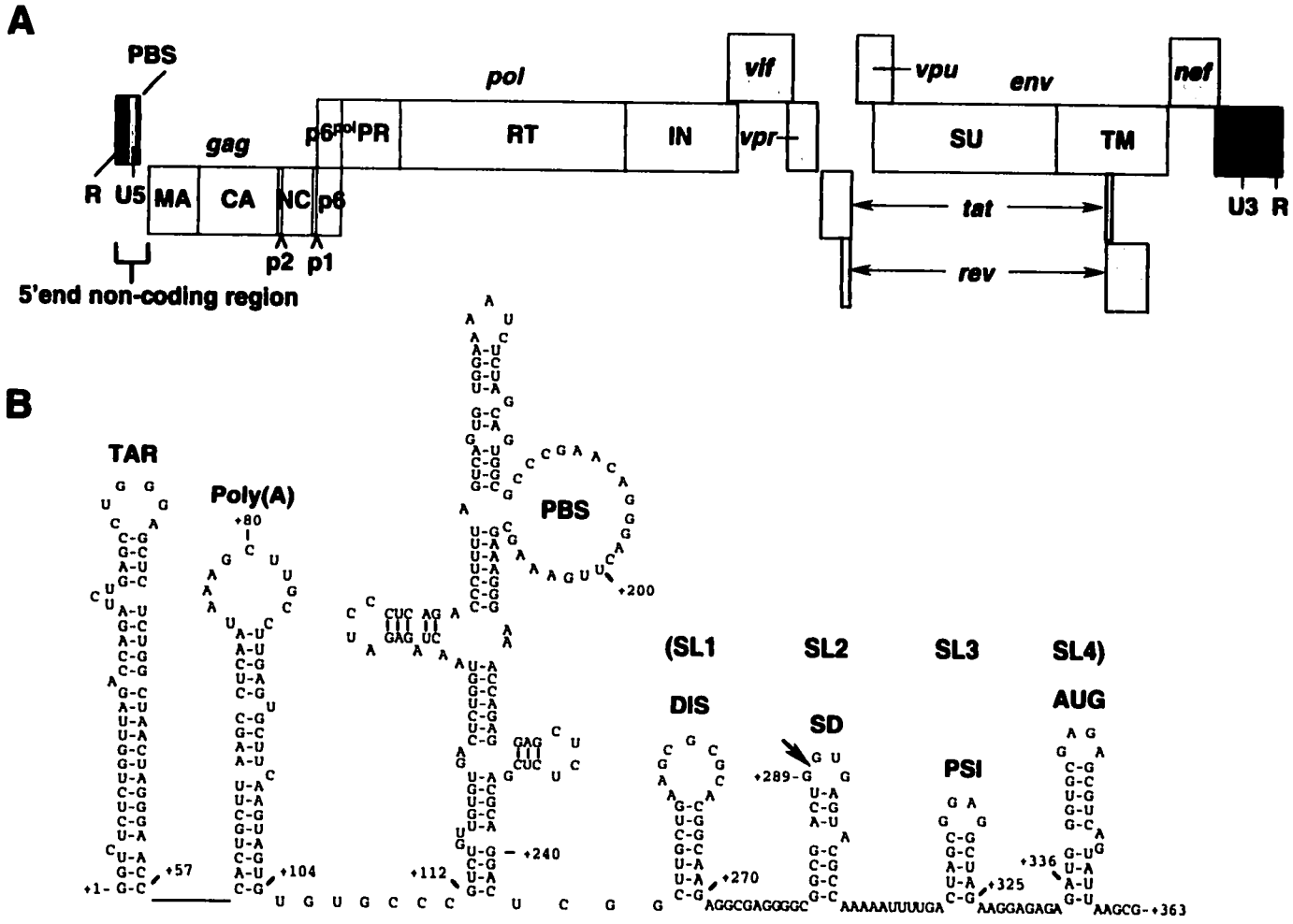

envelope proteins:
gp120 (SU), gp41 (TM)
internal structural protein: matrix (MA, p17),
capsid (CA, p24), nucleocapsid (NC, p7)
enhances
infectivity
degrades CD4;
enhances viral release
down-regulates CD4;
down-regulates MHC I;
enhances infectivity
PBS
5'LTR
gag
pol
vif
vpu
env
nef
3'LTR
p6pol
PR
RT
IN
vpr
SU
TM
U3 R U5
MA
CA
NC
p6
p2
p1
tat
rev
present in virion;
translocates PIC;
G2 cell cycle arrest
binding sites for host
transcription factors
enzymes: protease (PR, p10),
reverse transcriptase (RT, p55/p61),
integrase (IN, p32)
transactivates
LTR-directed
transcription
regulates nuclear RNA export

Figure 1-3. The organization of HIV-1 genomic RNA (A) and the secondary structure of its 5'end (B). (modified from Berkhout 1996a)


A
PBS
gag
pol
vif
vpu
env
nef
p6^pol
PR
RT
IN
vpr
SU
TM
R U5 MA CA NC p6
tat
U3 R
p2 p1
rev
5'end non-coding region
B
TAR
Poly(A)
PBS
(SL1
SL2
SL3
SL4)
DIS
SD
PSI
AUG
+80
+57
+104
+112
+200
+240
+289
+270
+325
+336
+363
U G U G C C C U C G G
AGGCGAGGGGC
AAAAAUUUUGA
AAGGAGAGA
AAGCG

TAR trans-activator protein (Tat) and other cellular factors (Garcia et al. 1988; Selby et al. 1989). (2) Transcription termination signal poly(A). The 3'end copy of this element is the signal for termination of transcription and poly(A) tail addition. However, the formation of the poly(A) hairpin at the 5'end of the genome RNA is also essential for packaging of viral genomic RNA and/or stability of the viral RNA (Das et al. 1997). (3) Primer binding site (PBS), where the primer tRNA is annealed and reverse transcription is initiated. Details will be discussed in section 1.3.2.2 of this chapter. (4) Dimerization initiation site (DIS), a hairpin structure whose loop contains a palindromic sequence, which is critical for initiation of genome dimerization via loop-loop interaction, *i.e.* a "loop-loop kissing" mechanism (Laughrea and Jette 1994; Paillart et al. 1994; Muriaux et al. 1995). (5) Major splice donor site (SD), to generate most of the subgenomic RNA transcripts (Purcell and Martin 1993). (6) Packaging signal (Ψ/E), a 46-base sequence located between the major SD and the *gag* gene, for selective encapsidation of full-length viral genome RNA (Kingsman and Kingsman 1996; Rabson and Wills 1997). There is also accumulating evidence that other regions of the HIV-1 genome are involved in packaging of viral genomic RNA. In particular, sequences in the TAR-U5 region (Vicenzi et al. 1994; Das et al. 1997; Helga-Maria et al. 1999; Liang et al. 2000), the DIS region (Kim et al. 1994; McBride et al. 1996, 1997), and the 5' part of the *gag* open reading frame (Luban and Goff 1994; Parolin et al. 1994) have been reported to contribute to the packaging function.

1.2.3 *gag* Gene Products

1.2.3.1 Gag Precursor Protein

As is typical of all retroviruses, HIV-1 initially synthesizes its structural proteins in the form of large Gag polyprotein precursors that are assembled to form virus particles. During or shortly after

virus budding, Gag precursors are specifically cleaved to yield mature structural proteins by the viral protease.

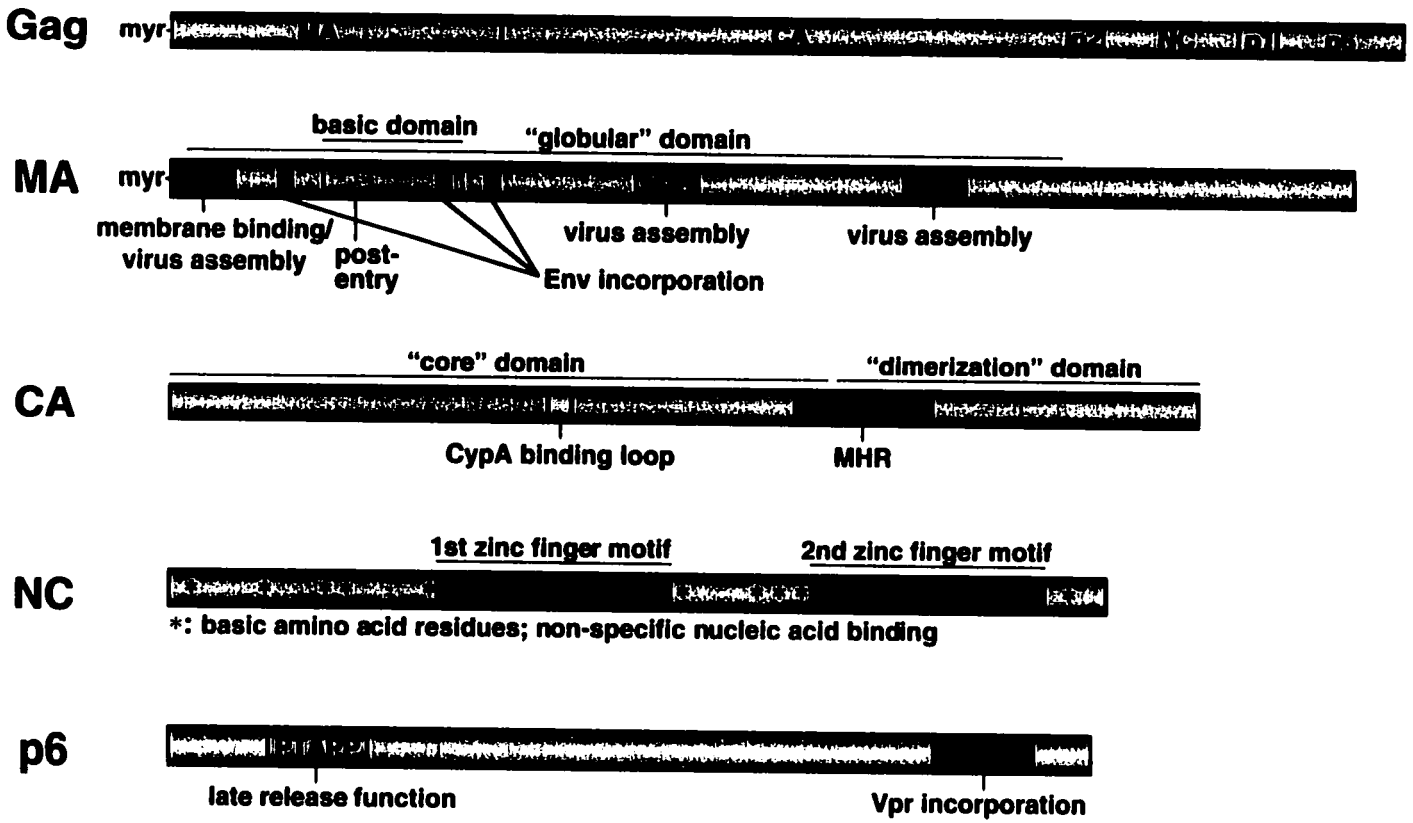
In the process of viral particle formation, Gag precursor protein is responsible for orchestrating the recruitment of viral proteins, *i.e.*, Gag, Gag-Pol, Env, Vpr, viral genomic RNA, and host cell-derived factors into virus particles. Gag is also involved in the efficient release of viral particles from the cell surface, as well as proper core morphogenesis during viral maturation. The regions responsible for these functions are distributed in the multiple domains of Gag. After proteolysis, each domain of Gag undergoes a transition in both shape and function. Upon de novo infection, the mature Gag proteins play new roles in the early steps of the viral life cycle. Figure 1-4 is a linear representation of major *gag* gene products. The major functions of each protein are also shown.

1.2.3.2 MA

MA proteins are trimerized in the crystal structure. NMR spectroscopy and X-ray crystallography studies indicate a hydrophobic "globular" domain, composed of α-helices and a three-stranded β-sheet at the amino-terminus of MA, with a small carboxy-terminal helix projecting away.

As the N-terminal domain of Gag precursor, MA is important in the targeting of Gag to the plasma membrane. Myristylation at the amino-terminus, as well as basic residues mainly located in a basic domain between residues 17 and 31, are essential for the membrane binding property (Göttlinger et al. 1989; Zhou et al. 1994). Furthermore, the specific interaction between MA domain of Gag and cytoplasmic domain of gp41 is also involved in the incorporation of Env protein into virions (Hill et al. 1996; Ono et al. 1997). A recent report demonstrated that there is a nuclear export signal (NES) in the basic domain of MA, which is required to direct unspliced viral RNA to the plasma membrane, ensuring the cytoplasmic availability of the components for virion assembly (Dupont et al. 1999).

Figure 1-4. Linear representation of major *gag* gene products. The major functions of each protein are also shown.


Gag
myr-
basic domain
"globular" domain
MA
myr-
membrane binding/
virus assembly
post-
entry
Env incorporation
virus assembly
virus assembly
"core" domain
"dimerization" domain
CA
CypA binding loop
MHR
1st zinc finger motif
2nd zinc finger motif
NC
*: basic amino acid residues; non-specific nucleic acid binding
p6
late release function
Vpr incorporation

In the early postinfection stage, as a mature protein, MA is found associated with the preintegration complex (PIC) (Bukrinsky et al. 1993b; Gallay et al. 1995a,b; Miller et al. 1997), where MA may help to enhance the efficiency of reverse transcription (Yu et al. 1992a; Reicin et al. 1995, 1996; Casella et al. 1997), increase the stability of cDNA product (Kiernan et al. 1998), and finally transport the PIC to the nucleus through a nuclear localization signal (NLS) presented in the basic domain of MA (Bukrinksy et al. 1993a,b; von Schwedler et al. 1994; Dupont et al. 1999).

1.2.3.3 CA

The CA protein is composed of two domains: a highly helical N-terminal "core" domain, and a globular C-terminal "dimerization" domain. The latter contains a major homology region (MHR), which is the most conserved region of Gag that displays significant homology among different genera of retroviruses (Wills and Craven, 1991). The core domain consists of seven α-helices, two β-hairpins and an exposed loop; the highly conserved residues of MHR could form a hydrogen bond network in the crystal structure, which may facilitate CA dimerization and Gag oligomerization by stabilizing the conformation of the entire C-terminal domain (Gitti et al. 1996; Momany et al. 1996; Gamble et al. 1996, 1997).

As a domain of Gag polyprotein, CA is involved in the incorporation of Cyclophilin A (CypA), a cellular peptidyl-prolyl *cis-trans* isomerase, for the formation of fully infectious virus particles (Braaten et al. 1996; Franke and Luban 1996; Streblow et al. 1998). The CypA binding site within CA was mapped to the exposed loop in the N-terminal core domain (Luban et al. 1993; Franke et al. 1994b; Gamble et al. 1996). As a domain of Gag-Pol polyprotein, CA also mediates the interaction between Gag and Gag-Pol, a function that is essential for the encapsidation of Gag-Pol, and therefore the recruitment of RT, IN, and PR into virions (Park and Morrow 1992; Smith et al. 1993; Srinivasakumar et al. 1995). Although the specific region of Gag

involved in the interaction with Gag-Pol has not yet been precisely mapped, the critical determinants within Gag-Pol that mediate its entry into virions was found to be the MHR and the adjacent C-terminal sequences of CA (Smith et al. 1993; Reicin et al. 1995; Srinivasakumar et al. 1995; Huang and Martin 1997).

Modification of the interface between MA and CA during virion maturation is important for core morphogenesis. Upon PR-mediated cleavage at the MA-CA junction, the N-terminus of CA is released from MA and membrane, leading to N-terminal salt-bridge formation and the consequent CA N-terminus rearrangement that is pivotal for the subsequent capsid condensation (von Schwedler et al. 1998). This model was confirmed by *in vitro* assembly experiments, in which addition of as few as four C-terminal MA residues to the CA N-terminus would switch a normally assembled cylinder (resembling a mature core) back to an immature spherical particle (Gamble et al. 1997; Gross et al. 1998; Campbell and Rein 1999). Consistently, virions with N-terminal CA mutations were often found displaying marked infectivity defects, and aberrant core morphology as well (Wang and Barklis 1993; Dorfman et al. 1994; Pettit et al. 1994; Reicin et al. 1995, 1996; von Schwedler et al. 1998).

1.2.3.4 NC

HIV-1 NC protein contains two copies of a CCHC ($CX_2CX_4HX_4C$) type of Zn finger motif, which are flanked by multiple basic residues. Both basic residues and Zn finger motifs are important for efficient encapsidation of full-length viral RNA (Aldovini and Young 1990; Gorelick et al. 1990; Dorfman et al. 1993; Zhang and Barklis 1995, 1997; Schwartz et al. 1997; De Guzman et al. 1998), that, in turn, provides a scaffold along which Gag proteins can tightly condense and pack together (de Rocquigny et al. 1992; Campbell and Vogt 1995; Poon et al. 1996).

The NC domains of retroviral Gag proteins were recognized early on as playing a role in RNA binding and encapsidation; however, like the other Gag domains, a variety of additional functions have been

ascribed to the HIV-1 NC. These include: (1) promotion of RNA dimerization and maturation (Darlix et al. 1990; de Rocquigny et al. 1992; Sakaguchi et al. 1993; Fu et al. 1994; Muriaux et al. 1995; Feng et al. 1996); (2) mediating Gag-Gag interactions (Bennett et al. 1993; Franke et al. 1994a; Parent 1995; Zhang et al. 1998); (3) stimulation of reverse transcription, by facilitating tRNA incorporation and annealing to the PBS (de Rocquigny et al. 1992; Tsuchihashi and Brown 1994; Lapadat-Tapolsky et al. 1995; Huang et al. 1997a, 1998; Cen et al. 1999, 2000), promoting initiation (Liang et al. 1998a; Rong et al. 1998), stimulating strand transfer (Peliska et al. 1994; You and McHenry 1994; Guo et al. 1997; Wu et al. 1999), increasing processivity (Ji et al. 1996; Wu et al. 1996), and stabilizing the preintegration complex (Berthoux et al. 1997); (4) stimulating coupled integration *in vitro* (Carteau et al. 1997, 1999); and (5) enhancement of basal-level transcription activity of the LTR (Zhang et al. 2000).

1.2.3.5 p6

Unlike MA, CA, and NC domains, the localization of p6 in virions is unclear. In HIV-1, several roles for p6 have been described. First, p6 is essential for viral particle release from the cell surface. Truncation of p6 produces particles that are defective for release and accumulate at the plasma membrane. The domain responsible for the budding defect was mapped to a Pro-Thr-Ala-Pro-Pro (PTAP) sequence near the N terminus of the protein (Göttlinger et al. 1991; Huang et al. 1995).

An additional role for HIV-1 p6 is the encapsidation of the accessory protein Vpr into virion. This function maps to a domain near the C terminus of p6 (Paxton et al. 1993; Lu et al. 1995; Kondo and Göttlinger 1996).

Other functions reported for p6 include: incorporating or retaining *pol*-derived proteins in the assembling virus particles (Yu et al. 1998), and as the most critical region for determining HIV-1 particle size (Garnier et al. 1998, 1999).

1.2.3.6 p2 and p1

Cleavage of HIV-1 Gag also generates two short spacer peptides, p2 and p1. The function of p1 is still unclear. The presence of p2 in Gag appears to regulate rates of proteolytic cleavage at both its ends and thereby influences the order of processing and virion core morphogenesis (Pettit et al. 1994; Kräusslich et al. 1995; Accola et al. 1998; Wiegers et al. 1998; Gross et al. 2000).

1.2.4 *pol* Gene Products

1.2.4.1 Gag-Pol Precursor Protein

HIV-1 encodes enzymes that are assembled into viral particles and catalyze essential steps in the infectious cycle. These enzymes are required for proteolytic maturation of the viral particle (*i.e.* protease, PR), reverse transcription of the viral RNA into DNA (*i.e.* reverse transcriptase, RT), and integration of the viral DNA into the host genome (*i.e.* integrase, IN). All of them represent important targets for antiviral therapy. A wealth of information about their structure and function have been obtained and have become the basis for the design and development of antiviral inhibitors.

HIV-1 encodes these enzymes in the *pol* gene and expresses them as part of the Gag-Pol polyprotein precursor via a -1 ribosomal frame shift from the *gag* gene reading frame. The frequency of the shift is about 5%, such that the relative abundance of the Gag and Gag-Pol precursor proteins is about 20:1 (Dickson et al. 1984; Jacks et al. 1988; Wilson et al. 1988). Through this mechanism as well as read-through suppression, a mechanism used by mammalian type-C retroviruses, retroviruses control the expression of the Gag protein at high levels relative to the enzymes, while retaining co-regulated expression.

1.2.4.2 Protease (PR)

All known retroviral PRs belong to the aspartic proteinase family, and share the family feature that two aspartic acid residues, each placed

in the highly conserved triplet Asp-Thr/Ser-Gly, coordinate a water molecule to hydrolyze the target peptide bond in the catalytic active site.

Like other retroviral PRs, the active form of HIV-1 PR is a dimer of two identical subunits, each of which is 99 amino acids long and contains a single triplet motif at the cleavage activity site. The two subunits of the dimer are linked by inter-subunit interactions, including two interlocking loops at the hydrophobic core, and a four-stranded anti-parallel β-sheet formed by both the amino and carboxyl termini of each subunit. Between the subunits exists a long cleft, which constitutes the substrate-binding pocket. On the floor of this cleft are the catalytically important aspartic acids. Projecting over the cleft are arms or "flaps", one from each subunit, which are composed of anti-parallel β-sheets with a β-turn, and are movable to allow the substrate to enter or cleavage products to leave the enzyme (Miller et al. 1989b; Gustchina and Weber 1990; Wlodawer and Erickson 1993).

Protease substrate recognition is highly specific, and this specificity comes from the side chain interactions between PR and substrate. The most important determinant is the four amino acids on either side of the scissile bonds of the substrate that are recognized by flaps of PR (Oroszlan and Luftig 1990; Pettit et al. 1993; Dunn et al. 1994; Katz and Skalka 1994). Under circumstances when multiple cleavage sites exist simultaneously, as in the Gag and Gag-Pol precursors, each cleavage site has its own primary sequence as well as a structural context that is not functionally equivalent with others, and thus is processed with a different rate by PR. This differential site recognition capability provides PR a very important mechanism to regulate PR activity and to control sequential precursor processing during maturation (Erickson-Viitanen et al. 1989; Gowda et al. 1989; Kräusslich et al. 1989; Loeb et al. 1989a; Partin et al. 1990; Pettit

et al. 1991, 1994; Tritch et al. 1991; Goobar-Larsson et al. 1995; Wiegers et al. 1998).

1.2.4.3 Reverse Transcriptase (RT)

Retroviral RTs exhibit three enzymatic activities: an RNA-dependent DNA polymerase (RDDP), Ribonuclease H (RNase H), and a DNA-dependent DNA polymerase (DDDP). These activities are employed in copying the plus-strand RNA genome to produce a minus strand of DNA, removal of the RNA template, and synthesis of the plus strand of DNA using the minus-strand DNA as a template.

HIV-1 RT is a heterodimer consisting of a 66-kD subunit (p66) and a 51-kD subunit (p51). The p51 subunit is derived from p66 by proteolytic cleavage of the RNase H domain at the C-terminal. Both of them have common N-termini, which are folded into four domains, referred to as "fingers", "palm", "thumb", and "connection" (Kohlstaedt et al. 1992). The individual fingers, palm, and thumb domains in both p66 and p51 are folded similarly, but their spatial arrangement within each subunit is quite different, thus forming an asymmetric dimer interface (Wang et al. 1994; Hughes et al. 1996). Genetic studies demonstrated that only the p66 subunit contributes directly to the polymerase activity of the RT heterodimer (Le Grice et al. 1991; Hostomsky et al. 1992; Lederer et al. 1992). The p51 subunit appears to have a structural role within the p66/p51 heterodimer.

In the palm of p66, three catalytically essential aspartic acid residues (110, 185, 186) form the polymerase active site, while the RNase H active site is defined by three acidic residues within the C-terminal domain of p66 (Larder et al. 1987; Boyer et al. 1992; Joyce and Steitz 1994; Yang and Steitz 1995). The physical distance between the two active sites is 15-18 bases (Furfine and Reardon 1991; Gopalakrishnan et al. 1992; Kohlstaedt et al. 1992; Jacobo-Molina et al. 1993). The cocrystals of HIV-1 RT with duplex DNA showed that the DNA in the vicinity of the polymerase active site has an A-like structure, whereas the DNA near the RNase H site is B-like in form; the

A- to B-form transition is accompanied by an overall bend of about 40° (Jacobo-Molina et al. 1993; Huang H. et al. 1998).

The structure of RT bound with its substrates has two states: "open" or "closure". Upon binding with template:primer, RT shows an "open" structure, which contains a deep cleft between fingers and thumb, with the polymerase active site at its base. After dNTP binding, a significant conformational change takes place: the fingers domain closes in toward the palm, so that various residues near the fingertips form part of the dNTP-binding site. In this "closed" state, the deep cleft closes down, trapping both the template strand and dNTP, with the DNA duplex binding along a groove stretching from the polymerase active site to the RNase H active site. After the reaction, the fingertips likely bend back to their open position, leading to release of pyrophosphate and allowing the enzyme to bind the next dNTP (Huang H. et al. 1998). The structure of unliganded HIV-1 RT, however, may represent another defined stage in the normal catalytic pathway, but only during initiation or reinitiation and not during processive synthesis.

1.2.4.4 Integrase (IN)

HIV-1 IN is a 32 kDa protein derived from the C-terminus of the Gag-Pol polyprotein. It has evolved to catalyze two separate reactions that are required to accomplish the integration of viral DNA into the host genome. One is a hydrolytic reaction termed "processing", resulting in the excision of GT dinucleotides from the 3'ends of the viral DNA (Bushman et al. 1990). Another is a trans-esterification reaction, referred to as "joining" or "strand transfer", enabling the processed 3' termini to be covalently joined to a host DNA target site (Engelman et al. 1991; Katz and Skalka 1994). A cell-free study also ascribed DNA-dependent DNA polymerase activity to HIV-1 IN; this might ensure that IN can affect the efficient repair of short gaps that flank the integrated proviral DNA (Acel et al. 1998).

IN contains three independently folding regions: a HHCC domain, a core domain, and a DNA-binding domain, from N- to C-terminus respectively. The HHCC domain has a Zn-binding motif composed of two histidine and two cysteine residues. This motif is essential for promoting IN multimerization, and increasing catalytic activity of the enzyme (Burke et al. 1992; Bushman et al. 1993; Ellison et al. 1995; Zheng et al. 1996; Lee et al. 1997). The core domain contains a "DD35E" motif, a triad of conserved acidic residues with stereotyped spacing in the primary sequence, which form the catalytic site of the enzyme (Engelman and Craigie 1992; Kulkosky et al. 1992; Drelich et al. 1993; Dyda et al. 1994). The C-terminal domain possesses sequence-independent DNA binding ability, which may potentially influence the binding of the target DNA into which the viral DNA integrates (Khan et al. 1991; Mumm and Grandgenett 1991; Vink et al. 1993; Woerner and Marcus-Sekura 1993). The interaction between the HHCC domain and core domain can induce a metal-dependent conformational change of IN, which, in turn, facilitates protein multimerization, forming a functional integration complex (Jones et al. 1992; Engelman et al. 1993; van Gent et al. 1993; Ellison et al. 1995; Asante-Appiah and Skalka 1997; Asante-Appiah et al. 1998; Asante-Appiah and Skalka 1999).

Nuclear import of the HIV-1 preintegration complex (PIC) is a prerequisite of integration. This active process need an array of nuclear localization signal (NLS) sequences provided by the MA, Vpr, and also IN. The NLS of IN was found to be located both in the core domain and in the C-terminal domain (Gallay et al. 1997).

1.2.5 *env* Gene Products

The HIV-1 *env* gene is located downstream from the *gag* and *pol* genes, and expresses Env polyprotein via a single-spliced mRNA. After glycosylation, the precursor polyprotein is oligomerized and then proteolytically cleaved into two subunits by a cellular convertase. The resulting surface subunit (gp120 or SU) and transmembrane subunit (gp41

or TM) remain noncovalently associated and trimerize on the surface of the virion (Chan et al. 1997; Weissenhorn et al. 1997).

The gp120 and gp41 glycoproteins have several important features in their sequences and structures. The gp120 sequence consists of five variable regions (V1-V5) interposed among more conserved regions. Variable regions V1-V4 form exposed loops anchored at their bases by intra-molecular disulfide bonds, while the more conserved regions fold into a gp120 core (Starcich et al. 1986; Leonard et al. 1990; Moore et al. 1994; Kwong et al. 1998; Wyatt et al. 1998). The gp41 has an N-terminal ectodomain containing a hydrophobic fusion peptide and two heptad repeats with a coiled coil character, a transmembrane segment, and a C-terminal cytoplasmic region. Synthetic peptides derived from the two heptad repeat regions are termed N (amino-terminal) and C (carboxy-terminal) peptides. The gp120 and gp41 are maintained in the assembled trimer by noncovalent interactions between the gp41 ectodomain and discontinuous structures composed of N- and C-terminal gp120 sequences (Helseth et al. 1991; Chan et al. 1997; Weissenhorn et al. 1997).

Envelope glycoproteins mediate the entry of HIV into cells through sequential interactions of gp120 with the CD4 and chemokine receptors on the surface of susceptible cells, followed by gp41 conformational changes that mediate fusion of the viral membrane with the target cell membrane (Chan and Kim 1998). CD4 binds in a recessed pocket on gp120. Phe43 of CD4 occupies the opening of a deep cavity in the CD4-gp120 interface, interacting with surrounding gp120 residues important for CD4 binding. In addition, Asp368 of gp120 and Arg59 of CD4 form a salt bridge; main-chain atoms on gp120 and CD4 form hydrogen bonds bridging the two proteins (Kwong et al. 1998; Wyatt and Sodroski 1998). The interaction of gp120 with the chemokine receptor is triggered by the gp120-CD4 binding (Wyatt et al. 1995; Wu et al. 1996; Trkola et al. 1996). Most of the gp120 residues involved in the chemokine receptor binding are located in the V3 loop and CD4-induced (CD4i) epitopes (Moore and Binley 1998; Rizzuto et al. 1998).

The gp120 contains most of the surface-exposed elements of the envelope glycoprotein complex, and hence presents most of the neutralization epitopes (Wyatt and Sodroski 1998). However, the conserved structures of gp120 are poorly exposed to the humoral immune system. The moieties involved in gp120-gp41 association are buried inside of the envelope glycoprotein spike (Moore and Sodroski 1996; Wyatt et al. 1997). The CD4-binding pocket is recessed in gp120, and the chemokine receptor-binding site is masked by V2 and V3 loops (Wyatt et al. 1993; Kwong et al. 1998; Rizzuto et al. 1998). Even in the relatively conserved gp120 core, the outer domain exhibits an extensively glycosylated surface, which appears as "self" to the immune system (Kwong et al. 1998; Wyatt et al. 1998). Therefore, gp120 shows poor immunogenicity and antibody reactivity, which is a challenge for vaccine developers.

1.2.6 Accessory/Regulatory Gene Products

In addition to three usual replicative genes *gag*, *pol* and *env*, the HIV-1 genome also encodes six accessory/regulatory proteins, *i.e.* Tat, Rev, Nef, Vif, Vpr, and Vpu. Each small protein acts as a molecular connector between two macromolecules to recruit cellular metabolic pathways for the purpose of efficient viral replication and *in vivo* pathogenesis (Cullen 1995, 1998; Emerman and Malim 1998).

Tat is a potent trans-activator that regulates high-level LTR-directed transcription via binding to a TAR RNA element presented at the 5'end of viral mRNA. During early transcriptional elongation, cellular RNA polymerase II (Pol II) synthesizes only short RNA products, including the TAR region, and is then stalled. Tat acts through assembly with two other cellular cofactors Cyclin T (CycT) and Cyclin T dependent kinase 9 (Cdk9) onto the bulged stem loop structure of TAR, with Tat binding to the bulge, CycT binding to the loop and bridging TAT and Cdk9. Cdk9 then phosphorylates the C-terminal domain (CTD) of Pol II, which stimulates efficient transcriptional elongation

of the nascent viral mRNA (Yang et al. 1996, 1997; Zhu et al. 1997; Wei et al. 1998; Gatignol et al. 2000).

HIV-1 transcribes only a single, genome-length primary transcript, which is exported into the cytoplasm either as an unspliced mRNA for the synthesis of Gag and Pol proteins or as a genomic RNA for virion assembly. The full-length viral RNA can also be singly spliced to produce Vif, Vpr, Vpu, and Env, or multiply/completely spliced to synthesis Tat, Rev, and Nef proteins. To avoid the retention of incompletely spliced mRNAs in the nucleus, HIV-1 employs host nucleo-cytoplasmic shuttling machinery via the viral Rev protein that binds to a viral Rev response element (RRE) within the *env* gene (Malim et al. 1989). Regulated by G protein Ran, Rev or RRE-associated Rev can bind to either import factor Importin β (Imp β) or export factor Crm1 (chromosomal region maintenance). Both factors belong to the nucleo-cytoplasmic transport protein family and mediate the shuttling of cargo proteins by direct interaction with nuclear pore component nucleoporins (Fischer et al. 1995; Fornerod et al. 1997; Henderson and Percipalle 1997; Neville et al. 1997; Stade et al. 1997; Pollard and Malim 1998).

Many distinct activities are associated with HIV-1 Nef protein. First of all, Nef can remove CD4 molecules from the cell surface by recruiting CD4 into clathrin-coated pits (CCPs), followed by internalization and transport into degradative lysosomes. In this endocytosis process, Nef may act as a connector between CD4 and the AP-2 complex, whose function in cells is to recruit transmembrane proteins to CCPs (Aiken et al. 1994; Le Gall et al. 1998; Piguet et al. 1998). A recent report revealed that Nef also connects the internalized CD4 with β-COPI in endosomes, thereby targeting CD4 to lysosomes for degradation (Piguet et al. 1999). In addition to CD4 down-regulation, Nef also induces specific down-regulation of cell surface MHC I receptors. The mechanisms involved include both the internalization of cell surface MHC I and the sorting of MHC I molecules from the trans-Golgi network (TGN) into AP-1-mediated clathrin-coated vesicles for ultimate degradation in lysosomes (Greenberg et al. 1998; Le Gall et al. 1998).

Nef can also enhance virion infectivity in a CD4-independent manner in specific cell types. By affecting the virion assembly process, viruses produced in the absence of Nef are less able to complete proviral DNA synthesis in the new round of infection, and this defect can not be complemented by Nef protein expressed *in trans* in target cells (Spina et al. 1994; Swingler et al. 1997).

Likewise, Vif can also modulate virion assembly in a manner that facilitates post-entry events, such as virion disassembly, reverse transcription, and stability and function of the reverse transcription complex (RTC)/preintegration complex (PIC) in the target cells (Dornadula et al. 2000; Ohagen and Gabuzda 2000). This biological activity of Vif can only be observed when primary T cells or "non-permissive" cell lines like H9 are used as virus producing cells (Gabuzda et al. 1992; Simon and Malim 1996). Direct interaction between Vif and the NC domain of the Gag precursor was demonstrated *in vitro* as well as in infected cells, and this interaction turned out to be a critical determinant of Vif function (Bouyac et al. 1997; Huvent et al. 1998).

Mediated by p6 protein, Vpr is incorporated into the virion nucleocapsid in molar amounts equivalent to those of the Gag protein (Paxton et al. 1993; Cohen et al. 1996). Vpr has two distinct activities. First, it can induce the arrest of infected cells in G2 phase, in which period the viral LTR is more active than in other phases of the cell cycle (Emerman 1996; Goh et al. 1998). Second, by interaction with nucleoporins and cellular import factor Importin α (Imp α), Vpr also plays an important role in mediating the import of HIV-1 PIC into the nucleus of non-dividing cells (Heinzinger et al. 1994; Vodicka et al. 1998; Popov et al. 1998).

As a unique accessory protein to HIV-1 and SIVcpz, Vpu selectively targets CD4 from the endoplasmic reticulum (ER) to proteasomes for degradation. Therefore, it prevents the simultaneously synthesized Env and CD4 from forming Env-CD4 complex in the ER. In this process, Vpu serves as a connector between CD4 and a cellular factor β-TrCP, which, in

turn, binds to the proteasome-targeting factor Skp1 (Willey et al. 1992; Margottin et al. 1998). In addition, Vpu also has a relatively non-specific function to enhance virion release, by preventing the budding of virions from the intra-cytoplasmic membrane, and by facilitating the release of budding virions from the membrane (Klimkait et al. 1990).

1.3 LIFE CYCLE OF HIV-1

An overview of major steps in HIV-1 replication is illustrated schematically in Figure 1-5.

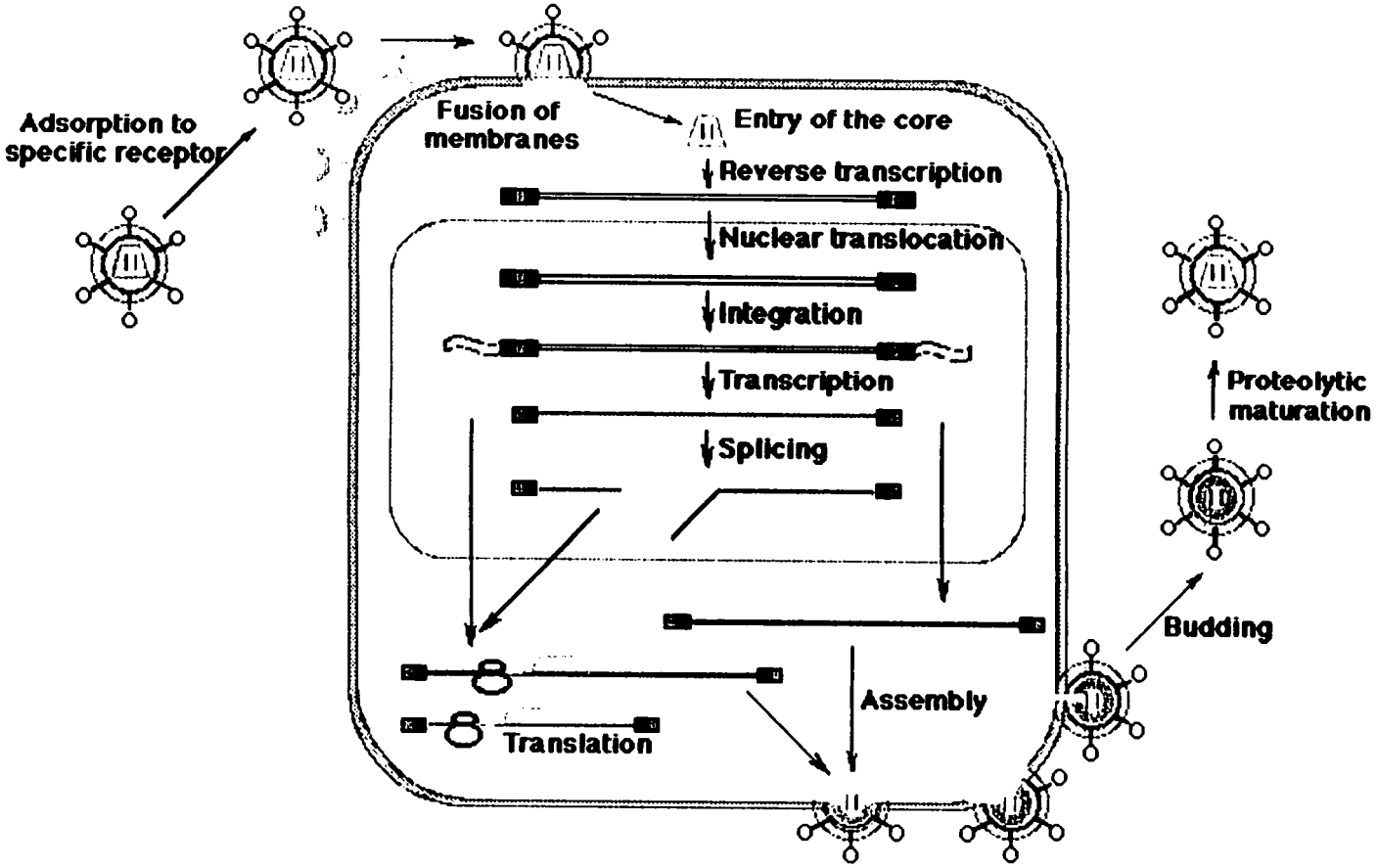
1.3.1 Viral Entry and Receptors

HIV-1 entry is the first step in the viral infection cycle, involving a series of multiple protein interactions and finally the fusion of viral and cell membranes. Through X-ray crystallography as well as extensive biochemical studies, a relatively clear understanding of this complex, multi-conformational process has been achieved.

The entry process is initiated by binding of HIV-1 exterior envelope glycoprotein gp120 to CD4, a cellular receptor expressed on a variety of cell types, including T lymphocytes, monocytes/macrophages, and other phagocytic cells. The structure of CD4 contains four extracellular immunoglobulin-like domains, a membrane-spanning region, and a charged cytoplasmic domain (Maddon et al. 1985). The gp120 glycoprotein binds to the most N-terminal extracellular domain of CD4. This binding induces many conformational changes in gp120, some of which involve the exposure of V3 loop and CD4i epitopes, which form the binding site for specific chemokine receptors (Kwong et al. 1998; Wyatt and Sodroski 1998).

For most HIV-1 isolates that are transmitted and that predominate during the early years of infection, chemokine receptor CCR5 is an obligate receptor for viral entry. Rare CCR5Δ32 homozygous individuals have been reported to be relatively resistant to HIV-1 infection (Zhang et al. 1996; Connor et al. 1997; Stewart 1998). HIV-1 isolates arising

Figure 1-5. Schematic overview of major steps in the HIV-1 replication cycle.


Adsorption to specific receptor
Fusion of membranes
Entry of the core
Reverse transcription
Nuclear translocation
Integration
Transcription
Splicing
Translation
Assembly
Budding
Proteolytic maturation

later in the course of infection use variable additional chemokine receptors, frequently CXCR4 (Feng et al. 1996; Littman 1998). The specificity of the gp120/chemokine-receptor interaction defines HIV-1 tissue tropism. The CCR5-using strains are M-tropic, and can replicate in macrophages and Th cells, but not in transformed T cell lines; while CXCR4-using viruses are T-tropic that replicate well both in transformed T cell lines and activated primary Th cells, but not in monocytes and macrophages. The differences in tropism between these two types of viruses are mapped to the V3 region of gp120 (Clapham et al. 1993).

Both CCR5 and CXCR4 are G protein-coupled receptors (GPCRs), with seven membrane-spanning segments predicted from the amino acid sequence (Premack and Schall 1996; Bieniasz and Cullen 1998). Multiple chemokine receptor regions, including both the N-terminus and the extracellular loops, contribute to the interaction with envelope glycoproteins. Coupled G proteins appears to be dispensable for viral entry; however, they may play important roles in post-entry events, and may have pathogenic consequences in uninfected cells (Davis et al. 1997; Weissman et al. 1997; Farzan et al. 1999).

Some cultured HIV-1 and HIV-2 isolates, as well as several primary SIV isolates, no longer depend on CD4 for efficient entry, and bind to chemokine receptors without prior CD4 interaction (Endres et al. 1996; Edinger et al. 1997; Dumonceaux et al. 1998). On the contrary, there is no example of infection that is independent of chemokine receptors. Therefore, it is possible that the chemokine receptors represent the primary, obligate receptors. The use of CD4 as a receptor may have evolved subsequently, allowing the chemokine receptor binding site to be sequestered from host immune surveillance (Wyatt and Sodroski 1998).

As most of the chemokine receptors are encased in the host membrane, binding with gp120 will move the gp120 bridging sheet (which connects the inner domain and outer domain of the gp120 core) close to the target membrane (Kwong et al. 1998). More importantly, the

interaction of the gp120-CD4 complex with the appropriate chemokine zreceptor is likely to trigger additional conformational changes in the HIV-1 envelope glycoprotein trimer, that leads to exposure of the gp41 fusion peptide.

Specifically, in free virions, gp41 exists in a native, nonfusogenic conformation, with the fusion peptide buried within (Chan and Kim 1998). Upon interaction of gp120 with a chemokine receptor, the conformational change in gp120 alters the gp120-gp41 interaction, which, in turn, triggers gp41 to undergo transition to a prehairpin intermediate conformation, in which the fusion peptide is exposed and subsequently inserted into a target membrane. Minutes later, the C peptide region binds to the N peptide coiled coil and adopts a helical conformation; the prehairpin intermediate then resolves to the fusion-active hairpin structure to further mediate membrane apposition. Subsequent molecular events involved in the merging of the apposing membranes remain unknown. Nevertheless, the formation of an effective "fusion-pore" within the membrane by glycoprotein/receptor oligomers seems necessary. After fusion is completed, the viral core is released into the host cell cytoplasm, while the envelope glycoproteins are left on the surface of the cell membrane.

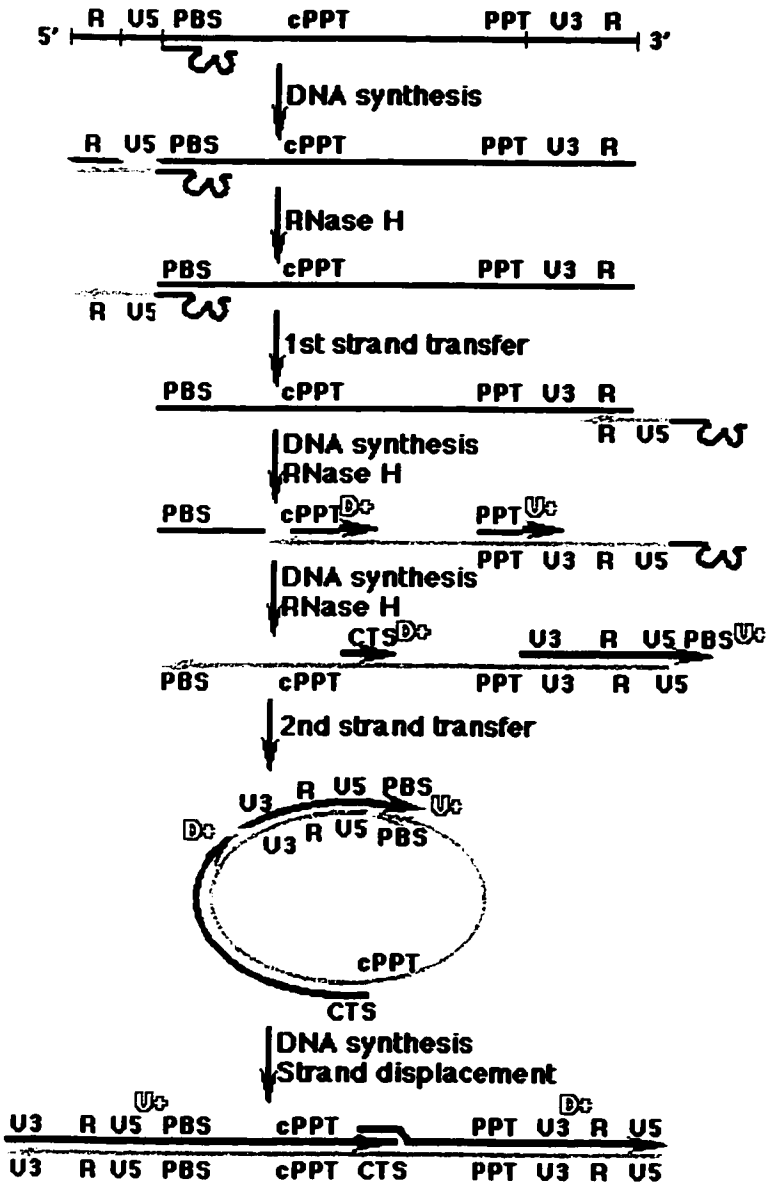
1.3.2 Reverse Transcription

After penetration into the cell, the viral RNA genome, still contained in a core complex of non-glycosylated proteins and associated with the virion reverse transcriptase, is transcribed into double-stranded proviral DNA. This reverse transcription complex (RTC) is also referred to loosely as the preintegration complex (PIC) (Fassati and Goff 2001).

1.3.2.1 Overview of the Reverse Transcription Scheme of HIV-1

The process of HIV-1 reverse transcription is believed to follow the scheme outlined in Figure 1-6 (Götte et al. 1999).

Figure 1-6. The current model of HIV-1 reverse transcription.


R U5 PBS
cPPT
PPT U3 R
5'
3'
DNA synthesis
RNase H
1st strand transfer
DNA synthesis
RNase H
DNA synthesis
RNase H
2nd strand transfer
CTS
cPPT
DNA synthesis
Strand displacement

1. Reverse transcription is initiated as the cognate tRNA primer (which is $tRNA^{Lys.3}$ in the case of HIV-1) anneals to the primer binding site (PBS) located ~200 nucleotides (nt) downstream from the 5'end of viral genomic RNA. Subsequent minus-strand DNA synthesis proceeds until the 5'end of RNA template, generating a DNA intermediate termed minus-strand strong-stop DNA or (-)ssDNA.

2. Concomitant with DNA synthesis, the RNA strand of the RNA:(-) ssDNA duplex is degraded by the RT-associated RNase H activity. In this process, the PBS region of the RNA template remains intact, because it forms an RNA/RNA homoduplex with $tRNA^{Lys.3}$, which is resistant to RNase H degradation.

3. RNase H degradation of the 5'end of the RNA releases the R region of (-)ssDNA, which is annealed to the R sequence presented at the 3'end of genomic RNA. This process is called the first strand transfer, which is an obligate step to allow continuous minus-strand DNA synthesis.

4. As minus-strand DNA synthesis proceeds, the RNA template of the RNA:DNA hybrid is again cleaved by RT-associated RNase H, leaving two purine-rich fragments that are highly resistant to RNase H degradation. These two regions are located in close proximity to the 3'end, as well as in the center of the viral RNA, and are referred to as the polypurine tract (3'PPT) and the central PPT (cPPT), respectively. Both of them serve as the primers for plus-strand DNA synthesis, and the two distinct plus-strand DNA segments thus formed are called upstream (U+) and downstream (D+) segments respectively.

5. Minus-strand DNA synthesis advances to the end of the RNA template, and forms a region at its 3' terminus that is complementary to the PBS. At this stage, the $tRNA^{Lys.3}$ primer is still attached to the 5' terminus of the minus-strand DNA. When RT encounters the tRNA sequence during U+ DNA synthesis, DNA polymerization continues until the first modified tRNA base is met. In this way, the first 18 nt of the tRNA sequence are copied to regenerate the PBS sequence at the 3'end of the U+ DNA. This discrete plus-strand DNA product is termed

plus-strand strong-stop DNA ((+)ssDNA). Specific RNase H action then removes the tRNA and PPT primers.

6. Facilitated by the complementary copies of the PBS sequence, presented on both nascent U+ and minus-strand DNAs, the second-strand transfer occurs as an essential step to continue DNA synthesis of either strand.

7. Completion of both D+ and minus-strand DNA synthesis requires RT strand displacement activity to break up the U3-R-U5 DNA duplex formed during U+ synthesis. In contrast, synthesis of the U+ segment requires the displacement of 99 nt of the D+ sequence and is then terminated when RT encounters the central termination sequence (CTS).

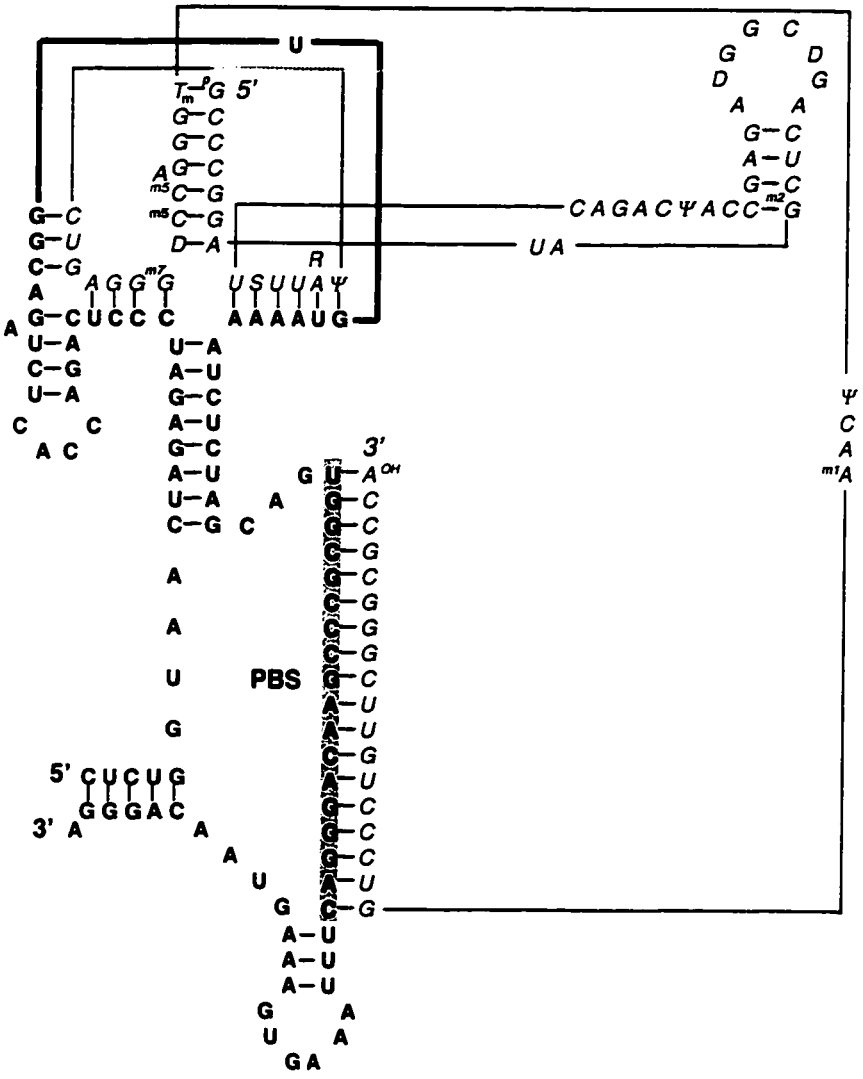
Thus, the final product of HIV-1 reverse transcription is a linear DNA molecule with a LTR at both ends, and bears a stable 99 nt long plus-strand overlap in its center, which is usually referred to as the central DNA flap.

Following completion of reverse transcription, the RTC may undergo a conformational change characterized by decapsidation, forming a mature PIC with a size compatible with translocation through nuclear pores (Zennou et al. 2000).

1.3.2.2 Initiation of Reverse Transcription

Upon placement of $tRNA^{Lys,3}$ onto the PBS sequence of HIV-1 genomic RNA (vRNA), the original highly structured stems and loops of both molecules are dissolved and replaced with a tRNA/vRNA binary complex structure which is characterized by appearance of specific intermolecular interactions. As shown in Figure 1-7, base-pairing of the PBS sequence occurs with the 3'-terminal region of $tRNA^{Lys,3}$. However, this interaction also involves also other intermolecular connections, including one between the tRNA anticodon loop and an A-rich loop (GUAAAA) located 12-17 nucleotides upstream of the PBS (Isel et al. 1993, 1995). Alterations of the PBS, as well as the A-rich loop, or other vRNA regions responsible for interactions with tRNA, result in

Figure 1-7. HIV-1 reverse transcription initiation complex. The viral RNA template sequence is in regular style; the $tRNA^{Lys,3}$ sequence is in italicized form (modified from Isel et al. 1995).


PBS
5'
3'
CAGACΨACC
UA
Ψ
C
A
m1A

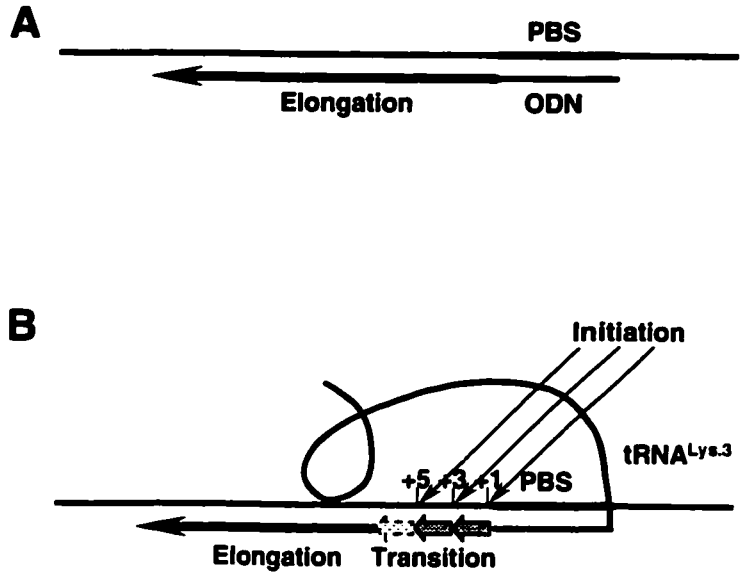
viruses with reduced infectivity; however, prolonged cell culture can lead to progressive restoration of the mutated sequences (Li et al. 1994; Das et al. 1995; Wakefield et al. 1995; Liang et al. 1997; Zhang et al. 1998a,b). When the PBS and A-rich loop are mutated simultaneously to be complementary to other tRNA species, *e.g.* $tRNA^{His}$ or $tRNA^{Met}$, the mutated HIV-1 virus can stably use an alternative tRNA as primer (Kang et al. 1997; Wakefield et al. 1996).

Since $tRNA^{Lys.3}$ involves extensive inter-molecular interactions within the tRNA/vRNA binary complex, while an 18 mer oligodeoxyribonucleotide (ODN) primer with a sequence complementary to the PBS does not, reverse transcription from $tRNA^{Lys.3}$ or from an ODN should be different. Indeed, (-) strand DNA synthesis from an ODN had little specificity, whereas a similar reaction from $tRNA^{Lys.3}$ did not, when heterologous RT was used in reverse transcription. The effect of Mn^{2+} on reverse transcription primed from these two primers is also different. Both these facts indicate that there exists a specific initiation stage of reverse transcription, which is represented by the reaction primed from $tRNA^{Lys.3}$; while reactions from ODN resemble the elongation stage which can be distinguished functionally from initiation (Isel et al. 1996). Due to its rapid dissociation rate and low nucleotide incorporation efficiency, the processivity of RT during initiation is four orders of magnitude lower than that during elongation (Lanchy et al. 1996a,b). A transition from initiation to elongation can be observed when $tRNA^{Lys.3}$ is used as primer, which is caused by dissociation of RT from the initiation complex after three or five nucleotides are incorporated; this was shown as short intermediate products, *i.e.*, +3, +5 products. Extended primer:template interactions are able to facilitate such a transition (Isel et al. 1996). Furthermore, post-transcriptional modifications of $tRNA^{Lys.3}$ were found important for efficient initiation, by favouring the formation of the specific initiation complex without affecting the polymerization rate of RT, a similar role to that played by the σ factor in transcription directed by *E. coli*. RNA Polymerase (Isel et al. 1996; Lanchy et al.

1996a,b). Based on the above studies, a model of initiation of HIV-1 reverse transcription has been proposed, as illustrated in Figure 1-8.

Several clues have also been obtained in regard to very early events during the initiation of reverse transcription. First, HIV-1 RT shows an ability to discriminate against non-self tRNA primers; this tRNA priming specificity is lost upon extension of the tRNA molecule by two nucleotides (Oude Essink et al. 1996). *In vitro* labeling of tRNA/vRNA complexes, extracted from HIV-1 virus particles, also revealed that primer $tRNA^{Lys,3}$ has already been extended by two nucleotides prior to de novo infection (Oude Essink et al. 1996; Huang et al. 1997b). Although it appears that RT does not dissociate from RNA template upon the second nucleotide addition, this two-base-extended complex undoubtedly represents an important initiation phase that may involve conformational rearrangement of RT. Another phase of initiation was evidenced by the appearance of pausing after the first nucleotide incorporation, especially when reverse transcription was performed at low dNTP concentrations, *e.g.*, 160 nM (Liang et al. 1998a). The presence of a +1 intermediate product was quite specific, and was not observed when an ODN was used to prime reverse transcription. In addition, the presence of NC protein in the reaction helped RT to escape this pausing. Both of these two facts are unique to the one-base-extended initiation complex but not to a multiply-extended complex, *e.g.*, a +3 intermediate product, implying that the +1 pausing event is a distinct stage during initiation. Although neither vRNA nor tRNA displays a significant change at the level of secondary structure upon the one base extension (Lanchy et al. 2000), RT was shown to undergo a conformational change as detected by different distances between its polymerization and RNase H sites (Götte et al. 1995). This conformational change may enable RT to adapt to a newly-appeared deoxy-ribonucleotide 3'-OH instead of a ribonucleotide 3'-OH at the 3'end of the tRNA primer, and, thus, may cause primer-specific pausing at the +1

Figure 1-8. A model of initiation of HIV-1 reverse transcription. (modified from Isel et al. 1996).


A
PBS
Elongation
ODN
B
Initiation
tRNA^Lys.3
+5
+3
+1
PBS
Elongation
Transition

position. Therefore, before RT reaches the end of initiation and dissociates from the complex at the +3 or +5 position, reverse transcription of HIV-1 might undergo at least two very early initiation phases, representing one- and two-nucleotide-incorporated tRNA/vRNA complexes respectively.

1.3.3 Nuclear Localization and Integration

1.3.3.1 Nuclear Localization

To integrate reverse transcribed viral cDNA into the host genome, retroviruses employ different strategies to make their PICs accessible to cellular DNA. For oncoviruses like RSV and MLV, viral replication is restricted to dividing cells, which enables the nuclear membrane barrier to be disrupted and host chromatin to be exposed to viral DNA during mitosis. Conversely, lentiviruses, notably HIV-1, have evolved a host nuclear transport pathway to import PICs into the nucleus via nuclear pore complexes (NPCs). This strategy allows lentiviruses to productively infect nondividing cells such as tissue macrophages (Fouchier and Malim 1999).

Nuclear translocation of the HIV-1 PIC requires its interaction with import factors through NLSs carried by PIC component proteins, including IN, MA, and Vpr. NLSs provided by IN and MA are of a classical basic-type, and interact with importin-β via the adapter importin-α in the cytoplasm (Gallay et al. 1995b, 1997; Fouchier and Malim 1999). In this process, IN-NLS plays a major role, whereas the function of MA-NLS is more controversial issue (Freed et al. 1995; Bukrinskaya et al. 1996; Dupont et al. 1999). Through direct interaction of importin-β with NPC nucleoporins, PIC is translocated into the nucleus, where the import factors are dissociated from PIC upon the binding of RanGTP to importin-β. This cycle is finally completed with the import factors being exported to the cytoplasm and the subsequent Ran dissociating in its GDP-bound form. Vpr also harbors NLS and augments the efficiency of PIC nuclear import. However, the

Vpr-NLS accesses the importin-α/β pathway in a non-conventional manner. By interacting with importin-α at a different binding site, Vpr-NLS could direct importin-α to the NPC in a importin-β-independent process, via the direct interaction of Vpr with nucleoporins (Popov et al. 1996, 1998; Fouchier et al. 1998; Vodicka et al. 1998).

In addition, the central flap of viral DNA formed at the final stage of reverse transcription is also a necessary element for the nuclear import of HIV-1 DNA. Flap-defective linear DNA molecules were found to associate with the nuclear membrane. The flap could act as a viral determinant for the initiation of the crawling of viral DNA filaments through nuclear pores, a situation similar to the nuclear export of mRNA through the pore guided by its 5' cap structure (Zennou et al. 2000).

1.3.3.2 Integration

The integration of proviral DNA into the host genome is mediated by viral IN in the context of the PIC. The composition of the PIC varies significantly, depending on different isolation conditions. The virion protein most stably associated with HIV-1 PIC appears to be IN itself (Farnet and Haseltine 1991). RT, MA and Vpr are also readily detected under a number of conditions, whereas NC, low amounts of CA, and PR are detected only under more limited circumstances (Bukrinsky et al. 1993; Karageorgos et al. 1993; Heinzinger et al. 1994; Gallay et al. 1995, 1997; Miller et al. 1997). A number of cellular proteins, including importin-α, HMG I (Y), and histones, have also been found associated with the PIC (Karageorgos et al. 1993; Gallay et al. 1996, 1997; Farnet and Bushman 1997).

Soon after completion of HIV-1 reverse transcription, usually while still in the cytoplasm, IN cleaves viral DNA endonucleolytically, eliminating the terminal two bases from each 3'end. Upon nuclear import and binding of the PIC with host DNA, IN catalyzes a concerted one-step cleavage-ligation reaction in which the 3'-OH groups at the viral DNA

ends are used to attack phosphodiester bonds on opposite strands of the target DNA. The reacting sites on the host DNA are staggered by five bases in the 5′ direction, and are on the same face of the double helix, separated by the major groove. Finally, DNA synthesis, possibly guided by viral IN, extends from the host DNA 3′-OH groups, filling in the gaps that flank the viral DNA, displacing the mismatched viral 5′ends, and joining the new 5′ends of viral DNA to the host DNA (Katz and Skalka 1994; Brown 1997; Acel et al. 1998). After integration, the provirus of HIV-1 gains the status of a cellular gene. It is replicated along with host cell DNA and is expressed by utilizing host gene expression machinery.

1.3.4 Expression of the Viral Genome

Like other retroviruses, HIV-1 contains a large array of *cis*-acting elements in its proviral DNA intermediate that are characteristic of cellular sequences, and thus employs host cell RNA pol II to produce viral mRNA transcripts. Most of these *cis*-acting elements, including the promoter and multiple enhancer sequences, lie in the U3 region of the 5′end LTR of the proviral DNA. With their help, transcription initiation from the LTR promoter is well controlled in different cell types or at different times, which likely determines whether a provirus is quiescent or actively replicating (Nabel and Verma 1993; Siebenlist et al. 1994; Miyamoto and Verma 1995). As a complex retrovirus, HIV-1 also encodes a *trans*-acting viral protein, Tat, which serves as an activator of LTR-directed transcription through binding with the 5′ TAR element of the nascent viral RNA. CycT also binds with the TAR RNA element, bridging Tat with Cdk9, which, in turn, phosphorylates the CTD of Pol II to stimulate the efficient transcriptional elongation of nascent viral RNA (Yang et al. 1996, 1997; Zhu et al. 1997; Wei et al. 1998).

HIV-1 generates only a single genome-length primary transcript that is subject to the same processing events as cellular RNAs,

including cap addition at the 5'end, and cleavage and polyadenylation at the 3'end. This full-length mRNA has two functions: it serves as the translation template for the Gag and Gag-Pol polyproteins, and it is packaged into progeny virion particles as genomic RNA. A fraction of the primary transcripts are spliced to form subgenomic-sized RNA molecules: some of these are singly-spliced mRNAs encoding Vif, Vpr, Vpu, and Env, while others are multiply/completely spliced mRNAs encoding Tat, Rev and Nef. Both unspliced and singly-spliced mRNAs are transported into the cytoplasm through binding of the viral protein Rev to the RRE sequence located within the *env* gene (Malim et al. 1989). The G protein Ran-coupled cellular nucleo-cytoplasmic shuttling machinery is involved in Rev-mediated viral mRNA exportation in HIV-1 (Emerman and Malim 1998).

Synthesis of the Gag and Gag-Pol polyproteins takes place on free ribosomes in the cytosolic spaces of the cell. Since HIV-1 mRNA is capped at its 5'end, it is generally believed that the typical ribosome scanning mechanism would be used for the initiation of viral protein translation at the first AUG encountered by the ribosome in a favorable context: (A/G)CCAUGG. Recently, evidence has been found for an internal ribosome entry site (IRES) within the RNA of SIV, suggesting that ribosomes may be able to avoid the extensive secondary structure of the leader sequence upstream of the *gag* gene (Ohlmann et al. 2000). Because of sequence similarity between SIV and HIV, the existence of an IRES in HIV-1 is also expected. The biological relevance of IRES in HIV-1 may rely on the high-level LTR-directed gene expression in the G2 phase, during which cap-dependent translation is diminished (Buck et al. 2000).

Two important modifications take place on the Gag protein, concurrent with its translation. One is myristylation at the N-terminal glycine, a residue located at the second position from the N-terminus that is exposed after removal of the initiator methionine (Henderson et al. 1983; Rein et al. 1986; Göttlinger et al. 1989). The result is the addition of myristate, a rare 14-carbon fatty acid, which is required

for the binding of Gag to the plasma membrane. Another modification is the phosphorylation of tyrosine and serine on the MA domain of Gag, that is associated with the nuclear transport of PICs (von Schwedler et al. 1994; Gallay et al. 1995a,b, 1996; Bukrinskaya et al. 1996).

The Gag-Pol fusion protein is transcribed from the same RNA species as that of Gag. Some retroviruses, *e.g.*, mammalian type-C retroviruses, can occasionally misread the *gag* termination codon as a sense codon in order to bypass it (Felsenstein and Goff 1992). However, for most retroviruses, including HIV-1, translation from the *gag* initiation codon undergoes a -1 ribosomal frameshifting from the *gag* reading frame to an overlapping portion of that of *pol*. In the case of HIV-1, the frameshift site is at the beginning of the p1 sequence, and the frequency of the shift is about 5%. Therefore, the molar ratio of the Gag vs Gag-Pol in HIV-1 virions is about 20:1 (Dickson et al. 1984; Jacks et al. 1988; Wilson et al. 1988).

Env is synthesized from a singly-spliced mRNA by employing the cellular synthesis machinery used for the surface and secreted proteins. Translation is initiated on free ribosomes. With the help of a signal recognition peptide (SRP), that is synthesized thereafter, the N-terminus of peptide is targeted to the membrane-bound ribosomes to continue the synthesis. After being released into the lumen of the rough endoplasmic reticulum (RER), nascent Env protein undergoes two important modifications in the fashion of a typical cellular glycoprotein: removal of the N-terminus leader sequence by a cellular protease and glycosylation followed by a series of modifications with sugars. As the ribosomes approach the end of the *env* mRNA, the hydrophobic membrane anchor is synthesized to prevent the Env protein from being fully released into the ER. Since Env and CD4 are simultaneously synthesized and may interact prematurely within the ER, HIV-1 also encodes a unique accessory protein, Vpu, which can selectively target CD4 from the ER to proteasomes for degradation. Env trimerization also occurs in the ER, then, the complex is transport to the Golgi apparatus, where the SU and TM sequences are separated by the action of a host-encoded protease (Swanstrom and Wills 1997).

1.3.5 Assembly and Budding

After the synthesis of HIV-1 viral polyproteins and genomic RNA, they are transported to the plasma membrane where viral assembly and budding occur. The Gag and Gag-Pol polyproteins are targeted to the cytoplasmic face of the cell membrane, whereas the Env glycoproteins are transported to the external surface of the cell via the secretary pathway and then move laterally to the budding site (Kräusslich and Welker 1996).

1.3.5.1 Principles of Viral Assembly and Budding

HIV-1 Gag protein is the main structural component of virions, and synthesis of Gag protein alone is sufficient to establish the normal assembly pathway and to form and release virus-like particles (VLP), the analogue of authentic immature retroviral particles (Gheysen et al. 1989; Craven and Parent 1996; Swanstrom and Wills 1997).

Three types of interactions are involved in Gag assembly, *i.e.*, membrane targeting and binding, Gag-Gag interaction, and functions needed at the late stage of budding, including envelopment and efficient release of the particle. The relevant functional elements are mapped exclusively within the Gag protein, designated as M domain, I domain, and L domain respectively. In the case of HIV-1, they are MA, NC, and p6. Interactions between individual Gag molecules may occur before their targeting to the plasma membrane, and may involve the formation of a detergent-resistant complex (DRC). After Gag myristylation, a late assembly intermediate complex that is detergent sensitive (DSC) can also be identified, which represents the products of extensive proteolytic processing of polyprotein precursors (Lee et al. 1998, 1999).

In addition to the protein compartment, RNA, but not necessarily viral genomic RNA, is also an essential component for Gag assembly, helping the formation of the core with proper morphology (Clavel and Orenstein 1990; Zhang and Barklis 1997). *In vitro* assembly of virion

cores from recombinant proteins depends on the presence of nonspecific nucleic acid, whereas RNase treatment disrupts these core structures and Gag-Gag interactions (Campbell and Vogt 1995; Gross et al. 1997; Burniston et al. 1999; Campbell and Rein 1999; Ganser et al. 1999). These studies support an assembly model, in which the RNA acts as the scaffold for the formation of highly regular protein arrays, and Gag polymerization and virion assembly are promoted by nonspecific interactions between NC and RNA (de Rocquigny et al. 1992; Bennett et al. 1993; Fuller et al. 1997; Bowzard et al. 1998; Dawson and Yu 1998; Cimarelli et al. 2000).

As the "particle-making machine", Gag also has the ability to incorporate other essential viral proteins including Gag-Pol and Env, and two copies of genome RNA as well, into virions. The protein component of Gag required for the Gag-Pol precursor incorporation is mapped to the CA domain (Smith et al. 1993; Srinivasakumar et al. 1995; Huang and Martin 1997). The specific interaction between the MA domain of Gag and the cytoplasmic domain of TM subunit is involved in the incorporation of Env protein (Hill et al. 1996; Ono et al. 1997; Dupont et al. 1999). The *trans*-acting element needed for specific packaging of viral RNA is also located within Gag, as discussed below.

1.3.5.2 Genomic RNA Packaging and Dimerization

As an essential step in the retroviral life cycle, viral RNA encapsidation of HIV-1 is a highly specific process, resulting in the selective packaging of unspliced viral mRNA into virions from a high background of cellular mRNAs and subgenomic viral RNAs. The specificity of viral genomic RNA packaging relies on two components: (1) the *cis*-acting RNA packaging elements in the 5′ leader sequence, including Ψ (PSI) or the E (encapsidation) signal; and (2) the *trans*-acting factors, namely, the NC domain of Gag polyprotein, which specifically interacts with the *cis*-acting elements and plays a critical role in directing viral RNA encapsidation.

The *cis*-acting sequences required for HIV-1 RNA encapsidation are multipartite, and are composed of discrete functional hairpin structures. Initial studies indicated that the region between the major splice donor (SD) and the start of *gag* were important for encapsidation (Lever et al. 1989; Aldovini and Young 1990; Clavel and Orenstein 1990). Such a placement of the encapsidation signal would provide the virus with the means to specifically recognize and preferentially encapsidate genomic viral RNA over spliced RNA species. Subsequent analyses revealed that the proximal sequences, which include an upstream region containing the dimerization initiation site (DIS) and the 5'end of the *gag* gene, also play a critical role in encapsidation (Kim et al. 1994; Luban and Goff 1994; McBride et al. 1996, 1997; Paillart et al. 1996; Harrison et al. 1998).

Through biochemical and enzymatic probing, as well as free-energy minimization algorithms, various RNA secondary-structure models of the HIV-1 leader sequence were established (Clever et al. 1995; Berkhout 1996; Damgaard et al. 1998). Despite some relatively minor differences, the exon-1 leader sequence consists of four distinct stem-loop RNA motifs flanking SD, termed SL1 through SL4. Among them, SL1 contains the DIS and is the only motif located upstream of the SD, while SL3 forms the so-called Ψ or E signal. Both SL1 and SL3 have been shown to be important for Gag protein binding *in vitro*, and the recruitment of viral RNA into virions (Clever et al. 1995; McBride et al. 1996, 1997; Damgaard et al. 1998; Harrison et al. 1998). In addition, there are three other hairpin structures in the R-U5 region, namely TAR, Poly(A), and the PBS; alteration of each of these structures could result in decreased viral RNA packaging (Rizvi and Panganiban 1993; Berkhout 1996; Das et al. 1997; Damgaard et al. 1998; Helga-Maria et al. 1999; Liang et al. 2000). However, their effects on encapsidation might be indirect: they may facilitate viral RNA incorporation through stabilizing and presenting the proper structures of SL1 and SL3.

Recognition of the HIV-1 packaging signal is largely carried out by the NC domain of the Gag polyprotein (Aldovini and Young 1990;

Gorelick et al. 1990; Jowett et al. 1992; Clever et al. 1995). The interaction of NC with its *cis*-acting packaging signal is highly specific. Exchange of the NC domain between HIV-1 and M-MuLV resulted in altered packaging specificity (Berkowitz et al. 1995; Zhang and Barklis 1995). *In vitro* experiments showed that the binding specificity relies on intact Zn finger motifs, especially the N-terminal one (Berkowitz et al. 1993, 1994; Damnull et al. 1994). Indeed, by switching of the Zn finger positions, it was found that the first Zn finger plays a more prominent role in RNA selection and packaging (Gorelick et al. 1993). Charged amino acid residues are also necessary for efficient HIV-1 RNA packaging, though they were generally believed to possess non-specific nucleic acid binding activity in this regard (Ottmann et al. 1995; Poon et al. 1996).

A three-dimensional structure of the HIV-1 NC protein bound to SL3, bas been determined by NMR spectroscopy, which provides further evidence in regard to specific interactions between SL3 and NC protein. In this model, high-affinity binding is mediated by specific interactions between the two Zn fingers of NC protein and the G^7 and G^9 nucleotides of the GGAG RNA tetra-loop. N-terminal residues of NC protein were also found to form a helix that binds to the major groove of the RNA stem (De Guzman et al. 1998).

Host and viral proteins, other that NC, have also been implicated in specific RNA packaging. For example, exchange of the NC domain between HIV-1 and MMTV did not change the specificity of RNA packaged into virions, indicating that HIV-1 NC may not be the exclusive determinant of RNA selectivity (Poon et al. 1998). Interestingly, the inclusion of the HIV-1 p2 domain, in addition to NC, in the context of HIV-2, significantly enhanced HIV-1 vector RNA encapsidation, suggesting that residues within p2 may contribute to specific recognition of the HIV-1 packaging signal (Kaye and Lever 1998). Consistent with this notion, compensatory mutations of DIS mutations were also found within Gag polyprotein, including the p2 and NC domains (Liang et al. 1999a,b). Furthermore, human Staufen (hStau), a double-

stranded RNA-binding protein important in mRNA transport, was found to be associated with viral genomic RNA, and is incorporated into HIV-1 virions. Over-expression of hStau enhances its virion incorporation level, as well as encapsidation of HIV-1 genomic RNA, indicating that it may participate in retroviral genome selection and packaging as well (Mouland et al. 2000).

Encapsidated full-length retroviral RNAs are found in homodimeric form in virus particles. A "kissing-loop" dimerization model has been proposed on the basis of *in vitro* studies (Laughrea and Jette 1994; Skripkin et al. 1994; Clever et al. 1996; Mujeeb et al. 1998). In this model, the dimerization initiation site (DIS) is mapped to the six-base palindromic sequence in the loop region of SL1. Dimerization is initiated by base-pairing of the DIS loops between two RNA molecules, and then the two DIS stems are melted and reannealed to form a stable intermolecular duplex. The overlapping of the packaging and dimerization signals suggests that these two processes are likely to be intricately associated. However, more convincing evidence is still needed to clarify the nature of dimer formation in virions, as well as the relationship between encapsidation and dimerization.

1.3.5.3 Primer tRNA Incorporation and Placement

Before the primer tRNA is placed onto the complementary PBS sequence in the viral genome, it undergoes an enrichment process during viral assembly to form a free tRNA pool in virions that is distinct from that of host cells. In the case of HIV-1, the virion tRNA subset includes $tRNA^{Lys.3}$ and $tRNA^{Lys.1,2}$, which are incorporated into virus particles with equal efficiency (Jiang et al. 1993). Among them, the cognate primer tRNA which is placed onto the PBS sequence of the genome is $tRNA^{Lys.3}$ (Ratner et al. 1985; Jiang et al. 1993). Surprisingly, the tRNAs are not directed into the virion as a result of being bound to viral RNA, since the absence of PBS in the virus genome does not affect tRNA incorporation (Jiang et al. 1993). On the other hand, HIV-1 RT is shown to be able to discriminate against non-self-tRNA primers *in vitro*,

suggesting that the RT domain of Gag-Pol may be involved in the selective encapsidation of tRNA (Li et al. 1994; Oude Essink et al. 1996). This hypothesis was confirmed by *in vivo* experiments, and the processing of Gag-Pol was shown to be unnecessary for tRNA packaging (Mak et al. 1994, 1997). In addition to RT, the NC domain within the Gag protein was also found to participate in this process by facilitating Gag-Pol incorporation, and by direct binding to $tRNA^{Lys,3}$ (Huang et al. 1997a).

HIV-1 NC protein is able to promote extensive and rapid annealing of primer tRNA to the PBS *in vitro* (de Rocquigny et al. 1992). The motif of NC essential for this activity is mapped to the basic amino acid residues flanking the first Zn finger, and not to the Zn finger structure itself (de Rocquigny et al. 1992; Huang et al. 1998). Consistently, the NC domain of Gag protein was reported to play a major role in $tRNA^{Lys,3}$ placement onto the genome (Feng et al. 1999; Cen et al. 1999, 2000). Again, processing of the polyprotein precursor is not a prerequisite for tRNA placement, although fully-processed NC protein was reported to be able to help increase priming efficiency of de novo reverse transcription (Huang et al. 1997a; Cen et al. 2000).

A conflicting notion was raised in regard to the relationship between primer tRNA incorporation and placement. In HIV-1, alterations of the PBS and the U5-stem-A-rich loop were made at the same time, so that they were complementary in respect to the 3'-terminal nucleotides and anticodon loop of other tRNA species, *e.g.*, $tRNA^{His}$ or $tRNA^{Met}$. These mutations resulted in the stable usage of other tRNAs as primer, without evidence of an increase in their relative concentration in the virion (Wakefield et al. 1995; Kang et al. 1997). However, if selective packaging of primer tRNA is not required for placement, as indicated by the above experiments, other biological evidence concerning tRNA selective enrichment is needed to elaborate this process, which is observed in most retroviral and retro-transposon systems (Mak and Kleiman 1997).

1.3.5.4 Maturation

Newly released viral particles are immature, and they must undergo proteolysis associated internal structure reorganization, which is characterized by the condensation of the inner core and capsid shell, to become mature, infectious viruses (Kaplan et al. 1993; Vogt 1996). Maturation is initiated when virally encoded PRs are activated by dimerization of Gag-Pol polyproteins during assembly, and then followed by an ordered pathway of sequential cleavages, which is controlled by the rate of proteolysis at individual sites (Debouck et al. 1987; Kaplan et al. 1994; Wiegers et al. 1998). For example, the p2-NC cleavage site is processed approximately 10-fold faster than other sites within the Gag polyprotein. Therefore, the initial cleavage of Gag happens at the C-terminus of p2, resulting in release of the RNA-binding NC protein and condensation of the ribonucleoprotein (RNP) core. Subsequently, CA is separated from the membrane by cleavage between the MA and CA, followed by formation of a new CA-CA interface at the N-terminus of CA. Cleavage between CA and p2 appears to be the last event in the proteolytic cascade, which liberates the C-terminus of CA and triggers capsid condensation for maturation (Pettit et al. 1994; Kräusslich et al. 1995; Gitti et al. 1996; Fuller et al. 1997; Accola et al. 1998; Wiegers et al. 1998; Gross et al. 2000).

The genomic RNA itself also undergoes a dramatic change in structure as a consequence of maturation. In newly-budded virus particles, genomic RNAs occur as a variable mixture of monomers and dimers, and even that fraction of dimeric RNAs are held together more loosely, as evidenced by slower mobility in electrophoresis and lower thermal stability (Fu et al. 1993, 1994). In contrast, in mature virions, genomic RNAs are tightly packed dimers that move faster in non-denaturing agarose gels and dissociate into monomers at a higher temperature. The maturation of genomic RNA was found to be dependent on the action of the viral PR, and the NC domain within the Gag polyprotein may participate in this process (Fu et al. 1993, 1994; Feng et al. 1996; Takahashi et al. 2000). In addition, maintenance of the Gag/Gag-Pol ratio was also found important for the efficiency and stability of RNA dimers (Shehu-Xhilaga et al.

2001). Interestingly, in PR$^-$ HIV-1 virus, tRNA placement was found to be incomplete, and the priming efficiency from the primer was also lower than that of wild-type virus. This indicates that a PR-mediated conformational alteration, in genomic RNA, $tRNA^{Lys,3}$, or both, occurs simultaneously with virus maturation (Liang et al. 1997; Cen et al. 2000).

1.4 HIV-1 NUCLEOCAPSID PROTEIN: A POTENTIAL TARGET OF ANTIRETROVIRAL THERAPY

1.4.1 HIV-1 NC Functions and Activities

The NC proteins of all retroviruses are small, highly basic proteins, containing one or two $CX_2CX_4HX_4C$ Zn finger-like motifs; exceptions are spumaviruses (Maurer et al. 1988). Deletions or substitutions of the Zn finger motif of the HIV-1 NC protein result in the absence of viral RNA in virions or alterations of the specificity of RNA packaging; this indicates that this motif of NC specifically recognizes and interacts with the packaging sequences and thus plays a critical role in viral genomic RNA packaging (Aldovini and Young 1990; Berkowitz et al. 1995; Zhang and Barklis 1995). On the other hand, non-specific binding of Gag to RNA via NC basic residues could drive Gag accumulation and create a favorable environment for the formation of protein-protein contacts among CA and possibly MA domains; this could foster virion assembly (Zhang and Barklis 1997; Bowzard et al. 1998; Dawson and Yu 1998; Cimarelli et al. 2000). Many NC mutants have been found to be noninfectious, though they were not significantly impaired in either genomic RNA packaging or in virion assembly; this implies that other essential functions of NC are involved, probably during the production of proviral DNA (Déméné et al. 1994; Berkowitz et al. 1996; Tanchou et al. 1998; Gorelick et al. 1999).

Presumably, most NC biological functions are mediated through its nucleic acid binding activity. The presence of basic residues is critical in this regard (Darlix et al. 1995; Schmalzbauer et al. 1996; Urbaneja et al. 1999). Mutation of seven of the nine basic amino acid

residues will reduce the binding of RNA by 50- to 90-fold. Direct electrostatic interactions between RNA and four basic residues of HIV-1 NC protein, *i.e.* Arg7, Lys20, Lys26 and Lys41, were also observed in a NC-poly(εA) complex. In contrast, though dispensable for the RNA-binding activity of NC, the intact Zn finger structures are essential for the recognition of specific viral RNA motifs during viral genome packaging (Berkowitz et al. 1993, 1994; Dannull et al. 1994; Allen et al. 1996; Berglund et al. 1997; Fisher et al. 1998). A NMR structure of the HIV-1 NC protein bound to the SL3 packaging sequence revealed that both Zn fingers were engaged in highly specific interactions with purine bases of the RNA loop, whereas the four N-terminal conserved basic residues only participated in non-specific, electrostatic interactions with RNA stem phospho-diester groups (De Guzman et al. 1998).

The most unusual biochemical property of HIV-1 NC protein is its RNA-chaperone activity; it facilitates RNA folding into an optimally base-paired structure, by preventing misfolding or by resolving species that are kinetically trapped in alternative conformations (Herschlag 1995; Rein et al. 1998). The minimum amount of NC required to perform this function is the saturation concentration, *i.e.*, 1 protein molecule per ~7 nucleotides. Again, the highly basic character of NC was shown to be essential in this process, whereas the Zn finger structures may provide specificity for the interaction between NC and nucleic acid (Rein et al. 1998).

Using recombinant or synthetic HIV-1 NC protein, a series of *in vitro* systems have been developed, in which NC was proved to function as an RNA-chaperone protein in various steps during retroviral replication. For example, during viral RNA dimerization and the subsequent maturation of the dimer, NC triggers the formation of a kissing complex between the DIS loops of two RNA molecules and then converts the complex into a more stable mature form (Muriaux et al. 1995; Feng et al. 1996, 1999). NC protein can also promote rapid annealing of primer tRNA to the PBS of the viral genome. The resulting

tRNA:RNA complex involves extensive intermolecular interactions, and shows a special pausing pattern in the subsequent reverse transcription initiation process (Prats et al. 1988; Barat et al. 1989; Lapadat-Tapolsky et al. 1995; Huang et al. 1998; Liang et al. 1998a; Rong et al. 1998). During reverse transcription, overall RT processivity can be increased by NC, via bridging of additional RT/RNA interactions to stabilize RT on the RNA template, or through alteration of template secondary structures as an RNA-chaperone protein (Rodríguez-Rodríguez et al. 1995; Tanchou et al. 1995; Ji et al. 1996; Wu et al. 1996; Druillennec et al. 1999; Lener et al. 1999). HIV-1 NC protein is also involved in minus-DNA strand transfer, by drastically reducing self-priming and catalyzing the annealing of the nascent intermediate DNA to acceptor RNA (Peliska et al. 1994; You and McHenry 1994; Guo et al. 1997); an alternative mechanism of NC in this process is via stimulating synthesis-independent RNase H activity to dissociate RNA hydrolysis products from (-)ssDNA (Peliska et al. 1994; Cameron et al. 1997). During plus DNA strand transfer, NC protein was reported to destabilize the tRNA-DNA hybrid for efficient RNase H function, to destabilize an internal (-)PBS DNA hairpin, and to stimulate annealing of the PBS sequence in (+)ssDNA to the acceptor DNA template (Wu et al. 1999; Johnson et al. 2000).

In summary, HIV-1 NC is a highly conserved protein and functions at various steps during the viral life cycle; this makes it an attractive target for the design of anti-HIV compounds. Since the CCHC type of Zn finger motif is critical for NC function and is relatively rare among cellular proteins (Berg and Shi 1996), it might be possible to develop compounds that specifically target NC Zn fingers without blocking the function of cellular proteins.

1.4.2 Compounds That Specifically Target HIV-1 NC

One of the greatest difficulties in the pharmacological intervention of HIV-1 infection and AIDS is the rapid selection of drug resistance due

to continuous viral replication and the huge viral genetic variability acquired through nucleotide misincorporation and recombination during reverse transcription. To date, the development of chemo-therapeutic agents has focused primarily on RT and PR. Regimens targeted at these enzymes are effective at reducing viral load as well as morbidity and mortality of the patients within one year. However, the effectiveness of long-term therapy is abrogated by the appearance and spread of resistance. To overcome this obstacle, new anti-retroviral agents are required, and HIV-1 NC protein has emerged as a potential novel target for antiviral therapy.

Using mass screening method, actinomycin D (Act D), a well-characterized anti-cancer antibiotic drug, was identified as a potent inhibitor of HIV-1 strand transfer (Gabbara and Peliska 1996). Further studies showed that this ssDNA-binding compound inhibits the annealing step in HIV-1 minus-strand transfer *in vitro*, while NC protein is unable to overcome this effect (Davis et al. 1998; Guo et al. 1998). Therefore, this activity interferes with NC-catalyzed events without interacting with the protein itself.

Many hydrophobic and mild oxidizing agents, including C-nitrosos, disulfides, disulfoxides and thiurams, have also been identified as able to interact with retroviral Zn fingers directly, causing Zn^{2+} to be ejected and NC function to be destroyed. This inactivation is irreversible, due to the formation of inter- and intra-molecular disulfide crosslinks between Zn-finger Cys residues following Zn^{2+} ejection (Rice et al. 1995, 1997; Tummino et al. 1996, 1997; Turpin et al. 1996, 1999; Domagala et al. 1997a,b; McDonnell et al. 1997; Prasad et al. 1998; Berthoux et al. 1999). Several compounds that can selectively target the retroviral NC Zn fingers without affecting cellular Zn finger proteins have also been reported (Huang M. et al. 1998; Basrur et al. 2000). These oxidizing NC inhibitors are directly virucidal and demonstrate broad anti-retroviral activity, including inhibiting interaction with HIV-1 Ψ RNA, preventing Gag precursor processing, and blocking initiation of reverse transcription. Because

of the highly conserved nature of the CCHC motif in retroviral NC proteins, and the fact that mutations in this motif are lethal for the virus, the possibility for the virus to be able to generate resistant mutants to these NC inhibitors is low. Indeed, drug-resistant isolates were not detected after more than two years in culture with one of the typical agents, and two compounds have already been introduced to Phase I/II clinical trials for the treatment of AIDS presently (Vandevelde et al. 1996; Frank-D et al. 2001).

1.4.3 Rationale and Objectives of the Research

We have summarized the multiple roles of HIV-1 NC protein in viral replication. The Zn finger motifs are indispensable for some NC functions (*e.g.* specific viral RNA encapsidation) while dispensable for other functions (*e.g.* $tRNA^{Lys,3}$ placement). To develop compounds that specifically attack the Zn fingers of NC protein, further understanding of the involvement of these motifs in various specific NC activities is necessary.

This thesis has two major objectives: (1) functions of NC and its Zn finger motifs in initiation and elongation of reverse transcription; (2) roles of NC in viral genomic RNA packaging. To pursue the first goal, we developed an *in vitro* reverse transcription system, in which synthetic NCp7 was employed to place natural $tRNA^{Lys,3}$ onto an HIV-1 RNA template that includes the PBS and flanking sequences. Low dNTP concentrations in our RT system also enabled us to detect very early pause sites, *e.g.* +1 and +3, in initiation of reverse transcription. Our experiments demonstrate that intact Zn fingers are crucial elements in the HIV-1 NC protein to promote the formation of an active tRNA:vRNA complex that is favored in initiation as well as the subsequent transition from initiation to elongation during the synthesis of (-) strand DNA.

NC protein is also known to participate in specific viral RNA packaging through tight binding of its Zn finger motifs with the SL1

and SL3 packaging signals. As a second objective of this thesis, we deleted the SL1 and SL3 sequences respectively, and identified and studied compensatory mutations in the Gag protein (including within the p2 and NC motifs). We found that hydrophobic amino acids in the HIV-1 p2 and NC proteins can contribute to the rescue of deleted viral RNA packaging signals. Furthermore, compensation of deletions in either SL1 or SL3 involves different second-site mutations in p2 and NC. This suggests that RNA elements up- or down-stream of the SD interact with the Gag protein in different fashion during viral RNA packaging.

Chapter 2

Roles of the Human Immunodeficiency Virus Type 1 (HIV-1) Nucleocapsid Protein in Annealing and Initiation vs Elongation in Reverse Transcription of Viral (-) Strand Strong-Stop DNA

This chapter was adapted from an article that appeared in the Journal of Virology (1998), Vol.72, pp. 9353-9358. The authors of this paper were Rong, L., C. Liang, M. Hsu, L. Kleiman, P. Petitjean, H. de Rocquigny, B. P. Roques, and M. A. Wainberg. All data presented in this chapter were from experiments performed by myself under the supervision of Dr. Wainberg. Dr. B. P. Roques provided synthetic HIV-1 NC protein. Dr. C. Liang provided assistance in the planning of the experiments and analysis of results.

2.1 Preface

The nucleocapsid protein (NC) of HIV-1 is a small basic protein that can bind to single-stranded nucleic acid. It has two Zn finger motifs, each of which contains Zn ion binding residues, *i.e.* CCHC, and forms a tight, rigid loop within the protein. NC protein exhibits nucleic acid chaperone activity *in vitro*: it catalyzes the rearrangement of nucleic acid molecules into the most thermodynamically stable conformation. With this activity, NC participates in reverse transcription at many steps and thereby enables RT to accomplish highly specific and efficient viral cDNA synthesis. For example, it stimulates the annealing of the tRNA primer to the primer binding site (PBS); it greatly reduces pausing during reverse transcription by transiently eliminating secondary structures in template RNA, at which the RT stalls. Mutagenesis studies have shown that the highly basic residues of NC protein, and not the Zn finger motifs, are essential for this activity. In our cell-free reverse transcription assay, synthetic NC was employed to perform tRNA placement, and was then removed by Proteinase K digestion and phenol:chloroform extraction. We found that a NC-annealed tRNA:template complex showed elevated elongation efficiency as compared with one formed by heat-annealing, even when NC was no longer present in the reverse transcription reaction. This is the first study to show that the manner in which tRNA is annealed to template RNA can affect the efficiency of subsequent reverse transcription. In addition, Zn fingers were shown to be essential in this regard, which provides clues toward understanding the molecular mechanisms that underlie the nucleic acid chaperone activity of the NC protein.

2.2 Abstract

HIV-1 nucleocapsid protein (NCp7) is a major structural protein found within the virion nucleocapsid where it is tightly associated with genomic RNA and the $tRNA^{Lys,3}$ primer. One of the functions of NCp7 is to promote the annealing of the tRNA primer onto the viral RNA template, a process that has been shown to occur through the activity of NC protein. To further study this subject, we have used a functional cell-free assay, in which NCp7 was employed to place tRNA primer onto the viral template RNA, and then was eliminated from such annealed complexes by Proteinase K digestion and phenol:chloroform extraction; this permitted the investigation of the annealed complex in the absence of NC protein. As a control, heat-annealing was also utilized to generate complexes between $tRNA^{Lys,3}$ and viral genomic RNA. The results showed that wild-type NCp7 at saturating concentrations caused formation of an active tRNA:template complex that gave rise to enhanced yield of full-length (-) strand strong-stop DNA ((-)ssDNA). In contrast, the use of heat-annealing to produce primer:template complexes resulted in lower yield of full-length (-)ssDNA than seen with NC protein, and the addition of NC protein to such pre-formed primer:template complexes was only able to reverse this defect to a marginal extent. Furthermore, NC protein, containing a point mutation in the first Zn finger or devoid of both Zn fingers, yielded primer:template complexes that are inefficient to generate full-length (-)ssDNA, although reactions could be normally initiated as seen in one-base extension assays. Therefore, two Zn finger motifs that are characteristic of NCp7 were essential in regard to generation of this tRNA:RNA complex that could participate in efficient elongation of reverse transcription reactions. The increased generation of full-length (-)ssDNA in the presence of NC-initiated primer:template complexes is partly explained by elevated switch from initiation to elongation of DNA polymerization.

2.3 Introduction

The human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein (NC) is processed from Gag precursors and is able to bind tightly to viral genomic RNA (Darlix et al. 1990; Henderson et al. 1990, 1992; Wondrak et al. 1993; Tanchou et al. 1995). Accordingly, NC can function as a portion of the Gag polyprotein precursor, essential for viral genomic packaging, and as a fully processed protein in reverse transcription steps that occur during de novo infection (Aldovini and Young 1990; Déméné et al. 1994; Berkowitz et al. 1995; Zhang and Barklis 1995; Berkowitz et al. 1996; Tanchou et al. 1998; Gorelick et al. 1999). At least three distinct roles are associated with NC in the context of reverse transcription: 1. it facilitates the annealing of HIV-1 cognate primer, *i.e.* $tRNA^{Lys,3}$, to the primer binding site (PBS) of the viral RNA template (Prats et al. 1988; de Rocquigny et al. 1992; Barat et al. 1993; Lapadat-Tapolsky et al. 1995; Huang et al. 1997a, 1998; Cen et al. 1999; Feng et al. 1999); 2. it stimulates specific viral DNA synthesis by reducing self-primed reverse transcription (Li et al. 1996; Lapadat-Tapolsky et al. 1997) or by enhancing the processivity of RT (Wöhrl et al. 1993; Ji et al. 1996; Wu et al. 1996); 3. it promotes the template switch in RT reactions to yield a full-length (-) or (+) strand DNA product (Peliska et al. 1994; You and McHenry 1994; Rodríguez-Rodríguez et al. 1995; Cameron et al. 1997; Guo et al. 1997; Auxilien et al. 1999; Wu et al. 1999).

The structures of retroviral nucleocapsid proteins are highly conserved, and, with the exception of spumaviruses, characteristically contain one or two copies of a CCHC motif that can bind Zn^{2+} with high affinity to form Zn fingers (Maurer et al. 1988; Darlix et al. 1995). The NC of HIV-1 contains two such Zn fingers flanked by regions rich in basic residues. Mutagenesis in the Zn fingers affects both the specificity of genomic RNA packaging and viral infectivity (Aldovini and Young 1990; Déméné et al. 1994; Berkowitz et al. 1995, 1996; Zhang and Barklis 1995; Tanchou et al. 1998; Gorelick et al. 1999).

The role of NC in the annealing of tRNA to the PBS is attributed to its RNA-chaperone activity to form an optimally base-paired RNA structure (Herschlag 1995; Rein et al. 1998). However, the structure/ function relationship in this process is not well understood. NC proteins without the Zn finger regions retain ability to bind and anneal complementary RNA sequences *in vitro* (de Rocquigny et al. 1992; Lapadat-Tapolsky et al. 1995; Rein et al. 1998), whereas point mutation in the first Zn finger was shown to compromise primer annealing efficiency (Déméné et al. 1994; Remy et al. 1998).

We have further studied the role of a synthetic form of NC, *i.e.* NCp7, a naturally observed cleavage product of the Gag precursor protein, in the annealing of primer $tRNA^{Lys.3}$ to viral genomic RNA. In doing so, we have distinguished the activities of this protein in the $tRNA^{Lys.3}$ annealing process from that of (-) strand strong-stop DNA ((-) ssDNA) synthesis. This research has involved the removal of NCp7 from viral RNA template through use of Proteinase K digestion and phenol: chloroform extraction of annealed primer:template complexes that have subsequently been studied in reverse transcription reactions.

Our results showed that initiation complexes formed between HIV-1 genomic RNA and $tRNA^{Lys.3}$ in the presence of NCp7 resulted in higher efficiency in generation of full-length (-)ssDNA than those formed by other methods, such as heat-annealing. We found that NC proteins lacking Zn fingers were able to anneal the tRNA primer onto RNA template with almost the same efficiency as that of wild-type NCp7. However, the elongation efficiency of reverse transcription reactions performed from such initiation complex was significantly reduced. This observation is consistent with an *in vivo* study that mutations within the CCHC motif are relatively unimportant in the genomic placement of $tRNA^{Lys.3}$ but are critical for extension from the tRNA primer (Huang et al. 1998).

2.4 Materials and Methods

2.4.1 Chemicals and reagents

All chemicals and radioisotopes were obtained from ICN Inc. (Montreal, Quebec, Canada) unless otherwise specified. Restriction enzymes, modifying enzymes, and RNA-guard (RNase inhibitor) were obtained from Pharmacia, Inc., Montreal, Canada. $tRNA^{Lys,3}$ was purified in our laboratories from human placenta as previously described (Jiang et al. 1993). Wild-type HIV-1 RT was prepared in our laboratory as previously described (Tsuchihashi et al. 1994). The anti-HIV-NC IgG monoclonal antibody (mAb) was a gift of Dr. J.-L. Darlix, ENSL, France.

2.4.2 Wild-type and modified nucleocapsid protein (NCp7)

The HIV-1 NCp7 peptides utilized consisted of 72 amino acids and were prepared by solid phase chemical synthesis as previously described (de Rocquigny et al. 1991). In addition to wild-type NCp7, modified proteins were also generated, including a variant containing a His to Cys modification in the first Zn finger (*i.e.* $H^{23}C$ NC) and a variant termed ddNC, that is deleted of both of the Zn fingers (Figure 2-1). Previous studies showed that a truncated form of NC, containing amino acids 13-64 and including both the Zn finger motifs as well as the $H^{23}C$ substitution, could no longer participate in annealing (Déméné et al. 1994). In contrast, ddNC that was devoid of both Zn fingers did retain both nucleic acid binding and annealing activities (de Rocquigny et al. 1992; Lapadat-Tapolsky et al. 1995).

2.4.3 Construction of RNA expression plasmids and preparation of HIV-1 RNA transcripts

The PBS/WT construct was generated as previously described (Arts et al. 1994), linearized by BssH II, and used as template in an Ambion Mega-Scripts kit (Austin, TX) to produce an RNA transcript. The RNA template thus generated is 251 nucleotides (nt) in length and includes HIV sequences (nt 17-254) as well as a short 13 nt stretch derived from the

PBS/WT vector. *In vitro* reverse transcription initiated by $tRNA^{Lys.3}$ from this template yielded a full length (-) ssDNA product of 178 nt joined to the 76 nt tRNA primer. The integrity of the RNA transcripts was routinely checked by denaturing gel electrophoresis (5% polyacrylamide-7M urea) prior to use in reverse transcription assays.

2.4.4 Placement of primer $tRNA^{Lys.3}$ onto the RNA template by NCp7 or by heat-annealing

The placement of primer $tRNA^{Lys.3}$ onto the viral RNA template was performed in a 10 µl reaction mixture containing 50 mM Tris-HCl [pH 7.2], 50 mM KCl, 5 mM $MgCl_2$, 1 pmol template RNA and 1 pmol $tRNA^{Lys.3}$. Various concentrations of NCp7 were added into the reaction mixtures that were then incubated at 37°C for 1h. When placement was carried out by heat-annealing, the reaction mixtures were incubated as described for 5 min at 85°C and then for 10 min at 55°C (Liang et al. 1997). Bovine serum albumin (BSA) was used as a control protein in both heat-annealing and NC placement reactions and did not have either a positive or negative effect (results not shown). The efficiency of placement is revealed by reactions in which $tRNA^{Lys.3}$ is extended at 37°C for 15 min in the presence of 50 ng wild-type HIV-1 RT (p66/51), 50mM Tris-HCl [pH 7.2], 50 mM KCl, 5 mM $MgCl_2$, 10 mM dithiothreitol, 10 units of RNA-guard and 1 µl dCTP[α-^{32}P] (specific activity, 4500Ci/mmol) in a total volume of 20 µl. Under these conditions, only one nucleotide is extended from annealed $tRNA^{Lys.3}$. The quantity of RT enzyme, *i.e.* 50 ng, was chosen on the basis of preliminary studies showing that higher enzyme concentrations resulted only in marginal increases in the amount of cDNA product (not shown). The ratio of enzyme to primer:template (*i.e.* 0.4 pmol RT:1 pmol template and 1 pmol tRNA) was sufficient to ensure that RT remained bound to primer:template in these reactions.

In the case of certain experiments, we wished to obtain pure tRNA:template complexes that had been formed by either the NC protein or heat-annealing methods. Accordingly, the NC protein in relevant

reactions was digested by addition of 1 µl Proteinase K (5 mg/ml) at 37°C for 15 min, following which both the Proteinase K and undigested residual NC protein were extracted with phenol:chloroform. Then, the binary tRNA:RNA template complexes in the liquid phase were precipitated at -20°C for 6 hours using an equivalent volume of isopropanol. Recovered $tRNA^{Lys.3}$:RNA template complexes were then redissolved in the initial reaction buffer. As a control, primer: template complexes that had been formed by heat-annealing were treated in the same way. Reactions were then initiated by addition of RT in the presence of dCTP[α-^{32}P].

2.4.5 *In vitro* reverse transcription

After the placement of $tRNA^{Lys.3}$ onto the viral RNA template by either NCp7 or heat-annealing, and single nucleotide extension mediated by 50 ng RT (see above), *i.e.* dCTP[α-^{32}P], 1 µl of a 2 mM mix of all four dNTPs were added to achieve further extension to generate full-length (-)ssDNA. Reactions were terminated at different times (*i.e.* 1, 4, 16, 32 min and 1h) by adding EDTA [pH 8.0] at a final concentration of 50 mM. After extraction with phenol:chloroform, reaction mixtures were precipitated with an equal volume of isopropanol. Recovered products were boiled in formamide-denaturing buffer for 5 min and fractionated on 8% denaturing polyacrylamide gels containing 7 M urea. The amounts of both full-length (-)ssDNA and total cDNA products were quantified by molecular imaging using a program provided by the manufacturer (BioRad, Mississauga, Ontario, Canada), in which results are expressed in units that approximate cpm.

2.4.6 Western blot

Various amounts of NCp7 were used to incubate with 1 pmol of template RNA and 1 pmol of primer $tRNA^{Lys.3}$ at 37°C for 1h. With or without Proteinase K and phenol:chloroform treatment, they were fractionated on 12% (w/v) SDS-polyacrylamide gels and transferred to nitrocellulose

filter. After being blocked with 5% (w/v) skim milk/0.05% Tween-20/PBS at 4°C for 16 hours, the filter was incubated with Rabbit anti-HIV-NC IgG monoclonal antibody (mAb) at 37°C for 1h. Following extensive washing with 0.05% Tween-20/PBS, secondary anti-Rabbit IgG, which is conjugated to horseradish peroxidase (HRP; Amersham Life Science, Toronto, Canada) was added for 1h at 37°C. After thorough washing, NCp7 was visualized by the ECL chemiluminescence detection kit (Amersham Life Science, Toronto, Canada).

2.5 Results

To study the role of NCp7 in synthesis of (-)ssDNA, we employed a cell-free reverse transcription system consisting of viral RNA template, human $tRNA^{Lys.3}$, RT (p66/51), and NCp7. The $tRNA^{Lys.3}$ primer was placed onto the RNA template by heat-annealing or through use of 30 pmol NCp7 to form a binary complex and was then extended by RT in the presence of dCTP[α-^{32}P] (specific activity, 4500Ci/mmol) for 15 min. Elongation from the one-base extended product was then monitored by incubation with cold dNTPs for varying periods.

Figure 2-2 (A to D) shows the results of these elongation experiments, and also demonstrates that early elongation products (+1 to +5) were extended over time into a full-length (-)ssDNA product. Band positions refer to the first nucleotide added to the 3'end of the tRNA primer. Full-length (-)ssDNA products were observed after 16 min in every case, at which time a variety of early DNA products were also observed. Increasing the time of incubation beyond 16 min led to a further accumulation of full-length product. In the case of reactions performed with the heat-annealing method, *i.e.* in the absence of NCp7, a number of non-specific products were seen, representing further extension from full-length (-)ssDNA (Figure 2-2A, lanes 4,5) (Guo et al. 1997). Studies with mutated forms of NCp7, containing alterations in the Zn finger regions, revealed only limited amounts of full-length (-)ssDNA product over long incubation times, *e.g.* 32 min-1h (Figure 2-2C, lanes 14,15; Figure 2-2D, lanes 19,20). We used a 16 min period to study elongation from single-base extended products in all subsequent experiments unless otherwise specified. The results of experiments with mutated NC proteins will be discussed below.

2.5.1 The manner of placement of $tRNA^{Lys.3}$ onto the HIV-1 RNA template can affect efficiency of elongation of reverse transcription

To further understand the role of NCp7 during synthesis of (-)ssDNA, we first employed varying concentrations of this protein (*i.e.* 5, 15, 30,

and 45 pmol, corresponding to 1 molecule of NCp7 per 50, 17, 8, and 6 nt residues, respectively) to promote the annealing of $tRNA^{Lys.3}$ onto the RNA template. The experimental strategy is described graphically in Figure 2-3B. The ratio of full-length (-)ssDNA product relative to total cDNA generated was calculated as a measure of the efficiency of elongation of reverse transcription. As shown in both Figure 2-3A (lanes 1-5) and 2-3C, the use of increasing concentrations of NCp7 in the placement of $tRNA^{Lys.3}$ onto viral RNA resulted in an increased ratio of full-length (-)ssDNA in comparison to total cDNA product. The data show an increase in the amount of total cDNA product up to a NCp7 concentration of 15 pmol (Figure 2-3A, lanes 1-3), followed by a decline when higher NCp7 concentrations were employed (Figure 2-3A, lanes 4,5). However, at these higher NCp7 concentrations, the production of non-specific DNA products was also decreased, and the amount of full-length (-) ssDNA relative to total cDNA product continued to increase. At a NCp7 concentration of 45 pmol, the ratio between full length (-)ssDNA to total cDNA even reached 0.69. Therefore, the efficiency of elongation was generally proportional to the amount of NCp7 used to form the $tRNA^{Lys.3}$:RNA template complex.

Because NCp7 is thought to exert its effect on the efficiency of viral cDNA synthesis through disruption of the secondary structure of the RNA template (Drummond et al. 1997), we also studied $tRNA^{Lys.3}$:RNA template complexes that had been generated by heat-annealing. In this circumstance, varying concentrations of NCp7 were only added later during the primer elongation stage of reactions (as illustrated in Figure 2-4B). The results of Figure 2-4A and 2-4C show that the presence of NCp7 in these reactions, even at very high concentrations, resulted in only a modest increase in the yield of full-length (-) ssDNA. Furthermore, as shown in Figure 2-4C, the addition of NCp7, at times after the heat-annealing of $tRNA^{Lys.3}$ to viral RNA, never led to the maximal ratio of (-)ssDNA over 0.25 compared with total cDNA synthesis. Therefore, the increased efficiency of elongation in synthesis of (-)ssDNA, in reactions in which NCp7 was used to promote

the formation of the primer:template complex (Figure 2-3A and 2-3C), cannot be attributed solely to the role of NCp7 during primer elongation, and must also involve aspects of NCp7 function during the placement of $tRNA^{Lys.3}$ onto the viral RNA template.

2.5.2 The role of NCp7 in formation of a $tRNA^{Lys.3}$:RNA binary complex with potential to yield high levels of full-length (-)ssDNA product

To further verify the role for NCp7 in primer placement and synthesis of (-)ssDNA, we next focused on the process of annealing. As the first step, $tRNA^{Lys.3}$ was placed onto the viral RNA template through use of varying concentrations of NCp7 or by heat-annealing as described above. Next, the proteins in these reactions were eliminated through Proteinase K digestion and phenol:chloroform extraction as described in Materials and Methods. After precipitation, the $tRNA^{Lys.3}$:RNA template complex was redissolved in the initial reaction buffer, and reactions continued in the presence of RT and dCTP[α-^{32}P]. In so doing, NC protein will not be present in the reaction system during reverse transcription (Figure 2-5B).

The results of Figure 2-5A and 2-5C show that NCp7 had again acted in a concentration-dependent manner during $tRNA^{Lys.3}$ placement to produce a primer:template complex that had the potential to yield high levels of full-length (-)ssDNA product. Concentrations of NCp7 > 30 pmol, *i.e.* saturating levels, were especially efficient in this regard. When a sub-saturating concentrations of NC, *e.g.*, 15 pmol, were used, no full-length (-)ssDNA products were generated, and overall levels of total cDNA products dropped significantly as well.

2.5.3 The NC Zn fingers are important in the formation of primer:template complexes with the potential to participate in highly efficient elongation

We also examined whether structural elements within NCp7 might be involved in formation of the $tRNA^{Lys.3}$ primer:RNA template complex with potential to participate in efficient elongation reactions. Toward this

end, we studied the effects of two NC proteins mutated in the Zn finger motifs, as described above. Previous reports have shown by gel retardation analysis that the Zn fingers of NCp7 are not required to promote the annealing of complementary nucleic acid sequences between $tRNA^{Lys,3}$ and viral genomic RNA (de Rocquigny et al. 1992; Lapadat-Tapolsky et al. 1995).

Figure 2-4A shows results obtained when either wild-type or mutated NC was added to RT reactions in which primer:template complexes had been formed by heat-annealing. The data show that addition of either wild-type or mutated NC during elongation only slightly increased the ratio of full-length (-)ssDNA relative to total cDNA product. In contrast, when annealing of primer to template was performed in the presence of NC proteins, far less full-length (-)ssDNA was observed in reactions in which the mutated forms of NC were employed (Figure 2-3A, lanes 6-10, 11-15). This diminished elongation efficiency was also demonstrated in time-course experiments when mutated NC were used (see above, Figure 2-2C and 2-2D).

We next performed single-base extension experiments to determine whether the inefficient generation of full-length (-)ssDNA was due to decreased levels of $tRNA^{Lys,3}$:RNA template complexes that had been formed by the mutated NC proteins. The results of Figure 2-6A show that reactions using wild-type NCp7 (Figure 2-6A, lanes 6-10) or either of the two types of mutated NC (Figure 2-6A, lanes 11-15, 16-20) yielded similar levels of single-base extended product, although, in each case, less product was generated than that seen when heat-annealing was used for primer:template formation (Figure 2-6A, lanes 1-5). These findings are consistent with earlier reports that NC proteins with Zn finger deletions retain annealing activity (de Rocquigny et al. 1992; Lapadat-Tapolsky et al. 1995). These single-base extension experiments facilitate functional analysis of tRNA:RNA template complexes in comparison with physical assays.

To test the hypothesis that the NC proteins containing mutations in the Zn fingers might be less able than wild-type NC to promote

subsequent extension, reaction mixtures containing primer:template and NC were subjected to Proteinase K digestion and phenol:chloroform extraction as described above, in which both wild-type and mutated NC peptides were used at saturating conditions, *i.e.* 30 pmol per reaction (Figure 2-7B). The results of Figure 2-7A and 2-7C show that $H^{23}C$ NC, which contained a mutation in the first Zn finger, was about 2.5 times less efficient than wild-type NCp7 in regard to generation of a template:primer complex able to participate in the highly efficient synthesis of (-)ssDNA. In contrast, hardly any full length (-)ssDNA product was formed in reactions performed with the NC peptide deleted of both Zn fingers (Figure 2-7A, lane 3), and RT reactions were blocked after formation of short cDNA products. Significant arrest was noted in these reactions at nt positions +1 to +5.

Thus, these experiments provide evidence that the role of NC protein in synthesis of (-)ssDNA is at least two-fold, *i.e.*, placement of the $tRNA^{Lys.3}$ primer and DNA elongation. These results show that the Zn finger motifs are especially important for the formation of $tRNA^{Lys.3}$:RNA template complexes that are necessary for fully efficient reverse transcription reactions during the elongation of (-)strong-stop DNA synthesis.

Figure 2-1. Primary sequences of wild-type and mutated HIV-1 NC proteins. The positions of Zn fingers, N-terminus, and C-terminus of the NC proteins are indicated. Zn finger sequences are in italicized form, the CCHC motifs are underlined, the mutated NC sequences are indicated.

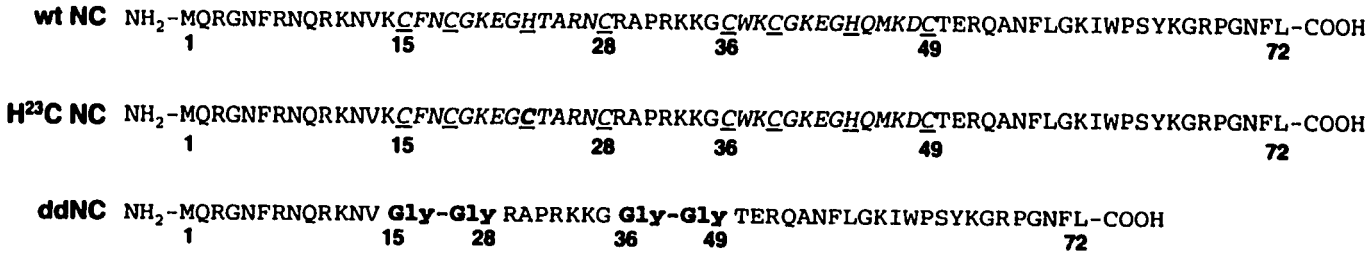

wt NC NH2-MQRGNFRNQRKNVKCFNCGKEGHTARNCRAPRKKGCWKCGKEGHQMKDCTERQANFLGKIWPSYKGRPGNFL-COOH
1 15 28 36 49 72
H23C NC NH2-MQRGNFRNQRKNVKCFNCGKEGCTARNCRAPRKKGCWKCGKEGHQMKDCTERQANFLGKIWPSYKGRPGNFL-COOH
1 15 28 36 49 72
ddNC NH2-MQRGNFRNQRKNV Gly-Gly RAPRKKG Gly-Gly TERQANFLGKIWPSYKGRPGNFL-COOH
1 15 28 36 49 72

Figure 2-2. Time course of (-)ssDNA synthesis. In (A), the $tRNA^{Lys.3}$ primer was placed onto the template by heat-annealing. (B) to (D) represent studies performed with primer:template complexes formed by wild-type NCp7, $H^{23}C$ NC protein and doubly-deleted Zn finger NC protein, respectively. Reactions were performed for periods ranging between 1 min (lanes 1,6,11,16), 4 min (lanes 2,7,12,17), 16 min (lanes 3,8, 13,18), 32 min (lanes 4,9,14,19), and 60 min (lanes 5,10,15,20), respectively. Band positions represent the first nt added to the 3'end of $tRNA^{Lys.3}$.

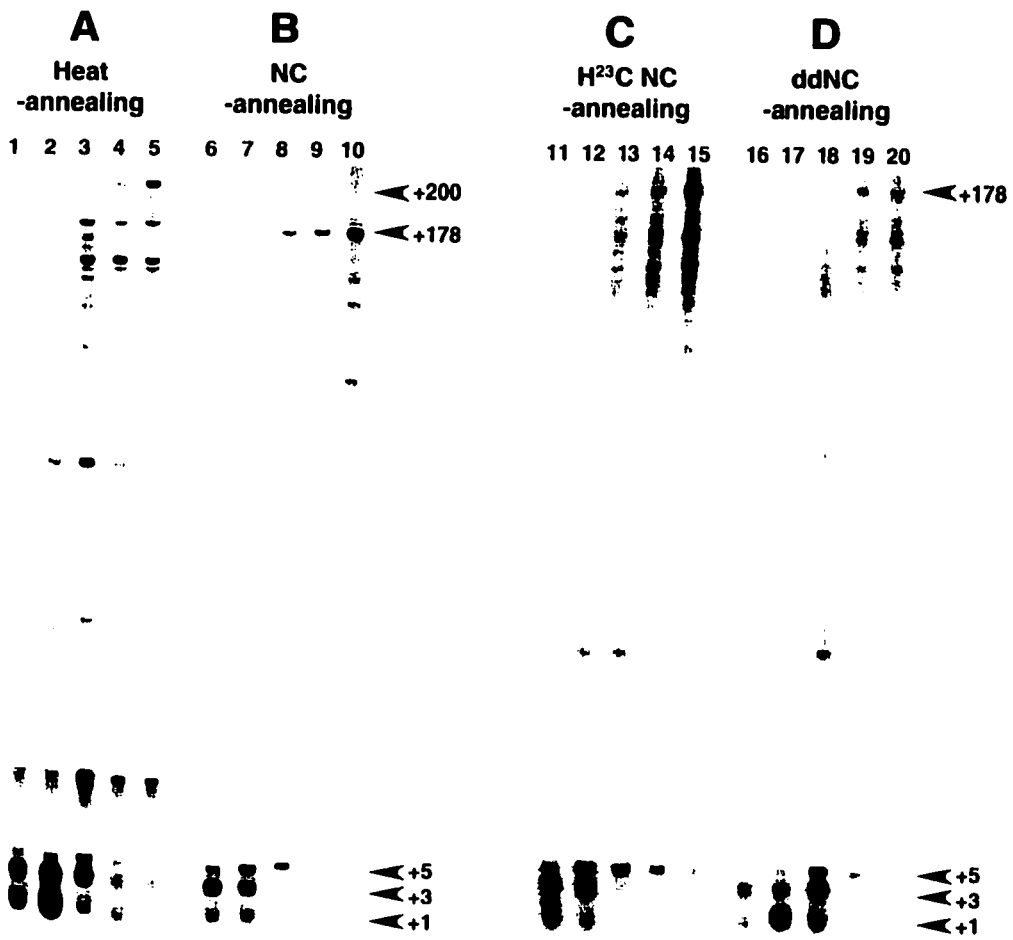

A
Heat
-annealing
B
NC
-annealing
C
H23C NC
-annealing
D
ddNC
-annealing
1 2 3 4 5
6 7 8 9 10
11 12 13 14 15
16 17 18 19 20
+200
+178
+178
+5
+3
+1
+5
+3
+1

Figure 2-3. Dose response curve of (-)ssDNA synthesis. Complexes of tRNA and viral RNA template were formed by either wt NCp7 (lanes 1-5), mutated $H^{23}C$ NC protein (lanes 6-10), or mutated ddNC protein (lanes 11-15) and reactions were carried out for 16 min as described in Materials and Methods. The concentrations of wt or mutated NC proteins used in these reactions were 0 pmol (lanes 1,6,11), 5 pmol (lanes 2,7,12), 15 pmol (lanes 3,8,13), 30 pmol (lanes 4,9,14), and 45 pmol (lanes 5,10,15) per reaction, respectively. Band positions represent the first nt added at the 3'end of $tRNA^{Lys,3}$, and relative quantification of amounts of product was by molecular imaging, using a program that provides cpm equivalents, as suggested by the manufacturer (BioRad Instruments, Mississauga, Ontario, Canada). The ratios of full length (-)ssDNA compared with total cDNA product in these reactions are shown in the graph.

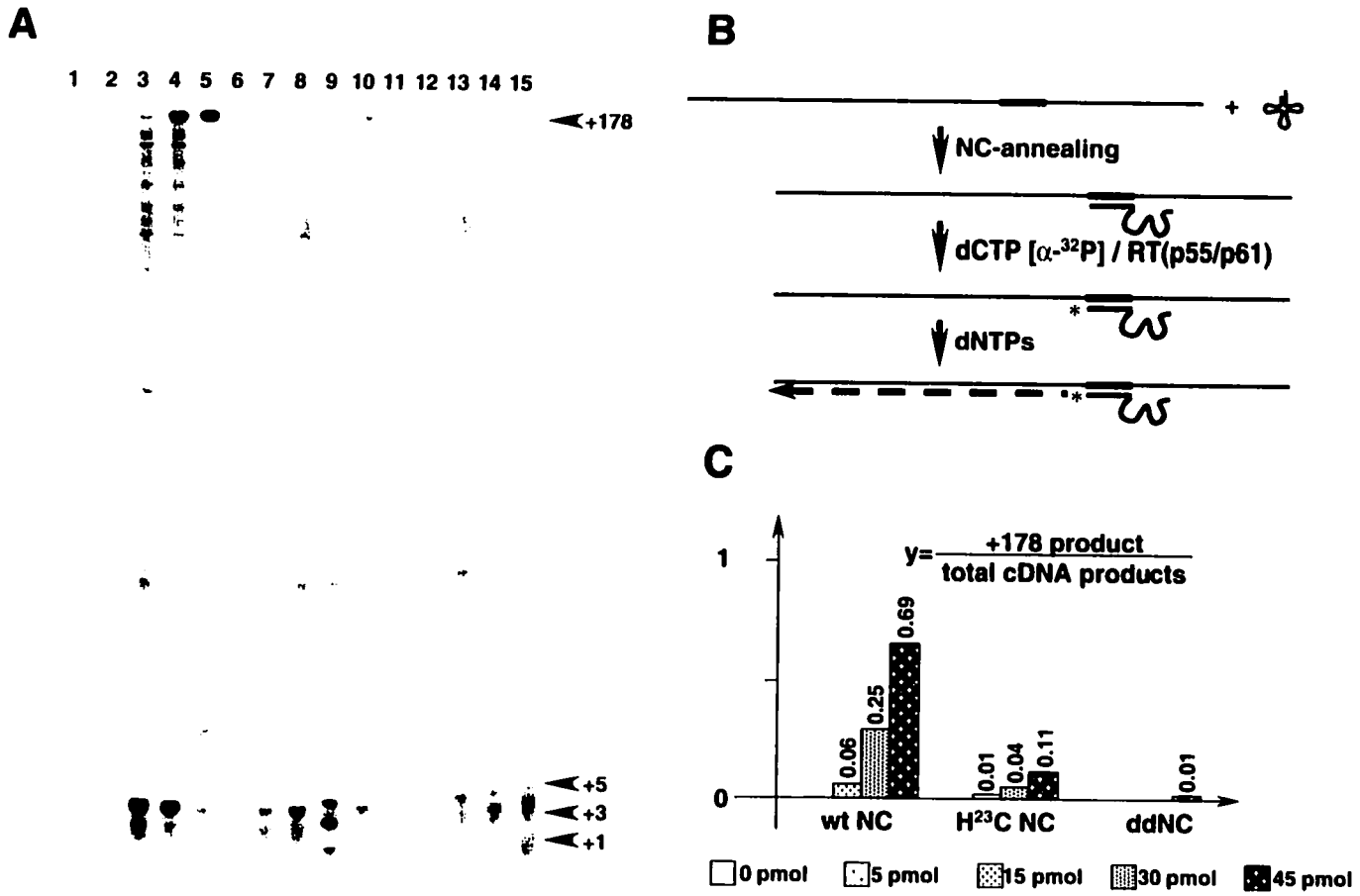

A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
+178
+5
+3
+1
B
+
NC-annealing
dCTP [α-32P] / RT(p55/p61)
*
dNTPs
*
C
1
0
y= +178 product / total cDNA products
0.06
0.25
0.69
0.01
0.04
0.11
0.01
wt NC
H23C NC
ddNC
0 pmol
5 pmol
15 pmol
30 pmol
45 pmol

Figure 2-4. Dose response curve of (-)ssDNA synthesis. Primer:template complexes were generated by heat-annealing. Varying amounts of wt NCp7 (lanes 2-5), mutated $H^{23}C$ NC protein (lanes 6-9) or mutated ddNC protein (lanes 10-13) were added during the primer elongation phase of these reactions. Lane 1 represents a control performed without NC protein; each group of lanes 2,6,10; 3,7,11; 4,8,12; and 5,9,13 represents reactions performed with 5, 15, 30, and 45 pmol of the different NC proteins per reaction, respectively. Band positions represent the first nt added at the 3'end of $tRNA^{Lys,3}$, and relative quantification of amounts of product was by molecular imaging, using a program that provides cpm equivalents, as suggested by the manufacturer (BioRad Instruments, Mississauga, Ontario, Canada). The ratio of full length (-)ssDNA compared with total cDNA product in these reactions are shown in the graph.

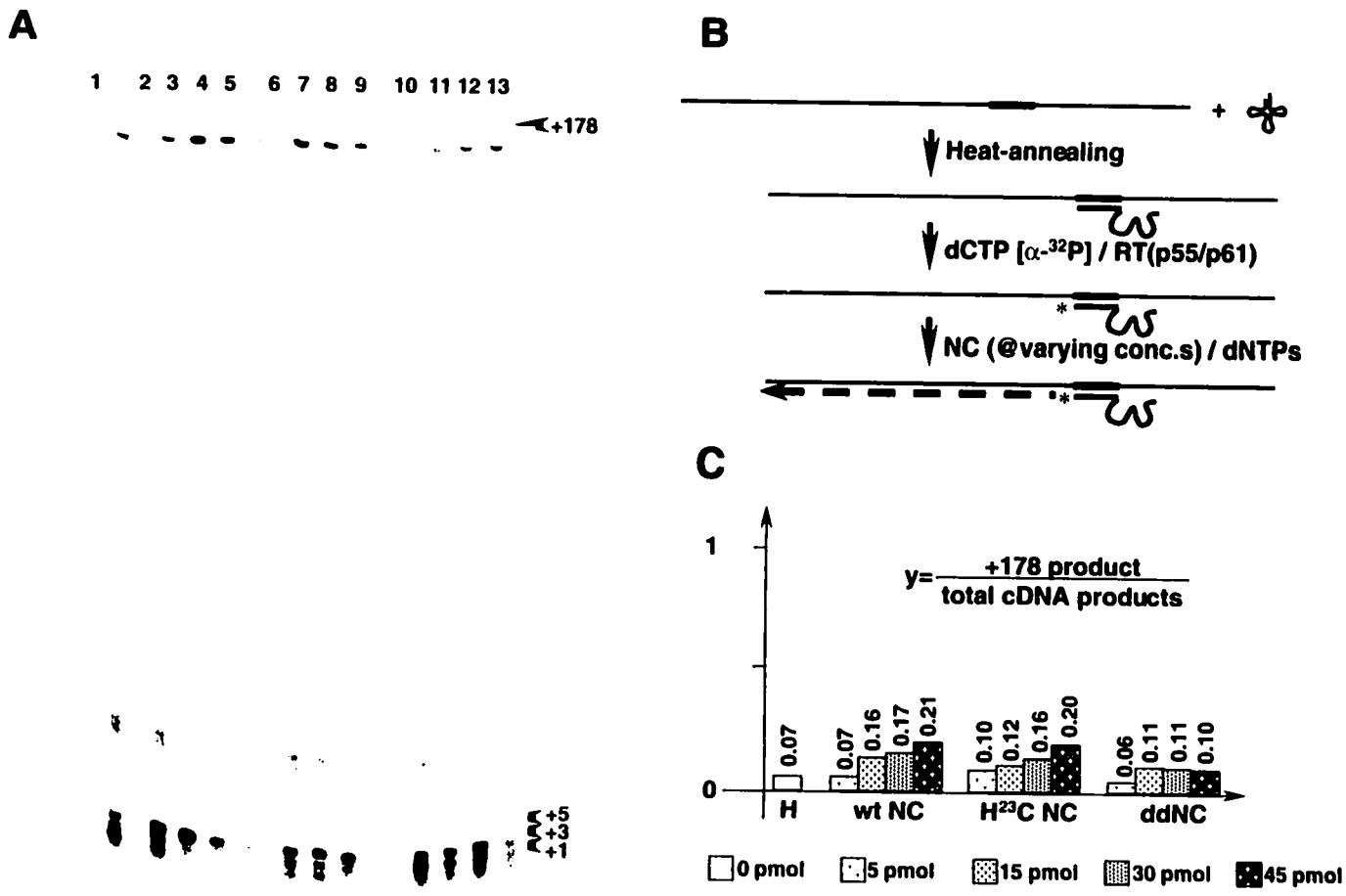

A
1 2 3 4 5 6 7 8 9 10 11 12 13
+178
+5
+3
+1
B
+
Heat-annealing
dCTP [α-32P] / RT(p55/p61)
*
NC (@varying conc.s) / dNTPs
*
C
1
0
y= +178 product / total cDNA products
0.07
0.07 0.16 0.17 0.21
0.10 0.12 0.16 0.20
0.06 0.11 0.11 0.10
H
wt NC
H23C NC
ddNC
0 pmol
5 pmol
15 pmol
30 pmol
45 pmol

Figure 2-5. Efficiency of elongation of tRNA:template complexes formed with varying concentrations of NC proteins. $tRNA^{Lys,3}$ was placed onto the viral RNA template in the presence of various concentrations of NCp7 to achieve the NCp7:nt ratios described in the legend to Figure 2-4. The primer:template complexes were then treated with proteinase K and phenol/chloroform as described in Materials and Methods, after which the reactions were reconstituted with recovered primer:template complex. Lane 1 is a control, representing the elongation efficiency of the primer:template complex formed by heat-annealing. Lanes 2 to 6 depict the elongation efficiency of complexes formed through addition of 0, 5, 15, 30, and 45 pmol of NCp7 per reaction, respectively. Band positions represent the first nt added to the 3'end of $tRNA^{Lys,3}$; relative quantification was by molecular imaging. The ratio of (-)ssDNA relative to the total amount of cDNA produced in each reaction is presented in the graph.

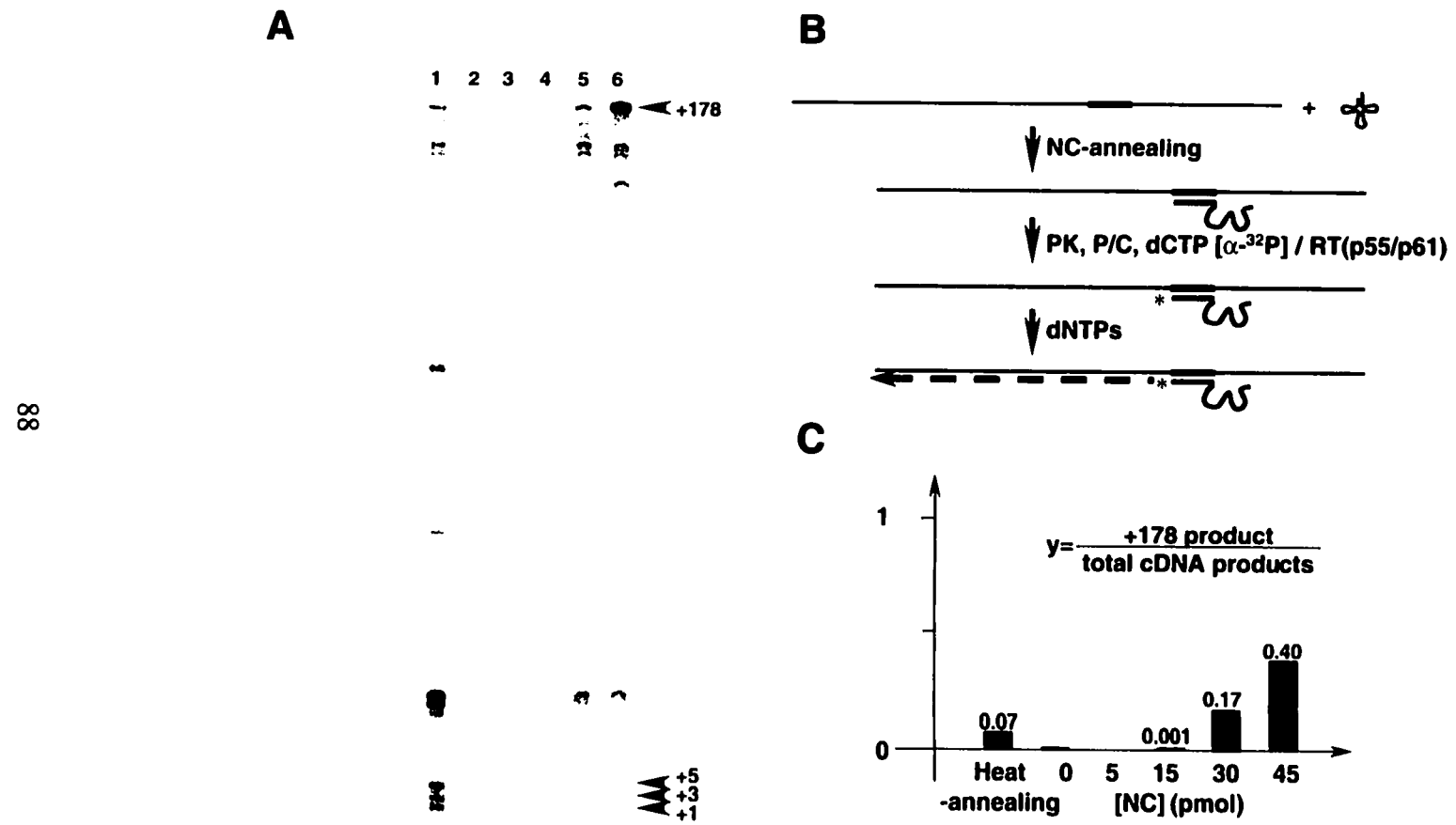

A
1 2 3 4 5 6
+178
+5
+3
+1
B
NC-annealing
PK, P/C, dCTP [α-32P] / RT(p55/p61)
dNTPs
C
1
0
y= +178 product / total cDNA products
0.07
0.001
0.17
0.40
Heat -annealing
0
5
15
30
45
[NC] (pmol)

Figure 2-6. Single-base extension of tRNA:template complexes. By monitoring incorporation of dCTP[α-^{32}P], reactions were followed for varying times, *i.e.* 0.5 min (lanes 1,6,11,16), 1 min (lanes 2,7,12,17), 4 min (lanes 3,8,13,18), 16 min (lanes 4,9,14,19), and 32 min (lanes 5, 10,15,20), respectively. We were able to distinguish the rates at which reactions had been initiated from different tRNA:template complexes. Complexes had been formed by either heat-annealing (lanes 1-5), or in the presence of wt NC (lanes 6-10), H^{23}C mutated NC protein (lanes 11-15), or mutated ddNC protein (lanes 16-20), at a concentration of 30 pmol per reaction. The densities of single-base extension products were determined on the basis of molecular imaging.

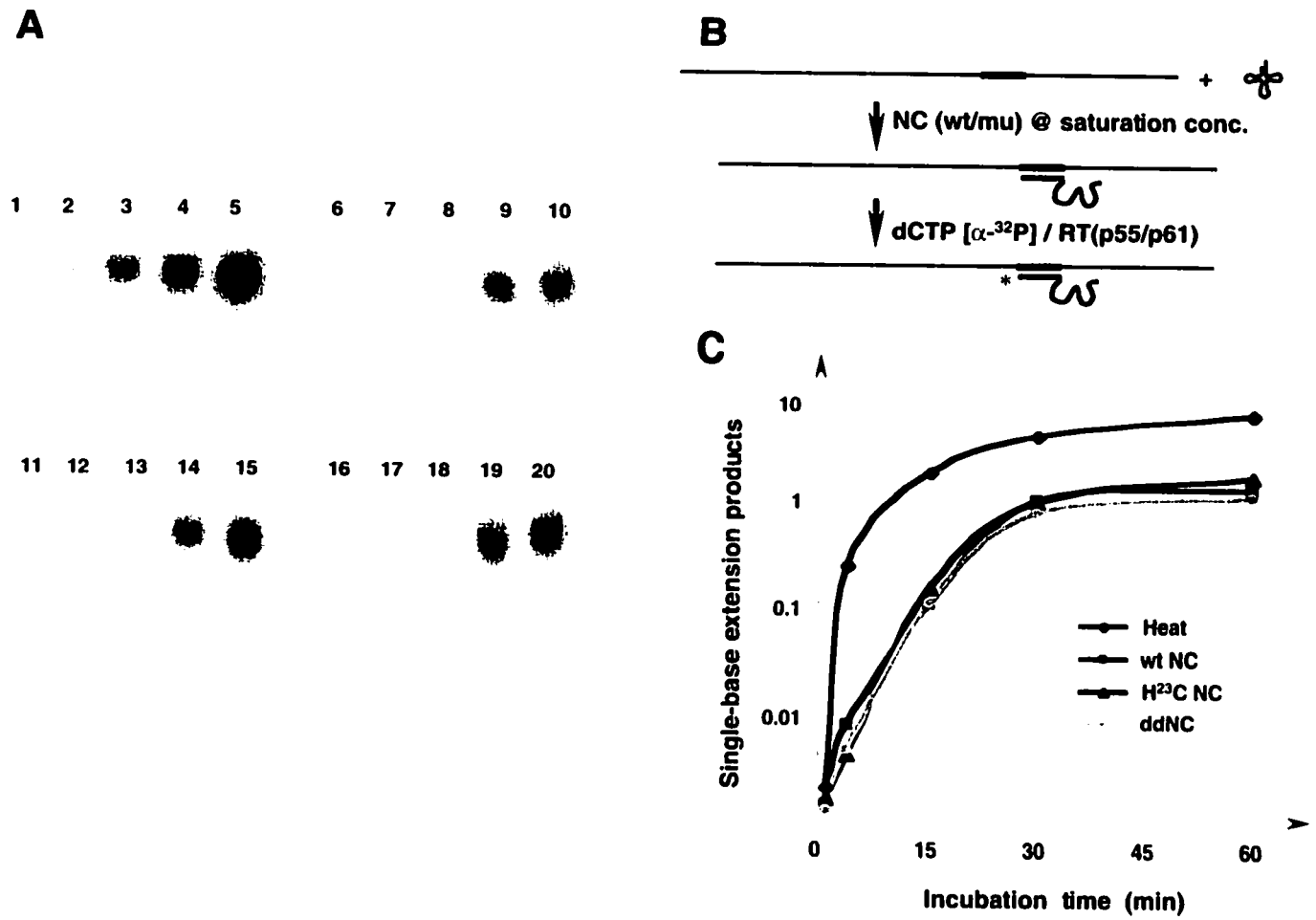
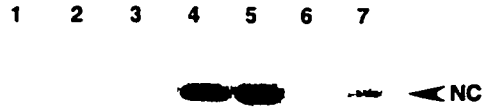

A
1 2 3 4 5 6 7 8 9 10
11 12 13 14 15 16 17 18 19 20
B
+
NC (wt/mu) @ saturation conc.
dCTP [α-32P] / RT(p55/p61)
*
C
Single-base extension products
10
1
0.1
0.01
0
15
30
45
60
Incubation time (min)
Heat
wt NC
H23C NC
ddNC

Figure 2-7. Effects of mutations within the Zn finger domains of NC on reverse transcription elongation. Either wt or mutated NC was used to generate primer:template complexes; these NC proteins were then eliminated from the complexes by digestion with proteinase K and extraction with phenol:chloroform as described in Materials and Methods. The primer elongation step was performed in the absence of NC protein. Quantification of products was as described above in the legend to Figure 2-3.

A
wt NC
H23C NC
ddNC
+178
B
NC (wt/mu) @ saturation conc.
PK, P/C, dCTP [α-32P] / RT(p55/p61)
dNTPs
C
y= +178 product / total cDNA products
1
0
0.20
0.07
wt NC
H23C NC
ddNC
+5
+3
+1

Figure 2-8. Analysis by western blot of residual HIV-1 NC protein within the tRNA:RNA complex after Proteinase K digestion and phenol:chloroform extraction. 1 pmol of tRNA and 1 pmol of vRNA were incubated at 37°C for 1h in the presence of 0 pmol (lane 1), 30 pmol (lanes 2, 4, 6), or 45 pmol (lanes 3, 5, 7) of NCp7 respectively. Lanes 2 and 3 represent samples after Proteinase K and phenol:chloroform treatment; lanes 4 and 5 represent samples without such treatment; in lanes 6 and 7, only one twentieth of the samples in lanes 4 and 5 were loaded. Western blot was performed as described in Materials and Methods.


1 2 3 4 5 6 7
NC

2.6 Discussion

NCp7 of HIV-1 is a basic protein that contains two Zn fingers, both of which are required to promote efficient packaging of viral genomic RNA, as demonstrated by mutagenesis studies (Aldovini and Young 1990; Berkowitz et al. 1995; Zhang and Barklis 1995). Certain mutations in the Zn fingers may affect viral infectivity and replication more dramatically than their impact on RNA incorporation into virions, suggesting that NCp7 is also involved in aspects of the HIV life cycle other than RNA packaging (Déméné et al. 1994; Berkowitz et al. 1996; Schwartz et al. 1997; Tanchou et al. 1998; Gorelick et al. 1999). *In vitro*, HIV-1 NCp7 has been shown to influence reverse transcription in several ways, including the placement of primer tRNA onto the viral genomic template, subsequent elongation, as well as the first and second strand transfer (Prats et al. 1988; Barat et al. 1989; de Rocquigny et al. 1992; Peliska et al. 1994; You and McHenry 1994; Lapadat-Tapolsky et al. 1995; Rodríguez-Rodríguez et al. 1995; Tanchou et al. 1995; Ji et al. 1996; Wu et al. 1996; Guo et al. 1997; Auxilien et al. 1999; Druillennec et al. 1999; Lener et al. 1999; Wu et al. 1999). The cell-free assays described herein distinguish between the roles of NC in the annealing process from that of primer extension, and reveal a greater understanding of the activity of NCp7 in regard to both of these events than that previously available.

The role of NCp7 in reverse transcription has been commonly believed to result from the denaturation of RNA template secondary structure during the elongation phase of the reaction (Drummond et al. 1997). However, we have shown that the use of NCp7 to promote complex formation between $tRNA^{Lys,3}$ and the RNA template led to a higher ratio of full-length (-)ssDNA than that obtained when a heat-annealing procedure was employed. Therefore, the increased elongation efficiency of RT reactions in the presence of NCp7 is not due solely to the activity of NCp7 during primer extension but is also related to the manner of placement of the $tRNA^{Lys,3}$ primer onto the HIV RNA template. The addition

of different amounts of NCp7 at times after the heat-annealed formation of primer:template complex had little or no effect on subsequent efficiency of elongation.

To provide direct evidence of this specific role of NC protein during the annealing process, we used Proteinase K digestion and phenol:chloroform extraction to recover a $tRNA^{Lys,3}$:RNA template complex from which NCp7 had been removed. Our results show that reverse transcription from tRNA placed by NC (at saturating concentrations) was carried out with higher elongation efficiency than from heat-annealed primer, even if the NCp7 was no longer present at later stages of the reaction. The fact that elongation efficiency was lower in such reactions than in those in which NCp7 remained associated with the $tRNA^{Lys,3}$:template complex throughout the reaction also suggests that NCp7 is involved both in the formation of an active binary complex during primer annealing and, as well, in the melting of RNA template secondary structure during the process of tRNA extension.

To confirm that the above reverse transcription reactions were performed in the absence of NC, we also used anti-HIV-NC mAb to analyze NC proteins after incubation with tRNA and RNA template and subsequent Proteinase K and phenol:chloroform treatment. As shown in Figure 2-8, after protein digestion and extraction, no NC protein can be detected in the western blot experiment. The sensitivity of blotting was proved by the observance of 3 pmol of NC protein that were loaded on the gel directly. At this concentration, NC protein was shown to have no effect on the increased ratio of full length (-)ssDNA (Figure 2-3, lane 2; Figure 2-4, lane2; Figure 2-5, lane 3).

Previous reports have indicated that one molecule of NC protein can occlude between 5-10 nt residues in viral genomic RNA (Darlix et al. 1995), and, indeed, the strongest effect in regard to synthesis of (-)ssDNA was observed at this ratio (Li et al. 1996). Thus, at saturating levels of NC protein, *e.g.* 30 pmol in our experiment, an RNA template molecule that is 251 nt in length should be completely covered by NC protein. At such NC concentrations, higher elongation efficiency

was observed, even when NC protein was not present during elongation (Figure 2-5A, lane 5). In contrast, the use of a lower NC concentration, *e.g.*, 15 pmol, resulted in more than a 50% drop in efficiency of (-)ssDNA synthesis (Figure 2-5A, lane 4). These findings suggest that the nature of the tRNA:template complex formed at sub-saturating concentrations of NCp7 may be different in conformation from that formed when saturating levels of NCp7 were employed. Indeed, at sub-saturating levels, NCp7 can perform its activity in the tRNA annealing process, and viral RNA may still be fully occluded by NC; however, specific interactions among individual NC molecules might no longer occur due to a relatively large distance between these units (Lapadat-Tapolsky et al. 1995). Therefore, the resultant complex under such conditions might be less structured and therefore less able to participate in synthesis of full-length (-)ssDNA.

Viral tRNA:RNA complexes formed by heat-annealing should, according to this logic, also be less structured. Furthermore, this defect should not be correctable through addition of saturating levels of NCp7, as seen in our experiments. The fact that much higher amounts of total cDNA were generated after heat-annealing than through use of NCp7 suggests that the former process can result in formation of the primer:template complex at high efficiency. In fact, the results of our single-base extension experiments (Figure 2-6) indicate that as much as ten times more primer:template complexes may be formed by heat-annealing than by NCp7 at saturating conditions.

We also studied NC proteins containing mutations in the Zn finger domains. One is $H^{23}C$ NC, in which a His at position 23 has been replaced by a Cys. This change caused both a conformational change in the first Zn finger and, as well, a modification in the spacial orientation of the short linker region of NC (Déméné et al. 1994). Viruses containing this substitution were non-infectious (Déméné et al. 1994). However, NCp7 that contained the aforesaid mutation retained annealing activity in our experiments, probably due to the effects of basic amino acids within the N- and C-terminals of the NC protein. These data are

consistent with results obtained with a second mutated form of NC in which the Zn fingers had been deleted and replaced by glycine-glycine linkages to maintain the same distance between basic regions as seen in wild-type NCp7. This mutated form of NC has also been reported to retain RNA annealing activity (Lapadat-Tapolsky et al. 1995).

Our experiments show that NCp7 containing the point mutation in the first Zn finger had lost a portion of its ability to generate (-) ssDNA, while retaining activity in regard to the annealing of tRNA onto viral template. In contrast, no full length (-)ssDNA was generated when both Zn fingers were deleted. This point becomes even clearer when one considers that twice as much sample formed in the presence of ddNC as opposed to wild-type NC was loaded onto gels in the experiment described in Figure 2-7. These observations are consistent with a recent report that mutations within the Cys-His motif are relatively unimportant in genomic placement of $tRNA^{Lys.3}$ *in vivo*, but important for the extension from the tRNA primer (Huang et al. 1998). Our data show that the Zn fingers are essential for the formation of primer:template complexes able to promote highly efficient elongation reactions. Therefore, the nature of the tRNA:RNA template complex formed in the presence of wild-type NCp7 is structurally and functionally distinct from that generated in the presence of the mutated NC proteins.

In order to attain full-length synthesis of HIV-1 (-)ssDNA from $tRNA^{Lys.3}$ annealed to the viral RNA template, at least two distinct reaction phases are necessary, *i.e.* initiation to generate products extended by 3-5 nt and elongation to yield longer cDNA products (Isel et al. 1996; Lanchy et al. 1996a,b). The switch from initiation to elongation has been shown to be rate-limiting, which includes a structural rearrangement of RT, and can be facilitated by extended primer:template interactions that involve an A-rich loop located 10 nt upstream of the viral PBS (Isel et al. 1996; Lanchy et al. 1996a,b). Our study shows that NCp7 also promotes the transition from initiation to elongation (Figure 2-2B). The quality of the tRNA:viral RNA template complex formed at saturating concentrations of NCp7 is key to the

transition from initiation to elongation (Figure 2-5, lanes 5,6). This also helps to explain why the tRNA:template complex formed by NCp7 yielded a higher ratio of full-length (-)ssDNA products than those formed by heat-annealing (Figure 2-5, lane 1). NC protein devoid of both Zn fingers resulted in a primer:template complex that could only yield initiation, *i.e.*, +3 to +5 nt, and other short DNA products, but not fully synthesized (-)ssDNA (Figure 2-7). These findings support the notion that the quality of the tRNA:vRNA complex formed by NCp7 is a key factor in regard to each of elongation efficiency as well as transition from the initiation to elongation stages of the RT reaction.

Chapter 3

Mechanistic Studies of Early Pausing Events during Initiation of HIV-1 Reverse Transcription

This chapter was adapted from an article that appeared in the Journal of Biological Chemistry (1998), Vol.273, pp. 21309-21315. The authors of this paper were Liang, C., L. Rong, M. Götte, X, Li, Y. Quan, L. Kleiman, and M. A. Wainberg. All data presented in this chapter were from experiments performed by myself and Dr. C. Liang under the supervision of Dr. Wainberg. Dr. C. Liang performed the construction of all the plasmids, and is responsible for Figure 3-2, 3-5, and 3-7 of this manuscript.

3.1 Preface

In the reverse transcription system described in Chapter 2, both full-length (-)ssDNA and various early cDNA products were observed in reactions performed for limited incubation times, *i.e.*, 16 min (Figure 2-2). According to previous reports, these early short (-) strand DNA products comprise the initiation stage of reverse transcription. In HIV-1, the initiation of (-) strand DNA synthesis can be distinguished from subsequent elongation by the different polymerization rate and dissociation rate of RT. Specifically, the presence of short cDNA products was due to the pausing and dissociation of RT from the initiation complex at +3/+5 nt positions; and the extensive inter-molecular interactions formed between $tRNA^{Lys.3}$ and viral RNA template were found to be essential for the efficient transition from initiation to elongation. In this chapter, we show an improvement of our *in vitro* reverse transcription system by decreasing the dNTP concentration to 160 nM, to allow the observation of early pause sites. Our results show that pausing at the +1 nt site represents a distinct step in the initiation of reverse transcription reactions performed with an HIV-1 RNA template and primer $tRNA^{Lys.3}$. On the other hand, the formation of the +3 pausing site was relative to sequences upstream of the PBS; deletion of an A-rich loop at the 5'end of the PBS caused a significantly diminished initiation rate and a strong block of reverse transcription at the +1 stage.

3.2 Abstract

We have investigated the role of sequences that surround the primer binding site (PBS) in the reverse transcriptase (RT)-mediated initiation of (-) strand DNA synthesis in HIV-1. In comparisons of reverse transcription initiated from either the cognate primer $tRNA^{Lys.3}$ or a DNA primer D-Lys.3, we observed that a +3 pausing site occurred in both circumstances. However, the initiation reaction with $tRNA^{Lys.3}$ was also characterized by a pausing event after incorporation of the first nucleotide. Alteration of sequences at the 5' instead of the 3'end of the PBS resulted in elimination of the +3 pausing site, suggesting that this site was template sequence-dependent. In contrast, the pausing event at the +1 nt position was still present in experiments that employed either of these mutated RNA templates. The mutations at the 5'end of the PBS also caused a severely diminished rate of initiation and the strong arrest of reactions at the +1 stage when $tRNA^{Lys.3}$ was used as primer. Therefore, we propose that the +1 pausing event is an initiation-specific event in regard to reactions primed by $tRNA^{Lys.3}$ and that sequences at the 5'end of the PBS may facilitate the release of reverse transcription from initiation to elongation.

3.3 Introduction

Retroviruses employ specific tRNA molecules as primers to initiate the synthesis of (-) strand DNA (Skalka and Goff 1993; Mak and Kleiman 1997). These primer tRNAs bind to an 18 nt segment of viral genomic RNA termed the primer binding site (PBS). Although such binding is principally mediated by a stretch of 18 nt close to the 3'end of tRNA, other sequences within viral genomic RNA and the tRNA primer are also involved and can modulate the initiation of (-) strand DNA synthesis, as shown in both retroviral (Alyar et al. 1992, 1994; Isel et al. 1993, 1995; Skripkin et al. 1996) and other systems (Wang et al. 1992; Shimamoto et al. 1993; Wang et al. 1993; Wihelm et al. 1994; Zimmerly et al. 1995; Friant et al. 1996). In HIV-1, this includes a stretch of four nucleotides, *i.e.* 622-AAAA-625 (the A-rich loop) that interacts directly with positions 33-USUU-36 of the anticodon loop of primer $tRNA^{Lys.3}$. This A-rich loop is important for initiation of (-) strand DNA synthesis and generation of progeny virus (Liang et al. 1997).

In HIV-1, the initiation stage of synthesis of (-) strand DNA, primed by $tRNA^{Lys.3}$, can be distinguished from subsequent strand elongation in regard to both the binding and kinetic properties of reverse transcriptase (RT) (Isel et al. 1996; Lanchy et al. 1996a,b). Previous reports showed that initiation was characterized by both early short (-) strand DNA products, resulting from pausing at the +3 or +5 nt positions, and rapid dissociation of RT from the initiation complex. The secondary structure formed between $tRNA^{Lys.3}$ and the viral RNA template may play an important role in the efficient transition from initiation to elongation.

To pursue this subject, we developed an *in vitro* reverse transcription system in which low concentrations of dNTPs (*i.e.* 160 nM) were used to enhance our ability to detect very early pause sites, *e.g.* +1 and +3, in reactions primed with the $tRNA^{Lys.3}$ cognate primer. To study the roles of sequences flanking the PBS, we generated a series of mutated HIV-1 RNA template, that contained mutations at both the 5' and

3'ends of the PBS that may potentially disrupt the secondary structure of complexes of $tRNA^{Lys,3}$ and viral RNA template. Our data provide the first evidence for a +1 pausing event in reverse transcription initiated from primer $tRNA^{Lys,3}$. We have also demonstrated on the basis of mutagenesis studies that the +3 pausing site depends on the nature of sequences at the 5'end of the PBS and that deletion of an A-rich loop in this region or substitutions at the 5'end of the PBS may make it difficult for primer $tRNA^{Lys,3}$ to be extended beyond the +1 pausing site.

3.4 Materials and Methods

3.4.1 Plasmids construction

The RNA expression plasmid is PBS/WT that contains HIV-1 DNA sequences between nt positions 473 to 1417 (Arts et al. 1994). The deletion of the A-rich loop (HIV/del-A) and the construction of mutation HIV/A2 have been previously described (Li et al. 1996; Liang et al. 1997). We also engineered a substitutional mutation (HIV/HUA) by replacing the sequence 624-AATCTCTAGCAG-635 with 5'-GAACACCCAACATT-3'; this was done by PCR using an antisense primer 5'-CCTGTTCGGGCGCCAAATGTTGGGTGTTC TTCCACACTGACTAAAGG-3' (650-606), containing the above substitutions (underlined) and a Nar I restriction site (GGCGCC), together with a sense primer 5'-AGACCAGATCTGAGAATGG-3' (468-486), that contained a Bgl II restriction site (AGATCT) (Figure 3-2). Substitutional mutations (HIV/Lys.1,2) within the PBS region were generated as described (Li et al. 1994). Partial deletions of sequences downstream of the PBS, *i.e.* HIV/LD1, HIV/LD2 and HIV/LD3, were also generated as described previously (Li et al. 1997). A sequence (230-754) from pUC18 (Promega, Madison, WI) was cloned into pSP72 by employing EcoR I and Aat II restriction sites to generate the plasmid pSP/UC.

3.4.2 Preparation of RNA template

An Ambion Mega-Scripts kit (Austin, Texas) was employed to generate RNA template. To prepare the wild-type or mutated HIV-1 RNA template, plasmid DNA was linearized by digesting with Acc I (Figure 3-1). To prepare the RNA template SP/UC, the plasmid pSP/UC was linearized with Aat II (Figure 3-9).

3.4.3 *In vitro* reverse transcription

These reactions were performed as previously described (Liang et al. 1997). Briefly, $tRNA^{Lys.3}$ or $tRNA^{Lys.1,2}$, prepared from human placenta (Jiang et al. 1993), or synthetic DNA primer D-Lys.3 (5'-GTCCCTGTTCGGGCGCCA-3') or D-Lys.1,2 (5'-GCCCCACGTTGGGCGCCA-3') that is complementary to

wild-type or HIV/Lys.1,2 PBS respectively, was annealed onto RNA template by denaturing at 85°C for 5 min and annealing at 55°C for 10 min in a reaction mixture containing 50 mM Tris-HCl [pH 7.2], 50 mM KCl and 5 mM $MgCl_2$. Reverse transcription reactions were performed in a volume of 20 µl containing 1 pmol of primer:RNA template complex, 50 mM Tris-HCl [pH 7.2], 50 mM KCl, 5 mM $MgCl_2$, 10 mM dithiothreitol, 10 units of RNA-guard (Pharmacia, Montreal, Canada), 160 nM of dNTPs and 45 ng of HIV-1 reverse transcriptase at 37°C for 15 min unless specified, after which reactions were terminated by adding EDTA [pH 8.0] to a final concentration of 50 mM. The cDNA products were fractionated on 8% denaturing polyacrylamide gels containing 7 M urea. The RT preparations used include wild-type HIV-1 enzyme (p66/51) prepared as described (Wöhrl et al. 1993), and wild-type RTs of avian myeloblastosis virus (AMV) (Pharmacia) and murine leukemia virus (MuLV) (Pharmacia). Since wild-type HIV-1 RT (p66/51) possesses RNase H activity, which might interfere in assays that used a DNA primer, a control experiment was also performed with an E478Q mutant RT, *i.e.* RNase H$^-$ RT (Götte et al. 1995). The results indicated that RNase H activity did not affect the pausing patterns in our reaction system (data not shown).

3.5 Results

3.5.1 The initiation of HIV-1 (-) strand DNA synthesis from $tRNA^{Lys,3}$ is characterized by rate-limiting pause sites at nt positions +1 and +3

Initiation of HIV-1 reverse transcription is a distinct step from that of elongation (Isel et al. 1996; Lanchy et al. 1996a,b). To further investigate this subject, we developed an *in vitro* reaction system that employed a template that contained only G, A, and C among the 5 nt at its 5'end (Figure 3-1A). Therefore, when dCTP, dTTP and dGTP were included in these reactions, only early products of reverse transcription were generated. Reactions were also performed with only dCTP (Figure 3-1A, lane 1) or both dCTP and dTTP (Figure 3-1A, lane 2) to provide information on the positions of the first and second bands seen on gels. The results of Figure 3-1A show that reaction products were observed at both the +1 and +3 positions in addition to a final expected product at the +5 site (lane 3). These reactions did not pause at either the +2 or +4 positions, indicating that they were rate-limiting only after the first and third nt were added. To prove that the above-mentioned pause sites (*i.e.* +1 and +3) were not due to an absence of dATP in the reactions, experiments performed with all four dNTPs were terminated at different times (5, 15 or 45 min), with the result that the +1 and +3 pause sites were still present (Figure 3-1A, lanes 4,5,6).

We also performed similar reactions with varying concentrations of RT enzyme. When $tRNA^{Lys,3}$ was used as primer, extension was commonly observed at the +1 and +3 positions. At very low concentrations of RT (*i.e.* 5-15 ng), the most common pause site was at position +1 (Figure 3-1B, lanes 1,2). At increased concentrations of RT, the $tRNA^{Lys,3}$ primer could be further extended to generate +3 and +5 products. However, strong pausing was still observed at the +3 position, even when high amounts of RT enzyme (*i.e.* 405 ng) were employed (lane 5). Similar findings were also obtained in time-course studies when reactions were performed for only 1-4 min, *i.e.* primer extension from $tRNA^{Lys,3}$ occurred

with pausing at nt positions +1 and +3 (Figure 3-1C, lanes 1,2). In contrast, when reactions proceeded for longer times, the +3 and +5 nt products accumulated. In fact, strong pausing at the +3 position was detected even after 64 min of incubation. Therefore, the +1 and +3 pause sites are common events observed during extension of (-) strand DNA from a $tRNA^{Lys.3}$ primer.

We next observed that the +1 and +3 pause sites were also present when increased concentrations of dNTPs, *i.e.* 80 nM, 160 nM, 320 nM, 640 nM, 1.28 μM and 2.56 μM were employed in reactions that yielded higher levels of (-) strand DNA products (Figure 3-1D). However, the +1 nt pausing site became increasingly faint with addition of higher concentrations of dNTP while the +3 nt pause site did not diminish in intensity. This suggests that both pause sites are integral features of HIV-1 reverse transcription reactions and are seen most clearly in reactions performed at dNTP concentrations of 160 nM (Figure 3-1D, lane 2).

3.5.2. The +3 pause site is dependent on sequences at the 5'end but not the 3'end of the PBS

To study the role of sequences at the 5'end of the PBS, we generated a mutated RNA template termed HIV/del-A that contained a deletion of the A-rich loop (622-AAAA-625) and a mutated RNA template HIV/HUA described above (Figure 3-2). The latter construct contained only T, A, and C within the 13 nt at the 5'end of the PBS; hence, when only dATP, dTTP and dGTP are included in reactions with the HIV/HUA template, extension should only proceed to the +13 stage. In reactions performed with 45 ng HIV-1 RT, $tRNA^{Lys.3}$ as primer, and wild-type RNA genome, *i.e.* HIV/WT, three bands at positions +1, +3 and +5 were clearly observed (lane 9). However, when either HIV/del-A or HIV/HUA served as RNA template, only one band at position +1 was seen, even when three different dNTPs were included (lanes 1-6); furthermore, this band was weaker than that seen at the same position with the wild-type RNA template HIV/WT. Considering the (-) strand DNA products at the +3 and +5 positions in

the case of wild-type RNA template HIV/WT, deletion of the A-rich loop or substitutions within the region nt 624-635 led to both greatly diminished efficiency of initiation as well as an arrest of extension at the +1 nt position.

To investigate the effects of HIV/del-A and HIV/HUA on the +3 pausing event, higher quantities of RT, *i.e.* 405 ng, were used to extend reactions beyond the +1 stage. In this circumstance, extended products (both +3 and +5) were observed in reactions performed with the HIV/del-A mutated RNA template (Figure 3-2B, lanes 1-3). However, when HIV/HUA was used, we detected extended products at the +5 and +13 sites and no pausing at the +3 site (lanes 4-6). Therefore, the presence of the A-rich loop (622-625) and maintenance of sequences at positions 624-635 are necessary for a release from the +1 nt pause site to occur; in addition, the strong pausing at the +3 position is dependent on nt sequences 624-635 at the 5'end of the PBS.

We also investigated the 3'end of the PBS in the initiation of (-) strand DNA synthesis through use of appropriate deleted RNA templates, *i.e.* HIV/LD1, HIV/LD2 and HIV/LD3, containing deletions between wild-type positions 654-671, 672-691, and 692-707, respectively (Figure 3-3). When $tRNA^{Lys.3}$ was used as primer, similar band patterns were observed in reactions that used either wild-type RNA template (HIV/WT) or the mutated RNA templates (HIV/LD1, HIV/LD2 and HIV/LD3), although reactions proceeded less efficiently with the latter constructs (Figure 3-3).

3.5.3. Reverse transcription does not pause at the +1 site when initiated from a DNA primer

As shown above, reverse transcription initiated from $tRNA^{Lys.3}$ still paused at the +1 position, even when sequences at both the 5' and 3'ends of the PBS were changed. When the first nt at the 5'end of the PBS was changed from G to T (*i.e.* template HIV/HUA), the addition of the first dATP still represented a rate-limiting step (Figure 3-2A, lanes 4-6). To study whether this was unique to RNA primers, reactions

were also performed with an 18 nt DNA primer, *i.e.* D-Lys.3, bound to the PBS. When only dCTP was included in the reaction mixture, one band was seen at position +1 (Figure 3-4A, lane 1). When both dCTP and dTTP were present, a band corresponding to nt position +2 was observed and that at the +1 position had disappeared (lanes 2-6). When dCTP, dTTP and dGTP were included, two bands corresponding to the +3 and +5 nt pause sites were observed (lanes 7-11). Thus, when a DNA oligomer was utilized as primer, reactions did not pause at the +1 site but did at position +3.

On the contrary, when utRNA$^{Lys.3}$ was used as primer, pausing at the +1 nt position can be seen (Figure 3-4B). In reactions which were incubated with dCTP, dTTP and dGTP for various times, the +3 and +5 nt products were observed and pausing occurred at the +3 position but less frequently than from tRNA$^{Lys.3}$ (Figure 3-4B, lanes 8-10). As a control for our experiments with the 18 nt DNA primer, we also employed an 18 nt RNA primer complementary to the PBS. Consistent with results obtained with tRNA$^{Lys.3}$ or utRNA$^{Lys.3}$ as primer, we found that these reactions also paused at the +1 nt position (data not shown). Hence, the +1 nt pausing event is associated with use of RNA primers during initiation of synthesis of (-) strand DNA.

As stated, the +3 pause site was dependent on the sequences of the template but not the primer (*i.e.* DNA or RNA) used in these reactions. To further verify such template dependence, experiments were performed with the DNA primer D-Lys.3 and the mutated viral RNA templates. The results of Figure 3-5 show that extension from the DNA primer occurred normally in reactions performed with either wild-type (HIV/WT) or the mutated (HIV/del-A, HIV/HUA, HIV/LD1, HIV/LD2, HIV/LD3) RNA templates. In particular, when HIV/HUA was used, no strong pausing was observed at the +3 position (Figure 3-5A, lane 6), consistent with results obtained with the cognate primer, tRNA$^{Lys.3}$ (Figure 3-2B).

3.5.4. Sequences within the PBS affect the efficiency of initiation of (-) strand DNA synthesis

To study whether sequences within the PBS itself were involved in initiation of (-) strand DNA synthesis, we mutated this region to make it complementary to the 18 nt segment located at the 3'end of $tRNA^{Lys.1,2}$, *i.e.* HIV/Lys.1,2. The results show that very little initiation of synthesis of (-) strand DNA occurred from a $tRNA^{Lys.1,2}$ primer when 45 ng HIV-1 RT was used in reactions containing the mutated PBS (Figure 3-6A, lanes 1-3); only a small amount of the +1 product was observed, suggesting that the reactions were totally arrested at the +1 stage (Figure 3-6A, lane 1, 2, 3). Differences in migration rates between $tRNA^{Lys.3}$ and $tRNA^{Lys.1,2}$ in these studies are probably due to sequence differences. When 405 ng HIV-1 RT enzyme was used with $tRNA^{Lys.1,2}$ primer for various times, the +3 and +5 products were observed and pausing occurred at the +3 position but less frequently than from $tRNA^{Lys.3}$, particularly after 45 min (Figure 3-6B, lanes 3, 6).

The initiation of (-) strand DNA synthesis from the mutated RNA template, HIV/Lys.1,2, was also investigated through use of the DNA primer, D-Lys.1,2, (5'-GCCCCACGTTGGGCGCCA-3') that was complementary to the above mutated PBS. We found that this modified DNA primer, D-Lys.1,2, that had annealed to the RNA template, HIV/Lys.1,2, could be extended to the +5 nt stage and that pausing occurred at the +3 position (Figure 3-6C, lane 3) as did reactions that employed wild-type RNA template HIV/WT and the DNA primer, D-Lys.3 (lane 6). Differences in sequences between the two DNA primers used; *i.e.* D-Lys.1,2 and D-Lys.3, were responsible for the more rapid appearance of product from the former than the latter. In addition, the efficiency of initiation from primer $tRNA^{Lys.1,2}$, annealed onto the mutated RNA template, HIV/Lys.1,2, was at least 10 times less than that from the cognate primer $tRNA^{Lys.3}$ annealed onto wild-type RNA template HIV/WT (Figure 3-6A). In assays with DNA as primer, the efficiency of extension from D-Lys.1,2 annealed onto the mutated RNA template, HIV/Lys.1,2 was approximately 5 times lower than that from D-Lys.3 annealed onto wild-type RNA template HIV/WT (Figure 3-6C, lanes 1, 4). Since all reactions were performed with equal amounts of RNA template (1 pmol), it can be

concluded that both the nature of the PBS sequence as well as the structure of the tRNA primer play important roles in the efficiency of initiation of (-) strand DNA synthesis.

3.5.5. Pausing at A-rich loop positions during synthesis of (-) strand DNA

When a still higher dNTP concentration was used, *i.e.* 2.56 μM, four additional pause sites were observed at positions +11, +12, +13 and +14, corresponding to the four As found within the A-rich-loop (622-AAAA-625). These four bands diminished in intensity when reactions were incubated for longer times, *e.g.* 45 min (Figure 3-7, lanes 4-6).

To confirm that these four bands were due to the presence of the A-rich loop in wild-type genomic RNA, the mutated template, HIV/del-A, deleted of these As, was studied. The results of Figure 3-7 show this resulted in elimination of these bands (Figure 3-7, lanes 7-9). When a different RNA template was used, *i.e.* HIV/A2 (deleted of the 4As at positions 622-625 but containing As instead of Gs at positions 620 and 621, thus partially reconstituting a A-rich region), the result was reappearance of the pause sites at positions +11, +12 and +13 (lane 10), although that at the +11 site gradually disappeared as reactions were incubated for longer times (*e.g.* 45 min) (lanes 10-12). Therefore, the A-rich loop is apparently responsible for specific pause sites during synthesis of (-) strand DNA.

3.5.6. Template sequences play a role in determining pausing positions from a DNA primer during initiation of (-) strand DNA synthesis

To understand whether pausing at the +3 position is unique to HIV-1 RT, we performed similar reactions with either AMV RT or MuLV RT in the presence of the DNA primer, D-Lys.3. The results of Figure 3-8 reveal that the +3 pause site was indeed present in reactions performed with these other enzymes (lanes 3, 4, 5 for AMV RT; lanes 8, 9, 10 for MuLV RT); for comparison, see reactions with HIV-1 RT (lanes 13, 14, 15). Therefore, pausing at the +3 site might have been due to the nature of

the complex formed between the RNA template and the primer used in these reactions.

The previous data point to a role for both the PBS and sequences upstream of the PBS in pausing during early stages of (-) strand DNA synthesis. To further study this subject, we performed RT assays using a novel DNA primer, SD, that annealed with sequences located approximately 80 nt downstream of the PBS in wild-type viral RNA HIV/WT. The results of Figure 3-9A showed that reactions paused at almost every step and especially at positions +2 and +5 (lane 3). We also used an alternative RNA template generated from plasmid pUC18, together with a DNA primer, ANI (Figure 3-9B). The results show that only one strong pause site at position +4 occurred when dATP[α-^{32}P], dTTP and dCTP were utilized in the reactions and that little or no pausing occurred except at position +7, that corresponded to the final reaction product (Figure 3-9B, lane 3). Thus entirely different pause sites occur, depending on the type of RNA template utilized.

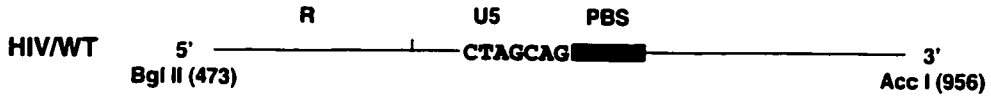
Figure 3-1. Synthesis of HIV-1 (-) strand DNA from primer $tRNA^{Lys,3}$. The wild-type RNA template, HIV/WT, is illustrated. Bands are labeled in regard to number of nucleotides extended from the 3'end of the $tRNA^{Lys,3}$ primer.

(A) Reactions performed with wild-type HIV-1 RNA template (HIV/WT) at a dNTP concentration of 160 nM. Lanes 1-3: reactions performed with 45 ng HIV-1 RT (p66/51) at 37°C for 15 min with dCTP[α-^{32}P] only (lane 1); both dCTP[α-^{32}P] and dTTP (lane 2); dCTP[α-^{32}P], dTTP and dGTP (lane 3). Lanes 4-6: reactions performed with 45 ng HIV-1 RT and all four dNTPs (i.e. dCTP[α-^{32}P], dTTP, dGTP, and dATP) for 5min (lane 4), 15 min (lane 5) and 45 min (lane 6).

(B) Reactions performed at 37°C for 15 min in the presence of 160 nM of dNTPs (including dCTP[α-^{32}P], dTTP and dGTP) and 5 ng (lane 1), 15 ng (lane 2), 45 ng (lane 3), 135 ng (lane 4), 405 ng (lane 5) of RT (p66/51), respectively.

(C) Reactions performed at 37°C with 45 ng HIV-1 RT and 160 nM of dCTP[α-^{32}P], dTTP and dGTP for 1 min (lane 1), 4 min (lane 2), 16 min (lane 3), 32 min (lane 4), and 64 min (lane 5), respectively.

(D) Reactions performed at 37°C for 15 min with 45 ng HIV-1 RT and 80 nM (lane 1), 160 nM (lane 2), 320 nM (lane 3), 640 nM (lane 4), 1.28 uM (lane 5), 2.56 uM (lane 6) of dNTPs (including dCTP[α-^{32}P], dTTP and dGTP).


R
U5
PBS
HIV/WT
5'
CTAGCAG
3'
Bgl II (473)
Acc I (956)

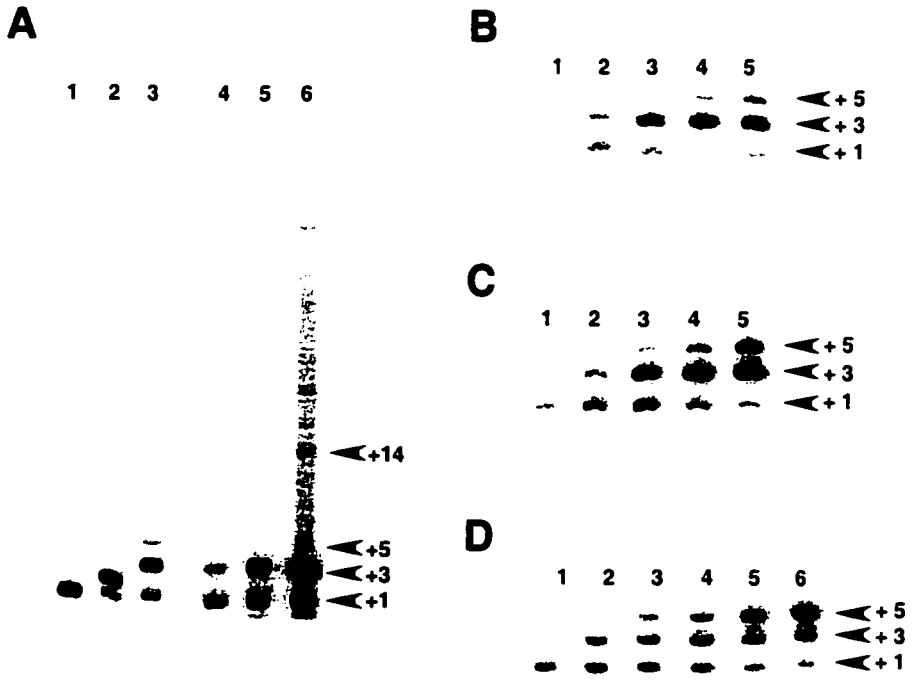
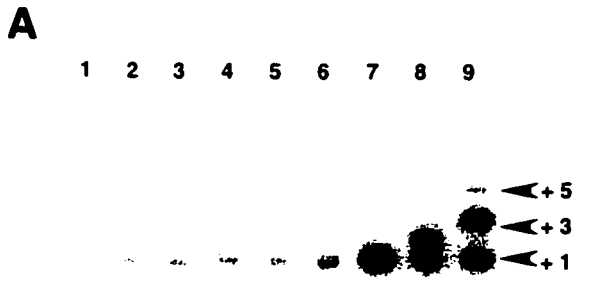

A
1 2 3 4 5 6
+14
+5
+3
+1
B
1 2 3 4 5
+5
+3
+1
C
1 2 3 4 5
+5
+3
+1
D
1 2 3 4 5 6
+5
+3
+1

Figure 3-2. Reverse transcription reactions performed with mutated (HIV/del-A or HIV/HUA) or wild-type (HIV/WT) RNA templates at 37°C in presence of 160 nM dNTPs. The deletion of the A-rich loop is indicated by the dashed line and the substituted nucleotides in HIV/HUA are underlined. The positions of pausing sites are labeled in respect to number of nucleotides extended from the 3'end of the primer.

(A) Reactions were performed with $tRNA^{Lys.3}$ primer using 45 ng HIV-1 RT for 15 min. Lanes 1-3: reactions with mutated RNA template HIV/del-A in presence of dCTP[α-^{32}P] (lane 1), dCTP[α-^{32}P] plus dTTP (lane 2), or each of dCTP[α-^{32}P], dTTP plus dGTP (lane 3). Lanes 4-6: reactions with mutated RNA template HIV/HUA and dATP[α-^{32}P] alone (lane 4), dATP[α-^{32}P] plus dTTP (lane 5), or each of dATP[α-^{32}P], dTTP plus dGTP (lane 6). Lanes 7-9: same order as lanes 1-3 except that wt RNA, i.e. HIV/WT RNA, was used as template.

(B) Reactions using $tRNA^{Lys.3}$ primer and 405 ng HIV-1 RT for various times, i.e. 5 min, (lanes 1, 4, 7), 15 min (lanes 2, 5, 8), and 45 min (lanes 3, 6, 9) in the presence of dCTP[α-^{32}P], dTTP and dGTP for each of the HIV/del-A and HIV/WT templates or dATP[α-^{32}P], dTTP, and dGTP for the HIV/HUA template. In the case of HIV/HUA, reactions can reach the +13 stage even when dCTP is absent, because the 13 nt upstream of the PBS do not include any Gs.


R
U5
PBS
HIV/del-A
5'
TGG----TCTCTAGCAG
3'
Bgl II (473)
Acc I (956)
HIV/HUA
5'
TGGAAGAACACCCAACATT
3'
HIV/WT
5'
TGGAAAATCTCTAGCAG
3'


A
1 2 3 4 5 6 7 8 9
+5
+3
+1

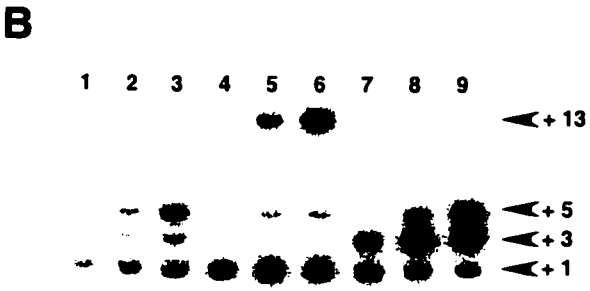
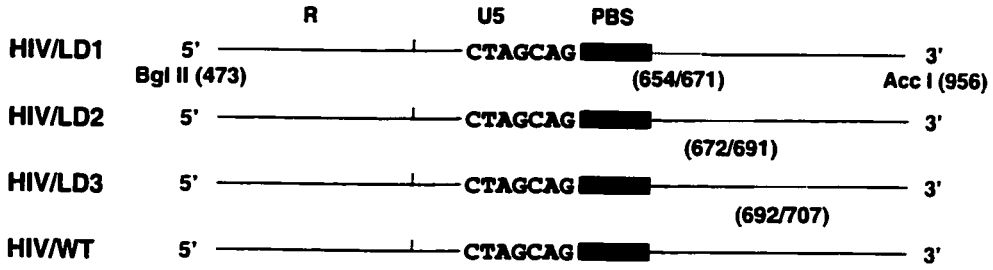

B
1 2 3 4 5 6 7 8 9
+13
+5
+3
+1

Figure 3-3. Effects of sequences downstream of the PBS on initiation of (-) strand DNA synthesis. The RNA templates employed include HIV/LD1 (lanes 1-3), HIV/LD2 (lanes 4-6), HIV/LD3 (lanes 7-9), and HIV/WT (lanes 19-12). Primed by $tRNA^{Lys,3}$, reactions were incubated with 45 ng HIV-1 RT (p66/51) and 160 nM dNTPs at 37°C for 15 min. In lanes 1, 4, 7, and 10, only dCTP[α-^{32}P] was added. In lanes 2, 5, 8, and 11, both dCTP[α-^{32}P] and dTTP were added. In lanes 3, 6, 9, and 12, each of dCTP[α-^{32}P], dTTP, and dGTP was present in the reactions.


R
U5
PBS
HIV/LD1
5'
CTAGCAG
3'
Bgl II (473)
(654/671)
Acc I (956)
HIV/LD2
5'
CTAGCAG
3'
(672/691)
HIV/LD3
5'
CTAGCAG
3'
(692/707)
HIV/WT
5'
CTAGCAG
3'

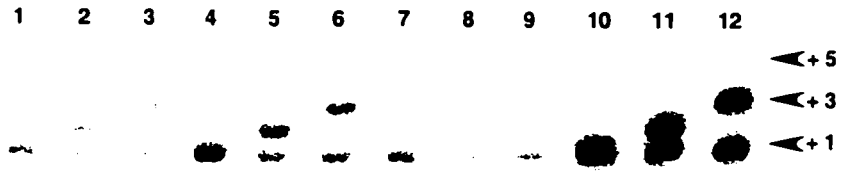
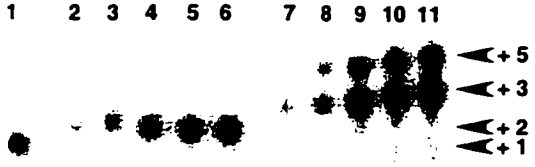

1
2
3
4
5
6
7
8
9
10
11
12
+5
+3
+1

Figure 3-4. Reverse transcription reactions performed with D-Lys.3 or utRNA$^{Lys.3}$. Bands are labeled in respect to number of nucleotides extended from the 3'end of the primer.

(A) Reactions incubated at 37°C with D-Lys.3, 45 ng HIV-1 RT and 160 nM dNTPs. Lane 1: reaction performed for 16 min with only dCTP[α-^{32}P]. Lanes 2-6: reactions performed with both dCTP[α-^{32}P] and dTTP for 1 min (lane 2), 4 min (lane 3), 16 min (lane 4), 32 min (lane 5), and 64 min (lane 6), respectively. Lanes 7-11: reactions performed with each of dCTP[α-^{32}P], dTTP and dGTP for 1 min (lane 7), 4 min (lane 8), 16 min (lane 9), 32 min (lane 10), and 64 min (lane 11), respectively.
(B) Reactions incubated at 37°C with utRNA$^{Lys.3}$, 45 ng HIV-1 RT and 160 nM dNTPs. Lanes 1-5: reactions performed with both dCTP[α-^{32}P] and dTTP for 1 min (lane 1), 4 min (lane 2), 16 min (lane 3), 32 min (lane 4), and 64 min (lane 5), respectively. Lanes 7-11: reactions performed with each of dCTP[α-^{32}P], dTTP and dGTP for 1 min (lane 6), 4 min (lane 7), 16 min (lane 8), 32 min (lane 9), and 64 min (lane 10), respectively.

A



B

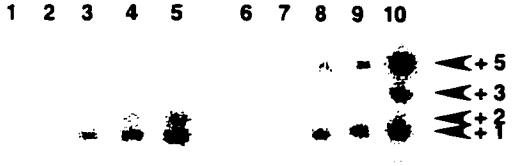
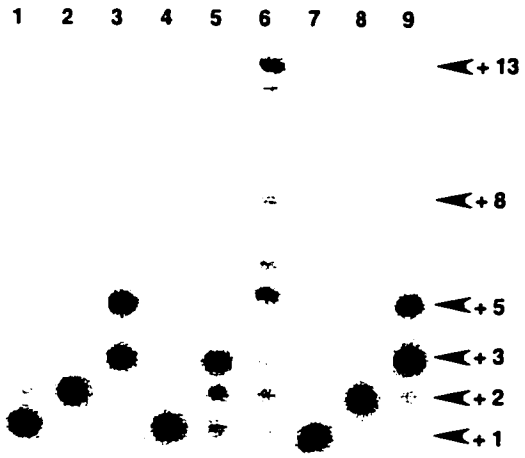


Figure 3-5. Reverse transcription reactions performed with D-Lys.3 and RNA templates with mutations at the 5′ or 3′ end of the PBS. Reactions were initiated at normal levels in spite of various mutations introduced into the RNA templates.

(A) The RNA templates employed included HIV/del-A (lanes 1-3), HIV/HUA (lanes 4-6), and HIV/WT (lanes 7-9). Primed by D-Lys.3, reactions were incubated with 45 ng HIV-1 RT (p66/51) and 160 nM dNTPs at 37°C for 15 min. The order of lanes 1-9 is the same as that of Figure 3-2A.
(B) The RNA templates employed include HIV/LD1 (lanes 1-3), HIV/LD2 (lanes 4-6), HIV/LD3 (lanes 7-9), and HIV/WT (lanes 19-12). Primed by D-Lys.3, reactions were incubated with 45 ng HIV-1 RT (p66/51) and 160 nM dNTPs at 37°C for 15 min. In lanes 1, 4, 7, and 10, only dCTP[α-^{32}P] was added. In lanes 2, 5, 8, and 11, both dCTP[α-^{32}P] and dTTP were added. In lanes 3, 6, 9, and 12, each of dCTP[α-^{32}P], dTTP, and dGTP was present in the reactions.

A



B

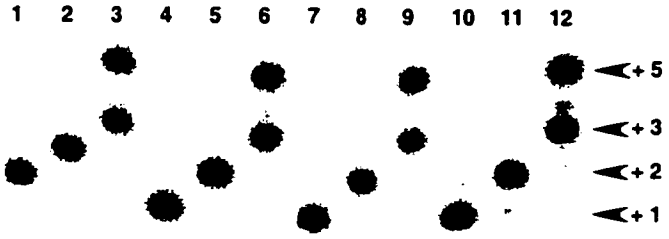
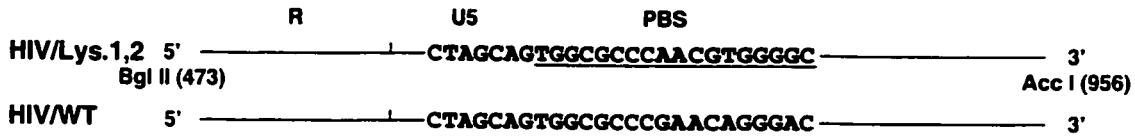


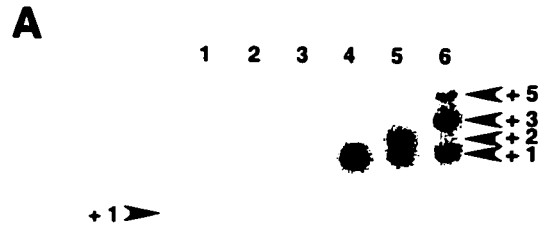
Figure 3-6. Reverse transcription reactions performed with PBS-mutated RNA template (*i.e.* HIV/Lys.1,2). The RNA templates HIV/Lys.1,2 and HIV/WT are illustrated. Bands are labeled in respect to numbers of nucleotides extended from the primer.

(A) Reactions were performed with 45 ng HIV-1 RT for 15 min using $tRNA^{Lys.1,2}$ to prime reactions with the HIV/Lys.1,2 template and $tRNA^{Lys.3}$ to prime reactions with HIV/WT template in the presence of dCTP[α-^{32}P] alone (lanes 1, 4), dCTP[α-^{32}P] plus dTTP (lanes 2, 5), or each of dCTP[α-^{32}P], dTTP and dGTP (lanes 3, 6).

(B) Reactions performed with 405 ng HIV-1 RT for 5 min (lanes 1, 4), 15 min (lanes 2, 5) or 45 min (lanes 3, 6) using $tRNA^{Lys.1,2}$ to prime reactions with the HIV/Lys.1,2 template and $tRNA^{Lys.3}$ to prime reactions with HIV/WT template in presence of dCTP[α-^{32}P], dTTP and dGTP.

(C) Reactions performed with DNA primers, D-Lys.1,2 (lanes 1-3) or D-Lys.3 (lanes 4-6), using 45 ng HIV-1 RT at 37°C for 15 min; nucleotide conditions as in (A).


R
U5
PBS
HIV/Lys.1,2 5'
CTAGCAGTGGCGCCCAACGTGGGGC
3'
Bgl II (473)
Acc I (956)
HIV/WT 5'
CTAGCAGTGGCGCCCGAACAGGGAC
3'


A
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+3
+2
+1
+1


B
1 2 3 4 5 6
+5
+3
+1
+5
+3
+1

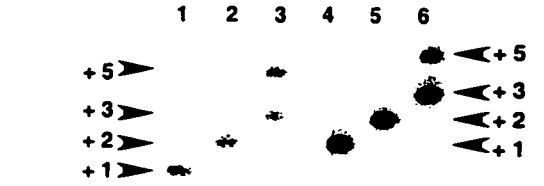
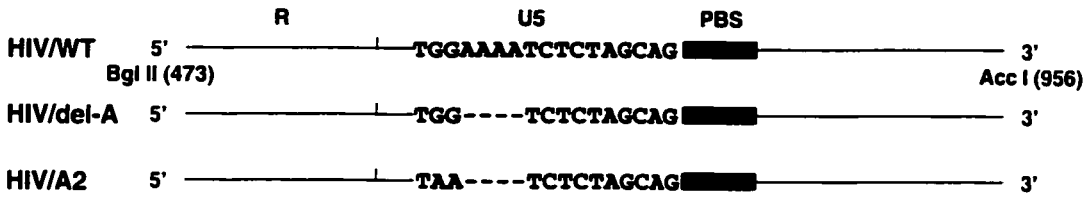

C
1 2 3 4 5 6
+5
+3
+2
+1
+5
+3
+2
+1

Figure 3-7. Pausing sites due to the A-rich loop region (622-AAAA-625) in the synthesis of (-)ssDNA using $tRNA^{Lys,3}$ as primer. The mutated RNA templates, HIV/del-A and HIV/A2, are illustrated. The deletion of the A-rich loop (622-AAAA-625) is indicated by the dashed line. In the mutated RNA template HIV/A2, nucleotides 620-GG-621 have been changed to 620-AA-621. Bands are indicated in respect to number of nucleotides extended from the primer $tRNA^{Lys,3}$. Lanes 1-3: reactions performed with low concentrations of dNTPs, i.e. 160nM, to show positions of early pause sites; dCTP was labeled with [α-^{32}P] (lane 1), both dCTP[α-^{32}P] and dTTP (lane 2), each of dCTP[α-^{32}P], dTTP and dGTP (lane 3). Lanes 4-6: reactions performed with wild-type RNA template HIV/WT in the presence of 2.56 μM dNTPs (including 160 nM [α-^{32}P]dCTP) for various times, i.e. 5 min (lane 4), 15 min (lane 5) and 45 min (lane 6). The full-length (-)ssDNA product was +178 nt. Lanes 7-9: reactions performed with mutated HIV-1 RNA template HIV/del-A containing the deletion of the A-rich loop sequence for 5 min (lane 7), 15 min (lane 8), and 45 min (lane 9). The full-length (-)ssDNA product in this case was +174 nt. Lanes 10-12: reactions performed with mutated HIV-1 RNA template HIV/A2 for 5 min (lane 10), 15 min (lane 11), and 45 min (lane 12). The full-length (-) ssDNA product was +174 nt.


R
U5
PBS
HIV/WT
5'
TGGAAAATCTCTAGCAG
3'
Bgl II (473)
Acc I (956)
HIV/del-A
5'
TGG----TCTCTAGCAG
3'
HIV/A2
5'
TAA----TCTCTAGCAG
3'

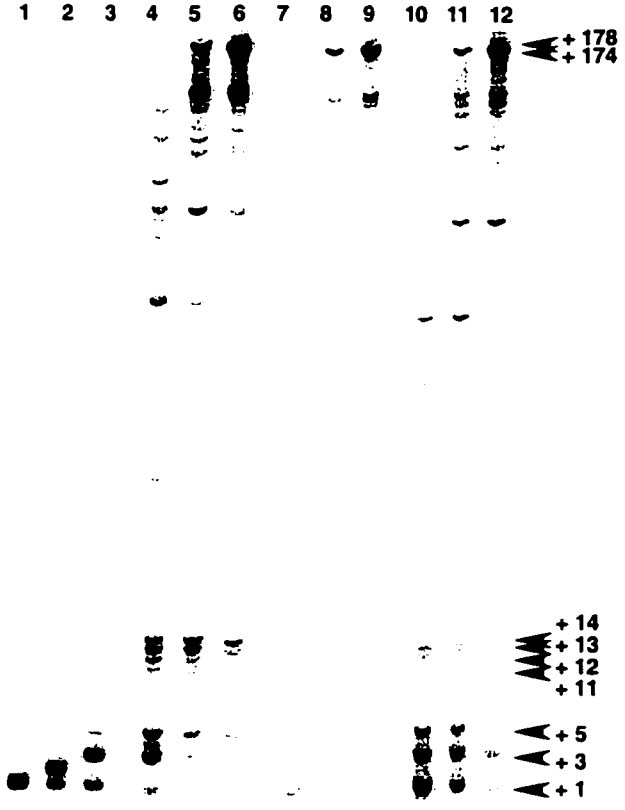

1 2 3 4 5 6 7 8 9 10 11 12
+ 178
+ 174
+ 14
+ 13
+ 12
+ 11
+ 5
+ 3
+ 1

Figure 3-8. Reactions performed at 37°C for 15 min using AMV RT (lanes 1-5), MuLV RT (lanes 6-10) or HIV-1 RT (lanes 11-15). Lanes 1, 6, 11: dCTP[α-^{32}P]; lanes 2, 7, 12: dCTP[α-^{32}P] plus dTTP; lanes 3, 4, 5, 8, 9, 10, 13, 14, 15: dCTP[α-^{32}P] plus dTTP plus dGTP. Reactions in lanes 1, 2, 3, 6, 7, 8, 11, 12, 13, 5 ng RT; lanes 4, 9, 14, 15 ng RT; lanes 5, 10, 15, 45 ng RT.

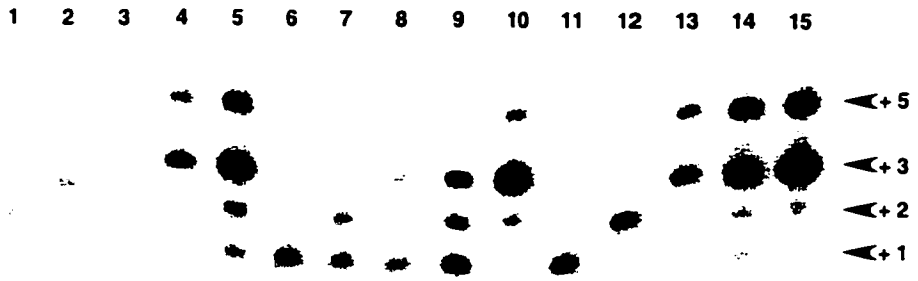
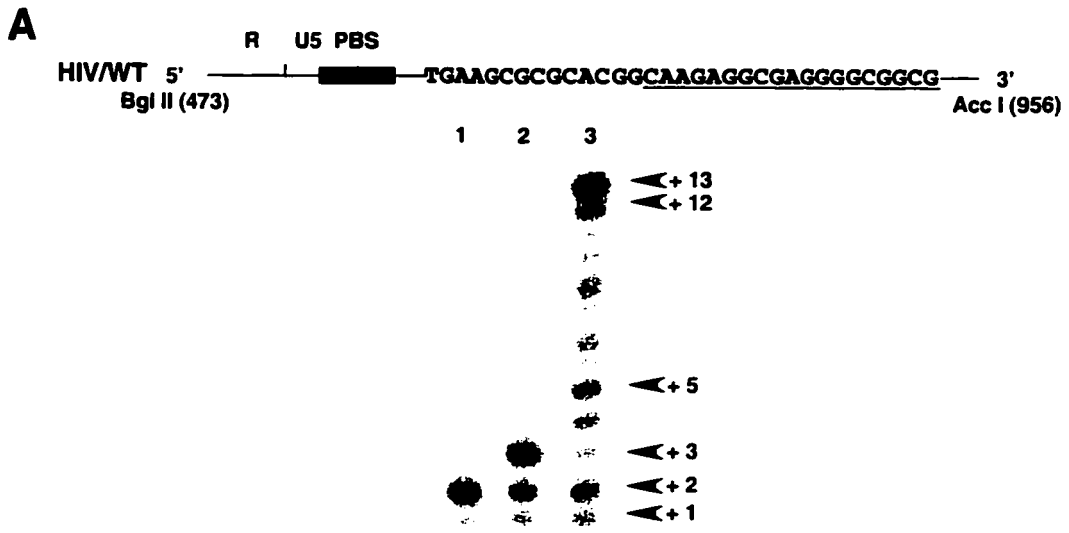
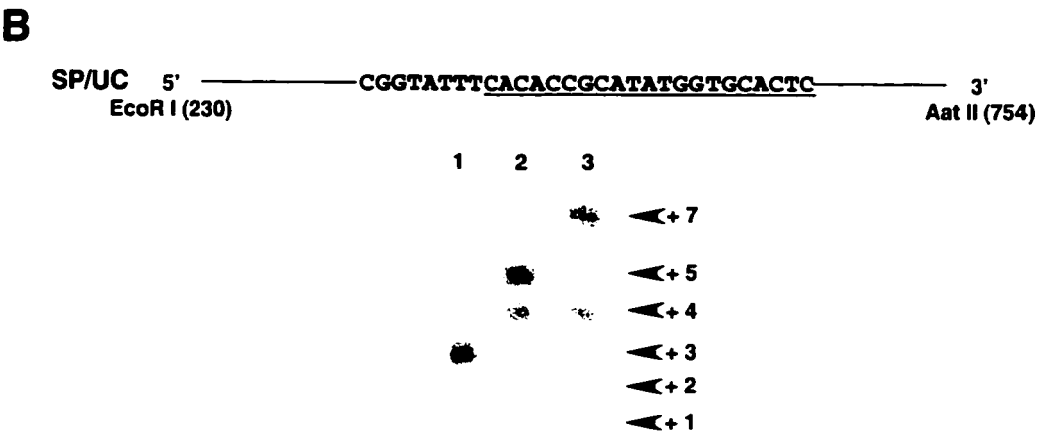

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
+5
+3
+2
+1

Figure 3-9. Reverse transcription reactions initiated from different origins.

(A) Reverse transcription performed with HIV-1 wild-type RNA template (HIV/WT) and antisense primer SD (5'-CGCCGCCCCTCGCCTCTTG-3') (736-718), located downstream of the PBS, using 45 ng HIV-1 RT (p66/51) at 37°C for 15 min. The DNA sequences in viral RNA to which the primer could bind are underlined. Bands are labeled in respect to number of nucleotides extended from the 3' terminus of primer SD. The dCTP was labeled with [α-^{32}P] in each case.

(B) Reverse transcription reactions performed with the RNA template SP/UC derived from plasmid pUC18 (230-754) and the antisense primer ANI (5'-GAGTGCACCATATGCGGTGTG-3') (510-490) using 45 ng HIV-1 RT (p66/51) at 37°C for 15 min. The DNA segment to which the primer ANI could bind is underlined. dATP was labeled with [α-^{32}P] in each case.


A
R U5 PBS
HIV/WT 5'
TGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCG
3'
Bgl II (473)
Acc I (956)
1 2 3
+ 13
+ 12
+ 5
+ 3
+ 2
+ 1


B
SP/UC 5'
CGGTATTTCACACCGCATATGGTGCACTC
3'
EcoR I (230)
Aat II (754)
1 2 3
+ 7
+ 5
+ 4
+ 3
+ 2
+ 1

3.6 Discussion

Biochemical analysis has shown that the initiation of HIV-1 reverse transcription can be distinguished from subsequent elongation (Isel et al. 1996; Lanchy et al. 1996a,b). The biological relevance of this observation is suggested by the fact that RT can lose its ability to discriminate against a non-self tRNA primer when the latter was extended by two nt (Oude Essink et al. 1996). Second, *in vitro* labeling revealed that primer $tRNA^{Lys.3}$ was extended by two nucleotides within virus particles that had engaged in synthesis of (-) strand DNA (Huang et al. 1997b). In our cell-free RT reaction system performed at low dNTP concentration (*i.e.* 160 nM), initiation of HIV-1 (-) strand DNA synthesis has been characterized on the basis of several early pause sites, including +1 and +3.

This is the first demonstration that pausing at the +1 nt site represents a rate-limiting step in reverse transcription reactions performed with an HIV-1 RNA template and the cognate primer $tRNA^{Lys.3}$. This is not an unexpected finding since HIV-1 RT possesses both RNA-dependent DNA polymerization (RDDP) and DNA-dependent DNA polymerization (DDDP) activities. During reverse transcription, the enzyme is bound to either RNA-DNA or DNA-DNA hybrids during RDDP and DDDP, respectively, except at the initiation of synthesis of (-) strand strong-stop DNA ((-)ssDNA), when the enzyme is bound to a RNA-RNA hybrid and employs $tRNA^{Lys.3}$ as primer for production of cDNA. After initiation takes place, the role of primer is effectively replaced by the newly-made DNA from which further extension will occur (Skalka et al. 1993). Therefore, the initiation of (-)ssDNA synthesis is a distinct stage of reverse transcription especially in regard to incorporation of the first dNTP.

It is generally believed that a conformational change of RT must precede the chemical step, resulting in a rate-limiting event (Ratner 1985; Kati et al. 1992; Hsie et al. 1993; Reardon 1993; Rittinger et al. 1995). When the first C deoxyribonucleotide from the dNTP pool is

added to the 3'-OH of the A ribonucleotide at the 3'end of $tRNA^{Lys.3}$, displacement of a ribonucleotide-ribonucleotide pair (A-U) must occur in favor of a newly formed deoxyribonucleotide-ribonucleotide pair (dC-G) at the RT polymerization active site. Due to the absence of a 2'-OH residue in dC, the two nucleotide-pairs (A-U and dC-G) may assume different conformations. Therefore, a structural rearrangement of the polymerization active site is required for the RT enzyme to adapt to the new dC-G pair before adding the next deoxyribonucleotide (dT) to the one-base extended primer. Our results demonstrate that this conformational rearrangement occurs as soon as the first dC has been added, and this rate-limiting step results in the pause site at the +1 position. In contrast, the use of the DNA primer, D-Lys.3, necessitates that the duplex to which RT is bound will always be a DNA-RNA hybrid, thus permitting the enzyme to catalyze the extension reaction without a change in conformation. Consequently, reactions primed by the DNA primer, D-Lys.3, do not need to pause after addition of the first dC.

Other studies have also indicated that a conformational rearrangement might be required for RT to proceed after addition of the first nt. Direct evidence for this comes from studies of the relationship between the RNase H cleavage site and the polymerization active site. Generally, a constant distance of 18 nt must exist between the RNase H cleavage site and the nascent primer 3' terminus. However, a distance of 19 nt instead of 18 nt was observed between these sites after incorporation of the first dC at the 3'end of primer $tRNA^{Lys.3}$ (Götte et al. 1995). Since the spatial relationship between the functional RNase H cleavage site and the polymerization active site serves as an indication of RT conformation, the 19 nt distance suggests a novel conformation for RT after incorporation of the first nt. Further supporting this notion, mutagenesis studies of the HIV-1 RT palm subdomain have shown that RNA and DNA primers may be differentially recognized by RT, *i.e.* RT may be associated with RNA vs DNA primers in different conformations (Ghosh et al. 1997). This helps

to account for the importance of the +1 pause site demonstrated in this paper.

We have also noted that the synthesis of (-) strand DNA paused at the +3 nt site, regardless whether $tRNA^{Lys,3}$ or a DNA primer was employed. We found that the sequence of the viral RNA template played an important role in the specification of this pause site. The results of Figure 3-2B and Figure 3-5A showed that when a mutated RNA template, HIV/HUA, containing substitutional changes at the 5'end of the PBS (624-635) was employed, pausing no longer occurred at the +3 position. In addition, the use of a different DNA primer (*e.g.* SD) or altered RNA templates (*e.g.* SP/UC) also resulted in a different set of pausing sites (Figure 3-9). Similar findings have been obtained in studies performed with mutated feline immunodeficiency virus (FIV), equine immune IA virus (EIAV), and HIV-1 RNA templates (Arts et al. 1996), suggesting that template sequence is also responsible for the nature of pausing seen during initiation of (-) strand DNA synthesis.

Our data add significantly to the notion that the initiation represents a distinct phase in HIV-1 reverse transcription reactions. First, we have documented that a pause site at the +1 position is rate-limiting, which may be caused by the conformational rearrangement of RT required to adapt the newly formed RNA-DNA hybrid structure. The failure of other groups to have previously detected this site might be due to their use of higher concentrations of dNTPs (*e.g.* 50 μM). Also, the $tRNA^{Lys,3}$ employed in our experiments is from human placenta rather than from other animal species. Since all retroviruses use tRNAs as primers, the +1 pausing site may be common to all RTs. Second, on the basis of our studies, the +3 pause site strongly depends on the sequence of the viral RNA template, especially the region at 5'end of PBS. Conceivably, the +1 position may represent the first point of transition, and the +3 pause site may be involved in the arrest of reactions at late initiation stages.

Through mutagenesis studies, more details were provided about the essential role of the intermolecular interactions formed between primer

$tRNA^{Lys,3}$ and the viral RNA template on the efficient initiation of (-) strand DNA synthesis. When sequences that flank the PBS were mutated, the result was a diminution in efficiency of initiation of synthesis of (-) strand DNA (Li et al. 1996, 1997; Liang et al. 1997; this study). This effect was especially pronounced when the A-rich loop was mutated, resulting in a virtual arrest of DNA synthesis from $tRNA^{Lys,3}$ at the +1 stage (Figure 3-2). Our data also confirm that the PBS is itself important for efficient initiation of (-) strand DNA synthesis (Figure 3-6). In other systems (*e.g.* avian retroviruses, hepadnaviruses, mitochondrial plasmids, bacteria, group II introns, and the yeast retrotransposon Ty1), the initiation of reverse transcription also depends on specific interactions between a primer and genomic RNA (Alyar et al. 1992, 1994; Friant et al. 1996; Shimamoto et al. 1993; Wang et al. 1993; Wang et al. 1992; Wilhelm et al. 1994; Zimmerly et al. 1995). In these cases, both the PBS and its flanking sequences serve as an origin of reverse transcription, by forming a specific secondary structure with primer tRNA, a situation similar to DNA replication in which specific sequences form an origin for DNA polymerase.

The role of the A-rich loop in HIV-1 reverse transcription and viral replication is controversial (Berkhout 1997). Mutations in the A-rich loop resulted in severely diminished initiation efficiency of HIV-1 reverse transcription, trapping primer extension from $tRNA^{Lys,3}$ at the +1 stage, as well as defective transition from initiation to elongation (Isel et al. 1996; Liang et al. 1997; Figure 3-2 of this study). However, our use of viral RNA template deleted of the A-rich loop, HIV/del-A, resulted in only a modest decrease in amount of final (-) strand DNA product (174 nt) (Figure 3-7). Consistently, viruses containing a deletion of the A-rich loop were only moderately impaired in replicative capacity (Liang et al. 1997). The present studies show that the A-rich region at positions 622-625 is important for both the efficient initiation of (-) strand DNA synthesis, as well as pausing that subsequently occurs at the +11-+14 positions. Deletion of the A-

rich loop resulted in impairment of initiation but also in a compensatory effect due to loss of the aforementioned pause sites. This may help to explain the ability of viruses deleted of the A-rich loop to sustain initial rounds of viral replication until the development of compensatory mutations in the region of this deletion (Liang et al. 1997).

Chapter 4

The Zn Finger Motifs of HIV-1 Nucleocapsid Protein Are Necessary for the Formation of a $tRNA^{Lys,3}$:vRNA Binary Complex that Is Favored during Initiation of (-) Strand DNA Reverse Transcription

This chapter was adapted from a manuscript in preparation. All data presented in this chapter were from experiments performed by myself under the supervision of Dr. Wainberg. Dr. B. P. Roques provided synthetic HIV-1 NC protein. Dr. C. Liang provided assistance in the planning of some experiments and analysis of results.

4.1 Preface

In Chapter 2, we demonstrated that NC facilitated the formation of an active tRNA:vRNA complex which showed efficient transition from initiation to elongation in the synthesis of (-)ssDNA. In Chapter 3, we described the early pausing events during the initiation of HIV-1 reverse transcription at nt positions +1 and +3, respectively. Here, we further characterize the role of synthetic HIV-1 NC protein in the initiation of reverse transcription. We found that a tRNA:vRNA binary complex, formed in the presence of NC, can overcome the +1 nt pausing during initiation of the RT reaction; the intact Zn finger structure was required for NC to perform this function. In contrast, the +3 nt pausing site was shown to be dependent on the stability of a 8-base-pair stem structure 3 nt upstream of PBS, that is formed solely by template RNA sequences within the tRNA:vRNA complex. These observations suggest that the +1 and +3 pausing events represent two distinct phases during initiation of reverse transcription. The former may involve a conformational change in RT to adapt to a newly present deoxy-ribonucleotide 3'-OH instead of a ribonucleotide 3'-OH at the 3'end of the tRNA primer. The occurrence of the latter is due to the dissociation of RT from the initiation complex, when it encounters the first stem structure on the RNA template.

4.2 Abstract

In HIV-1, $tRNA^{Lys,3}$ and viral RNA template form a specific complex that is characterized by extensive inter- and intra-molecular interactions. The initiation of (-) strand DNA synthesis from this complex can be distinguished from the subsequent elongation stage based on reverse transcriptase binding and kinetic properties (Lanchy et al. 1996a,b). Reverse transcription initiation can be detected by the early pausing events at +1, +3 and +5 nt positions (Isel et al. 1996; Liang et al. 1998a; Chapter 3 of this paper). In this study, we tried to examine the role of NCp7 at the initiation stage of reverse transcription. Surprisingly, we found that the use of wild-type NC protein with intact Zn finger motifs helped RT to escape pausing at the +1 but not at the +3 nt position. This observation indicates that the +1 pausing event represents a distinct step during initiation of reverse transcription that may involve a conformational change in RT. To further pursue the cause of the accumulation of the +3 nt intermediate product, we focused on a stem structure at the 5'end of the PBS that is formed by the template RNA sequence within the tRNA:vRNA complex. A series of mutations was introduced to disrupt the stability of this stem structure, and the results show that the formation of the +3 nt pausing event was due to the need to dissolve a lower C-G base pair in the aforementioned stem structure before incorporation of dCTP at the +4 nt position during initiation of reverse transcription.

4.3 Introduction

HIV-1 reverse transcription is initiated as its cognate primer $tRNA^{Lys,3}$ is annealed onto the primer binding site (PBS) of viral RNA template (vRNA). In addition to the base-pairing between the PBS sequence and the 3'-terminal region of $tRNA^{Lys,3}$, the resulting tRNA:vRNA binary complex is also characterized by other extensive but specific inter- and intra-molecular interactions (Isel et al. 1993, 1995; Skripkin et al. 1996). When $tRNA^{Lys,3}$ was employed as primer, reverse transcription by homologous RT showed fast and efficient initiation as compared with the inefficient initiation by heterologous RTs. However, when an 18 mer oligodeoxyribonucleotide (ODN) primer, with a sequence complementary to the PBS, was used to prime (-) strand DNA synthesis, the advantage of homologous RT in the reverse transcription was lost. This observation suggests a specific initiation stage of reverse transcription primed from $tRNA^{Lys,3}$, while the reaction primed from ODN resembles the non-specific elongation stage that can be distinguished functionally from initiation (Isel et al. 1996). A transition from initiation to elongation occurs when $tRNA^{Lys,3}$ is used as primer, which is caused by dissociation of RT from the initiation complex after incorporation of three or five nucleotides that are seen as short intermediate products, *i.e.*, +3 and +5 products (Isel et al. 1996).

Before RT reaches the end of initiation and dissociates from the complex at the +3 or +5 position, reverse transcription of HIV-1 might also undergo other very early initiation states. One of these is the pausing event after incorporation of the first nucleotide, especially when reverse transcription is performed at low dNTP concentrations, *e.g.*, 160 nM (Liang et al. 1998a; Chapter 3 of this thesis). Unlike the +3 and +5 pausing, the +1 pausing event is quite specific, since it can not be observed when an ODN is used to prime reverse transcription; this implies that +1 pausing event is a distinct and unique stage during initiation of reverse transcription. This notion has been further pursued in this study.

NCp7 plays an essential role in the stimulation of rapid primer annealing (Prats et al. 1988; Barat et al. 1989; de Rocquigny et al. 1992; Lapadat-Tapolsky et al. 1995). The resultant tRNA:vRNA complex shows an elevated switch from initiation to elongation in the subsequent synthesis of (-)ssDNA (Rong et al. 1998; Chapter 2 of this thesis). In this study, we studied NC-derived $tRNA^{Lys.3}$:vRNA in our *in vitro* reverse transcription reactions in the presence of 160 nM of dNTPs. Our results show that use of NC protein can help RT escape from the +1 but not +3 pausing event, and that intact Zn finger motifs in NCp7 are necessary in this regard.

The binding of the $tRNA^{Lys.3}$ primer to the PBS results in a complex with extensive secondary structure in the region of the PBS and its flanking sequences. The three nt (GAC) at the 5'end of the PBS are looped out, whereas the immediately upstream eight nt (+171-+178) bind to another upstream complementary template sequence (+134-+141) to form a stable stem structure, as illustrated in Figure 4-1A (Isel et al. 1993, 1995; Skripkin et al. 1996). Therefore, when reverse transcription from the primer reaches the +3 stage (dG), the hydrogen bonds of the bottom base pair of the stem has to be disrupted before the forth nucleotide (dC) can be incorporated; this may result in pausing at the +3 nt position. To test this hypothesis, we introduced a series of mutations into the bottom 1, 3, or 5 base pairs of the stem to destabilize this structure. The effects of these mutations on +3 pausing were studied in an *in vitro* reverse transcription system, in which early cDNA products can be detected.

4.4 Materials and Methods

4.4.1 Plasmids construction

Primers used in the mutagenesis studies are listed in Table 4-1. The mutated HIV-1 RNA templates N1-N8 were generated by PCR using primer pairs Bgl-S/Nar-A(1-8). After digestion with Bgl II and Nar I, the PCR products were inserted into an RNA transcription vector PBS/WT that contains a HIV-1 DNA sequence between nt positions 473 to 1417 (Arts et al. 1994). Because the mutated sequences of N2 and N6 include a Bgl II restriction site, a partial digestion method was employed for their construction. The same mutations were also introduced into BH10 using Hpa-S/Nar-A(1-8) as primer which includes the Hpa I and Nar I restriction sites. The sequences and structures of wild-type and mutated RNA templates are illustrated in Figures 4-1A, 4-1B, 4-4A, 4-5A, 4-7A, and 4-8A, respectively.

4.4.2 Preparation of synthetic NCp7 and RNA template

A series of HIV-1 NCp7 peptides, including the wild-type form of 72 amino acids as well as $H^{23}C$ NC and ddNC, with mutated Zn fingers, was prepared by solid phase chemical synthesis as described in Chapter 2 (de Rocquigny et al. 1991). The RNA transcription plasmids were linearized by BssH II, and used as template in an Ambion Mega-Scripts kit (Austin, TX) to produce RNA transcripts. The integrity of the RNA transcripts was routinely checked on 5% polyacrylamide gels containing 7M urea prior to use in reverse transcription assays.

4.4.3 Viral RNA isolation

COS-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum, and were transfected with HIV-1 DNA constructs by the calcium phosphate method (Sambrook et al. 1989). Progeny virus was harvested 48h after transfection. Culture supernatants (20 ml) were clarified in a Beckman GS-6R centrifuge at 3,000 rpm for 30 min at 4°C and quantified on the basis of viral CA

antigen (Ag) levels by enzyme-linked immunosorption assay (Abbott Laboratories, Abbott Park, Ill.). Virus particles were then pelleted through a 20% sucrose cushion at 40,000 rpm for 1h at 4°C, using an SW41 rotor in a Beckman L8-M ultracentrifuge. Total viral RNA was extracted from viral pellets by Trizol Reagent (GIBCO BRL, Montreal, Quebec, Canada), and dissolved in DEPC-treated double distilled water at 50 ng of the CA Ag/µl (final concentration).

4.4.4 *In vitro* reverse transcription

1 pmol of $tRNA^{Lys.3}$ that was prepared from human placenta (Jiang et al. 1993) was annealed onto 1 pmol of RNA template by incubation with 30 pmol of NCp7 at 37°C for 1h or, alternatively, by denaturing at 85°C for 5 min and annealing at 55°C for 10 min, in a 10 µl reaction mixture containing 50 mM Tris-HCl [pH 7.2], 50 mM KCl, 5 mM $MgCl_2$. In order to determine whether NC protein functioned during initiation of reverse transcription at the primer placement or at primer extension phase, Proteinase K digestion and phenol:chloroform extraction was also performed in some cases (Rong et al. 1998; Chapter 2 of this thesis). In the case of RNA isolated from virus, $tRNA^{Lys.3}$ is already naturally annealed onto the viral RNA template (Huang et al. 1997b); therefore, these tRNA:vRNA complexes were directly subjected to the following *in vitro* reverse transcription reactions.

Primer tRNA was extended by reverse transcriptase in a volume of 20 µl containing 50 mM Tris-HCl [pH 7.2], 50 mM KCl, 5 mM $MgCl_2$, 10 mM dithiothreitol, 10 units of RNA-guard (Pharmacia, Montreal, Canada), and 160 nM dNTPs at 37°C for 15 min unless specified, after which reverse transcription reactions were terminated by adding EDTA [pH 8.0] to a final concentration of 50 mM. The cDNA products were fractionated on 8% denaturing polyacrylamide gels containing 7 M urea. The RTs used in this study were prepared as described (Wöhrl et al. 1993), including wild-type HIV-1 enzyme (p66/51), mutated HIV-1 RT containing a mutation at codon 89 (*i.e.* E89G), or mutated HIV-1 RT with a mutation at codon

184 (*i.e.* M184V). 45 ng wild-type RT or 50 ng E89G RT were used in the reactions. Since M184V RT exhibits reduced processivity during reverse transcription (Back et al. 1996; Boyer and Hughes 1995), higher concentration, *e.g.*, 250 ng of M184V RT, was employed to achieve similar cDNA synthesis level as that of wild-type enzyme (data not shown).

4.5 Results

4.5.1 NC protein helps reverse transcription escape from the +1 pausing event

To study whether NC protein could affect the +1 and +3 pausing events in reverse transcription reactions primed with $tRNA^{Lys,3}$, 30 pmol of NC protein were added to our 20 µl reaction system for tRNA placement. Subsequent RT reactions were carried out for various periods (1, 4, 16, 32, and 64 min) either in the presence of NC (Figure 4-2, lanes 5-10), or in the absence of NC, which was achieved by Proteinase K and phenol: chloroform treatment prior to reverse transcription (Figure 4-2, lanes 11-15). As a control, similar time course experiments were performed with heat-annealed tRNA:vRNA complex (Figure 4-2, lanes 1-5). The data show that NC significantly diminished the pausing event at the +1 nt position, while not affecting the + 3 nt pausing. Interestingly, even though NC was removed before the start of reverse transcription, +1 pausing was still dramatically decreased in reactions initiated from the tRNA:vRNA complex preformed by NC (Figure 4-2, lanes 11-15). Therefore, NC carries this specific function through the formation of an active tRNA:vRNA complex, and not by direct action in RT reactions. Furthermore, more +5 nt product was generated in the presence of NC protein, suggesting that NC had contributed toward driving initiation of reverse transcription from early phase to late phase.

4.5.2 The role of NC protein in initiation of reverse transcription is dependent on intact Zn finger motifs

Our previous work had already shown that the Zn fingers in NCp7 were essential for the formation of primer:template complex able to efficiently elongate RT reactions (Rong et al. 1998; Chapter 2 of this thesis). We next examined two mutated NC proteins in the initiation of RT reactions: one is $H^{23}C$ NC, in which a His at the 23 position was substituted by a Cys, and another is ddNC, in which both of the Zn fingers have been replaced by Gly-Gly linkages. Primer tRNA was placed

onto viral RNA template either by the heat-annealing method, or by various concentrations of the NC proteins, *i.e.*, 5 pmol, 15 pmol, 30 pmol and 45 pmol. Thereafter three of the four of dNTPs were added to initiate reverse transcription. Results of Figure 4-3 show that neither the $H^{23}C$ NC nor the ddNC can achieve release from the +1 pausing site in these reactions (lanes 5-12). Therefore, wild-type Zn fingers are important for NC to promote the formation of an active tRNA:vRNA initiation complex.

4.5.3 The +3 nt pausing event is caused by a viral RNA template stem structure located upstream of the PBS within the tRNA:vRNA complex

When HIV-1 cognate primer $tRNA^{Lys.3}$ anneals with viral RNA to form a tRNA:vRNA complex, a stem structure involving eight base-pairings of template sequences can be observed 3 nt upstream of the PBS (Isel et al. 1993, 1995; Skripkin et al. 1996). A simplified secondary structure of tRNA:vRNA complex showing the sequence composition of this stem is illustrated in Figure 4-1A. The sequences of mutated template RNAs that we designed to destabilize this stem structure are listed in Figure 4-1B.

Since NC protein does not affect the pause event at the +3 nt position, we only employed heat-annealing in the following *in vitro* reverse transcription systems, in which 160 nM of three (*i.e.*, dCTP, dTTP, and dGTP) or all four dNTPs were added to reactions that used mutated RNAs as templates. As a control, wild-type RNA templates were also annealed with $tRNA^{Lys.3}$ by the heat method, following which, one, two, three or four of the dNTPs were added. As shown in Figure 4-4B, lanes 1-3, incorporation of one or two dNTPs gave products at the +1 nt or both the +1 and +2 nt positions. In the presence of dCTP, dTTP and dGTP, pausing at both the +1 and +3 nt positions was seen in addition to the expected five-base extended product. The presence of all three intermediate products, with the addition of all four dNTPs, indicates that their presence in lane 3 is not due to the absence of dATP. Longer cDNA products in lane 4 were labeled at positions +27 nt, +28 nt, and

+40 nt. Because low concentration of dNTPs (*i.e.* 160 nM) were used in these reactions, it is difficult for longer reverse transcription products, *i.e.* > +40 nt, to be seen on the gel.

The mutations introduced into the viral RNA template can be divided into four groups. The first group includes N1 and N2, in which 3 or 5 bases in the left part of the stem were substituted, such that the lower 3 or 5 base-pairs were disrupted (Figure 4-4A). The effect of this change on the +3 nt pausing event is shown in Figure 4-4B, lanes 5-8. In the case of N1, the pausing site at the +3 nt position disappeared (lanes 5, 6), while pausing at the +5 nt site was diminished but still evident; at the same time, more longer cDNA products (*e.g.*, +27 nt, +28 nt, +40 nt) were produced compared with wild-type template. Even stronger perturbation of the stem structure by the N2 mutation caused both a significantly decreased efficiency of initiation as well as of transition from early phase to late phase of initiation, *i.e.* only one base extension product is seen in lanes 7 and 8.

The second group of RNA templates, *i.e.*, N3 and N4, involves mutations in the right half of the stem. In N3, an original G, located at the forth nt upstream of PBS, was changed to a C, such that the bottom hydrogen bond of the stem was destroyed. In N4, a GAU, *i.e.*, the fourth to sixth nt upstream of the PBS, were changed into CGA; therefore, the lower 3 base-pairs were disrupted and replaced by two new hydrogen-bonds (Figure 4-5A). These two mutations both gave rise to relatively complicated pausing patterns in initiation reactions. When all four dNTPs were present in the reactions, neither the +3 nt nor the +5 nt products were seen on the gel (Figure 4-5B, lanes 6 and 8). When only three dNTPs (*i.e.*, dCTP, dGTP, dTTP) were added, pausing at the +3 nt site disappeared as well. However, cDNA products longer than +16 nt were observed alongside the expected +5 nt and +7 nt products (Figure 4-5B, lanes 5 and 7). Therefore, nucleotide misincorporation and elongation from the misincorporated nucleotide must have occurred at template sequences wherever a U was met.

This result was confirmed by use of two mutated forms of RT associated with higher than average base incorporation fidelity, *i.e.*, E89G, and M184V (Drosopoulos and Prasad 1996; Pandey et al. 1996; Wainberg et al. 1996). During reverse transcription of the N4 RNA template, nucleotide misincorporation was either diminished or eliminated in reactions performed with either the E89G or M184V RT (Figure 4-6).

The third group of mutations includes N5 and N6, that are generated by flipping the lower 3 and 5 base pairs (Figure 4-7A). Because the stem structure were preserved, the initiation specific intermediate product at the +3 nt position was, as expected, not affected (Figure 4-7B, lanes 4-8). The efficiency of initiation in reactions performed with these mutated RNA templates was also near wild-type level. However, this group of mutations significantly diminished the efficiency of the switch from the +3 to the +5 stage; cDNA products beyond +3 nt can hardly be seen on the gel.

We also generated N7 and N8, in which the distance between the stem structure and the PBS was increased by the insertion of CAGs (Figure 4-8A). In the case of N7, only one CAG repeat was inserted, and the pausing event at the +3 nt position, associated with wild-type template, moved to a higher position at +6 nt (Figure 4-8B, lanes 5 and 6). When more insertions were introduced into the template, *e.g.* N8, the reaction became defective at the initiation stage. As shown in Figure 4-8B, lanes 7 and 8, the reactions were arrested after the incorporation of first nucleotide.

4.5.4 Initiation of reverse transcription from the virion-derived tRNA:vRNA complex

COS-7 cells were transfected with HIV-1 cDNA constructs containing N1, N3, N4, N5 and N7 mutations. Then, virus particles were harvested, and viral RNA was isolated as described in Materials and Methods. Initiation from the virion-derived tRNA:vRNA complexes was studied by incubation with 45 ng RT, and 160 nM of dCTP, dTTP, dGTP, at 37°C for

15 min. The results of Figure 4-9 show that both the +3 and +5 nt products were observed with RNA from wild-type virus, indicating that initiation is an essential step of reverse transcription, and that this fact can even be detected in virion-derived tRNA:vRNA complexes.

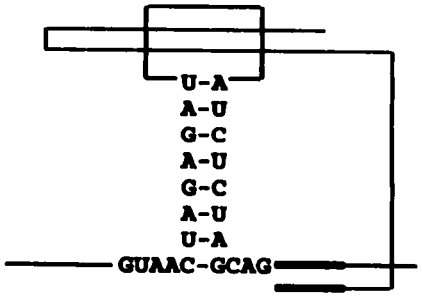
When we compare the pausing pattern in reactions that used either virion-derived tRNA:vRNA complexes, or complexes formed through heat-annealing or NCp7, we found that the virion-derived RNA complexes produced the highest level of final cDNA at +5 nt or above, and NC-annealed tRNA gave rise to the most +3 nt product. In contrast, initiation from the heat-annealed complex was mostly blocked after the first nt incorporation (Figure 4-9).

Table 4-1. Primers utilized in the experiments.

Name	Sequence	Location
Bgl-S	5'-AGACCAGATCTGAGAATGG-3'	468-486
Hpa-S	5'-CTGCAGTTAACTGGAAGGGCTAATTCACTCCC-3'	1-21
Nar-A1	5'-TCGGGCGCCACTGCTAGAGATTTTCCACACTGACTAAAAGGGTCTGAGGGATCTCATCTTACCAGAGTC-3'	646-577
Nar-A2	5'-TCGGGCGCCACTGCTAGAGATTTTCCACACTGACTAAAAGGGTCTGAGGGATCAGATCTTACCAGAGTC-3'	646-577
Nar-A3	5'-TCGGGCGCCACTGGTAGAGATTTTCC-3'	646-620
Nar-A4	5'-TCGGGCGCCACTGGCTGAGATTTTCC-3'	646-620
Nar-A5	5'-TCGGGCGCCACTGGATGAGATTTTCCACACTGACTAAAAGGGTCTGAGGGATCTCATCTTACCAGAGTC-3'	646-577
Nar-A6	5'-TCGGGCGCCACTGGATCTGATTTTCCACACTGACTAAAAGGGTCTGAGGGATCAGATCTTACCAGAGTC-3'	646-577
Nar-A7	5'-TCGGGCGCCACTGCTGCTAGAGATTTTCC-3'	646-620
Nar-A8	5'-TCGGGCGCCACTGCTGCTGCTAGAGATTTTCC-3'	646-620

Figure 4-1. (A) Illustration of the secondary structure of the complex formed between $tRNA^{Lys-3}$ and HIV-1 viral RNA template. (B) Lists of wild-type as well as mutated viral RNA template sequences employed in these reactions.

A


U-A
A-U
G-C
A-U
G-C
A-U
U-A
GUAAC-GCAG

B

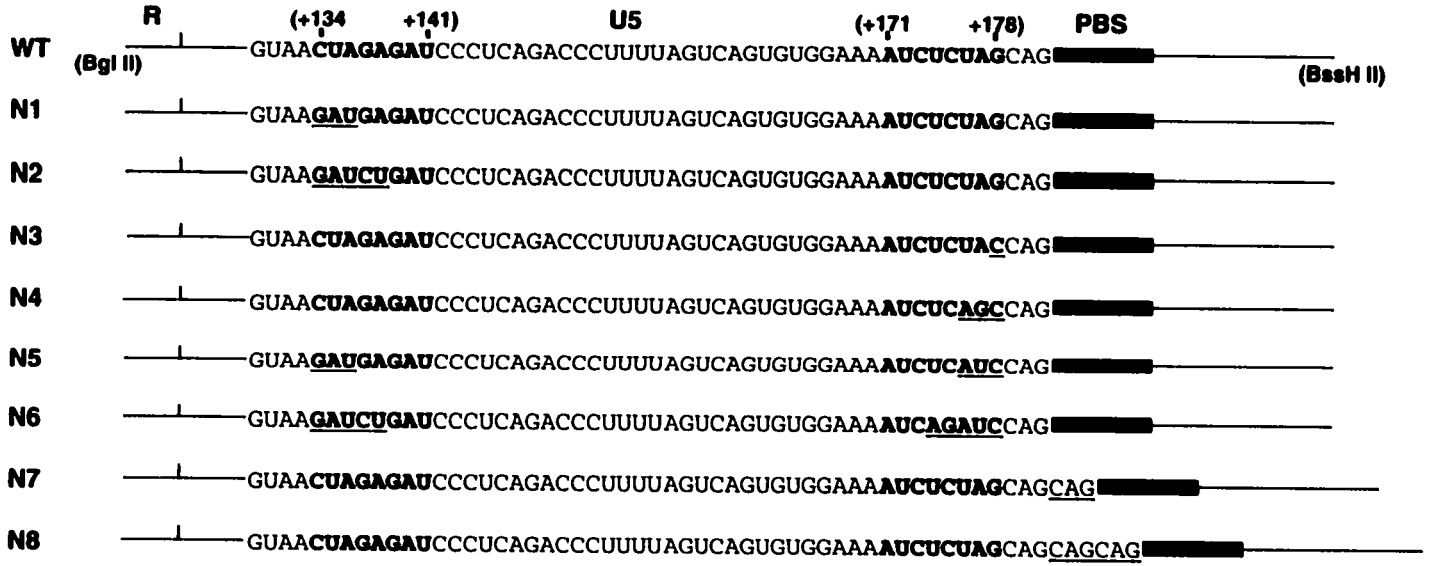

R
(+134
+141)
U5
(+171
+178)
PBS
WT
(Bgl II)
GUAACUAGAGAUCCCUCAGACCCUUUUAGUCAGUGUGGAAAAUCUCUAGCAG
(BssH II)
N1
GUAAGAUGAGAUCCCUCAGACCCUUUUAGUCAGUGUGGAAAAUCUCUAGCAG
N2
GUAAGAUCUGAUCCCUCAGACCCUUUUAGUCAGUGUGGAAAAUCUCUAGCAG
N3
GUAACUAGAGAUCCCUCAGACCCUUUUAGUCAGUGUGGAAAAUCUCUACCAG
N4
GUAACUAGAGAUCCCUCAGACCCUUUUAGUCAGUGUGGAAAAUCUCAGCCAG
N5
GUAAGAUGAGAUCCCUCAGACCCUUUUAGUCAGUGUGGAAAAUCUCAUCCAG
N6
GUAAGAUCUGAUCCCUCAGACCCUUUUAGUCAGUGUGGAAAAUCAGAUCCAG
N7
GUAACUAGAGAUCCCUCAGACCCUUUUAGUCAGUGUGGAAAAUCUCUAGCAGCAG
N8
GUAACUAGAGAUCCCUCAGACCCUUUUAGUCAGUGUGGAAAAUCUCUAGCAGCAGCAG

Figure 4-2. Initiation of reverse transcription from NC-annealed $tRNA^{Lys.3}$ primer. $tRNA^{Lys.3}$:wild-type template RNA complexes were achieved either by heat-annealing (lanes 1-5) or by incubation with synthetic HIV-1 nucleocapsid protein (lanes 6-15), as described in Materials and Methods. Reverse transcription reactions were performed with 45 ng HIV-1 RT (p66/51) and 160 nM dCTP[α-^{32}P], dTTP and dGTP, and were terminated at 1 min (lanes 1, 6, 11), 4 min (lanes 2, 7, 12), 16 min (lanes 3, 8, 13), 32 min (lanes 4, 9, 14), and 64 min (lanes 5, 10, 15), respectively. Lanes 11-15: NC proteins were removed after primer placement; therefore, reverse transcription reactions were initiated in the absence of NC. Bands are labeled in respect to numbers of nucleotides extended from the primer.

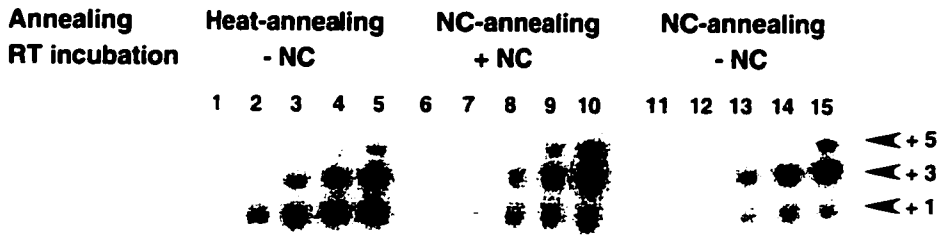

Annealing
RT incubation
Heat-annealing
- NC
NC-annealing
+ NC
NC-annealing
- NC
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
+5
+3
+1

Figure 4-3. Initiation of reverse transcription from $tRNA^{Lys.3}$:vRNA complexes formed by Zn finger mutated NC proteins. Primer:template complexes were achieved by wild-type NCp7 (lanes 1-4), $H^{23}C$ NC (lanes 5-8), and ddNC (lanes 9-12), as described in Materials and Methods. The quantities of NC proteins involved in each reaction are 5 pmol (lanes 1, 5, 9), 15 pmol (lanes 2, 6, 10), 30 pmol (lanes 3, 7, 11), and 45 pmol (lanes 4, 8, 12), respectively. The heat-annealing method was used as a control and these results are presented in the left lane, which is labeled as H. The initiation complexes were then incubated with 160 nM of dCTP[α-^{32}P], dTTP, dGTP, and 45 ng of HIV-1 RT (p66/51) at 37°C for 15 min. The intermediate cDNA products were fractionated on gels and the bands are labeled in respect to numbers of nucleotides extended from $tRNA^{Lys.3}$.

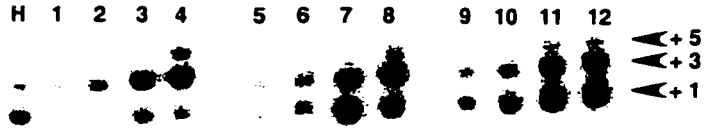

H 1 2 3 4 5 6 7 8 9 10 11 12
+5
+3
+1

Figure 4-4. (A) Structures of mutated N1 and N2 RNA templates within the tRNA:vRNA complexes. (B) Initiation of reverse transcription from the aforementioned complexes. Lanes 1-4 represent experiments performed with wild-type RNA template; lanes 5 and 6 are reactions using N1 template; lanes 7 and 8 are reactions using N2 mutated template. The heat-annealed tRNA:vRNA complexes were incubated with 45 ng of HIV-1 RT (p66/51) at 37°C for 15 min, in the presence of dCTP[α-^{32}P] only (lane 1); both dCTP[α-^{32}P] and dTTP (lane 2); dCTP[α-^{32}P], dTTP and dGTP (lanes 3, 5, 7); and all four dNTPs (lanes 4, 6, 8). The final concentration of each of the dNTPs was 160 nM.

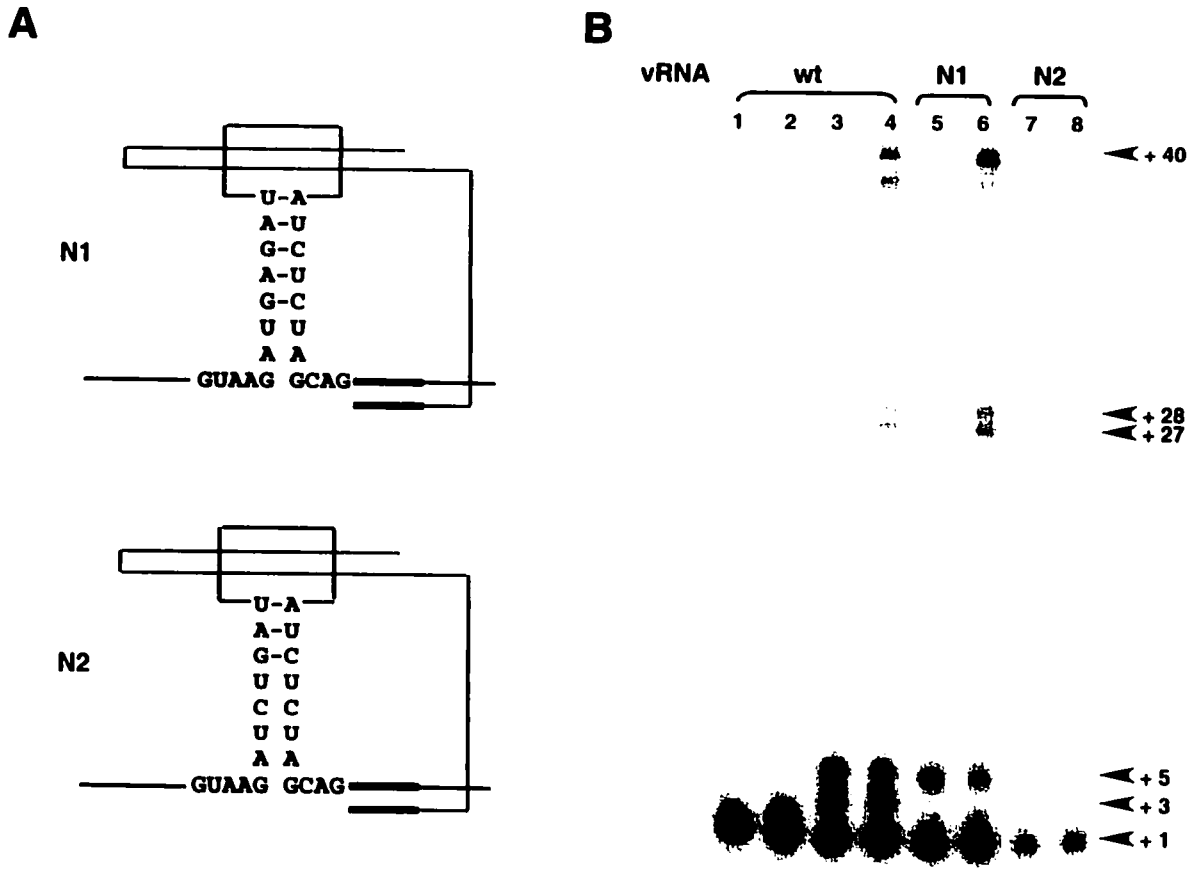

A
N1
U-A
A-U
G-C
A-U
G-C
U U
A A
GUAAG GCAG
N2
U-A
A-U
G-C
U U
C C
U U
A A
GUAAG GCAG
B
vRNA
wt
N1
N2
1 2 3 4 5 6 7 8
+ 40
+ 28
+ 27
+ 5
+ 3
+ 1

Figure 4-5. (A) Structures of mutated N3 and N4 RNA templates within the tRNA:vRNA complexes. (B) Initiation of reverse transcription from these complexes. The order of lanes 1-8 is the same as that of Figure 4-4, except that lanes 5 and 6 are reactions using N3 template; lanes 7 and 8 are reactions using N4 mutated template.

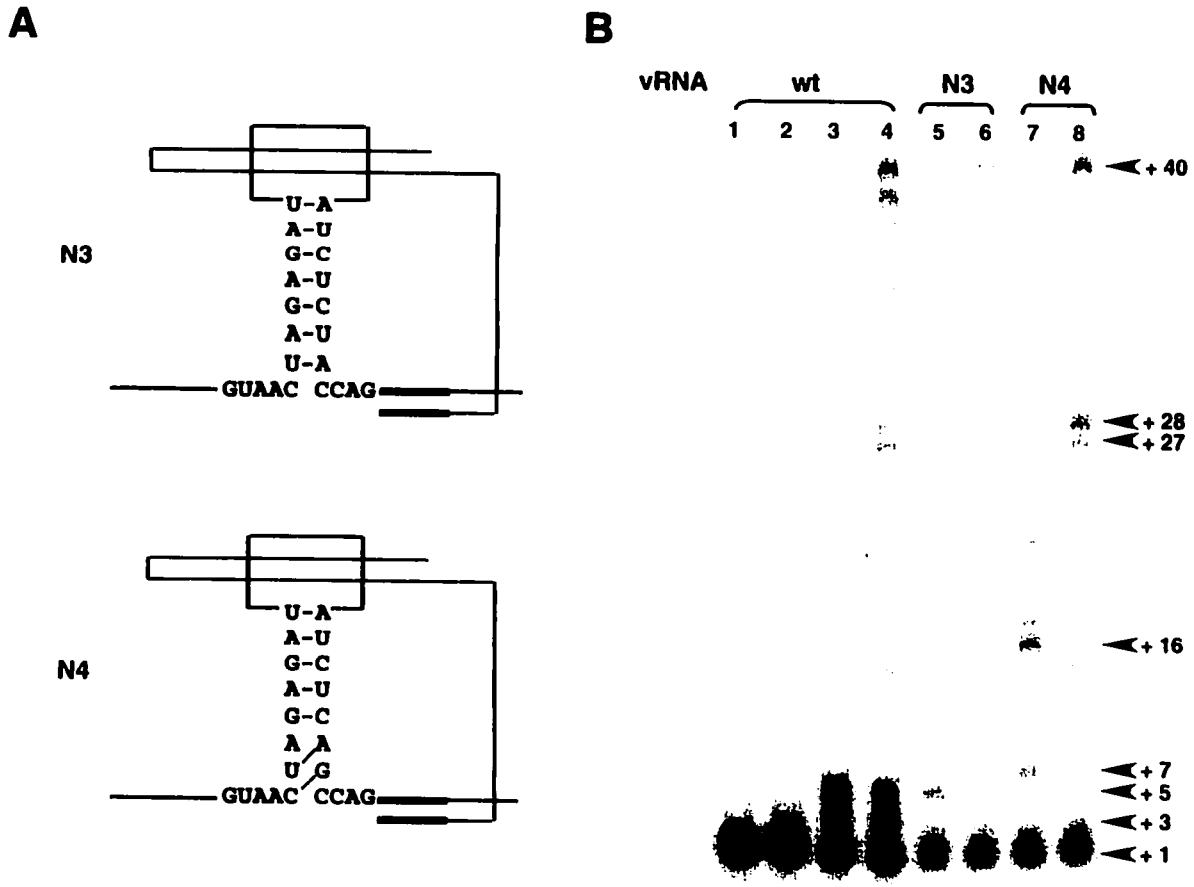

A
N3
U-A
A-U
G-C
A-U
G-C
A-U
U-A
GUAAC CCAG
N4
U-A
A-U
G-C
A-U
G-C
A A
U G
GUAAC CCAG
B
vRNA
wt
N3
N4
1 2 3 4 5 6 7 8
+ 40
+ 28
+ 27
+ 16
+ 7
+ 5
+ 3
+ 1

Figure 4-6. Initiation of reverse transcription of M4 using E89G (A) and M184V (B) mutated RTs. Lanes 1-4 and 9-12 employed 45 ng of wild-type RT (p51/p66) and 160 nM of dCTP[α-^{32}P] (lanes 1, 9), dCTP[α-^{32}P] and dTTP (lanes 2, 10), dCTP[α-^{32}P], dTTP, and dGTP (lanes 3, 11), and all four dNTPs (lanes 4, 12), respectively. The orders of lanes 5-8 and 13-16 are the same as those of lanes 1-4 or 9-12 except that 45 ng of E89G RT (lanes 5-8) or 250 ng of M184V RT (lanes 13-16) were present in the reactions.

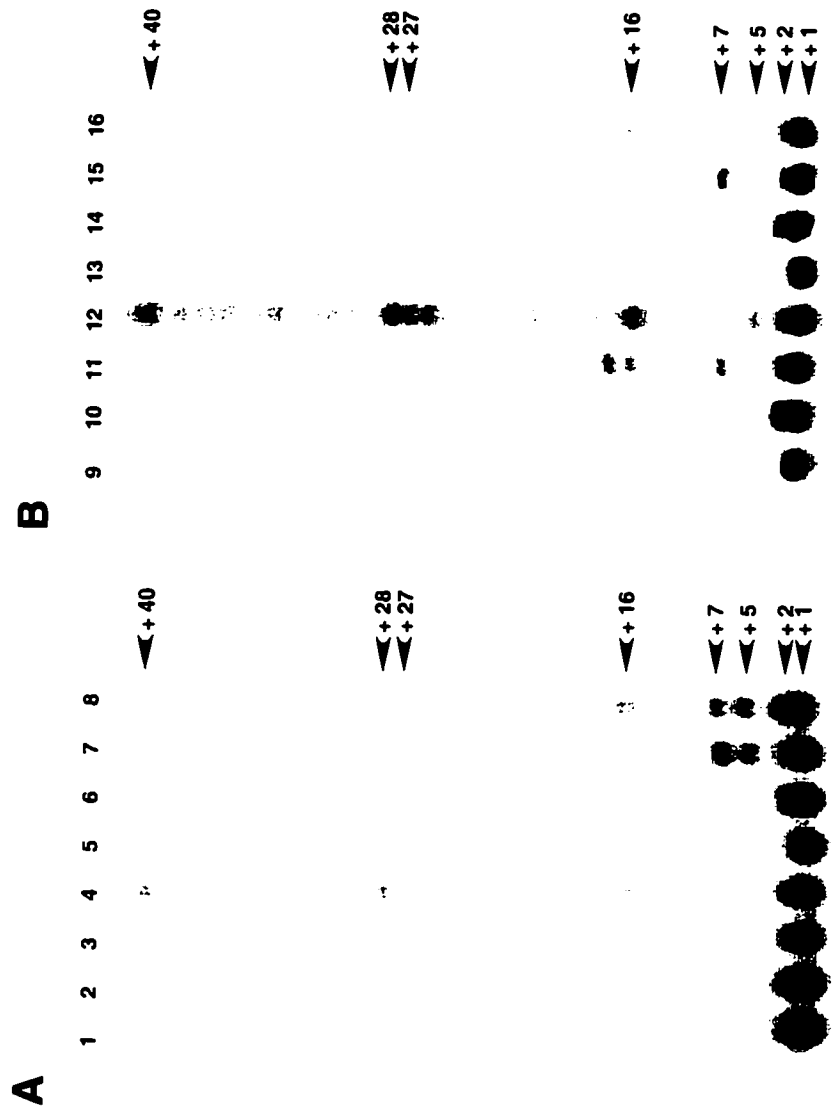

A
B
+40
+28
+27
+16
+7
+5
+2
+1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

Figure 4-7. (A) Structures of mutated N5 and N6 RNA templates within the tRNA:vRNA complexes. (B) Initiation of reverse transcription from these complexes. The order of lanes 1-8 is the same as that of Figure 4-4, except that lanes 5 and 6 are reactions using N5 template; lanes 7 and 8 are reactions using N6 mutated template.

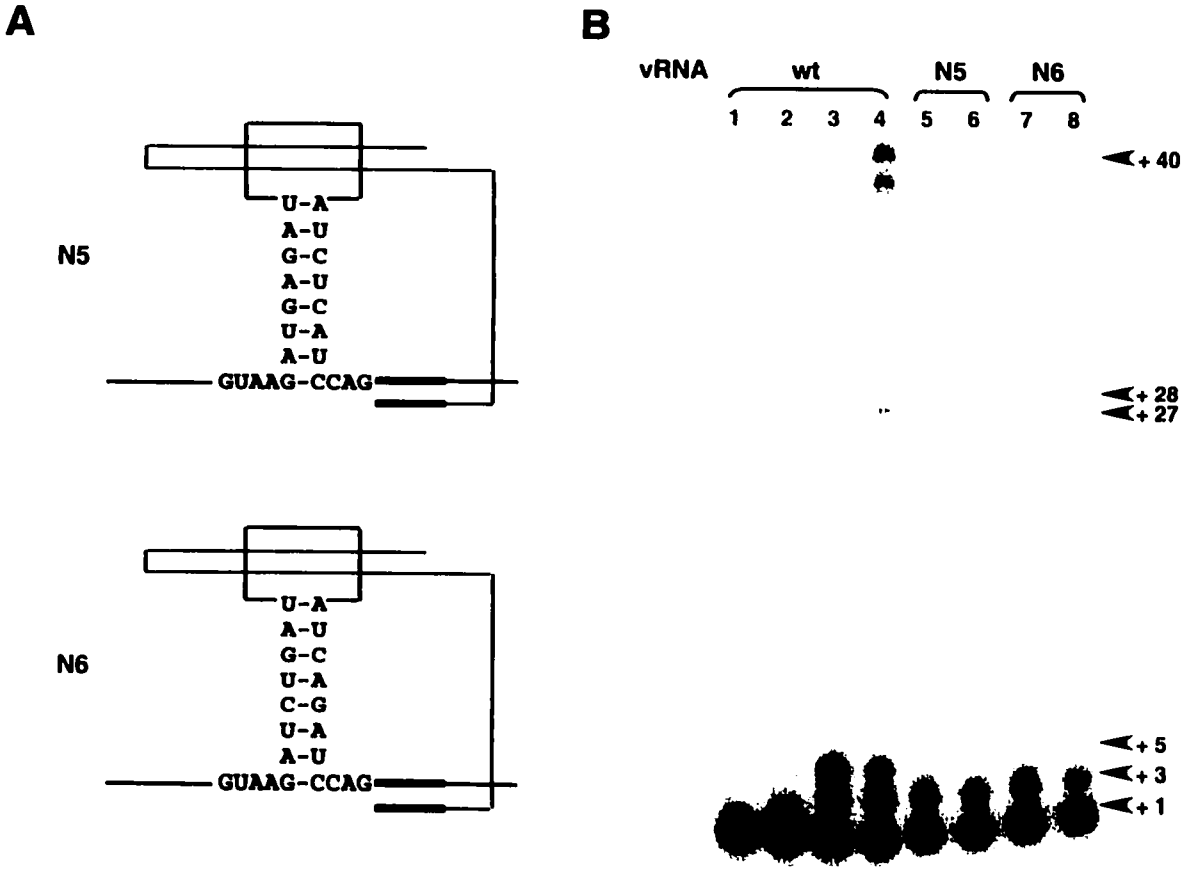

A
N5
U-A
A-U
G-C
A-U
G-C
U-A
A-U
GUAAG-CCAG
N6
U-A
A-U
G-C
U-A
C-G
U-A
A-U
GUAAG-CCAG
B
vRNA
wt
N5
N6
1 2 3 4 5 6 7 8
+ 40
+ 28
+ 27
+ 5
+ 3
+ 1

Figure 4-8. **(A) Structures of mutated N7 and N8 RNA templates within the tRNA:vRNA complexes. (B) Initiation of reverse transcription from these complexes.** The order of lanes 1-8 is the same as that of Figure 4-4, except that lanes 5 and 6 are reactions using N7 template; lanes 7 and 8 are reactions using N8 template.

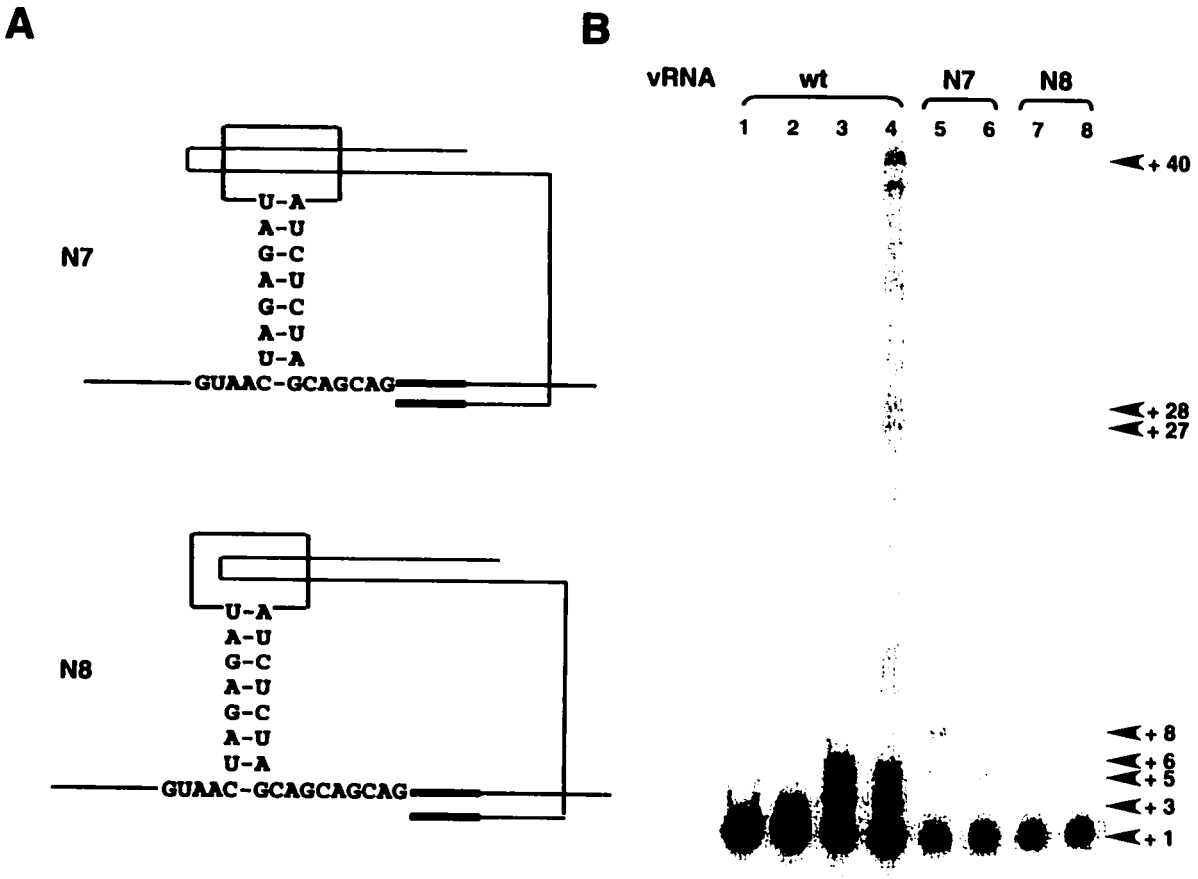
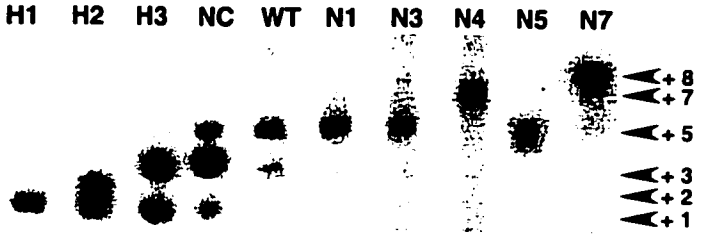

A
N7
U-A
A-U
G-C
A-U
G-C
A-U
U-A
GUAAC-GCAGCAG
N8
U-A
A-U
G-C
A-U
G-C
A-U
U-A
GUAAC-GCAGCAGCAG
B
vRNA
wt
N7
N8
1 2 3 4 5 6 7 8
+ 40
+ 28
+ 27
+ 8
+ 6
+ 5
+ 3
+ 1

Figure 4-9. Initiation of reverse transcription from virion-derived tRNA:vRNA complexes. Viral RNAs were isolated as described in Materials and Methods from viral pellets containing 50 ng of CA Ag. As controls, 1 pmol of $tRNA^{Lys,3}$ was annealed with 1 pmol of wild-type synthetic vRNA by either the heat method (H1, H2, H3) or by 30 pmol of NCp7 (NC). The primer tRNA was then extended by 45 ng RT (p51/p66) in the presence of 160 nM of dCTP[α-^{32}P] (H1), dCTP[α-^{32}P] and dTTP (H2), or dCTP[α-^{32}P], dTTP and dGTP (H3, NC, N1, N3, N4, N5, N7), respectively.


H1
H2
H3
NC
WT
N1
N3
N4
N5
N7
+ 8
+ 7
+ 5
+ 3
+ 2
+ 1

4.6 Discussion

HIV-1 NC protein (NCp7) is a small, basic protein, containing two characteristic $CX_2CX_4HX_4C$ Zn finger-like motifs in its structure. This protein has been shown to function at different steps during the viral life cycle, including viral RNA packaging and dimerization, virion assembly, reverse transcription, integration, and transcription (Darlix et al. 1995; Chapter 1 of this thesis). Because of these features, NC has become an attractive target for the design of anti-HIV compounds.

One essential role of NC during reverse transcription is to facilitate placement of primer tRNA onto the PBS of viral genomic RNA. Basic amino acid residues instead of Zn finger structures of NC were found to be critical in this regard (de Rocquigny et al. 1992; Lapadat-Tapolsky et al. 1995). However, NC was also found to participate in the formation of an active tRNA:vRNA complex, which possessed elevated efficiency in the switch from initiation to elongation in the synthesis of (-)ssDNA; the Zn finger motifs were required in this regard (Rong et al. 1998; Chapter 2 of this thesis). In this study, we have further shown that an intact Zn-finger-containing HIV-1 NC protein is necessary for formation of a tRNA:vRNA binary complex that is favored during initiation of (-) strand DNA reverse transcription, *i.e.*, the use of intact NC protein helps RT to escape from the +1 nt pause position. These experiments provide direct evidence that the NC Zn fingers are indispensable for the proper conformation of the RNA complex in the primer annealing process. Initiation of reverse transcription and the subsequent switch to elongation may represent targets that can be specifically blocked by anti-NC as well as anti-RT compounds.

In contrast, the pause event at the +3 nt position, was not affected by the presence of NC protein in the annealing process (Figure 4-2). Similar differences in formation of the +1 nt and +3 nt pause sites were also observed in our previous studies. For example, initiation of reverse transcription paused at the +3 nt site whenever $tRNA^{Lys,3}$ or a DNA primer was employed; however, the +1 nt intermediate

product disappeared when RT reactions were primed from an oligo DNA (Liang et al. 1998a; Chapter 3 of this thesis). *In vitro* probing data have described the existence of a 8-base-pair stem structure located 3 nt upstream of the PBS within the tRNA:vRNA complex (Isel et al. 1993, 1995; Skripkin et al. 1996). Accordingly, we reasoned that reverse transcription must have paused after the third nucleotide was incorporated in order to dissolve the base pairs of the stem structure. In this study, we introduced a series of mutations into the viral RNA template, such that the stem structure was deliberately destabilized. Our results show that reverse transcription of RNA templates containing these mutations no longer paused at the +3 nt position.

HIV-1 RT initiates reverse transcription as $tRNA^{Lys,3}$ is placed onto the PBS. The resulting tRNA:vRNA complex represents as a reverse transcription initiation complex, that is characterized by specific inter- and intra-molecular interactions. In contrast, a DNA primer annealed to the PBS represents a non-specific elongation complex, in which there are only intramolecular interactions with the exception of base-pairing at complementary sequences between primer and template. Once the tRNA primer is extended by 3 or 5 bases, RT dissociates from the initiation complex, and can then rebound with the complex in an elongation mode. This RT transition process is detected on gels by the early short (-) strand DNA products at the +3 and +5 nt positions (Isel et al. 1996).

Our mechanistic studies of early pausing events and the role of NC protein in this process contribute further to an understanding of the initiation of reverse transcription. First, we have demonstrated that the +1 pausing event is a distinct rate-limiting step during initiation, since it can only be observed when RNA is used to prime reverse transcription, and because the addition of NC proteins containing intact Zn finger motifs can help RT to escape this pause site (Figure 3-4A, Figure 4-2, Figure 4-3). Second, the presence of the +3 nt pausing event is solely due to the specific secondary structure of the tRNA:vRNA complex.

The sequence and structure of the upstream PBS region can affect reverse transcription in many ways. In addition to its role with respect to the +3 intermediate product, the structure and the position of the stem can also influence the efficiency of priming. Changing a greater numbers of nucleotides resulted in less efficient initiation, and this effect was most pronounced in the case of the N2 and N8 mutated templates (Figure 4-4B,4-8B). The sequence of the right half of the stem structure was also found to participate in efficient switching from the +3 nt pause site to a even later initiation intermediate at the +5 nt position. Reverse transcription of the N5 and N6 templates, which exchanged base-pair sequences without disturbance of the stem structure, barely proceeded beyond the +3 stage, although the reaction was initiated in near normal fashion (Figure 4-7B). The fidelity of reverse transcription was also affected, when sequences on the right half of the stem were mutated (Figure 4-5B). Finally, the early pause events at the +3 and +5 nt positions were observed when tRNA:vRNA was prepared from virus particles, indicating that similar initiation events also transpire in the virus. However, comparison of relative amounts of each short cDNA product revealed that the initiation complex, isolated from the virus, is far more similar to a NC-annealed complex than to one prepared by heat-annealing (Figure 4-9).

The biological relevance of the +1 and +3 pausing events in reverse transcription may rely on their regulatory roles that may be similar to that seen during transcription by *E. Coli.* RNA polymerase. In the latter context, initiation begins in an abortive mode and pauses near the start site, and a regulatory subunit, σ, is required for transcription to proceed to elongation (von Hippel et al. 1984; McClure 1985). In HIV-1, the NC protein may play a role analogous to that of the σ factor. Our finding that the tRNA:vRNA complex formed in the presence of NC was favored in the initiation of reverse transcription and in the subsequent switch from initiation to elongation provides important evidence on this subject.

Chapter 5

Hydrophobic Amino Acids in the Human Immunodeficiency Virus Type 1 p2 and Nucleocapsid Proteins Can Contribute to the Rescue of Deleted Viral RNA Packaging Signals

This chapter was adapted from an article in preparation. All data presented in this chapter were from experiments performed by myself under the supervision of Dr. Wainberg. Dr. C. Liang provided assistance in planning certain experiments and analysis of results.

5.1 Preface

As a domain of the Gag polyprotein, HIV-1 NC also participates in selective encapsidation of viral genomic RNA through specific interactions with *cis*-acting RNA packaging elements in the 5' leader sequence, including SL1 and SL3. Different deletions in the SL1 region can be compensated for by various second-site mutations in the Gag protein. Interestingly, they all share the same second-site mutations in p2 and in the NC protein, *i.e.*, a T12I substitution in p2 (MP2) and a T24I substitution in NC (MNC). In this chapter, we changed the T12 of p2 or the T24 of NC to each of 19 other amino acids; the results show that amino acids with long hydrophobic side chains (including V, L, I and M) at the above two positions are favored in compensation of for the SL1 deletions. When SL3 was deleted, compensation mutations were identified that involved substitutions at two novel sites, *i.e.* A11V in p2 and I12V in NC. Therefore, compensation of deletions in either SL1, which is located upstream of the major splice donor site (SD), or in SL3, downstream of the SD, involved different second-site mutations in p2 and NC. This suggests that RNA elements that flank the SD have qualitatively different type of interactions with Gag proteins during viral RNA packaging.

5.2 Abstract

The leader sequence of human immunodeficiency virus type 1 (HIV-1) contains four distinct stem-loop RNA motifs termed SL1 through SL4. Both SL1 and SL3 have been shown to participate in specific encapsidation of viral RNA. Compensation studies have identified two second-site mutations, including MP2 (*i.e.* T12I substitution in p2) and MNC (*i.e.* T24I substitution in NC protein), that are involved in the rescue of various deletions (*e.g.* BH-D1, BH-D2 and BH-LD3) in the SL1 RNA region located between PBS and the 5′ major splice donor (SD) site. In this study, we have deliberately changed the T12 in p2 or the T24 in NC to each of 19 other amino acids. We found that amino acids with long hydrophobic side chains, *i.e.* V, L, I and M, are favored either at position 12 in p2 or at position 24 in NC to compensate for the above-mentioned deletions. Further studies showed that only a few amino acids cannot be used at these two sites by the wild-type virus, due to decreased RNA levels in the virion or abnormal Gag protein processing. In this case, W, D and E cannot substitute for T12 in p2, and S, D and N cannot substitute for T24 in NC, without affecting viral infectivity. We also examined the SL3 RNA structure, located downstream of the 5′ SD site, and identified two novel second-site mutations, *i.e.* A11V in p2 and I12V in NC, that can compensate for the deleted SL3 RNA sequence. It is concluded that compensation of deletions either in SL1 or in SL3 involves different second-site mutations in p2 and NC. This indicates that SL1 and SL3 likely play different roles during viral RNA packaging.

5.3 Introduction

Human immunodeficiency virus type 1 (HIV-1) encapsidates two copies of full-length viral RNA that form a dimer through non-covalent linkage at the 5'end (Berkowitz et al. 1996). The *cis*-acting elements that are involved in the specific packaging and dimerization of viral RNA are located in the 5' viral RNA leader sequence. Complex secondary structures have been proposed to exist in the leader region, among which SL1 and SL3 can bind to nucleocapsid (NC) protein with high affinity and are thought to be the major RNA elements responsible for viral RNA encapsidation (Harrison and Lever 1992; Kaddrick et al. 1996; McBride and Panganiban 1996, 1997; Baudin et al. 1997; Clever and Parslow 1997; Liang et al. 1998). SL1 has also been shown to function as the dimerization initiation site (DIS) for viral RNA (Awang and Sen 1993; Fu et al 1994; Laughrea and Jette 1994, 1996a,b; Marquet et al. 1994; Paillart et al. 1994, 1996a,b, 1997; Skripkin, et al. 1994; Muriaux et al. 1995; Berkhout and van Wamel 1996; Clever et al. 1996; Kaddrick et al. 1996). In addition, although the TAR, poly(A) and PBS hairpins, that are located in the R-U5 region at the 5'end of the leader sequence, bind to NC protein with low affinity, they have also been shown to contribute to the packaging process (Das et al. 1997, 1998; McBride et al. 1997; Helga-Maria et al. 1999; Harrich et al. 2000; Liang et al. 2000).

We have been particularly interested in the roles of RNA sequences, located upstream of the 5' major splice donor (SD) site, in encapsidation of viral RNA. These RNA sequences are contained in both spliced and unspliced viral RNA, yet allow the virus to selectively recruit unspliced RNA while excluding spliced RNA (Clever and Parslow 1997). To shed light on this issue, a number of deletion mutations, termed BH-D1, BH-D2, and BH-LD3, have been constructed to selectively remove RNA sequences in this region (Liang et al. 1998b, 1999a,b, 2000). These deletions had adverse impacts on both viral RNA encapsidation and viral replication. Interestingly, when the mutated

viruses were cultured for a prolonged period, revertants with wild-type replication kinetics arose and the results of sequencing analysis revealed two substitutional mutations, *i.e.* MP2 (T12I in p2) and MNC (T24I in NC), that were able to rescue all of the above mutated viruses. Therefore, exploration of the mechanisms by which the MP2 and MNC mutations are able to compensate for the functions of the deleted RNA sequences will add new insights into the roles of RNA sequences upstream of the 5′ SD site in viral RNA encapsidation and viral replication.

The first question we sought to answer was whether the I residue is the only amino acid at either position 12 in p2 or at position 24 in NC that can rescue the above deletions. Answers to this will shed light on the strictness of steric interactions at the above two sites in the rescue process. Toward this end, we have substituted either the T12 in p2 or the T24 in NC with each of 19 other amino acids, and have screened the resultant constructs for rescue ability, packaging efficiency, viral assembly, and infectiousness. We have also identified compensatory mutations that appear after deletion of the SL3 sequence, *i.e.*, the packaging signal downstream of the 5′ SD site, and have compared them to the MP2 and MNC mutations.

5.4 Materials and Methods

5.4.1 HIV-1 DNA mutagenesis

The BH10 clone of infectious HIV-1 cDNA was employed as starting material to generate the following mutant constructs. The BH-D1, BH-D2 and BH-LD3 deletions eliminated sequences at nucleotides (nt) +200 to +226, +200 to +233, and +241 to +256, respectively, and were constructed as previously described (Liang et al. 1998b, 1999b, 2000). The BH-SL3-M1 deletion removed the sequence at nt +306 to +325, that constitutes the SL3 RNA structure. This was generated by PCR using primer pair pSL3-M1 (5'-CTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTAC GCCAAAAAAAGGAGAGAGAT-3' [706 to 791])/pAPA-A (5'-CCTAGGGGCCCTGCAATTTCTG -3' [2016 to 1995]), and BssH II/Apa I as restriction sites.

An amino acid T residue at position 12 in the p2 protein was substituted by each of 19 other amino acids through the use of primer p2-12X (5'-CCAAGTAACAAATTCAGCTNNNATAATGATGCAGAGAGGC-3' [1893 to 1932]), in which N represents A, C, G or T; this permits each of the 20 amino acids to appear at this site (Figure 5-1). PCR was performed with the primer pair p2-12X/pNC-A (5'-TTAGCCTGTCTCTCAGTACAATC-3' [2084 to 2062]) and the initial PCR product was used as a primer in a second round of PCR along with primer pSph-S (5'-AGTGCATCCAGTGCATGCAGGGCC-3' [1431 to 1454]). The final PCR product was digested with restriction enzymes Sph I and Apa I and inserted into BH-D1 or BH10 to generate clones D1-p2-12X or BH-p2-12X (X represents any of the 20 amino acids). A large number of bacterial colonies were screened to ensure representation of substitutions of all 20 amino acids at position 12 in p2.

The amino acid T residue at position 24 in NC was substituted by each of 19 other amino acids using primer pair pSph-S/pNC-24R (5'-CCTAG GGGCCCTGCAATTTCTGGCNNNGTGCCCTTCTTTGC-3' [2007 to 1966]) and restriction sites Sph I/Apa I to generate clones D2-MP2-NC24X or BH-NC-24X.

One of our clones, *i.e.*, BH-SL3-M1, contained seven more As in addition to the A-repeat upstream of the SL3 deletion after long-term culture in MT-2 cells. This construct was digested with Hpa I and BssH

II and inserted into BH10 to generate construct M1-12A. Either or both of the other two compensation mutations of BH-SL3-M1, *i.e.* A11V in p2 and I12V in NC, were also introduced into BH-SL3-M1 and M1-12A, by employing the same PCR and cloning strategies as for the construction of D1-p2-12X or BH-p2-12X. The primers used include p2-11V (5'-CCAAGTAA CAAATTCAGTTACCATAATGATGC-3' [1893 to 1924])/pNC-A/pSph-S, and pNC-12V (5'-GGAACCAAAGAAAGGTTGTTAAGTGTTTC-3' [1940 to 1968])/pNC-A/pSph-S, respectively.

5.4.2 Cell culture, transfection and infection

COS-7 and MT-2 cells were grown in Dulbecco's modified Eagle's medium (DMEM) and RPMI 1640 medium, respectively, each supplemented with 10% fetal calf serum. COS-7 cells were transfected with HIV-1 DNA constructs in the presence of either $CaCl_2$ or Lipofectamine (GIBCO BRL, Montreal, Quebec, Canada) (Sambrook et al. 1989). Progeny virus was harvested 48h after transfection and quantified by measuring levels of viral CA antigen (Ag) by enzyme-linked immunosorption assay (Abbott Laboratories, Abbott Park, Ill.).

For infectivity assays, similar amounts of virus (*i.e.* 3 ng of CA Ag) were used to infect $5x10^5$ MT-2 cells. After 2h, the cells were washed twice to remove unbound virus and grown in complete RPMI 1640 medium. Culture fluids were collected at various times and reverse transcriptase (RT) activity was measured (Liang et al. 1998b).

For those mutated viruses with diminished infectiousness, the infected cells were split upon confluence and kept in culture until extensive formation of syncytia and high levels of RT activity were observed. At this stage, culture fluids were used to infect fresh MT-2 cells. Viruses were passaged until the infections observed were as virulent as those caused by wild-type virus. Cellular DNA was purified and amplified by PCR through the use of primer pair Hpa-S (5'-CTGCAGTTAACTGGAAGGGCTAATTCACTCCC-3' [1 to 21])/Bcl-A (5'-CTATGAGTATCTGA TCATACTG-3' [2445 to 2424]). The PCR product was cloned and sequenced to confirm the presence of the original mutations and to identify novel

mutations that might have been present in non-coding leader RNA sequences and in the *gag* gene.

5.4.3. Western blot and immunoprecipitation assays of viral proteins

Culture fluids (10 ml) collected from COS-7 cells 48h after transfection were clarified on a Beckman GS-6R centrifuge at 3,000 rpm for 30 min at 4°C. Viral particles were then pelleted through a 20% sucrose cushion at 40,000 rpm for 1h at 4°C with an SW41 rotor in a Beckman L8-M ultracentrifuge. Viral pellets were suspended in 50 µl of NP40-lysis buffer. Transfected COS-7 cells were washed twice with cold phosphate-buffered saline (PBS) and also lysed in 200 µl of NP-40 lysis buffer. 10 µl volumes of protein samples were fractionated on 12% SDS-polyacrylamide gels and transferred to nitrocellulose filters. After being blocked with 5% skim milk-0.05% Tween 20-phosphate buffer at 4°C for 16 h, the filters were incubated with anti-HIV-p24 immunoglobulin G1 (IgG1) monoclonal antibody (mAb) (ID Labs Inc., London, Ontario, Canada) at 37°C for 1h. After extensive washing with 0.05% Tween 20-phosphate buffer, secondary anti-mouse IgG-horseradish peroxidase-conjugated antibody (Amersham Life Sciences, Oakville, Ontario, Canada) was added for 1h at 37°C. After thorough washing, viral proteins were visualized with an ECL chemiluminescence detection kit (Amersham Life Science, Amersham Place, England).

COS-7 cells that had been transfected with HIV-1 recombinant DNA constructs were starved in DMEM without L-Met and L-Cys at 37°C for 1h, after which cells were metabolically radiolabeled with [^{35}S]-L-Met and [^{35}S]-L-Cys (ICN) at a concentration of 100 µCi/ml for 30 min at 37°C. After extensive washing with complete DMEM, supplemented with 30 µg/ml L-Met and 60 µg/ml L-Cys, the cells were cultured for 1h. Virus particles from culture fluids were pelleted by ultra-centrifugation as described above and analyzed on 12% SDS-polyacrylamide gels. The cells were washed twice with cold phosphate-buffered saline (PBS) and lysed with 1 ml of lysis buffer. The cell lysates were clarified in a bench-

top Eppendoff centrifuge at 13,000 rpm for 10 min at 4°C, and then incubated with anti-HIV-1 CA mAb for 1h at 4°C, following which 5 µl of protein-A linked Sepharose 4B (Pharmacia, Montreal, Quebec, Canada) was added for a further 30 min incubation. The Sepharose 4B was then centrifuged and washed in turn with NET-gel buffer and Tris-NP40 buffer (Sambrook et al. 1989). Pellets were suspended in 20 µl of 1x SDS-containing gel-loading buffer, boiled and analyzed by 12% SDS-polyacrylamide gels. Viral proteins were visualized by exposure to X-ray film.

5.4.4 Encapsidation of viral RNA by RT-PCR and Northern blot assay

Supernatants from COS-7 cells that had been transfected with various HIV-1 recombinant DNA constructs were clarified in a Beckman GS-6R centrifuge at 3,000 rpm for 30 min at 4°C and quantified on the basis of CA Ag levels. Viral RNA was purified from an amount of virus containing 2 ng of CA Ag using an RNA extraction kit (QIAGEN, Germany). Viral RNA was dissolved in 50 µl of DEPC-treated water and treated with 20 units of RNase-free DNase I (GIBCO BRL, Montreal, Quebec, Canada) at 37°C for 30 min to remove any DNA contamination. 5 µl volumes of viral RNA were amplified for 20 cycles using a One-Tube RT-PCR kit (Boerinhger Mannheim, Mannheim, Germany) using the primer pair pGAG1/pST to analyze full-length viral RNA (Liang et al. 1998b). DNA products were analyzed on 5% native polyacrylamide gels and visualized following exposure to X-ray film.

For Northern blot assay, virus pellets harvested from culture fluids (80 ml) of transfected COS-7 cells were suspended in 100 µl of TN buffer (50 mM Tris-HCl [pH 7.5], 10 mM NaCl) and transfected COS-7 cells were lysed in 200 µl of NP-40 lysis buffer, of which a 5 µl volume of each sample was subjected to CA antigen measurement. All remaining samples were then treated with 20 units of RNase-free DNase I (GIBCO BRL, Montreal, Quebec, Canada) at 37°C for 30 min followed by Protease K digestion (GIBCO BRL, Montreal, Quebec, Canada) at 37°C for

30 min. Viral RNAs were extracted with water-saturated-phenol once and twice with phenol:chloroform. After precipitation in a 0.7-fold volume of isopropanol at -20°C for overnight, RNA samples were resuspended in DEPC-treated double distilled water at a final concentration of 50 ng of CA Ag/μl. 5 μl of RNA samples were fractionated on 0.9% agarose gels with 18% formaldehyde and 1X MOPS buffer (20 mM MOPS [pH 7.0], 8 mM NaAc, and 1 mM EDTA [pH 8.0]), and then transferred onto nylon membranes. After baking in a vacuum oven at 80°C for 1h, the membranes were prehybridized in buffer containing 50% formamide, 0.5% SDS, 6X SSPE (1X SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 M EDTA [pH 7.7]), 5X Denhardt's solution, and 0.1 mg of salmon sperm DNA (GIBCO BRL) at 42°C for 3h. HIV-1 proviral DNA was employed as a probe and was labeled with a nick translation kit (Boerinhger Mannheim, Mannheim, Germany). Hybridization was performed at 42°C overnight in buffer containing 50% formamide, 0.5% SDS, 6X SSPE, 0.1 mg/ml of salmon sperm DNA, and the DNA probe (10^6 cpm/ml). After extensive washing, the membranes were exposed to X-ray film.

5.5 RESULTS

5.5.1 Substitutions of the T amino acid with V, L, I, C or M can increase the infectiousness of the BH-D1, D2 and LD3 mutant viruses, while replacement with W, D, and E amino acids decreases the infectiousness of wild-type virus

We have previously shown that the BH-D1 deletion mutation, eliminating HIV sequences at nt +200 to +226, attenuated viral replication. Long-term culture of the mutated virus in MT-2 cells led to a revertant containing a mutation at position 12 in p2, *i.e.* T12I, that helped to restore viral infectiousness to near wild-type levels (Liang et al. 2000). We now sought to answer whether other amino acids at position 12 in p2 were also able to rescue the BH-D1 deletion. Toward this end, T12 in p2 in BH-D1 was substituted by each of 19 other amino acids (Table 5-1). Relevant recombinant DNA constructs were transfected into COS-7 cells and the progeny viruses thus generated were quantified on the basis of CA Ag levels. Equivalent amounts of virus were used to infect MT-2 cells and the infectiousness of each preparation was determined by the formation of syncytia and RT activity in culture fluids. The results of Table 5-1 show that four amino acids, including V, L, C and M, in addition to the previously detected I, could help to correct the adverse effects of BH-D1 on virus replication. This subject was further evaluated by replication kinetic studies (Figure 5-2A). Viruses containing either G, K, D, or E at position 12 were barely infectious. In contrast, when T12 was replaced with either V, L, I, C, or M, the recombinant viruses thus generated were able to generate high levels of RT activity (Figure 5-2A). Among the five amino acids that could play this role, C was the least efficient.

The T12I substitution in p2 was also identified in revertants of the BH-D2 and BH-LD3 mutated viruses (Liang et al. 1998b, 2000). We therefore wished to determine whether the same amino acids that had been shown to compensate for BH-D1 (see above), *i.e.* V, L, C and M, were also able to rescue the BH-D2 and BH-LD3 defective viruses. Toward

this end, the T12 in the p2 of BH-D2 or BH-LD3 was changed to either V, L, C, or M. In addition, a mutation at position 24 in the NC protein, termed MNC (T24I), was also included in these constructs, since it has been shown that this mutation works in synergy with the T12I mutation in p2 to rescue both BH-D2 or BH-LD3 (Liang et al. 1998b, 2000). The various constructs, *i.e.* D2-V-MNC, D2-L-MNC, D2-C-MNC, D2-M-MNC, LD3-V-MNC, LD3-L-MNC, LD3-C-MNC and LD3-M-MNC, were transfected into COS-7 cells and the progeny viruses thus generated were used to infect MT-2 cells. The results of Figure 5-2B and C show that all of the above constructs produced viable viruses in contrast to the non-infectiousness of BH-D2 and BH-LD3. Therefore, each of V, L, C, and M can help to rescue the BH-D2 and BH-LD3 deletions. The C and L substitutions were less efficient in this regard than either V, I, or M.

We examined the impact of various substitutional mutations of T12 in p2 on the infectivity of wild-type virus. Replication data show that the T12W substitution in p2 abolished viral replication and that each of the T12D and T12E mutations dramatically decreased viral infectiousness (Figure 5-3A, B). Substitutions to either L or F markedly diminished viral replication, yet were permissive for production of high levels of RT activity after 10 days, while each of I, P and Y only slightly delayed viral replication (Figure 5-3A, B). In terms of impact on viral replication, the various substitutions can be ranked as W>D, E>L, F>I, P, Y>G, A, V, S, C, M, H, K, R, N, Q.

5.5.2 Effects of T12 mutations in p2 on $Pr55^{Gag}$ proteolytic cleavage efficiency

T12 is one of eight amino acids in the HIV-1 p2 and NC proteins that are recognized and cleaved by the viral protease. Hence, mutations of T12 may affect the efficiency of cleavage at this site and decrease viral replication. To test this hypothesis, the processing of Gag in our various mutated viruses was examined through short-term radiolabeling and immunoprecipitation of viral proteins. For this

purpose, we employed anti-CA mAb to detect each of $Pr55^{Gag}$, intermediate cleavage products, including p41/p39 and p25, as well as mature p24 in cell lysates (Figure 5-4A). The intensities of the protein bands were quantified and the percentage of each viral protein was plotted to assess the efficiency of Gag processing (Figure 5-4B).

In the case of the BH-p2-12UAG construct, that produced a truncated Gag protein, (*i.e.* MA-CA-p2 but missing three amino acids at the C-terminus of p2 because of the UAG stop codon), a protein band of 39 kD was observed. When a number of aliphatic amino acids, *i.e.* G, A, V, L, and I, were substituted for T at position 12 in p2, we found that the levels of $Pr55^{Gag}$ diminished as the length of the side chain of the amino acid increased (Figure 5-4A, B). Yet, this did not directly correlate with viral infectiousness, since the G, A, V and I mutants had similar replication capacity (Figure 5-3). Of these, the L substitution resulted in a modest accumulation of p25 (CA-p2) fusion product in comparison with wild-type (T) (Figure 5-4A, B); this correlates with the decreased infectiousness of the L mutant (Figure 5-3).

Substitution of T12 in p2 by S, C, or M, the side chains of which contain hydroxyl or sulfur groups, did not affect the processing of Gag proteins (Figure 5-4A, B). Mutation of T to the cyclic amino acid P resulted in significant accumulation of $Pr55^{Gag}$, diminished levels of the intermediate p39 and p25 products, as well as enhanced cleavage of p24 from p25 (Figure 5-4A, B). Among substitutions involving the aromatic amino acids F, Y and W, W led to both enhanced accumulation of p25 and slightly different migration patterns for both the p25 and p24 proteins (Figure 5-4A, B), deficits that may account for the non-infectiousness of the T12W mutant. In contrast, mutations to the basic amino acids H, K, or R yielded differential results, with both H and K causing an accumulation of $Pr55^{Gag}$ while R facilitated cleavage between p2 and NC and led to the rapid appearance of the p25 and p24 products (Figure 5-4A, B). The acidic amino acids D and E resulted in enhanced accumulation of $Pr55^{Gag}$, appearance of the p41 intermediate protein, and

a slightly slower migration rate of p25. Both the N and Q mutants showed moderate accumulation of $Pr55^{Gag}$.

After radiolabeling, COS-7 cells were cultured for 1h in complete DMEM, after which culture supernatants were pelleted by ultra-centrifugation and virus particles were lysed and analyzed on 12% SDS-polyacrylamide gels. No viral protein signal was detected in the case of the BH-p2-12UAG construct; this confirms that this construct was unable to produce virus particles (Figure 5-4C). Among all amino acids, W resulted in the greatest accumulation of p25 (Figure 5-4C), and, thus, had the highest impact on processing of $Pr55^{Gag}$, consistent with elimination of viral infectivity.

We further examined levels of full-length viral RNA packaged by various recombinant virus particles. The majority of the 19 amino acids used to substitute for T at position 12 in p2 did not markedly affect RNA encapsidation (Figure 5-5A, B). However, W resulted in substantially decreased levels of viral RNA in virus particles (Figure 5-5A, B).

5.5.3 The V, L, M, F, and Y amino acid residues can play the same role as I at position 24 in NC to rescue the BH-D2 mutated virus, and the S, D, and N amino residues at position 24 in NC dramatically decreased the infectiousness of wild-type virus

We have previously shown that the MNC point mutation T24I together with the T12I in MP2 can compensate for the BH-D2 deletion, in which the nt +200 to +233 sequence has been eliminated (Liang et al. 2000). To determine whether amino acids other than I at position 24 in NC can also play this role, T24 was changed to each of the 19 other amino acids to generate constructs D2-MP2-NC24X. The infectiousness of the viruses from these constructs was examined by infecting MT-2 cells. The results of Figure 5-6 show that V, L, and M at position 24 in NC were able to rescue the BH-D2 mutated virus as well as I. F and Y were less efficient in this regard. Therefore, amino acid residues with long

hydrophobic side chains are favored at position 24 in NC to compensate for the BH-D2 deletion.

Next, we inserted the above-described substitutions of T24 into wild-type virus to examine their effects on viral replication. The results of Figure 5-7 show that most of the substitutions did not or only moderately affect viral infectivity. However, the S and N mutations substantially decreased viral replication, and D eliminated viral infectiousness. Therefore, wild-type virus can use most of the 20 amino acids at position 24 in NC without affecting viral replication. It is interesting that neither a C nor a H at position 24 in NC affected the viral growth. This implies that these residues did not interfere with the binding of Zn^{2+} ion by the CCHC motif in the first Zn finger.

NC is the major viral structural protein that determines viral RNA packaging efficiency; therefore, the effects of the above NC substitutions on packaging were analyzed by RT-PCR. The results of Figure 5-8 show that acidic amino residue D had the most adverse impact in this regard, while G, A, S, H, R, E and N only moderately diminished viral RNA packaging.

5.5.4 Deletion of SL3 is compensated by point mutations A11V in p2 and I12V in NC

The aforementioned BH-D1, BH-D2, and BH-LD3 deletions eliminated RNA packaging signals that were located upstream of the 5′ SD site; these deletions resulted in similar compensatory mutations in the p2 and NC proteins. We have now extended these studies to investigate the SL3 packaging signal that is located downstream of the 5′ SD site. Toward this end, the SL3 RNA motif was eliminated by deleting sequences at nt +306 to +325 in the BH-SL3-M1 construct. The results of infection assays showed that the BH-SL3-M1 mutant virus displayed diminished infectiousness in MT-2 cells (Figure 5-9A). Prolonged culture led to a revertant with wild-type replication kinetics. The results of cloning and sequencing experiments revealed that two point mutations were

present in the revertants, *i.e.* an A11V substitution in p2 and an I12V substitution in NC; furthermore, the reverted viruses contained varying numbers of As (from four to ten) that had been inserted into the virus, in addition to the five As in the wild-type sequence just upstream of the deletion.

To verify the role of these newly identified mutations in the rescue of the BH-SL3-M1 deletion, they were recombined with the M1 deletion to generate constructs M1-A11V, M1-I12V, M1-A11V-I12V, M1-12A (which involves an insertion of seven As), and M1-A11V-I12V-12A. The growth curves of these recombined viruses show that the A11V mutation in p2 was alone able to markedly stimulate the growth of BH-SL3-M1; A11V and I12V together further increased infectiousness to a higher level. The addition of the A-repeats neither corrected the attenuated replication of BH-SL3-M1, nor affected the elevated growth of M1-A11V-I12V (Figure 5-9B).

5.5.5 Effects of compensatory mutations A11V (p2) and I12V (NC) on viral RNA packaging and viral protein processing

Viral RNA splicing and packaging were checked through analysis of viral RNA in the transfected COS-7 cells as well as in viral particles by Northern blot as described in Material and Methods. As shown in Figure 5-10A, the relative amounts of non-spliced and spliced viral RNA in transfected COS-7 cells were not affected by the presence of either the SL3 deletion or the compensatory mutations A11V (p2) and I12V (NC). Nor, surprisingly, were levels of viral RNA that were encapsidated significantly different between the wild-type and mutated viruses (Figure 5-10B). This observation was further confirmed by RT-PCR (Figure 5-10C).

Viral proteins were also analyzed by Western blot using anti-CA antibody. No profound differences were observed between wild-type and mutated constructs in either viral particles or in cell lysates (Figure 5-11). Gag protein processing was also studied by radiolabeling and immunoprecipitation. The introduction of a p2 mutation at position 11

resulted in an intermediate processing product, p25, *i.e.*, p24 linked with p2, that was observed in virus particles 1h after radiolabeling (Figure 5-12).

Figure 5-1. Schematic illustration of mutations in the U5 region, CA, p2 protein and NC. The amino acid sequence of p2 as well as parts of the CA and NC proteins are shown. The arrows indicate viral protease cleavage sites that result in formation of p2. The Zn finger sequence of NC is in italicized form. The substituted nucleotide in U5 or amino residues in CA, p2 and NC are shown in darken form. Nucleotides in U5 are numbered from the beginning of the U3 region. Numbering of amino acid positions starts from the first residue of each protein.

T12 I (p2)
A11V (p2)
I12V (NC)
T24 I (NC)
KARVL AEAMSQVTNSATIM MQRGN--IVKCFNCGKEGHTARNCRA
+301 (A)12
Δ +306- +325 (SL3)
R
U5 (PBS)
MA
CA
p2
NC
p1
p6

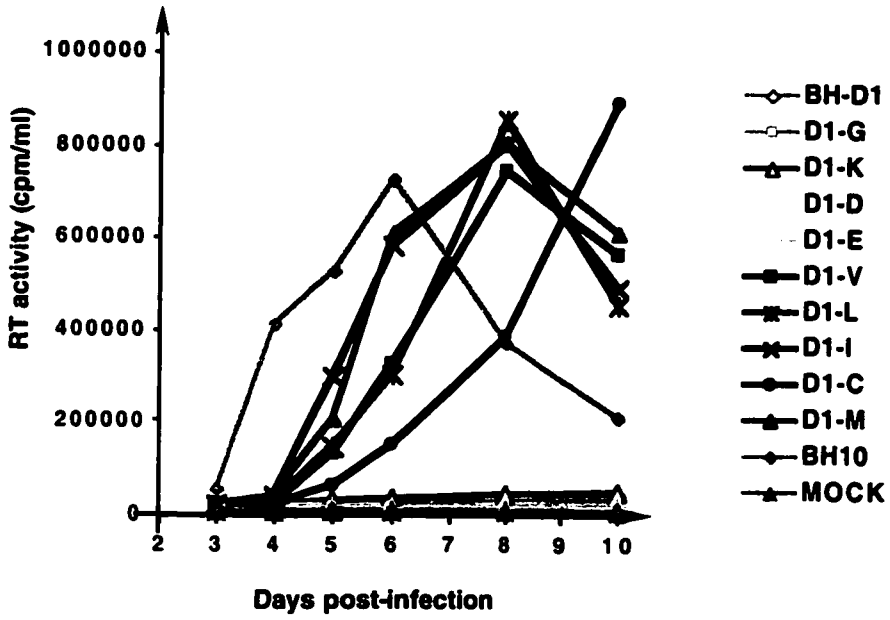
Table 5-1. Infectiousness of various D1-p2-12X viruses in MT-2 cells.

Amino Acid Substitutions	Genetic Codons	Infectivity*
Gly (G)	GGC	-
Ala (A)	GCC	-
Val (V)	GUU,GUG	+
Leu (L)	UUA	+
Ile (I)	AUU,AUA	+
Ser (S)	UCC,UCU	-
Thr (T)	ACU	-
Cys (C)	UGU	+
Met (M)	AUG	+
Pro (P)	CCU	-
Phe (F)	UUU	-
Tyr (Y)	UAU	-
Trp (W)	UGG	-
His (H)	CAC	-
Lys (K)	AAG	-
Arg (R)	CGA	-
Asp (D)	GAC	-
Glu (E)	GAG	-
Asn (N)	AAU	-
Gln (Q)	CAG	-
(Stop codon)	UAA	-
BH10(T)	ACC	+

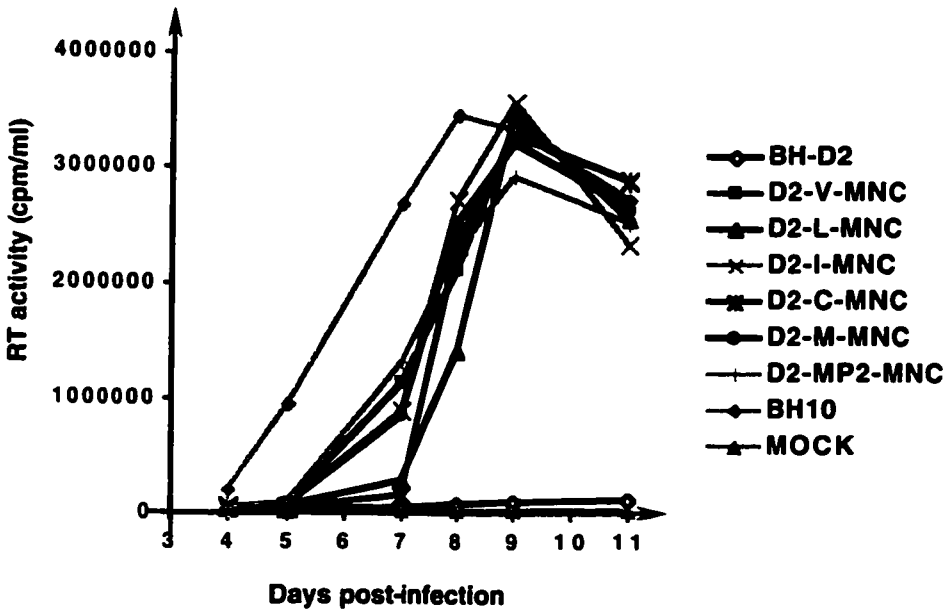
***: A value of 5.0×10^5 cpm RT activity from 50 ml of culture fluids was considered positive(+).**

Figure 5-2. (A) Replication kinetics of wild-type BH10 and mutated viruses containing the BH-D1 deletion and substitutions of T12 in p2. (B) Infectiousness of mutated viruses containing the BH-D2 deletion, substitutions of T12 in p2 as well as a T24I point mutation in NC. (C) Growth curves of mutated viruses possessing the BH-LD3 deletion, substitutions of T12 in p2 and a T24I point mutation in NC. In these studies, $5x10^5$ MT-2 cells were infected by viruses equivalent to 3 ng of p24 Ag. RT activity in culture fluids was determined at various times. The D2-MP2-MNC and LD3-MP2-MNC constructs, both containing a T12I substitution in p2 and a T24I mutation in NC, were generated as previously described (Liang et al. 1998b, 2000), and served as positive controls. Mock infections represent negative controls performed with heat-inactivated BH10 virus.

A


1000000
800000
600000
400000
200000
0
RT activity (cpm/ml)
2
3
4
5
6
7
8
9
10
Days post-infection
BH-D1
D1-G
D1-K
D1-D
D1-E
D1-V
D1-L
D1-I
D1-C
D1-M
BH10
MOCK

B


4000000
3000000
2000000
1000000
0
RT activity (cpm/ml)
3
4
5
6
7
8
9
10
11
Days post-infection
BH-D2
D2-V-MNC
D2-L-MNC
D2-I-MNC
D2-C-MNC
D2-M-MNC
D2-MP2-MNC
BH10
MOCK

C

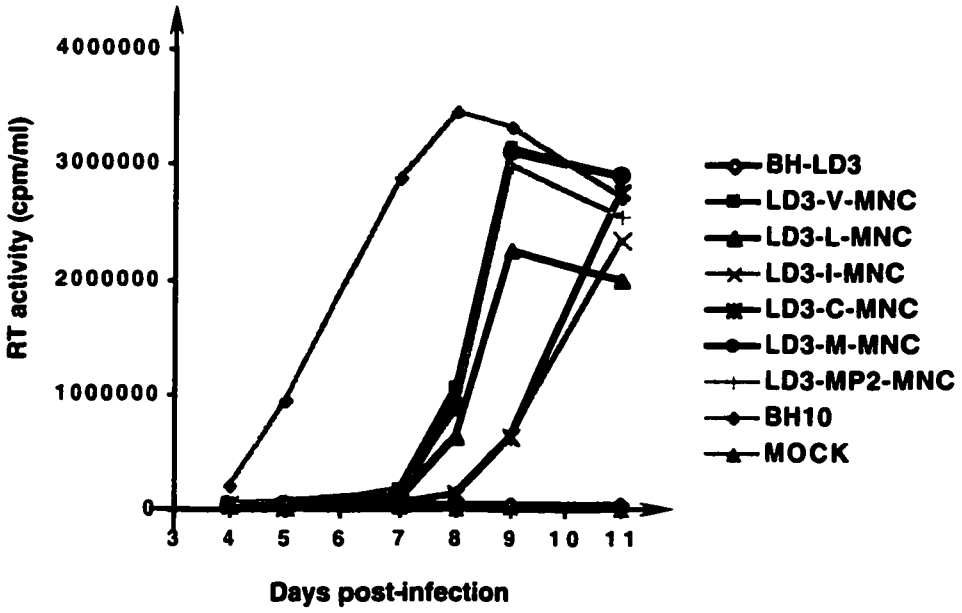
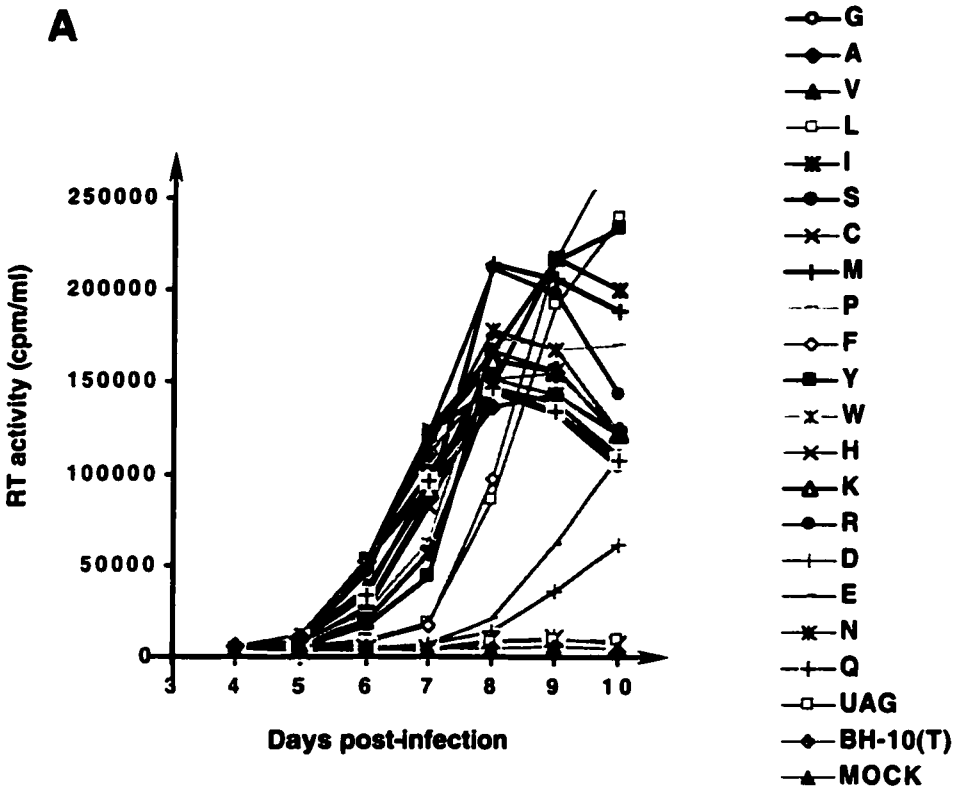

4000000
3000000
2000000
1000000
0
RT activity (cpm/ml)
3
4
5
6
7
8
9
10
11
Days post-infection
BH-LD3
LD3-V-MNC
LD3-L-MNC
LD3-I-MNC
LD3-C-MNC
LD3-M-MNC
LD3-MP2-MNC
BH10
MOCK

Figure 5-3. Replication capacities of wild-type BH10 and mutated viruses containing substitutions of T12 in p2. Mutations are represented by single letter abbreviations of amino acids. UAG is a stop codon that was inserted at position 12 in p2.

(A) Growth curves of wild-type and mutated viruses after infection of MT-2 cells.
(B) RT activity in culture supernatants at 7 days after infection. Mock represents a negative control performed with heat-inactivated wild-type virus.


A
RT activity (cpm/ml)
250000
200000
150000
100000
50000
0
3
4
5
6
7
8
9
10
Days post-infection
G
A
V
L
I
S
C
M
P
F
Y
W
H
K
R
D
E
N
Q
UAG
BH-10(T)
MOCK

B

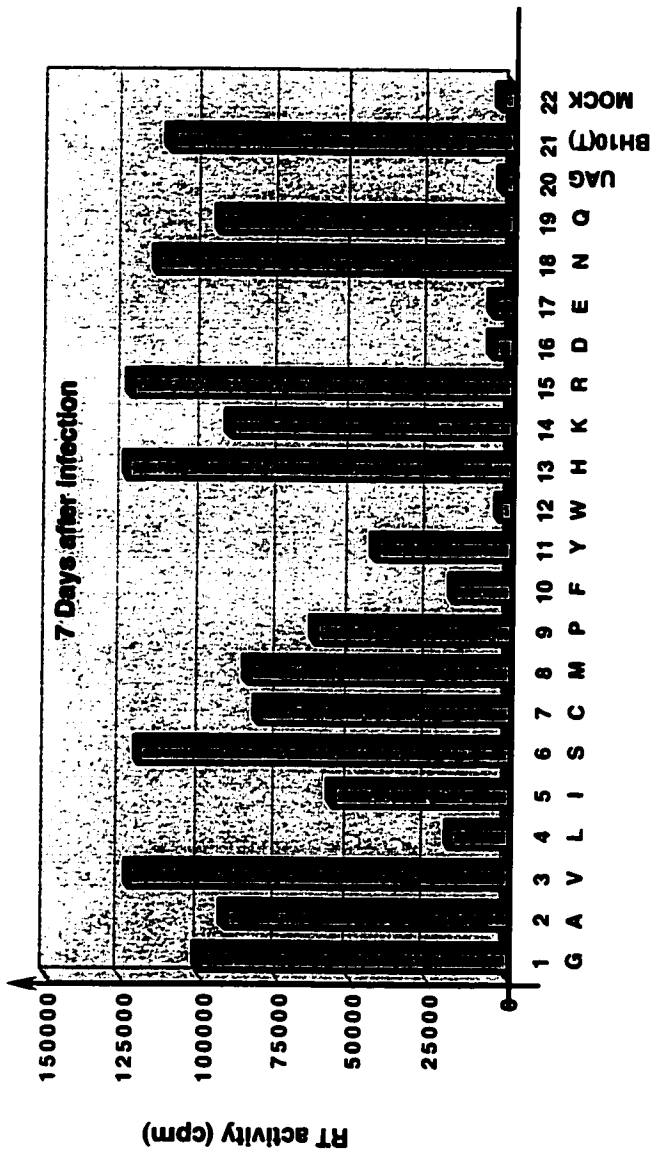

RT activity (cpm)
150000
125000
100000
75000
50000
25000
0
7 Days after infection
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
G A V L I S C M P F Y W H K R D E N Q
UAG
BH10(T)
MOCK

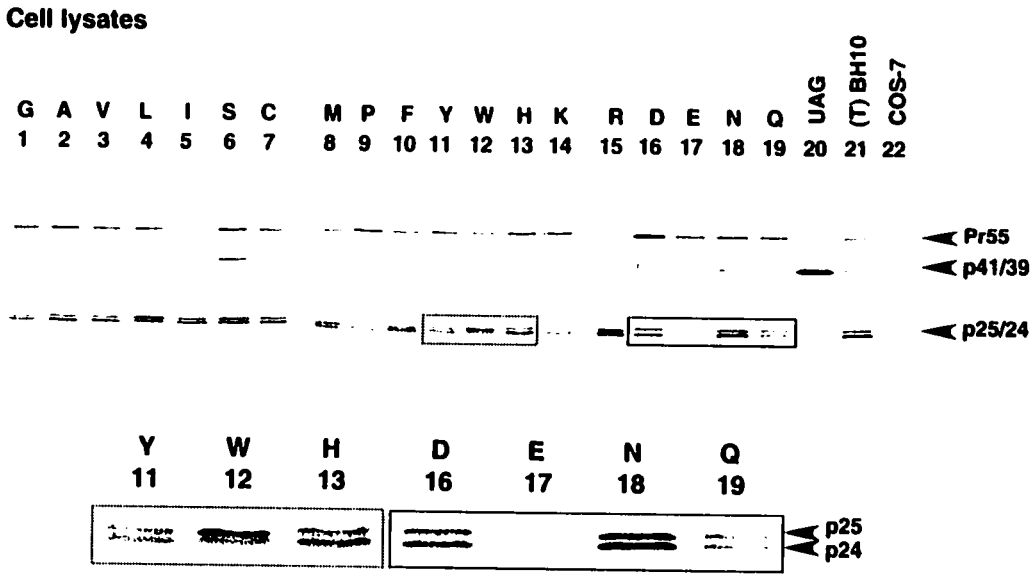
Figure 5-4. Processing of the Gag precursor protein $Pr55^{Gag}$ in wild-type BH10 and mutated viruses containing substitutions of the T12 amino acid in p2.

(A) Transfected COS-7 cells were labeled with [^{35}S]-L-Met and [^{35}S]-L-Cys for 30 min and cultured for 1h. Cells were then lysed and the lysates were subjected to immunoprecipitation through the use of mAb against CA (p24) Ag. The observed viral proteins, including $Pr55^{Gag}$, p41, p39, p25 and p24, are labeled on the right of the gels. To clearly visualize the p25 and p24 proteins in certain mutants, enlarged portions of the gels are shown.

(B) The intensities of viral protein bands were quantified through the use of the NIH Image Program, and the percentages of each viral protein in mutant or wild-type virus were plotted.

(C) After one hour in culture, labeled virus particles were pelleted from supernatants and analyzed on 12% SDS-polyacrylamide gels. A portion of the gel was enlarged to clearly show accumulation of p25 protein in the W mutant.

A


Cell lysates
G A V L I S C M P F Y W H K R D E N Q UAG (T) BH10 COS-7
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Pr55
p41/39
p25/24
Y W H D E N Q
11 12 13 16 17 18 19
p25
p24

B
Pr55
p41
p39
p25
p24
Percentage of various viral proteins
100%
80%
60%
40%
20%
0%
G A V L I S C M P F Y W H K R D E N Q UAG BH10(T)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

C

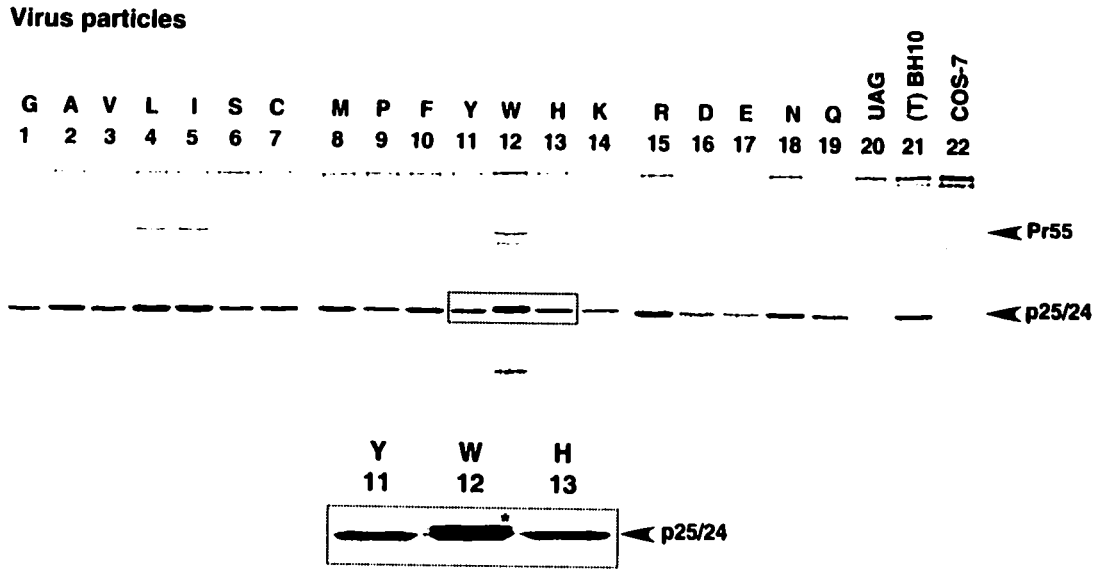
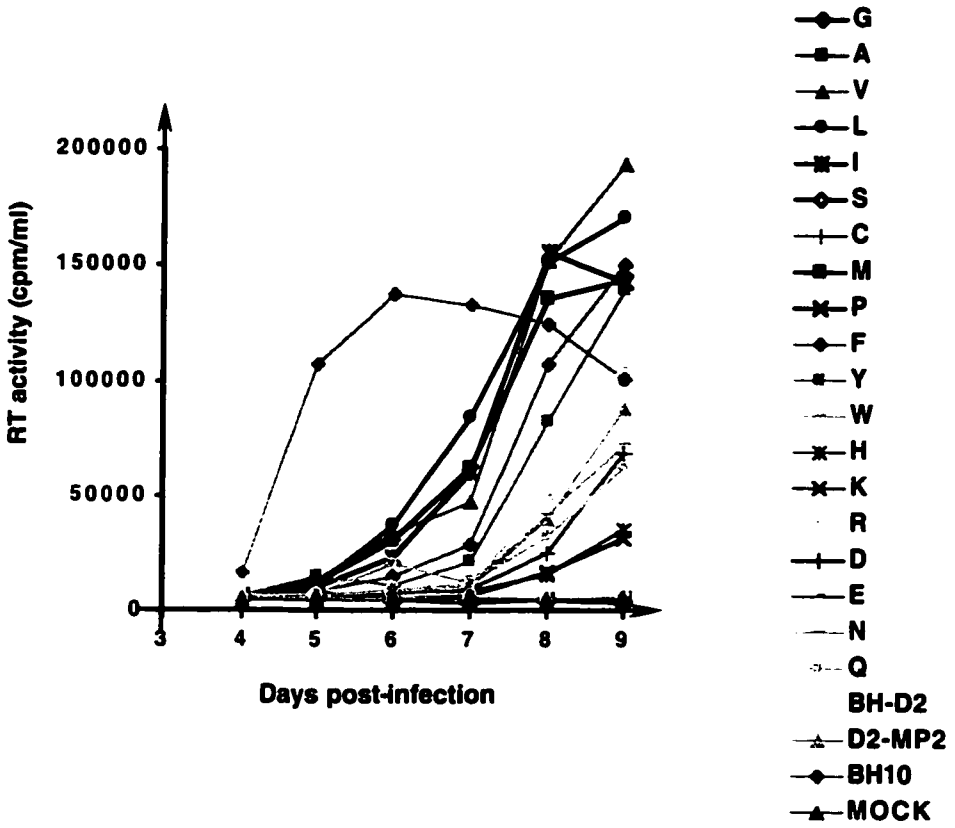

Virus particles
G A V L I S C M P F Y W H K R D E N Q UAG (T) BH10 COS-7
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Pr55
p25/24
Y W H
11 12 13
p25/24

Figure 5-5. Levels of viral RNA in wild-type virus and in mutated viruses harboring substitutions at position 12 in p2. Levels of full-length viral RNA were assessed by RT-PCR through the use of primer pair pGAG1/pST (Liang et al. 1998b). RNA samples were treated with DNase to remove any contaminating DNA. RNA samples were also digested with RNase A and then subjected to RT-PCR as a negative control to exclude the possibility of DNA contamination. A negative control of wild-type BH10 is shown in lane 1. 10 ng of BH10 plasmid DNA was also used in RT-PCR to serve as a positive control and to indicate the size of the PCR product (lane 25). RNA obtained from wild-type virus was diluted 1:2 and 1:4 before RT-PCR to ensure a linear range of these reactions. The results represent three independent experiments. Band intensities were quantified with the NIH Image Program and plotted. The amount of BH10 RNA was arbitrarily set at 1.0.

A
(-) G A V L I S C M P F Y W H K R D E N Q
UAG
BH10 (1)
BH10 (1:2)
BH10 (1:4)
(+)
B
Relative amounts of viral RNA
0
0.4
0.6
0.8
1.0
1.2
1.4
G A V L I S C M P F Y W H K R D E N Q
UAG
BH10 (1:1)
BH10 (1:2)
BH10 (1:4)

Figure 5-6. (A) Effects of various substitutions of the T24 residue in NC on the infectiousness of the BH-D2 mutated viruses. (B) The RT activity of each virus at day 7 after infection. Mutations are represented by single letter abbreviations of amino acids. Mock represents a negative control performed with heat-inactivated wild-type virus.

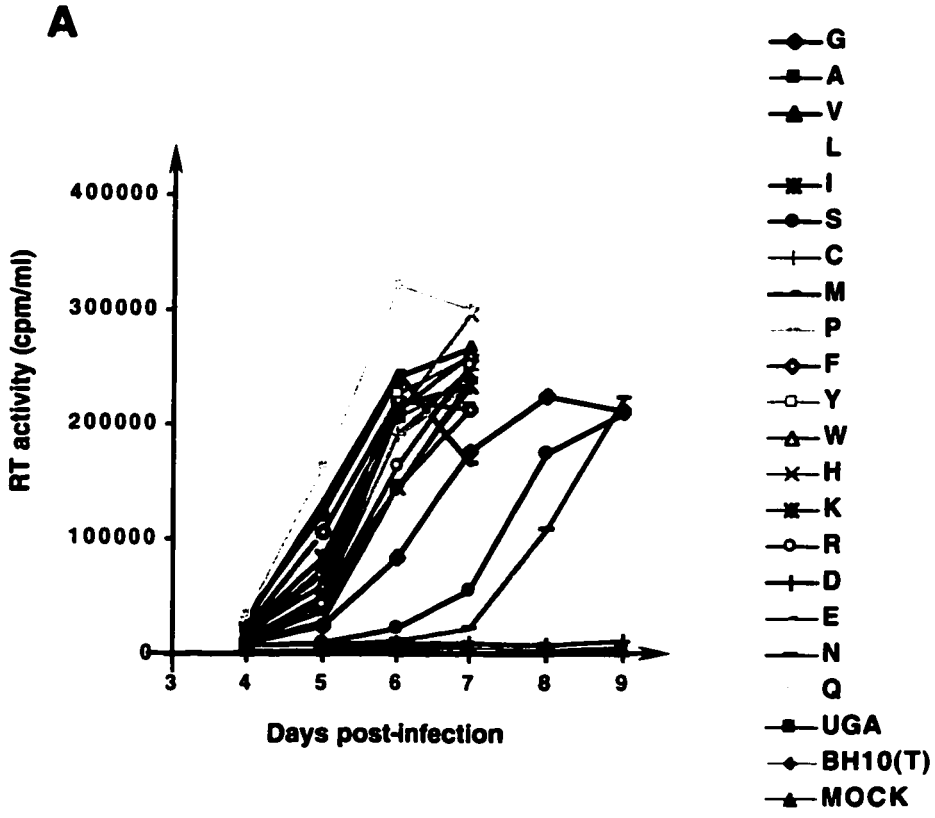
A


RT activity (cpm/ml)
200000
150000
100000
50000
0
3
4
5
6
7
8
9
Days post-infection
G
A
V
L
I
S
C
M
P
F
Y
W
H
K
R
D
E
N
Q
BH-D2
D2-MP2
BH10
MOCK

B

RT activity (cpm)
150000
120000
90000
60000
30000
0
7 Days after infection
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
G A V L I S C M P F Y W H K R D E N Q
BH-D2
D2-MP2
BH10
MOCK

Figure 5-7. (A) Growth curves of the BH10 viruses containing substitutions of the T24 in NC in MT-2 cells. (B) RT activity of each virus at day 6 after infection. Legends refer to Figure 5-6.


A
400000
300000
200000
100000
0
RT activity (cpm/ml)
3
4
5
6
7
8
9
Days post-infection
G
A
V
L
I
S
C
M
P
F
Y
W
H
K
R
D
E
N
Q
UGA
BH10(T)
MOCK

B

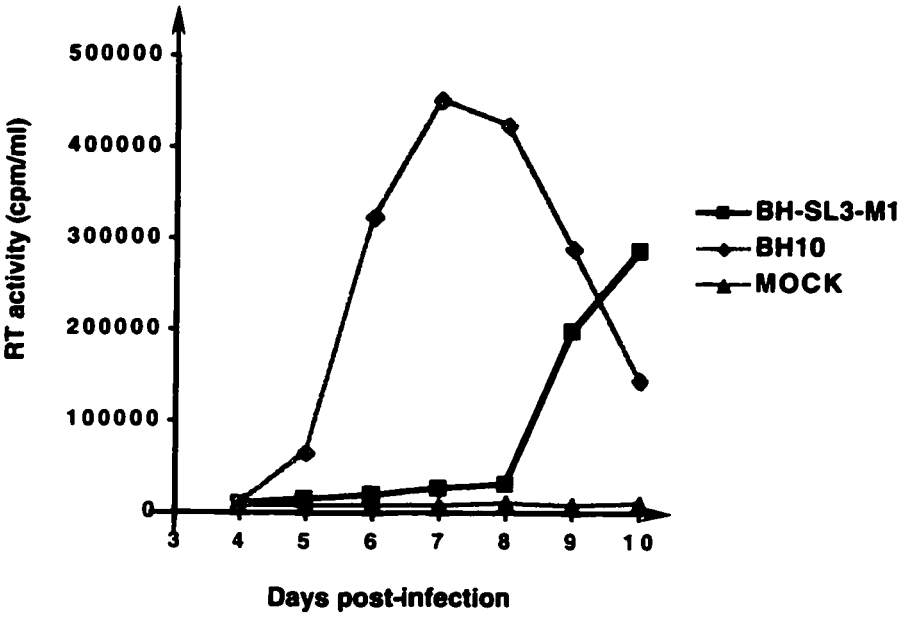
RT activity (cpm)
400000
300000
200000
100000
0
6 Days after infection
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
G A V L I S C M P F Y W H K R D E N Q
UAG
BH10(T)
MOCK

Figure 5-8. Viral genomic RNA levels in BH10 viruses containing various substitutions of the T24 residue in NC. Substitutions are represented by the single letters of amino acids. Viral RNA from viruses equivalent to 200 pg of CA antigen was subjected to RT-PCR using primer pair pGAG1/pST. The intensity of each band was quantified using the NIH Image program and plotted.

A
B
(+)
BH10(1:4)
BH10(1:2)
BH10(1)
Q N E D R K H W Y F P M C S I L V A G
Relative amount of viral RNA
0
0.4
0.6
0.8
1.0
1.2
1.4
BH10(1:4)
BH10(1:2)
BH10(1)
Q N E D R K H W Y F P M C S I L V A G

Figure 5-9. (A) Growth curves of the BH10 wild-type and BH-SL3-M1 mutated viruses in MT2 cells. (B) Effects of the A11V substitution in p2 and I12V in NC on the replication kinetics of the BH-SL3-M1 mutated virus.

A


500000
400000
300000
200000
100000
0
RT activity (cpm/ml)
3
4
5
6
7
8
9
10
Days post-infection
BH-SL3-M1
BH10
MOCK

B

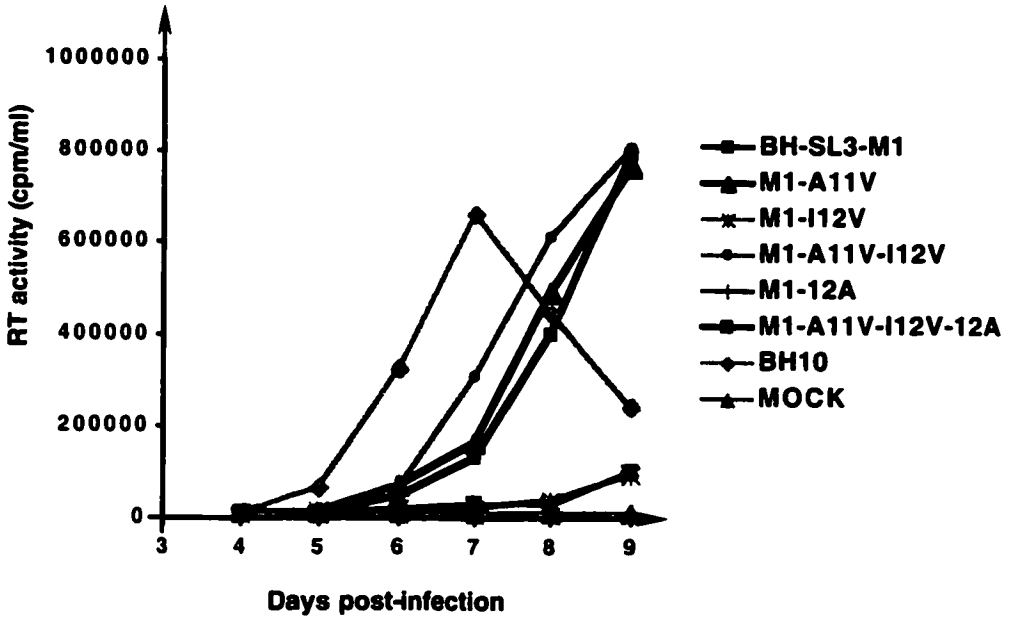
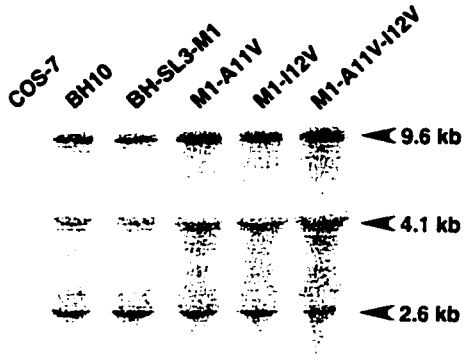

1000000
800000
600000
400000
200000
0
RT activity (cpm/ml)
3
4
5
6
7
8
9
Days post-infection
BH-SL3-M1
M1-A11V
M1-I12V
M1-A11V-I12V
M1-12A
M1-A11V-I12V-12A
BH10
MOCK

Figure 5-10. (A) Effects of the A11V substitution in p2 and I12V substitution in NC on viral RNA expression and splicing in transfected COS-7 cells. Transfected COS-7 cells were lysed in NP-40 lysis buffer. As described in Materials and Methods, viral RNA was extracted, fractionated on agarose gels, then transferred onto nylon membranes and hybridized with a labeled HIV-1 proviral DNA probe. The 9.6 kb band represents full-length viral genomic RNA; the 4.1 kb and 2.6 kb bands represent spliced viral RNAs.

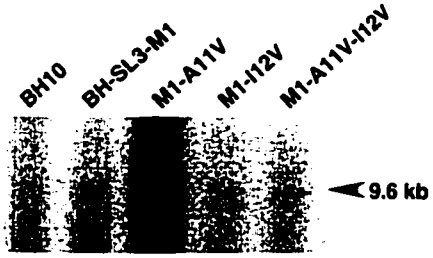
(B) Viral genomic RNA levels in the wild-type and aforementioned mutated viruses detected by Northern blot. Virus pellets were harvested from culture fluids of transfected COS-7 cells. Viral RNAs that are equivalent to 250 ng CA of virus pellets were subjected to Northern blot assay using a HIV-1 proviral DNA probe labeled with a nick translation kit.

(C) Viral genomic RNA packaging determined by RT-PCR. Using primer pair pGAG1/pST, RT-PCR was performed to detect levels of full-length viral RNA (Liang et al. 1998b). Viral RNAs employed in this experiment included BH10 (lanes 1, 6, 11), BH-SL3-M1 (lanes 2, 7, 12), M1-A11V (lanes 3, 8, 13), M1-I12V (lanes 4, 9, 14), and M1-A11V-I12V (lanes 5, 10, 15). RNA samples were digested with RNase A and then subjected to RT-PCR as a negative control to exclude the possibility of DNA contamination (lanes 1-5). RNAs were also diluted 1:4 before RT-PCR to ensure a linear range of these reactions (lanes 11-15). 10 ng of BH10 plasmid DNA was used in RT-PCR to serve as a positive control and to indicate the size of the PCR product (lane 16). The results represent three independent experiments.

A


COS-7
BH10
BH-SL3-M1
M1-A11V
M1-I12V
M1-A11V-I12V
9.6 kb
4.1 kb
2.6 kb

B


BH10
BH-SL3-M1
M1-A11V
M1-I12V
M1-A11V-I12V
9.6 kb

C

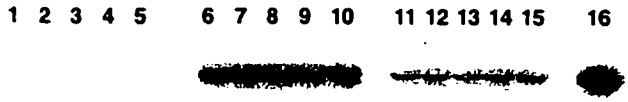
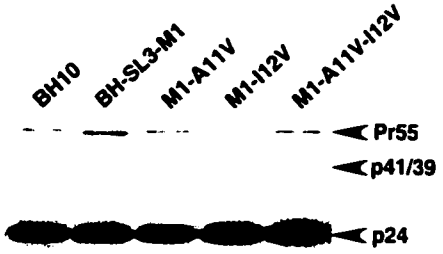

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Figure 5-11. Western blot of viral proteins in viral particles or cell lysates using anti-CA antibody. 48h after transfection, protein samples from viral pellets and transfected COS-7 cells were isolated as described in Materials and Methods. After fractionation on 12% SDS-polyacrylamide gels, they were transferred to nitrocellulose filters and sequentially incubated with anti-HIV-p24 immunoglobulin G1 (IgG1) monoclonal antibody (mAb) (ID Labs Inc., London, Ontario, Canada) and secondary anti-mouse IgG-horseradish peroxidase-conjugated antibody (Amersham Life Sciences, Oakville, Ontario, Canada). After thorough washing, viral proteins were visualized with an ECL chemiluminescence detection kit (Amersham Life Science, Amersham Place, England). The observed viral proteins, including $Pr55^{Gag}$, p41, p39, and p24, are labeled on the right of the gels.

Virus Particles



Cell Lysates

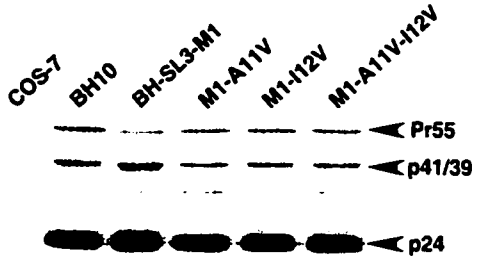
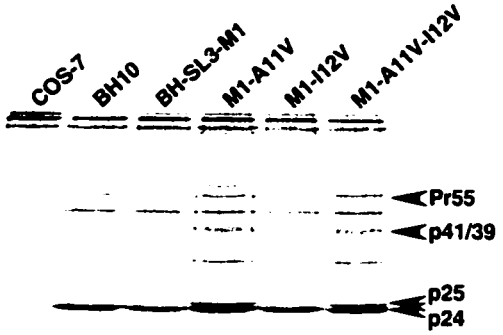


Figure 5-12. Processing of $Pr55^{Gag}$ in wild-type BH10 and mutated viruses containing substitutions of A11V in p2 and I12V in NC. Transfected COS-7 cells were labeled with [^{35}S]-L-Met and [^{35}S]-L-Cys for 30 min and cultured for 1h. Cells were then lysed and the lysates were subjected to immunoprecipitation studies through the use of mAb against CA (p24) Ag. Labeled virus particles were also pelleted from supernatants and were analyzed on 12% SDS-polyacrylamide gels. The positions of observed viral proteins, including $Pr55^{Gag}$, p41, p39, p25 and p24, are labeled on the right of the gels.

Virus Particles



Cell Lysates

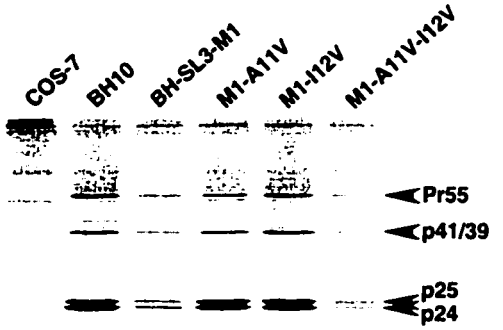


Figure 5-13. The positions and sequences of primary and compensatory mutations in this study.

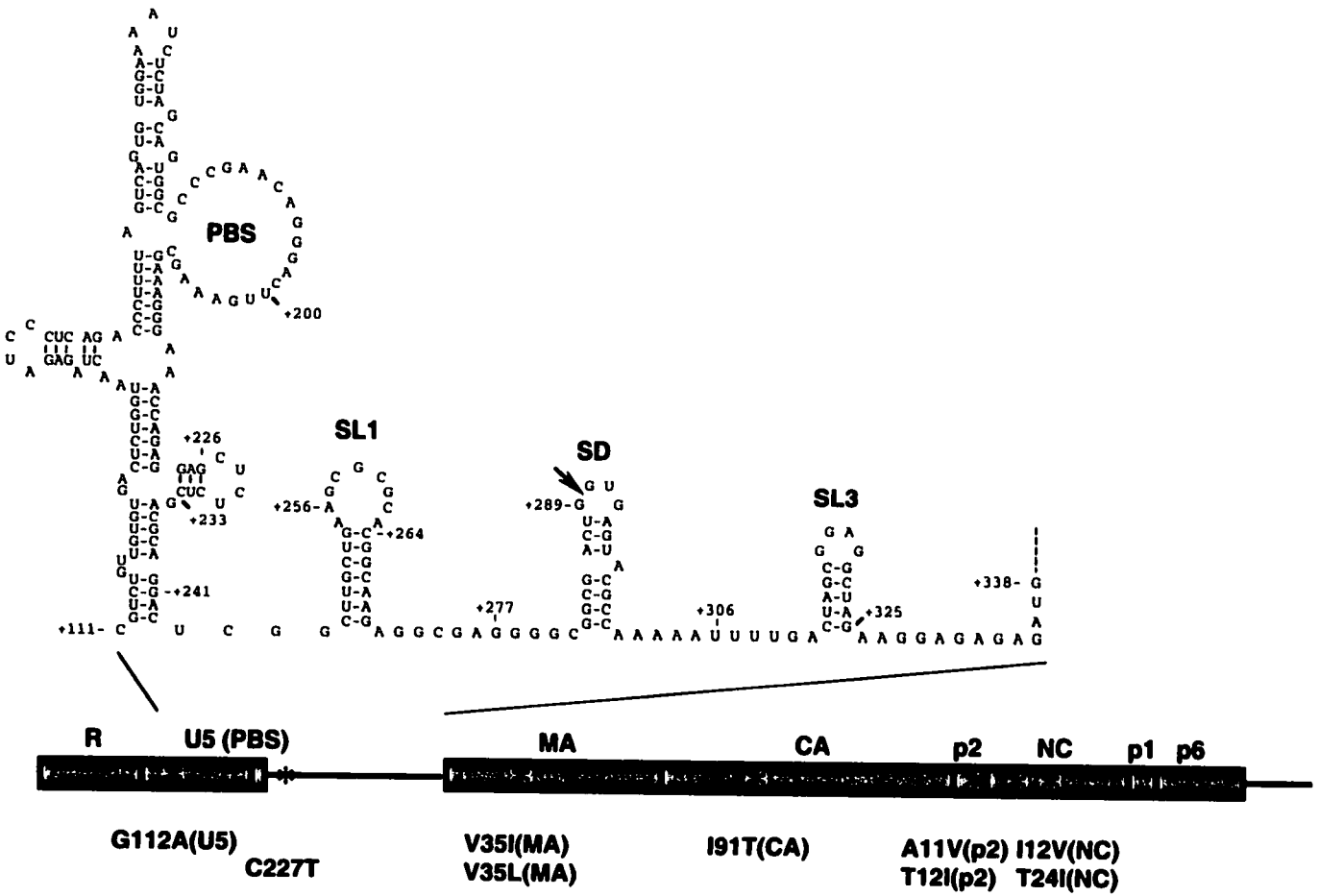

PBS
SL1
SD
SL3
+200
+226
+233
+241
+256
+264
+277
+289
+306
+325
+338
+111
R
U5 (PBS)
MA
CA
p2
NC
p1
p6
G112A(U5)
C227T
V35I(MA)
V35L(MA)
I91T(CA)
A11V(p2)
T12I(p2)
I12V(NC)
T24I(NC)

Table 5-2. Compensatory mutations of deletions of packaging signals up- or down-stream of the major SD site.

	U5 G112A	C227T	MA V35I	MA V35L	CA I91T	p2 A11V	p2 T12I	NC I12V	NC T24I
BH-D1:Δ+200-+226	+	+					+		+
BH-D2:Δ+200-+233	+						+		+
BH-LD3:Δ+241-+256			+		+		+		+
BH-LD4:Δ+264-+277				+			+		+
BH-SL3-M1:Δ+306-+325						+		+	

5.6 Discussion

We have previously deleted various sequences between the PBS and 5' SD site that impact on viral RNA packaging and dimerization. Interestingly, long term culture of all the mutated viruses gave rise to the MP2 and MNC compensatory mutations in the Gag protein (Liang et al. 1998b, 2000). In this study, we show that V, L, M, and C at position 12 in p2 can rescue the deletions, as can the MP2 (T12I in p2) substitution previously identified in culture. V, L, I, and M rescued the impaired infectivity of the mutated viruses by similar levels, while C contributed to a lesser extent. Similar findings were obtained with the MNC mutation (T24I in NC). Screening identified six amino acids at position 24 in NC, including V, L, I, M, F and Y, that helped to rescue the aforementioned deletions. Of these, F and Y were the least efficient.

These results demonstrate that the V, L, I and M residues were identified in both p2 and NC as the most efficient in the rescue process. Since V, L, I, and M all contain long hydrophobic side chains, we hypothesize that specific interactions of a hydrophobic nature must play a role in interactions between p2 and NC with other sequences of Gag and/or viral RNA in the rescue of the BH-D1, BH-D2 and BH-LD3 mutated viruses. The I residue may be the only one to have been identified in culture, because to achieve a T to I substitution involves only a single base change, *i.e.* ACC→AUC, whereas the other amino acid substitutions, *i.e.*, V, L, or M, require two or three nucleotide mutations simultaneously.

The packaging signal of HIV-1 possesses a multipartite nature: in addition to SL1, a region termed SL3, that is located downstream of 5' SD site, also contributes to viral RNA packaging process through its tight binding to Gag protein (Clever et al. 1995; McBride et al. 1996, 1997; Damgaard et al. 1998; Harrison et al. 1998). In this study, SL3 was deleted to generate construct BH-SL3-M1 (Δ+306-+325), which resulted in defective viral replication. We speculated that BH-SL3-M1

construct may have had altered interactions with Gag protein; evidence in support of this idea was provided by the identification of the compensatory mutations in p2 and NC, responsible for renewed growth of this mutated virus. Surprisingly, the SL3 deletion barely affected levels of viral RNA packaging (Figure 5-10), an observation that is differ from these of other groups, who reported significant decreases (Lever et al. 1989; Aldovini and Young 1990; Clavel and Orenstein 1990; Luban et al. 1994; Harrison et al. 1998). However, the sequence that we deleted is slightly shorter than that deleted by the others, such that only the SL3 hairpin and the adjacent six nucleotides upstream of SL3 were removed while the other hairpin structures were left intact. Therefore, the binding of our mutated leader sequence to Gag protein may also be different, leading to a differential influence on levels of viral RNA encapsidated into particles. Similarly, one of our SL1 mutations, *i.e.*, BH-LD4 (Δ+264-+277), produced a significant diminution in viral infectiousness, but only slightly affected levels of viral genomic RNA packaging, also related to compensatory mutations in p2 and NC (Liang et al. 1999b).

SL1 and SL3 may participate in viral genomic RNA encapsidation in different ways. For example, SL1 plays an essential role in viral RNA dimerization, a process that is associated with packaging; the placement of SL3 downstream of the 5′ SD site might provide the specificity of genomic RNA over spliced RNA for viral protein recognition and selection. Further proof supporting this hypothesis comes from a comparison of the types of compensatory mutations that occurred during long-term culture of the SL1 or SL3 mutated viruses. The positions and sequences of the primary mutations are illustrated in Figure 5-13, and the associated compensatory mutations are summarized in Table 5-2. In addition to two rare mutations, *i.e.*, G112A and C227T, that are involved in the rescue of D1/D2 and D1 deletions respectively, most of the compensation mutations were identified in the Gag protein. All the deletions that eliminated RNA elements upstream of the 5′ SD site gave rise to the same compensatory mutations in p2 and NC, while

deletion of SL3, which is located downstream of the major SD site, resulted in different mutations in these two proteins. This suggests that RNA elements up- and down-stream of the SD interact with Gag proteins differentially in the packaging process.

Nuclear magnetic resonance (NMR) analyses of the NL4-3 NC and the SL3 RNA complex indicated that direct contact occurs between the NC I24 residue and SL3 RNA; this implies that I24 participates in packaging (De Guzman et al. 1998). However, in BH10 the 24th position of NC is occupied by a T residue. Since multiple RNA elements are involved in the packaging process, interactions of NC with SL1 or other viral RNA elements may enable the virus to recruit wild-type levels of viral RNA during packaging. However, when the SL1 is deleted, a T24I change in NC appears necessary to strengthen the interactions between NC and SL3 RNA, such that the mutant virus can properly package viral RNA. Since V, L, and M contain similar hydrophobic side chains to I, their presence in NC can also correct defective viral RNA packaging. In support of this, BH10 viruses containing V, L, I or M at position 24 in NC packaged high levels of viral RNA (Figure 5-8).

Removal of the SL3 packaging signal may cause the mutated viruses to change NC amino residues, such that the affinity of Gag protein for other packaging signals (*e.g.* SL1) is increased. Since SL1 and SL3 differ both in nucleotide sequence and in secondary structure, it follows that NC can contact either SL1 or SL3 using different residues. Thus, other mutations in NC may be expected to compensate for the SL3 deletion. Supporting this hypothesis, a novel I12V substitution in NC was shown to compensate for the SL3 deletion. We speculate that this I12V mutation may increase the affinity of NC to the SL1 structure; and that this repairs the packaging deficit. NMR structure analysis of the NC and SL1 complex will be needed to clarify this point.

Compensation of both the SL1 and SL3 deletions involve point mutations in the p2 peptide. The T12I substitution is associated with the SL1 deletion, and the A11V substitution is identified with the SL3 deletion. p2 is critical for Gag processing and viral morphogenesis

(Pettit et al. 1994; Kräusslich et al. 1995; Accola et al. 1998; Wiegers et al. 1998). Recently, p2 was also shown to be involved in viral RNA packaging (Kaye and Lever 1998). Therefore, it is not surprising that point mutations in p2 can help to rescue deletions of the SL1 or SL3 packaging signals.

It is believed that RNA packaging may have already been accomplished before cleavage of Gag by protease, and that NC may mediate packaging while part of the Gag precursor protein. p2 is located immediately next to the amino-terminus of NC. It is possible that a part of p2, especially amino acids at its carboxyl-terminus, may be linked to NC and bind to viral RNA. In fact, amino acids ATIM (positions 11 to 14) at the carboxyl-terminus of p2 and amino acids MQ (positions 1 to 2) at the amino-terminus of NC can form a continuous α-helix on the basis of computer modeling (Morellet et al. 1999). In the NMR structure of NL4-3 NC and SL3 RNA, residues K3 to R10 form a 3_{10} helix of NC that binds within the RNA major groove (De Guzman et al. 1998). Thus, it is possible that the 3_{10} helix can extend to the p2 region, and that residues at the carboxyl-terminus of p2 can participate in the binding of viral RNA. On the other aspect, binding of p2 to viral RNA may also play an important role in Gag processing. This has been shown by cell-free experiments, in which the presence of RNA greatly facilitated the cleavage of the Gag precursor protein (Gross et al. 1997).

Chapter 6

General Discussion

RNA-chaperone activity of HIV-1 nucleocapsid protein and its Zn finger motifs

Proper RNA structure is often essential for efficient biological function. However, the tendency of RNA for alternate folds and the high thermodynamic stability of RNA duplexes make it possible for RNA molecules to assume a variety of conformations. Biochemical mechanisms must be able to control this process; these can include a "guide", matchmaker, or RNA-chaperone role, played by a protein (or RNA in certain cases) (Herschlag 1995). Through transient breaking of base pairs and subsequent re-pair of alternate combinations, an RNA-chaperone protein can lower the energy barrier required for unfolding of incorrect structures. Nonspecific interactions between RNAs and proteins may be key in this process; specific RNA-binding proteins may also use nonspecific interactions to facilitate the proper folding of a cognate RNA (Herschlag 1995).

HIV-1 nucleocapsid protein (NCp7) is a small basic protein, containing two Zn finger motifs of the $CX_2CX_4HX_4C$ form. It shows nucleic acid binding property *in vitro*, with a preference for single-stranded molecules (Darlix et al. 1995). NCp7 has been demonstrated to function as an RNA-chaperone protein in a wide variety of cell-free systems: it promotes annealing of complementary nucleic acid sequences (Dib-Hajj et al. 1993; Tsuchihashi and Brown 1994; You and McHenry 1994; Lapadat-Tapolsky et al. 1995); it enhances nucleic acid strand transfer from a less stable to a more stable hybrid (Tsuchihashi and Brown 1994; Lapadat-Tapolsky et al. 1995); it stimulates hammerhead ribozyme catalysis (Tsuchihashi et al. 1993; Bertrand and Rossi 1994; Herschlag et al. 1994; Müller et al. 1994).

This RNA-chaperone function of NC was also proved in more specific *in vitro* assays that mimic various steps during the HIV-1 viral replication cycle. Even as a domain of the Gag polyprotein, NC is able to catalyze the dimerization of retroviral genomes containing DIS sequences and the annealing of $tRNA^{Lys,3}$ to the PBS sequence of template RNA, either before or during viral assembly (Feng et al. 1999). After

virions are released from the cell, NC protein induces maturation of dimeric retroviral RNA, which is more thermostable and runs faster in gels than the non-mature viral RNA dimer (Fu et al. 1994; Feng et al. 1996). During reverse transcription, NCp7 is known to increase RT processivity, in part by altering template secondary structures (Rodríguez-Rodríguez et al. 1995; Tanchou et al. 1995; Ji et al. 1996; Wu et al. 1996). This protein can also enhance both minus and plus DNA strand transfers. In the first case, NC was found to drastically reduce self-priming, and catalyze the annealing of the nascent intermediate DNA to acceptor RNA (Peliska et al. 1994; You and McHenry 1994; Guo et al. 1997). In the second case, NC facilitates destabilization of the tRNA-DNA hybrid as well as an internal (-)PBS DNA hairpin, and stimulates annealing of the PBS sequence in (+)ssDNA to the acceptor DNA template (Wu et al. 1999; Johnson et al. 2000).

Our studies focused on the RNA-chaperone activity of HIV-1 NCp7 on the annealing of $tRNA^{Lys,3}$ to viral RNA template containing the PBS (Chapter 2, 4). Compared with a heat-annealing method, we found that the $tRNA^{Lys,3}$:vRNA initiation complex, formed in the presence of NC, resulted in higher efficiency of cDNA synthesis in subsequent reverse transcription reactions. This can be attributed, at least in part, to less pausing at the +1 nt position during the initiation stage, as well as to increased efficiency of the switch from initiation to elongation. In contrast to commonly accepted concepts in RNA-chaperone activity, several new facts emerged from work with this specific cell-free system:

1 An RNA complex with the most thermostable structure, *i.e.*, that formed by heat-annealing, may not always be the most functional.
2 The RNA-chaperone mechanism to explain the formation of the most stable RNA complexes includes a transient base-pair destabilization, followed by random re-pairing until the maximal number of base pairs is reached. However, in our system, NC was shown to assist in the formation of a functional RNA complex rather

than a thermostable one. This shows that a random or non-specific model does not fit well with our findings.

3 Zn finger motifs were found to be important; this provides further evidence that specific interactions between protein and RNA are involved in the proper folding of the tRNA:vRNA complex.

Therefore, the tRNA:vRNA complex formed in the presence of HIV-1 NC protein is a specialized one, which may involve both nonspecific and specific interactions between the protein and the RNA. The high levels of basic residues in NC could enable it to act as a nonspecific RNA-chaperone, and thus stimulate the formation of a tRNA:vRNA hybrid that resembles a heat-annealed complex. Subsequently, the RNAs involved might undergo a partial intra-complex rearrangement, until the correct conformation is achieved and trapped by specific interactions with the Zn finger motifs of the NC protein. This does not rule out the possibility that Zn finger motifs may act as small "guides" by trapping correctly folded subdomains in the second phase of this process.

Our findings are not isolated results; a Zn-finger dependent RNA-chaperone activity of NCp7 has also been reported in other *in vitro* studies. For example, an aromatic amino acid within the N-terminal finger, *i.e.* F16, is necessary for maturation of dimeric genomic RNA (Feng et al. 1996). Zn^{2+}-binding residues were also found to be indispensable for efficient minus and plus strand transfer during HIV-1 reverse transcription (Guo et al. 2000).

Initiation of HIV-1 reverse transcription

The current model of HIV-1 initiation of HIV-1 reverse transcription is based on studies of the $tRNA^{Lys.3}$:vRNA complex formed by the heat-annealing method. The secondary structure of this complex is characterized by extensive intermolecular interactions, including annealing of the 3'-terminal region of $tRNA^{Lys.3}$ with the PBS as well as base-pairing between the tRNA anticodon loop and an A-rich loop (GUAAAA) located 12-17 nucleotides upstream of the PBS (Isel et al. 1993, 1995).

Reverse transcription from $tRNA^{Lys,3}$ involves a specific initiation stage, during which the processivity of RT is four orders of magnitude lower than that of subsequent elongation, due to its rapid dissociation rate and low nucleotide incorporation efficiency (Lanchy et al. 1996a,b). A transition from initiation to elongation can be observed when $tRNA^{Lys,3}$ is used as primer, which is caused by dissociation of RT from the initiation complex after three or five nucleotides are incorporated; this was shown as short intermediate products, *i.e.*, +3, +5 products (Isel et al. 1996). Extended primer:template interactions are able to facilitate such a transition (Isel et al. 1996).

In our study, reverse transcription was performed at low dNTP concentrations, *i.e.*, 160 nM, and another phase of initiation was evidenced by the appearance of pausing after the first nucleotide incorporation. The presence of a +1 intermediate product was very specific: it disappeared when an 18 mer ODN with sequence complementary to the PBS was used as primer (Chapter 3); the presence of NC protein during tRNA primer annealing helped RT to escape this pausing (Chapter 4). These two findings are unique to the +1 but not +3 nt product, indicating that the former is a distinct stage during initiation. Since a deoxyribonucleotide 3'-OH appeared at the 3'end of the tRNA primer, rather than the original ribonucleotide 3'-OH after the first nucleotide incorporation, a conformational rearrangement of RT may be necessary; this might cause primer-specific pausing at the +1 position. Such a conformational change of RT was confirmed by the presence of a 19 nt instead of the usual 18 nt distance between the RNase H cleavage site and the polymerization active site after addition of the first dC at the 3'end of the $tRNA^{Lys,3}$ primer (Götte et al. 1995).

Through mutagenesis studies, we have also found that the presence of the +3 nt pause site is solely due to the specific secondary structure of the tRNA:vRNA complex, *i.e.* an 8-base-pair stem structure located 3 nt upstream of the PBS (Isel et al. 1993, 1995; Skripkin et al. 1996); the sequence of the right half of this stem also

participates in efficient switching from the +3 nt pause site to an even later initiation intermediate at the +5 nt position (Chapter 4).

Based on these findings, we proposed a model for the initiation of HIV-1 reverse transcription. As shown in Figure 6-1, HIV-1 RT initiates reverse transcription as $tRNA^{Lys.3}$ is placed onto the PBS. After the first dC is added to the 3'-OH of the ribonucleotide at the 3'end of $tRNA^{Lys.3}$, RT undergoes a structural rearrangement of the polymerization active site. This is required for the enzyme to adapt to the new dC-G pair before addition of the next deoxyribonucleotide (dT) to the one-base extended primer, and is the cause of the +1 nt pause site. In addition to the nature of the primer used, the presence of NC protein during primer annealing can also affect this specific initiation event.

Once the tRNA primer is extended by three bases, RT meets the first stem structure of the template, and disrupts it before the addition of the fourth deoxyribonucleotide. This leads to the dissociation of RT from the initiation complex and the pausing event at the +3 nt position. The resumption of the reaction requires the rebound of RT within the complex as well as the correct context of the CUAGA sequence, 4-8 nucleotides upstream of the PBS. Before the end of initiation, RT again dissociates from the initiation complex at the +5 nt position via an unknown mechanism. After five nucleotides are incorporated, a transition from initiation to elongation can be observed, which is caused by the dissociation of RT from the initiation complex and its rebound with the complex in an elongation conformation.

Another important early event, *i.e.* a two-base-extended product, is not included in this model. It appears that RT does not dissociate from RNA template upon addition of the second nucleotide, since no pausing has ever been observed at the +2 nt position in *in vitro* experiments. However, this intermediate can be detected in HIV-1 virus particles, which may indicate its important biological relevance in initiation of reverse transcription *in vivo* (Oude Essink et al. 1996; Huang et al. 1997b).

***Trans*-acting factors involved in HIV-1 genomic RNA packaging**

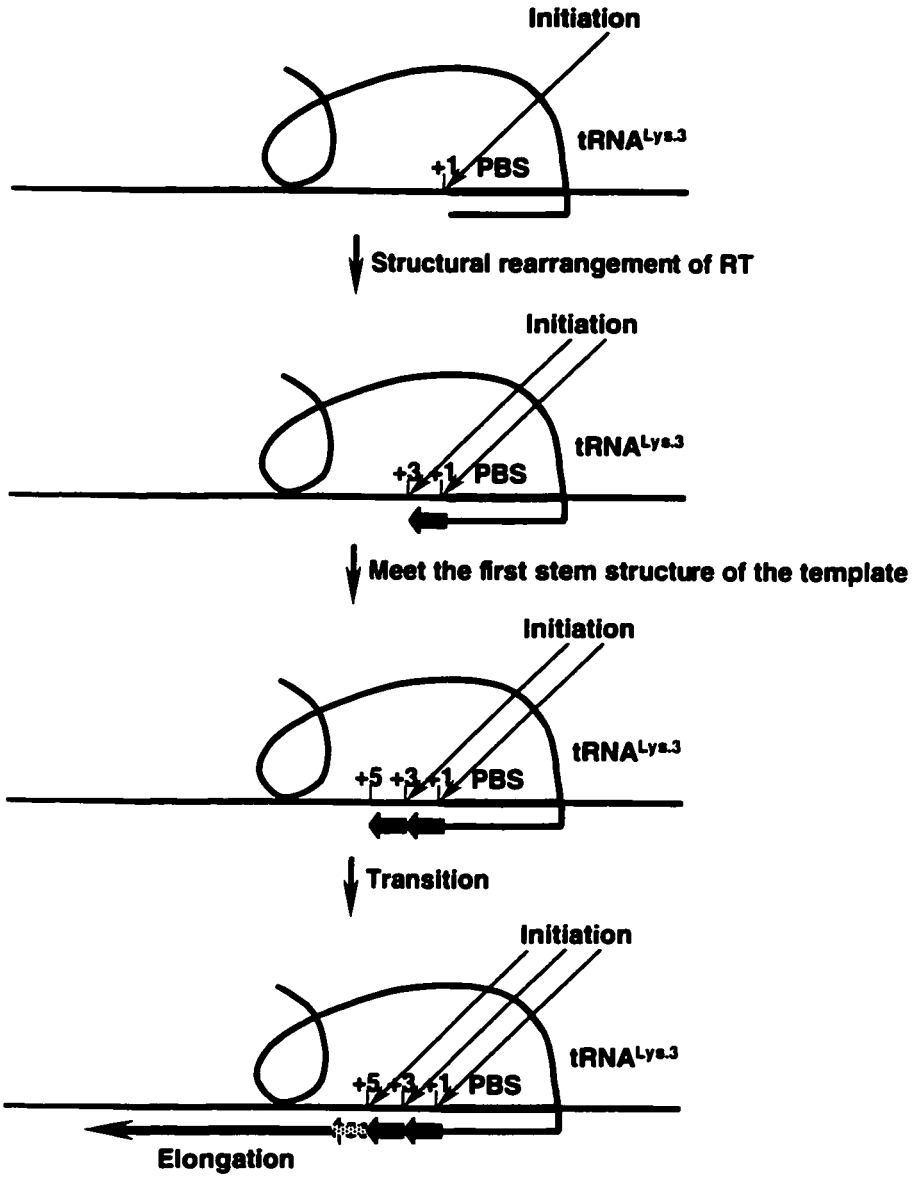
HIV-1 genomic RNA encapsidation is a highly specific process, resulting in selective incorporation of unspliced viral mRNA into virus particles from a high background of cellular mRNAs and various subgenomic viral RNAs. The *cis*-acting sequences involved in this process consist of discrete functional stem-loop structures in the 5' leader sequence; these mainly include SL1, SL3, and SL4 (Lever et al. 1989; Aldovini and Young 1990; Clavel and Orenstein 1990; Kim et al. 1994; Luban and Goff 1994; McBride et al. 1996, 1997; Paillart et al. 1996; Harrison et al. 1998). The major *trans*-acting factor involved was identified as the NC domain of the Gag polyprotein (Aldovini and Young 1990; Gorelick et al. 1990; Jowett et al. 1992; Berkowitz et al. 1995; Clever et al. 1995; Zhang and Barklis 1995). Through mutagenesis studies, Zn finger motifs of the NC protein were found to be responsible for specific recognition and interaction with the packaging sequences (Aldovini and Young 1990; Berkowitz et al. 1995; Zhang and Barklis 1995). Basic residues of NC protein were also found to be indispensable for efficient HIV-1 RNA packaging, though they were generally believed to contribute to this process by non-specific binding with nucleic acid (Ottmann et al. 1995; Poon et al. 1996). Consistent with these findings, a NMR structure of HIV-1 NC protein bound to the SL3 sequence, revealed that both Zn fingers were engaged in highly specific interactions with purine bases of the GGAG RNA tetra-loop, whereas the four N-terminal conserved basic residues only participated in non-specific, electrostatic interactions with RNA backbones (De Guzman et al. 1998).

Other viral proteins may also contribute to HIV-1 selective RNA encapsidation, since exchange of the NC domain between HIV-1 and MMTV did not change the specificity of the RNA molecules that were packaged (Poon et al. 1998). p2 is a prominent candidate, since the introduction of HIV-1 p2 in addition to the NC domain into HIV-2 significantly enhanced the encapsidation of HIV-1 vector RNA (Kaye and Lever 1998). Furthermore, compensation of both the SL1 (Liang et al. 1999a,b;) and SL3 (Chapter 5) deletions involve point mutations in the p2 peptide. In

the NMR structure of NC and SL3 RNA, mentioned above, residues K3 to R10 form a 3_{10} helix that binds within the RNA major groove (De Guzman et al. 1998). On the basis of computer modeling, amino acids ATIM (positions 11-14) at the carboxyl-terminus of p2 and amino acids MQ (positions 1-2) at the amino-terminus of NC can also form a continuous α-helix (Morellet et al. 1999). Therefore, the 3_{10} helix may extend to the p2 region within the Gag polyprotein, and residues at the carboxyl-terminus of p2 may participate in the interaction of Gag with viral RNA.

In addition, a dsRNA-binding protein, *i.e.* human Staufen (hStau), was also found to be incorporated into HIV-1 virions and to be associated with viral genomic RNA. Positive correlations between levels of hStau expression and of viral genomic mRNA encapsidation suggested a role for hStau in retroviral genome selection and packaging (Mouland et al. 2000).

Figure 6-1. Model of the initiation of HIV-1 reverse transcription.


Initiation
tRNA$^{Lys.3}$
+1 PBS
Structural rearrangement of RT
Initiation
tRNA$^{Lys.3}$
+3 +1 PBS
Meet the first stem structure of the template
Initiation
tRNA$^{Lys.3}$
+5 +3 +1 PBS
Transition
Initiation
tRNA$^{Lys.3}$
+5 +3 +1 PBS
Elongation

Contribution to Original Knowledge

The following is a summary of my original work and contributions to the scientific research community under the supervision of Dr. Mark. A. Wainberg.

Chapter 2. The HIV-1 NC protein is a major structural component of the virion nucleocapsid, where it is tightly associated with the genomic RNA dimer and primer $tRNA^{Lys,3}$. NC acts as a nucleic-acid-chaperone *in vitro*, *i.e.*, it stimulates the folding of nucleic acids into optimal conformations by preventing misfolding or by resolving misfolded species. Due to this unusual biochemical activity, NC plays a variety of crucial roles throughout the virus life cycle, including promoting the placement of the tRNA primer onto the viral RNA template, and destabilizing the secondary structure in the RNA template that RT encounters during elongation of reverse transcription. In this chapter, we employed synthetic NC protein to place natural $tRNA^{Lys,3}$ onto an *in vitro* transcribed viral RNA template. Then, NC was removed from this annealed complex by Proteinase K and phenol:chloroform treatment. We found that NC protein at saturating concentrations facilitated the formation of an active tRNA:vRNA complex that showed enhanced transition from initiation to elongation as well as an elevated efficiency of elongation during (-)ssDNA synthesis. This is the first study to show that the chaperone activity of NC is not only to form a thermostable RNA structure but is also functional. Furthermore, disputes about the function of the NC Zn fingers during primer annealing and the RT elongation process were also resolved in our studies. We found that NC that was mutated in the Zn fingers retained annealing activity, as shown in one-base extension assays; however, these motifs were indispensable to yield an RNA complex that was able to generate full-length (-)ssDNA with high efficiency.

Chapter 3. On the basis of different RT polymerization/ dissociation rates, reverse transcription of (-) strand DNA in HIV-1 can be divided into two stages: initiation and elongation. The initiation stage was detected on gels by the presence of short cDNA products at +3/+5 nt positions. The efficient transition from initiation to elongation was determined on the basis of proper secondary structure formed between $tRNA^{Lys.3}$ and the viral RNA template. In our cell-free RT reaction system described in this chapter, initiation of HIV-1 (-) strand DNA synthesis was characterized by early pause sites at the +1 and +3 nt positions. This is the first demonstration that pausing at the +1 nt site represents a rate-limiting step in initiation of HIV-1 reverse transcription. The +1 site is distinct from other early pausing sites in the initiation stage, since reactions primed by an oligo DNA, with sequences complementary to the PBS, did not pause after addition of the first nt. A conformational re-arrangement of RT may occur as soon as the first nt is incorporated, so that its binding mode can be switched from an RNA-RNA duplex to a DNA-RNA hybrid. Mutagenesis studies also showed that the formation of the +3 pausing site was dependent on sequences upstream of the PBS; deletion of an A-rich loop at the 5'end of the PBS resulted in the impairment of initiation as well as an arrest of DNA synthesis at the +1 stage.

Chapter 4. Based on the observations of the above two chapters, we further employed synthetic wild-type or Zn finger mutated NC proteins to place natural $tRNA^{Lys.3}$ onto HIV-1 viral RNA template, and then examined the resultant RNA complexes in the subsequent initiation of (-) strand DNA synthesis. We found that reverse transcription that was initiated from wild-type NC-annealed tRNA but not from mutated NC-annealed tRNA escaped the pausing at the +1 nt position. Therefore, the Zn finger motifs of HIV-1 NC protein are necessary for the formation of $tRNA^{Lys.3}$:vRNA binary complex that is favored during initiation of (-) strand DNA synthesis. On the basis of Chapter 3, we speculated that the

appearance of +3 nt pause site might be due to the stall and dissociation of RT when it encounters the first stem structure on the RNA template. This hypothesis was proved in this study by introduction of mutations into the RNA template. The disruption of the stem located 3 nt upstream of the PBS, within the tRNA:vRNA complex, led to disappearance of the +3 pause site. In this chapter, we also show that initiation from the virion-derived tRNA:vRNA complex can also be detected, indicating that reverse transcription in the virus undergoes an initiation stage as well. Furthermore, through calculations of the relative amount of each short cDNA product, we found that the virion-derived complex is more functionally related to a NC-annealed complex than to a heat-annealed one. This observation provides *in vivo* evidence for the functional chaperone activity of NC, that we have demonstrated in cell-free assays.

Chapter 5. The HIV-1 NC protein also participates in specific encapsidation of viral genomic RNA through tight binding with the *cis*-acting packaging elements SL1 and SL3. Both of these RNA elements are located in the HIV-1 5′ leader sequence, flanking the major splice donor (SD) site. Compensation studies have identified two second-site mutations that are involved in the rescue of various deletions in the SL1 region, *i.e.* a T12I substitution in p2 and a T24I substitution in the NC protein. In this chapter, we changed the T12 of p2 or the T24 of NC to each of 19 other amino acids, and found that V, L, I and M were the most efficient at rescue of viral replication capacity. These four amino acids have long hydrophobic side chains, which are essential in the correction of the defective specific interactions between p2/NC and viral RNA. This was further confirmed by studies of the SL3 deletion for which compensatory mutations also involved hydrophobic residue (V) substitutions in p2 and NC. However, these mutations appeared at two novel sites, *i.e.* A11V in p2 and I12V in NC, suggesting that RNA elements located up- or down-stream of SD may interact with Gag protein in different fashion during viral RNA packaging.

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Manly Smokes:
Tobacco Consumption and the Construction of Identities in Industrial Montreal, 1888-1914

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Abstract

This dissertation explores the cultural practice of smoking and its connection to social relations from the beginning of cigarette mass production in Montreal in 1888 to the First World War. It uncovers the norms of smoking etiquette and taste, their roots in gender, class and race relations and their use in reproducing these power relationships. It argues that these prescriptions reflected and served to legitimize beliefs about inclusion, exclusion and hierarchy that were at the core of nineteenth-century liberalism. Liberal ideals of self-control and rationality structured the ritual of smoking: from the purchase of tobacco; to who was to smoke; to how one was supposed to smoke; to where one smoked. These prescriptions served to normalize the exclusion of women from the definition of the liberal individual and to justify the subordination of the poor and cultural minorities. Furthermore, even while these prescriptions were at their height, an emergent group of beliefs began to recast notions of respectable smoking around new ideals of speed and ungendered universality. This challenge was not only part of the transition from bourgeois to mass consumption, it was the roots of a transformation of the liberal order in the years previous to the First World War.

Résumé

Le tabagisme, en tant que pratique culturelle en lien avec les relations sociales, constitue l'objet de cette thèse. L'étude couvre la période qui va des débuts de la production de masse de la cigarette à Montréal en 1888 jusqu'à la Première Guerre mondiale. Elle dégage les normes associées au tabagisme, les goûts des fumeurs et leur étiquette, leurs origines de genre, de classe et d'ethnicité, en plus de leur utilisation dans la reproduction de ces relations de pouvoir. L'hypothèse soutenue veut que ces normes, ces prescriptions, reflétaient et voulaient légitimer les valeurs d'inclusion, d'exclusion et la hiérarchie inhérentes au libéralisme du XIX^{e} siècle. Les idéaux libéraux de contrôle de soi et de rationalité ont effectivement structuré les rituels du tabagisme : quel tabac choisir et acheter, qui peut fumer, comment et où fumer. Ces normes ont servi à exclure les femmes de la définition de l'individu libéral et à justifier la subordination des pauvres et des minorités. Au moment même où les normes issues du libéralisme du XIX^{e} s'imposaient avec le plus de vigueur, un nouveau système normatif émergeait. Par les idéaux de vitesse et d'universalité non genrée qu'il valorisait, ce nouveau système a entraîné une timide modification des normes associées au tabagisme. Cette remise en question ne représente pas seulement un aspect de la transition de la consommation bourgeoise à la consommation de masse, elle marque les débuts d'une transformation de l'ordre libéral dominant au cours des années précédant la Première Guerre mondiale.

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Steve Watt, Suzanne Morton, and Brian Young have contributed to my intellectual development and taught me much about collective research and the politics of academia. Individually, Lorraine O'Donnell and Steve Watt provided useful suggestions on parts of the thesis on short notice. Karine Hébert translated my abstract and along with Julien Goyette, discussed organizational strategies at a crucial moment. Suzanne Morton has given me great teaching advice, read a version of the thesis and asked questions that pushed me in useful directions. My thesis supervisor, Brian Young, has given me far more than I ever really expected. Certainly he has furnished valuable criticism and advice, but he also put confidence in me "to find" my topic, and above all, he has been a good friend. Brian is the most recent of people who have kept me fascinated in history. This list includes Barry Tate, Phil Werstein, Pat Coling and Donald Davis, who at the University of Ottawa, originally suggested that I "work on tobacco."

While researching in Ottawa I stayed with Vincent Klassen, Frédérique Tsai and their family, sometimes for long periods of time. My gratitude to them goes well beyond food, shelter, and not unimportantly, a personal introduction to cigar smoking. When their daughter Emmanuelle was born I was named as her godfather, an honour that has made me think of them as family more than friends. In the depths of graduate work, spending time with Emmanuelle and her brother Alexandre, playing with dolls or telling bedtime stories, renewed my energy and allowed me to escape an unhealthy obsession with my own work.

My largest debt is to my own family. Montreal is a long way, in many senses, from where I grew up – the small but proud town of Tavistock. My brother, Jensen, and his wife, Heidi, have visited me on numerous occasions, helping to reduce the distance. My parents, Bob and Joan Rudy, have encouraged me in so many ways it is difficult to sum up in a few lines. They have celebrated my successes and sent care packages of chocolate-covered coffee beans and cigars when the thesis was not going well and it seemed like it would never end. I love them greatly and the thesis never would have been completed without their support. One of their great gifts was to raise my brother and me as part of a tightly knit extended family. Within this extended family I want to recognize three women who have shaped who I am. My grandma, Doris Rudy, has always been an enthusiastic supporter of my work and my intellectual and political journeys. She is an avid newspaper "clipper" – and the fact that newspapers are one of the key sources for this dissertation is, I think, no coincidence. Finally, my two great aunts, Helen Rudy and her late sister, Ethel Rudy, are my family's first historians. The stories they tell, and have told, reveal a passion for the everyday concerns of "ordinary" people. They personify the kind of historian and person I want to be. It is to these three women I dedicate this thesis.

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List of Abbreviations

ATCC	- American Tobacco Company of Canada
CCTJ	*-Canadian Cigar and Tobacco Journal*
CMA	-Canadian Manufacturers Association
CMIU	-Cigar Makers' International Union
CMOJ	*-Cigar Makers' Official Journal*
MTLF	-Montreal Trades and Labor Federation
RCLT	-Royal Commission on the Liquor Traffic
RCRLC	-Royal Commission on the Relations of Labor and Capital
RCTT	-Royal Commission on the Tobacco Trade in Canada
WCTU	-Women's Christian Temperance Union

Introduction

This dissertation explores the cultural practice of smoking and its connection to social relations from the beginning of cigarette mass production in Montreal in 1888 to the First World War. While people smoked for personal reasons, these rituals were shaped by norms of smoking etiquette and taste, what I call prescriptions. Smoking prescriptions were rooted in, and served to mold, gender, class and race relations. My thesis statement is twofold. First, prescriptions around smoking reflected and perpetuated beliefs about inclusion, exclusion and hierarchy that were at the core of nineteenth-century liberalism. These liberal ideals of self-control and rationality structured the ritual of smoking: from the purchase of tobacco; to who was to smoke; to how one was supposed to smoke; to where one smoked. As these liberal prescriptions were at their height, a new "structure of feeling" was emerging, aiming to redefine notions of "proper" smoking rituals.[1] The second element of my argument is that this challenge was not only part of the transition from bourgeois to mass consumption, it was part of the transformation of the liberal order in the years previous to the First World War.

Studies of liberalism in Quebec have, for the most part, concentrated on the political and economic ideology of political, religious and business elites. Discussion has revolved around the very existence of liberalism in the face of clerical-nationalists. And while its existence has been confirmed by Fernande Roy and others, significant questions

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[1]On "structures of feeling" and their transformation, see Raymond Williams, *Marxism and Literature* (Oxford: Oxford University Press, 1977), pp.121-135.

about gender, class and race remain.[2] As Ian McKay has pointed out, nineteenth-century liberalism was "something akin to a secular religion or a totalizing philosophy ... [rather than]... an easily manipulated set of political ideas" and notions of what constituted the "individual" were broadly internalized into everyday life.[3] According to McKay, the "individual" of nineteenth-century liberalism was only partially related to the concept of a "living human being." Nineteenth-century liberalism defined the "individual" as a "rational" and "self-possessed" person. These were ideals, built on gender, class and racial norms that provided the criteria for political inclusion and exclusion. Women, workers and numerous ethnic groups were often excluded from this definition of an "individual," furnishing the rationale for their political exclusion. This thesis is part of a growing number of investigations into the everyday rituals of political order which include John Kasson's study of manners, Keith Walden's analysis of the Toronto Industrial Exhibition, Mary Ryan's examinations of parades and public celebrations and David Scobey's investigation of promenading.[4]

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[2]Fernande Roy outlines many of the important issues within this debate in her *Progrès, Harmonie, Liberté. Le libéralisme des milieux d'affaires francophones de Montréal au tournant du siècle* (Montreal: Boréal, 1988). Since Roy's book, others have stayed within the parameters she sets out. See Yvan Lamonde, *Louis-Antoine Dessaulles, 1818-1895: un seigneur libéral et anticlérical* (Saint-Laurent: Fides, 1994); Yvan Lamonde (dir.) *Combats Libéraux au XX^e Siècle* (Montreal: Fides, 1995).

[3]Ian McKay, "The Liberal Order Framework: A Prospectus for a Reconnaissance of Canadian History," *Canadian Historical Review*, 81, 4 (December 2000), pp.624-625.

[4]John Kasson, *Rudeness and Civility: Manners in Nineteenth-Century Urban America* (New York: Hill and Wang, 1990); Keith Walden, *Becoming Modern in Toronto: The Industrial Exhibition and the Shaping of a Late Victorian Culture* (Toronto: University of Toronto Press, 1997); Mary P. Ryan, "The American Parade: Representations of the Nineteenth-Century Social Order," in Lynn Hunt, ed. *The New*

Studying the social practices and cultural symbolism of smoking in late nineteenth-century Montreal reveals the extent to which liberalism was internalized as a powerful male identity and created social hierarchies to justify subordinating people on the basis of their gender, class and ethnicity. This was most clear around questions of gender. Nineteenth-century notions of respectable smoking dictated that women were not supposed to smoke. The rationale went to the heart of liberal definitions of the individual – women did not have the power of self-control. Their health, their safety, the safety of others as well as the role for which women were most valued, that of reproduction, were all at stake. Nor did women have the capacity to be rational economic actors and choose a quality tobacco. Only men were respectable smokers. They saw themselves as having self-control and as connoisseurs. Smoking brought men together. It gave odour and visible shape to spaces socially constructed as male.[5] Women who entered not only risked infecting their clothes with its smell, they put their respectability into question.

Though the terms were often inseparable, by the end of the nineteenth century class related differently than gender to notions of the liberal individual. Once again, smoking provides several insights. Tobacco was inexpensive enough that almost all men

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Cultural History (Berkeley: University of California Press, 1989), pp.131-153, *Women in Public: Between Banners and Ballots, 1825-1880* (Baltimore: Johns Hopkins University Press, 1990) and *Civic wars: democracy and public life in the American city during the nineteenth century* (Berkeley: University of California Press, 1997); David Scobey, "Anatomy of the promenade: the politics of bourgeois sociability in nineteenth-century New York" *Social History*, (May 1992), pp.203-227.

[5]Mary Douglas and Baron Isherwood, *The World of Goods: towards an anthropology of consumption* (New York: Routledge, first published 1979, this edition 1996), p.45.

could smoke, regardless of class. Still, not all men could afford to smoke highly-esteemed tobacco and the value of the tobacco reflected on the character of the smoker. The symbolic consequences of smoking poorly regarded tobacco worked differently for the rich and the poor. A wealthy man could smoke a low quality tobacco and in the end still be rich whereas for a poor man to smoke an inferior tobacco was seen as a *reflection* of his character and a cause of his class position. Similarly, there were considerable material difficulties to following the gender prescriptions of space around smoking. Not everyone could provide a separate space for male smokers and the consequences of not being able to segregate the sexes by smoking reflected on the character of the smoker and any women present. Yet men could also perform their class by exhibiting self-control in public situations and refrain from smoking when in the presence of women. Self-control also became a class issue as the amount of time a man could spend smoking was limited by his job, making it difficult to live up to the ideal of the leisurely, self-controlled smoker. Conversely, working class poverty could be blamed on the individual through his excessive smoking. These prescriptions on smoking served to naturalize material inequalities as the fault or choice of the individual, rather than being precipitated by structural problems within the economy or the inequalities of class.[6]

People of other cultural backgrounds were also judged on their ability to abide by these liberal prescriptions around smoking. Smokers that were racially "othered" included

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[6]This is the same liberal ideal that stigmatized the unemployed (neo-liberal governments continue to invoke this reasoning), see James Struthers, *No Fault of Their Own: Unemployment and the Canadian Welfare State, 1914-1941* (Toronto: University of Toronto Press, 1983).

not only people from foreign lands like the Philippines and eastern Europe, but also Natives and farmers from rural Quebec. In this era of mass immigration to Montreal, these complaints about questionable smoking habits were symbolic of larger anxieties about the racial constitution of the nation. Transgressions were offered as proof of inferiority and unworthiness of citizenship. While female smoking was frowned upon in turn-of-the-century Montreal, women smokers elsewhere were not stigmatized. When these female smokers arrived in Montreal the fact that they smoked played a role in constructions of feminine incivility. The hierarchies of tobacco used to judge the character of smokers were also culturally specific and partially based on racial and gender ideologies naturalized through the structures of the market. Smokers who had formed their tastes elsewhere risked being labeled "tasteless."

It is important to clarify that while these liberal prescriptions were dominant, smoking was part of other symbolic systems operating in Montreal. Indeed, though French Canadian tobacco was disdained according to dominant hierarchies of taste, it was symbolic of a particularly rural vision of the French Canadian nation. Prostitutes and dandies also used smoking to create feminine and masculine identities outside of dominant norms. From a radically different point of view, the Women's Christian Temperance Union (WCTU), which was established in Montreal in 1883, contested the dominant notion that smoking was a symbol of respectable masculinity. Rather, they saw it as a threat to the race and nation and their beliefs in social gospel theology pushed them to organize campaigns for age restriction laws and prohibition of the cigarette. Outside the WCTU, others were attempting to transform the ritual in other directions. As part of their

campaigns to be included as liberal subjects, other women sought to retain the ideals of rationality and self-control that smoking signified and erase the gender exclusivity of the ritual. Smoking, more than any other consumer good, held particularly liberal symbolism even as liberalism transformed.

The tension between liberal preoccupations with self-possession and rationality, on one hand, and smoking's addictive nature, on the other, made smoking a particularly useful and tenacious ritual of liberal values. Anthony Arblaster writes that according to eighteenth and nineteenth-century liberal texts, the rational individual "is not the one who merely *uses* reason to guide and assist his desires. He is the man who through reason liberates himself from the tyranny of appetite and desire...."[7] Even in the nineteenth century there was an awareness of tobacco's "tyranny of appetite and desire," but its seemingly benign effects in comparison to alcohol or drugs meant it was a surmountable, though not insignificant risk, and thus a particularly meaningful display of self-control.[8]

In the period between 1888 and 1914, Montreal was a fascinating case study in the everyday workings of the liberal order because of its divided and rapidly transforming social and cultural landscape. The city was the industrial capital of Canada and boasted the most economically powerful bourgeoisie in the country. Both facts suggest

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[7]Anthony Arblaster, *The Rise and Decline of Western Liberalism* (New York: Basil Blackwell Inc., 1984), p.36.

[8]My comparison of these consuming rituals is drawn from Cheryl Krasnick Warsh, ed. *Drink in Canada: Historical Essays* (Montreal: McGill-Queen's University Press, 1993); Wolfgang Schivelbusch, *Tastes of Paradise: A Social History of Spices, Stimulants, and Intoxicants* (New York: Vintage Books, 1992); and Jordan Goodman, Paul E. Lovejoy and Andrew Sherratt, eds. *Consuming Habits: Drugs in History and Anthropology* (New York: Routledge, 1995).

particularly fruitful explorations into the diverse workings of class in relation to these liberal prescriptions and into how the ritual was transformed by industrial capitalism. Being the major Canadian metropolis of the period also meant that Montreal had significant women's groups, allowing for a more extensive gender analysis of opinion on smoking. With the city's division between language groups, Montreal provides a unique opportunity to explore the nature of social relations between Francophones and Anglophones outside the sphere of formal politics. Immigration also marked Montreal's urban landscape in several ways. Massive numbers of foreign immigrants arrived in Montreal during the period, and whether they stayed or not, their presence received comment. These derogatory remarks said more about the racial views of those already in Montreal than about the "civility" of those arriving. Similarly, the wave of rural French Canadian immigrants arriving in Montreal was not beyond the condescending eye of both Anglophone and Francophone Montrealers. Religious affiliations in Montreal were also greatly divided, between Roman Catholic and Protestant as well as between Protestant denominations.[9] The roles of these groups – business, organized labour, women's groups, Francophones and Anglophones, recent immigrants, and churches – in the construction of, or opposition to, the liberal order through the prism of smoking rituals is the subject of the thesis.

Montreal is also uncommonly valuable case study because it was the centre of the Canadian tobacco industry during this period and these businesses were powerful players

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[9]Paul-André Linteau, *Histoire de Montréal depuis la Conféderation* (Montreal: Boréal, 1992).

in the production of meaning around smoking products. In 1888, the Davis family, one of Montreal's most prominent cigar manufacturing families, introduced the Bonsack cigarette machine, allowing cigarettes to be made faster and cheaper than ever before. Soon after, a branch plant of James Buchanan Duke's American Tobacco Company dominated the Canadian cigarette industry with the eldest Davis son, Mortimer, at its head. Davis then proceeded to break into the pipe tobacco market monopolized by Sir William Macdonald. Part of Davis's challenge to Macdonald's stranglehold on the market was through the use of mass-advertising to convince Macdonald smokers to switch to an American Tobacco Company of Canada (ATCC) brand. While the technological innovations used in the cigarette and tobacco industries required large investments of capital, there were few new technologies in the cigar industry, keeping it relatively free of monopoly. Instead cigar manufacturers faced off with cigar makers over changes in work process and reduced wages in a turbulent period of labour relations. All of these issues of production, labour, distribution and marketing were important to perceptions of quality and the availability of a smokers' favourite tobaccos.

A cultural examination of smoking is particularly rich because, as well as being a ritual of the liberal order, smoking was also a ritual of consumption. Canadian historians have never entirely ignored questions of consumption during the era, usually portraying them as questions of the unequal bounties and failures of industrial capitalism. Social historian Terry Copp, for example, discussed consumption in terms of cost of living of

Montrealers and the poverty of the city's working class resulting from industrialization.[10] Subsequently, feminist historians of the working class like Bettina Bradbury have shown that women played the particularly important role of buying their family's basic necessities.[11] Yet it is beyond the problematiques of these working class historians to link what were admittedly modest purchases to structural changes in the economy. Culturally informed purchases, however, made industrial growth possible, most obviously in the consumer goods sector. By looking at the cultural underpinnings of demand we can humanize economic change, linking personal decisions to what in Quebec and Canada have been presented as culturally neutral market economies.[12] Anyone who has been involved in boycotting businesses for their labour practices in the third world should see themselves here.

Studying the cultural meanings and social uses of smoking is also important because of today's health concerns. Current research has shown that these too are framed by cultural issues. For example, research shows that the group most likely to take up smoking is young women who use tobacco as an appetite suppressant to control their weight. Among other reasons, smoking, for them, is used as a response to cultural ideals

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[10]Terry Copp, *The Anatomy of Poverty: The Condition of the Working Class in Montreal, 1897-1929* (Toronto: McClelland and Stewart, 1974).

[11]Bettina Bradbury, *Working Families: Age, Gender, and Daily Survival in Industrializing Montreal* (Toronto: McClelland and Stewart, 1993).

[12]Notable exceptions here are Ian McKay, *The Quest of the Folk: Antimodernism and Cultural Selection in Twentieth-Century Nova Scotia* (Montreal: McGill-Queen's University Press, 1994) and Keith Walden, *Becoming Modern in Toronto.*

of the female body.[13] And women are not the only ones whose tobacco habit is rife with broader cultural significance. Polls have shown that Quebeckers, for example, smoke more than people living in any other province, though we know little about why this is the case.[14] The social role and meanings of smoking differ from culture to culture, and within these cultures tobacco is smoked for different reasons, depending on the class, gender, age and cultural heritage of the smoker. This symbolism plays a decisive part in an individual's decision to begin smoking and successful campaigns to stop people from smoking must be sensitive to these cultural and social dimensions.

This concentration on the meanings people gave to smoking is part of the growing field of new cultural history. On the one hand, building on the work of anthropologists, new cultural historians study not only high culture or institutional cultural activities but popular culture. On the other, new cultural historians draw on literary theorists and have asserted that meanings and "truth" are not natural or self-evident but constructed by human activity. As Catherine Belsey explains "The project of cultural history is to identify the meanings in circulation in earlier periods, to specify the discourses, conventions and signifying practices by which meanings are fixed, norms 'agreed' and truth defined."[15]

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[13]The literature around this question is voluminous. Useful starts are Lorraine Greaves, *Smoke Screen: Women's Smoking and Social Control* (Halifax: Fernwood publishing, 1996); B. Jacobson, *The Ladykillers: Why Smoking is a Feminist Issue* (London: Pluto Press, 1981); and Rob Cunningham, *Smoke and Mirrors: The Canadian Tobacco War* (Ottawa: International Development Research Centre, 1996).

[14]Brenda Branswell, "Quebec and the High Cost of Smoking," *Maclean's*, 25 October 1999.

[15]Catherine Belsey, "Towards Cultural History - in Theory and Practice," *Textual Practice* 3,2 (Summer 1989) p.163.

This cultural approach is a new venture in Quebec historiography. For the most part, Quebec cultural history has focused on high culture rather than popular culture or questions of meaning. Indeed, the leading historiographer of Quebec cultural history, Yvon Lamonde, has shown that the subdiscipline has centered on the history of ideas, literary history and religious history and few excursions have been made into the new cultural history.[16] At worst this focus on "high culture" has led to claims by certain historians that entire regions of Quebec have lacked culture or that culture only arrived in smaller Quebec cities when opera companies toured there.[17] Understandably these sorts of claims have marginalized cultural history in Quebec, making it irrelevant to most people's lives in the past and to historiographic discussions about more recent transformations like industrialization.[18]

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[16]Yvan Lamonde, "L'Histoire Culturelle comme Domaine Historiographique au Québec," *Revue d'Histoire de l'Amérique Française* (Autumn 1997: Vol.51, No.2), pp.285-299. There are several recent exceptions to this: Michèle Martin, *"Hello Central?" Gender, Technology, and Culture in the Formation of Telephone Systems* (Montreal: McGill-Queen's University Press, 1991); Suzanne Marchand, *Rouge à Lèvres et Pantalon. Des pratiques esthétiques féminines controversées au Québec, 1920-1939* (Montreal: Éditions Hurtubise HMH Ltée, 1997); Patrice Groulx, *Pièges de la mémoire: Dollard des Ormeaux, les Amérindiens et nous* (Hull: Vents d'Ouest, 1998); H.V Nelles, *The Art of Nation-Building: Pageantry and Spectacle at Quebec's Tercentenary* (Toronto: University of Toronto Press, 1999); and Isabel-Caroline Caron, *Se créer des ancêtres. Les écrits historiques et généologiques des de Forest et des Forest en Amérique du Nord, 19e et 20e siècles* (Ph.D. Dissertation, McGill University, 2001).

[17]"Vie culturelle en milieu urbain," session held at 52nd Congrès de l'Institut d'histoire de l'Amerique française, Trois-Rivières, 23 October 1999.

[18]It is striking that in the fiery debates around Ronald Rudin's *Making History in Twentieth Century Quebec* (Toronto: University of Toronto Press, 1997), no one, including Rudin himself, has mentioned that the new cultural history has been slow to establish in Quebec. Native historians have long noted the cultural differences between European and native societies as well as the structural similarities. See, for example, Inga

Most studies of industrialization in Canada and Quebec have focused on its "motors" - "supply" questions of business and labour and its dislocations, rather than "demand." These interpretations are built on explanatory metaphors derived from orthodox Marxism and historians working in the *Annales* tradition. Orthodox Marxists posited that culture (superstructure) is determined by a society's economic organization (base), while *Annales* historians developed the framework of structure (economy and demography), conjunctures (social structure) and phenomenon (culture). In these schema, culture is a result of economic relationships and holds little or no explanatory power. As cultural historian Robert Darnton puts it "if we can get the social setting right the cultural content will somehow follow."[19] By linking the values upon which many of these purchases were made with the broader ideologies of identity construction we can develop a more human view of Montreal's industrialization and economic change more generally.[20]

Clendinnen, *Ambivalent Conquests: Maya and Spaniard in Yucatan, 1517-1570* (Cambridge: Cambridge University Press, 1987).

[19]Robert Darnton, *The Great Cat Massacre and Other Episodes in French Cultural History* (New York: Vintage Books, 195), p.259. For discussion of orthodox Marxism and the Annales approach, see Lynn Hunt, "Introduction: History, Culture, and Text," in her edited work, *The New Cultural History* (Berkeley: University of California Press, 1989), pp. 1-22. For an example of the treatment of "culture" as irrelevant to industrialization, see Paul-André Linteau, René Durocher, and Jean-Claude Robert *Quebec: A History, 1867-1929* (Translated by Robert Chodos) (Toronto: James Lorimer and Company, 1983).

[20]There is a growing literature on consumption and identity formation. See, for example, Pierre Bourdieu, *Distinctions: A Social Critique of the Judgment of Taste*, trans. Richard Nice (Cambridge: Cambridge University Press, 1984); Arjun Appadurai, ed., *The Social Life of Things: Commodities in cultural perspective* (Cambridge: Cambridge University Press, 1986) pushes the argument further and argues that these purchases effect economies. Victoria de Grazia's collection, *The Sex of Things: Gender and Consumption in Historical Perspective* (Berkeley, University of California Press, 1996) continues on the

Historians have argued that the social role of consumption changed over time, transforming from bourgeois to mass-oriented consumption.[21] Few historians have looked at this transition in Canada. One exception is Joy Parr who has explored the meanings of domestic goods in post-World War Two Canada. She situates her work within the historiographic debate over the question of how to characterize this transition. On one side are critics who argue that it was fueled by business interests that used advertising to create a consumer society. In this view, consumers were passive and readily consumed whatever was offered them. On the other side are academics who have maintained that the meanings business attempted to give to new goods were not accepted by consumers and that consumption has been used to subvert authority: "Meanings were fragmented, destabilized, and endlessly regenerated. Consumption became a process of energetic disruption rather than pliant subordination." For her part, Parr contends that there was more than one transformation in the cultural meaning of consumption and what is needed now are case studies, situating particular objects within time, geography and social space.[22]

While the historiography of smoking in other countries has been dominated by gentlemen scholars, a rare model for studying the history of smoking comes from Matthew

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same grounds and shows how gender identities are important to this process.

[21]For the international historiography of the arrival of "consumer culture," see Jean-Christophe Agnew, "Coming up for air: consumer culture in historical perspective," in John Brewer and Roy Porter, eds., *Consumption and the World of Goods* (New York: Routledge, 1993).

[22]Joy Parr, *Domestic Goods: The Material, the Moral, and the Economic in the Postwar Years* (Toronto: University of Toronto Press, 1999), pp.8-10.

Hilton's recently published *Smoking in British Popular Culture 1800-2000.*[23] Hilton's narrative, up until the First World War, turns primarily around the relationship between product choice and the construction of the smoker's identity. He argues that in the early twentieth century this relationship transformed from a bourgeois-liberal smoking ethic that valued individuality above all, especially in tobacco mixtures and cigars, to a technological-rationality, a more standardized mass culture individuality, most fully embodied in the cigarette. My dissertation builds on Hilton's work, with two important differences. Instead of studying an entire country I focus on one city, and in place of a longer temporal study, the thesis is limited to a period of 26 years. This allows me to expand on Hilton's insights on the relationship between purchasing and identity: rather than focusing only on "national tastes," I can include regional tastes and their relationship to local identities. I can also use other methodologies that expose the many dimensions of this ritual and the changing role smoking played in the construction of class, gender, ethnic and age identities.

There is a rich and diverse group of sources that make it possible to study changing thought on smoking. Such a conclusion may not seem evident at first glance. Indeed, evidence that today would give more precise information on what was being smoked and what it meant to smokers does not exist in the pre-World War One era. Polling, for example, is a relatively recent phenomenon that did not begin until the 1940s

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[23]The most famous works of "Gentlemanly scholarship" on smoking are G.L Apperson. *The Social History of Smoking.* (London: Martin Secker, 1914) and Count Corti. *A History of Smoking* (Guernsey: Guernsey Press Co., first published 1931, this edition 1996). Matthew Hilton, *Smoking in British Popular Culture 1800-2000* (Manchester: University of Manchester Press, 2001).

in Canada.[24] And because of today's tobacco wars, companies that may have done in-house research on their "markets" or had more precise production and distribution statistics, are not willing to open their records to researchers. Some historians have used the federal government's records of tobacco taxes, excise statistics, to understand what Canadians smoked. Jan Rogozinski, for example, used Canadian excise figures to estimate consumption patterns in 1920. He found that cigarettes were far less important in the Canadian tobacco market than in America or Britain. The cigarette made up only 19% of tobacco consumption, whereas pipe tobacco continued to dominate until the late 1920s.[25] Why this difference exists is a question beyond the scope of this thesis. For a study of one city, moreover, these statistics present further challenges. There is no way to tell whether they were representative of Montreal smokers. Excise statistics exist for the city but because the Canadian tobacco industry was based in Montreal and tobacco excised at the city's tobacco factories was consumed across the country, the statistics offer little precision on tobacco consumption in Montreal. What is more, they do not take into account untaxed pipe tobacco sold in the city, which I argue, was a significant quantity. Nor do they include the number of smokers who rolled their own cigarettes. To make excise statistics yet more imprecise as a measure of consumption, before 1920 chewing tobacco was included in statistics for pipe tobacco (I was forced to exclude chewing tobacco and snuff from this study because of a paucity of sources). Still, excise statistics

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[24]Daniel J. Robinson, *The Measure of Democracy: Polling, Market Research, and Public Life, 1930-1945* (Toronto: University of Toronto Press, 1999).

[25]Jan Rogizinski, *Smokeless Tobacco in the Western World, 1550-1950* (New York: Praeger, 1990).

are suggestive and can be weighed with other sources to get a sense, if not an accurate measurement, of consumption.

Numerous observers left evidence of what people were smoking and what meaning they and others assigned to tobacco consumption. My path into these sources began by reading through the Canadian tobacco industry's two primary trade journals, *Canadian Cigar and Tobacco Journal* and *Liqueurs et Tabacs,* the trade journal of Cigar Makers' International Union, the *Cigar Makers' Official Journal* and the numerous Montreal medical journals of the period. Not only do these journals give insights into the workings of the industry and tobacco's medical status, they also alerted me to public debates about smoking. The trail then led to government documents like parliamentary debates and prime ministers' papers as well as to personal and institutional papers and newspapers. Most significant were the papers of the Royal Commission on the Tobacco Trade (1902). I then culled numerous memoirs, etiquette guides, novels, poetry, cartoons in newspapers, collections of paintings and other cultural sources of the period to understand how smoking was used by their producers to build larger narratives. Occasionally evidence from beyond the temporal limits of this thesis became useful to highlight the distinctiveness of pre-War beliefs about smoking. From time to time it was also necessary to venture outside of Montreal to weigh the city's distinctive views towards tobacco, especially in the case of religious positions on smoking. Looking outside of Montreal is also essential when exploring the state's involvement in regulating and taxing tobacco since, because of the nature of Canadian federalism, discussions of taxing and regulating tobacco in Montreal largely happened in Quebec City and Ottawa. While most of these

are middle class sources and certainly offer less information on the smoking habits on the less powerful, a significant amount can be learned through middle class descriptions of improper and unmanly smoking, providing class judgements are exposed as such, rather than portrayed as truths about what was good and bad conduct.

The Montreal bourgeoisie, like their British counterparts, constructed a specific set of liberal prescriptions around smoking. The first three chapters outline these notions as well as underlining the material constraints of adhering to them. The first chapter argues that the immediate pre-war period was the height of the belief that smoking was an exclusively male pass-time. Liberal prescriptions around this male ritual played out spatially, inscribing social spaces with masculine identity. I trace the consequences of following these rules and breaking them and the roles of smoking in male sociability. Chapters two and three discuss the relationship between product choice and masculine identity. Men constructed themselves as connoisseurs of tobacco who could differentiate quality. Yet these hierarchies, I argue, were as much based on racial and gender stereotypes as on any intrinsic value within the tobacco itself. Chapter two uses a case study of the cigar to explore notions of connoisseurship and chapter three demonstrates that these beliefs were not universally accepted. Bourgeois notions of quality tobacco debased traditional French-Canadian tobacco, yet many French Canadians refused to accept these negative assessments.

Change over time becomes much clearer in chapters four and five as they chronicle challenges to the liberal notions delineated in the first three chapters. Chapter four examines the first anti-smoking movement in Montreal as well as its efforts in provincial

and federal anti-smoking campaigns. In addition to looking at the ideology of this anti-smoking movement, I also examine the diverse reasons for its failure. Finally, chapter five documents the more successful, though contested, challenges to nineteenth-century liberal notions of proper tobacco consumption. These centred on the transition to a new way that individuals related to their tobacco as well as new understandings of who could respectably smoke. These transformations were brought on both by changing views of who was included in the definition of an "individual," the relationship between speed and masculinity which had consequences for liberal notions of self-control, and by business and government campaigns to redefine "rational," quality tobacco. Eric Hobsbawm has argued that the liberalism of the nineteenth century succeeded in marginalizing its own creators as it eventually accorded political rights to women and the working class, but in doing so undermined the character of nineteenth-century bourgeois hegemony.[26] Indeed, in this age before the health concerns around tobacco were clear, the technological changes of the industrial revolution as well as the broader transformation of liberalism in pre-First World War Montreal began to challenge prescriptions around the ritual of smoking and to lay the foundations of today's consumer society.

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[26]Eric Hobsbawm, *Age of Empire, 1875-1914* (New York: Vintage books, 1987), pp.1-11.

Chapter One

Separating Spheres

From 1888 to 1914, smoking in Montreal was almost exclusively a male activity. Moral reformers who opposed smoking rarely discussed female offenders, not because they thought women smoking was less vice-ridden than men, but because it was not a frequent occurrence. Others in positions to monitor women's behaviour have left revealing silences around smoking. No nurses in training at the Montreal Maternity Hospital were reprimanded for smoking and patients at the Montreal Maternity Hospital were caught drinking alcohol but never smoking.[1] Doctors working in Montreal's insane asylums noted an enormous gender gap in smoking among their patients: according to Dr. Villeneuve of the Roman Catholic Longue Pointe Asylum, out of all his female inmates between 1894 and 1914, only seven smoked; and Dr. Burgess of the Protestant Verdun Asylum maintained that over 25 years there was only one female smoker in his asylum, while 50 per cent of the men smoked.[2] This gender exclusivity of smoking was not always the case in Quebec. Numerous rural women born in the first half of the nineteenth century took up pipe smoking. Similarly, women in the 1920s who smoked cigarettes were not anathematized like their counterparts at turn of the twentieth century. Etiquette guides

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[1]Montreal Maternity Hospital, Matron/Superintendent's Reports, 1889-1926. RG 95, McGill University Archives.

[2] "Proceedings and Evidence of the Select Committee appointed to Inquire and Report as to the expediency of making any amendment to the existing laws for the purpose of remedying or preventing any evils arising from the use of the cigarette." *Appendix to the Journals of the House of Commons*, No.3, 1914, pp.89-90. Hereafter referred to as "Proceedings."

after the First World War, for example, counseled that women be offered cigarettes after dinner and in Montreal, female students petitioned to gain their own smoking rooms at McGill University's women's residence, Royal Victoria College.[3]

The pre-War period was the height of the association between smoking and masculine identities. This connection was set within broadly shared standards of respectability and civility that differed for men and women. Women who did smoke were stigmatized differently depending on how their class and racial status was perceived. These assessments of character could have consequences for a woman's social standing, search for work and citizenship.[4] For men, beginning to smoke was nothing less than a rite of passage to manhood. It was a ritual that could bring together men of diverse cultural backgrounds. Much of the etiquette of smoking followed the prescriptive spatial metaphor of gendered spheres: women were associated with the private sphere of the home and family while men were linked to public sphere activities like politics and business, spatially making the male sphere anywhere outside of those spaces specifically set aside for a woman's reproductive tasks. "Social space," as Mary P. Ryan puts it, "serves as a

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[3]Margaret Visser, *The Rituals of Dinner: The Origins, Evolution, Eccentricities and Meaning of Table Manners* (Toronto: Harper-Collins Publishers Ltd., 1991), p.291; Administrative Records of the Warden, RG 42, MUA.

[4]Kate Boyer, "Re-Working Respectability: The Feminization of Clerical Work and the Politics of Public Virtue in Early Twentieth-Century Montreal," in Tamara Myers *et al. Power, Place and Identity: Historical Studies of Social and Legal Regulation in Quebec* (Montreal: Occasional Papers of the Montreal History Group, 1998) pp.151-169; Joan Sangster, "Softball Solution: Female Workers, Male Managers and the Operation of Paternalism at Westclox, 1923-1960," *Labour/Le Travail*, 32 (Fall 1993), 167-199.

scaffolding upon which both gender distinctions and ... identity are constructed."[5] These spatial and identity constructions not only embodied the unequal power relations between men and women, identities that not all women were content with, they were also impossible to follow for all but the most materially secure of middle class women. Within the male public sphere there were also structures of respectability – ideals of masculinity – that held consequences for men who transgressed them. Indeed, historians and social theorists have written that men were to be high minded, demonstrating a liberal ideal of rational critical thought.[6] According to these codes of respectability, smoking symbolically evoked a tone of thoughtfulness and made visible the boundaries of this male public sphere; and to adhere to these codes separating men and women was itself a public display of respectability. In mixed class situations, for a man to forfeit his "right" to smoke in the public sphere when a woman was present was a performance of gentility and mark of distinction. The relationship between gendered norms of respectability and class is complex. Indeed, historians have argued that separate spheres ideology originated and was promoted most by the middle class. Furthermore, because of its costliness, it was difficult for the less wealthy to follow. Still, historians have shown that working class men and women also used separate spheres prescriptions and those practices within their means

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[5]Ryan, *Women in Public*, p.59.

[6]Geoff Eley, "Nations, Publics, and Political Cultures: Placing Habermas in the Nineteenth Century," and Mary P. Ryan, "Gender and Public Access: Women's Politics in Nineteenth-Century America," in Craig Calhoun ed. *Habermas and the Public Sphere* (Cambridge: MIT Press, 1992).

to achieve levels of respect within their communities.[7] Such was the case with the etiquette of smoking in late nineteenth century Montreal. An individual's ability to follow liberal prescriptions of smoking depended both on one's finances as well as the cost of the particular smoking ritual.

I. Women Smokers

In the first half of the nineteenth century smoking was less of an exclusively male activity than it would be in the late nineteenth century and therefore it was less of a symbolic border between public and private spheres. Numerous accounts exist of elderly women smoking pipes in the early twentieth century. An article in the tobacconist trade journal *Liqueurs et Tabacs* maintained that older residents of Vaudreuil could remember a family of seven, mother, father, two sons and three daughters, from la Petite Côte who all smoked pipes. Older residents also told their grandchildren about a wedding procession in which both the bride and groom smoked their pipes. All the descendants of the family smoked, and one daughter who was pictured beside the article, at the age of 78, still enjoyed her pipe full of ATCC Red Cross Cut Tobacco.[8] Female pipe smokers were also

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[7]Suzanne Morton and Janet Guildford, "Introduction" as well as Sharon Myers, "'Not to be Ranked as Women'" in Morton and Guildford, eds. *Separate Spheres: Women's Worlds in the 19th-Century Maritimes* (Fredericton: Acadiensis Press, 1994), pp.9-21; Mark Rosenfeld, "'It was a hard life': Class and Gender in the Work and Family Rhythms of a Railway Town 1920-1950," *Historical Papers,* (1988), 237-279.

[8]"Le Tabac et la longévité," *Liqueurs et Tabacs*, April 1903, p.30. Though there is no reference to it, the picture of this old woman smoking may have been poking fun at the WCTU since this was the month they took their anti-smoking campaign to the Canadian Parliament.

found in other countries and provinces in the first half of the nineteenth century. Historians elsewhere have noted that there was less stigma on women smoking in Britain and the United States earlier in the century, especially in rural areas. Evidence from other Canadian provinces also suggests that some rural women born in the first half of the nineteenth century smoked the pipe.[9] While sources for Quebec and elsewhere are admittedly thin, that female smoking was more acceptable in early nineteenth century Quebec would make sense. According to Quebec historians gender roles were in transition in the 1830s and 1840s and early nineteenth-century codes of respectability may have frowned less on women who smoked.[10]

While we know less about popular-class attitudes, by the late nineteenth century, it probably was not acceptable for bourgeois women to smoke. 1875-1876 entries in the diary of Henriette Dessaulles, daughter of a notable rural Quebec family, serve as an example. At fifteen she had a crush on a local boy who had gone to classical college – a boy she would eventually marry. Yet she was unsure of the seriousness of her interest in him, whether it was love or friendship and decided it was only friendship. "The fact remains, though," she wrote, "that I would rather have been a boy, his best friend,"[11] but

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[9]Morton and Guildford, *Separate Spheres*, cover and p.7.

[10]Le collective Clio, *L'histoire des femmes au Québec depuis quatre siècles* (Montreal: Le Jour, éditeur, 1992); Bettina Bradbury, *Wife to Widow: Class, Culture, Family and the Law in Nineteenth-Century Québec* (Montreal: Programme d'études sur le Québec de l'Université McGill, 1997).

[11]Henriette Dessaulles, *Hopes and Dreams: The Diary of Henriette Dessaulles, 1874-1881* (Originally published 1971. Translated, 1986 by Liedewy Hawke), 18 April 1876, p.99.

the activities boys shared in were not acceptable for girls: "I can see Maurice. He is reading, and smoking as he reads. If at least I could smoke or swear! But I don't know how and it's not allowed."[12]

By late century women who smoked were disparaged. They were belittled in different ways depending on their race and class. When women of colour appeared smoking in Montreal cultural sources, smoking was part of the performance of incivility. For example, a 1905 story in the Montreal middle-class weekly *l'Album Universel* featured an interview with an American missionary, Miss Ida Plummer, who worked in the new American possession, the Philippines. She worked directly with a tribe known as the Igorrots who had been headhunters. The story tells of their tribe's marriage ceremonies and clothing, with both the interviewer and Plummer commenting on the Igorrots' incivility. Pictured in the centre of the article was a young Igorrot woman smoking a pipe, part of the visual construction of the incivility of the tribe (figure 1).[13]

Figure 1 Igorrot woman pictured in *l'Album Universel* (1905).

The link between racialized incivility and women smoking extended to new immigrants arriving in Montreal. A Montreal *Star* article entitled, "Greek Gypsies

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[12]*Ibid.*, 5 May 1875, p.44.

[13]"Le Mariage chez les Igorrotes," *l'Album Universel*, 25 March 1905, p.928.

Pictureque, But Not very Desirable as Citizens of Dominion" recounted the story of Gypsies arriving in Montreal after being refused entry into the United States. Homeless, they were housed in the immigrant quarters of Windsor Station in Montreal where they came under the eye of the journalist as well as the station manager. The journalist commented on the Gypsy women's inability to live up to Canadian standards of gendered civility: "They are filthy and unkempt [sic]; the women almost savage in their abandon; their little ones half nourished and all evidently without a particle of respect for the ordinary laws of cleanliness and sanitation." On top of their dirtiness and failure to take care of their children, both the men and the women were "inveterate cigarette smokers." The station manager, Mr. Miller, was disgusted by what the author called, "a most undesirable class of people with which to increase the population of Canada." Here again, women smoking were part of way the journalist recognized the Gypsies as uncivilized.[14]

The uncivilized did not necessarily have to come from a foreign land. An early historiographic debate on smoking habits of rural French Canadians serves as an example. In his *Histoire de la Seigneurie de Lauzon* (1904), historian J.-Edmond Roy took issue with an American traveler who wrote over a century before that "les Canadiens ... sont d'éternels fumeurs. On dirait que chaque homme, femme et enfant doit nécessairement avoir sa pipe et son sac à tabac, et s'en servier constamment." The traveler then claimed to have seen sixteen year old French-Canadian brunette girls working in the fields and puffing clouds of smoke. Roy, in defense of French-Canadian womanhood, responded that French-Canadian girls did not smoke, so he must have confused "les Français du

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[14]Montreal *Star*, 21 March 1903, p.7.

Canada, avec les descendants des Iroquois, des Hurons et des Micmac."[15] According to Roy, native women could smoke pipes because he saw them as primitive and women smoking was uncivilized behaviour. Some French Canadians were willing to accept that French- Canadian women smoked, but they made it part of the distant rural past. Dr. L. J. Lemieux, Sheriff of Montreal, physician and professor of the history of medicine at Laval, president of the Board of Censors of moving pictures, and organizer of the Montreal Juvenile Court told the commission that "we [Quebec] have some of those old people, but they are passing away now: they [women] are getting more civilized."[16]

In Montreal at the turn of the century women smoking were stigmatized as immoral. John Todd, a young McGill medical student from Toronto wrote his mother telling her of the bizarre people and events he saw in Montreal. Among them was a woman carrying a cigarette (though not smoking it). "Then I saw a female, with a half-smoked cigarette behind her ear, walking along the street. I had often read of this, but this was the first time I had seen it. No, I cannot see why people live in Montreal, when they can go to Toronto."[17] Lemieux equated women smoking cigarettes to prostitution claiming that 90 per cent of women in the Montreal women's jail smoked and many of these women were prostitutes since "being prostitutes they are degenerates and everything

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[15]J.-Edmond Roy, *Histoire de la Seigneurie de Lauzon* Volume 4, Lévis, pp.169-170. This debate is also recounted in George M. Wrong, *A Canadian Manor and its Seigneuries* (Toronto: Macmillan Co., 1908), p.181.

[16]Dr. L.J. Lemieux in "Proceedings," p.82.

[17]John Todd to his mother, 15 June 1895, Bridget Todd Fialkowski, ed. *John L. Todd Letters, 1876-1949* (self-published), McGill Archives.

that is bad they take up."[18] Whether the women were prostitutes or not Lemieux understood the cigarette to be a sign of moral deviance. Furthermore, it was possible that prostitutes smoked cigarettes and wore particular clothes and makeup to declare themselves to be prostitutes. This might explain the striking difference in numbers of female smokers in jail compared to the few in the asylum discussed earlier. As Mara L. Deire writes, "For prostitutes, their revealing dress and cosmetics were literal advertisements of who they were and what they were selling.... [D]istinctive trademarks of short skirts, cigarettes, a slow saunter, and bold eye contact, were 'professional' signifiers."[19] This is also suggested in a letter from Montreal poet Charles Gill to fellow poet and friend Louis-Joseph Doucet. Gill described in detail a prostitute he frequented whose behaviour, according to him, was not what he saw as that of a "normal" prostitute. She did not go into the brothel until after 9:30 at night and never if it rained and she refused half of her clients. When outside in the city she was honest, calm, reserved, dressed simply and wore no makeup. On top of these other issues of physical appearance, "Elle ne fume ni ne boit" all of which Gill linked to prostitution.[20]

Smoking could cause a woman to fail to live up to the goals society gave women: marriage and having children. Etiquette commentators and guides published in Montreal during the period counseled against women smoking using these rationales. Etiquette

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[18]Lemieux in "Proceedings," p.86.

[19]Mara L. Keire, "Dope Fiends and Degenerates: the Gendering of Addiction in the Early Twentieth Century," *Journal of Social History*, (Summer 1998), p.814.

[20]Réginald Hamel, editor. *Charles Gill: Correspondance* (Montreal: Éditions Parti Pris, 1969), letter from Charles Gill to Louis-Joseph Doucet, 23 July 1917, p.193.

guide author Madame Sauvalle gave, for example, "Un mot seulement pour les jeunes femmes qui s'aventurent de temps à l'autre à lancer quelques bouffées de fumées." While he acknowledged that smoking cigarettes was widespread among Europe's elite women, it was clear that this was a case of aristocratic decadence that should not be tolerated by the other classes: "il devient de très mauvais ton lorsqu'on n'approche pas des marches d'un trône." According to Sauvalle, a woman's priority was to find a husband and smoking put her success in jeopardy.[21]

As Sauvalle's advice suggests, responses to women smoking changed according to the class of women who smoked. Another group of women to smoke in Montreal before the First World War were a select group of "Society Women" who saw themselves as culturally linked to Europe. From the 1880s, Montreal newspapers and reviews published stories about elite European women who smoked. In 1889 *Le Monde Illustré* recounted a story first run in a royalist London newspaper about the Countess of Paris smoking a pipe as she walked around London. While Léon LeDieu, the author, could not verify the authenticity of the story, he commented in amazement, "Oui, une pipe, une vraie pipe, une pipe en plâtre, courte et noire, j'allais dire culottée!"[22]

While stories of European society women circulated from time to time in the Montreal press, the question of the extent to which Montreal society women smoked was rarely posed. Immediately before the First World War newspapers increasingly ran

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[21]Madame Sauvalle, *Mille Questions d'étiquette: Discutées, Resolues et Classées* (Montreal: Librairie Beauchemin Limitée, 1907), p.119.

[22]Léon Ledieu, *Le Monde Illustré*, 26 January 1887, p.307.

exposés on the smoking habits of Montreal's elite women. Even the reputations of these elite female smokers could be put into question for smoking, though in a different way than rural women smokers or women smokers of other races. Indeed, these society women were branded as irresponsible and careless smokers. In 1912 the Montreal *Herald* ran the headline, "Lady's cigarette caused one fire at Windsor Hotel: Fair Smokers Are Careless, and Window Blaze Was Result." Two fires had broken out at the Windsor in one week and "gave rise to sinister rumors...." One of the fires, the management countered, was started by a woman's cigarette:

> It is alleged that the ladies are very careless about their cigarette ends. They often choose, for instance, to deposit them on the edge of the ventilators right in the open draft.

The fire caught on a curtain and then destroyed the woodwork around the window.[23]

Whether female smoking was the cause of the fires is unknown, but the hotel management used the cultural image of careless women smokers as a cover up. In a similar sense, Bettina Bradbury has written that factory owners used the stereotype of women being naturally careless to explain girls injured while working in factories, especially when doing work that was previously considered male.[24] In the case of the Windsor fires, the Montreal Fire Department was never called so no investigation was ever undertaken.[25] What is more, there were few stories in the news about men being

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[23]"Lady's Cigarette Caused One Fire at Windsor Hotel: Fair Smokers Are Careless, and Window Blaze Was Result," Montreal *Herald*, 20 March 1912, p.6.

[24] See Bradbury, *Working Families*, p.135.

[25]The *Fire Register: Montreal Fire Department* lists no fires at the Windsor in the weeks before the report.

dangerous smokers and men clearly began many fires by smoking. In the same year as the Windsor incident, for example, the Montreal Fire Department responded to four fires caused by smoking on tramways alone, spaces historians have recognized as being dominated by men.[26]

This trickle of stories about women smokers became a flood in 1914 during hearings of the "Select Committee appointed to inquire and report as to the expediency of making any amendment to the existing laws for the purpose of remedying or preventing any evils arising from the use of cigarettes," or what was commonly called the "Commons Commission on the Evils of the Cigarette." Established in 1914 to avoid WCTU demands for cigarette prohibition, the Committee heard numerous witnesses who testified about the smoking habits of elite women. First W.L. Scott, President of both the Ottawa Children's Aid Society and the Union of Children's Aid Societies of Ontario, announced that, "women of the very best class" were beginning to smoke.[27] Then F.X. Choquet, judge of the Montreal Juvenile Court, agreed that Montreal had the same problem.[28] The Montreal witnesses were unanimous that, as the Montreal *Gazette* put it, the city's elite women were "Cigarette Slaves."[29] The Montreal *Star* sent out a reporter to interview local "society and

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[26]Donald F. Davis and Barbara Lorenzkowski, "A Platform for Gender Tensions: Women Working and Riding on Canadian Urban Transit in the 1940s," *Canadian Historical Review*, 79,3, September 1998. Pp.432-465. *Fire Register: Montreal Fire Department*, 1912 entries 253, 1157, 1832, 2103, AVM.

[27]W.L. Scott in "Proceedings," p.16.

[28]F.X. Choquet in "Proceedings," p.24.

[29]"Society Women Cigarette Slaves," Montreal *Gazette*, 17 April 1914, p.1.

club women" to get their opinions on women smoking. Many of these women couched their responses in terms of equality between men and women. Lady Julia Drummond opined, "I want to say, first of all, that I agree with Boyd-Carpenter, former Bishop of Ripon, who said: 'what isn't a sin in man, isn't a sin in woman.'" Lady Williams-Taylor, who, according to the reporter, "gave the viewpoint of typical English society women," declared, "I see no objection whatever to women smoking.... I see no reason why there should be two standards, one for us and one for our brothers and husbands."[30] While Lady Drummond and Lady Williams-Taylor believed women should have the right to smoke they did not admit to smoking themselves. The exposé in the Montreal *Star* noted, "except where they were opposed to women smoking the majority [of society women] asked that they be not quoted.... [They] hesitate to come out publicly in favor of 'smokes for women.'"[31]

Working class women may in fact have been more diligent than middle class women about following the custom of women not smoking. After all, if the price of respectability was abstaining from smoking, this was a symbol of respectability even the poorest could display. The testimony at the commission and the follow-up stories all made clear that it was always elite women who were picking up the cigarette. Working class women, according to witnesses at the commission, emphatically were not. Rose Henderson, a probation officer with the Montreal Juvenile Court and long-time activist among women's and labour groups testified that working class women "are not the class

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[30]"Favor Feminin Smoking," Montreal *Star*, 17 April 1914, p.1.

[31]*Ibid.*

of people among whom the mothers are smokers" because they could not afford tobacco.[32] As late as 1919, while on a trip to Europe, Montreal union leader Gustav Francq was scandalized by the number of women workers who smoked, suggesting that smoking among respectable working class women was a rarity.[33]

II. The Rituals of Manhood

By the end of the nineteenth century respectable smoking was only possible by men. Many likened smoking to a ritual that symbolized a boy's transition to manhood. It was expected that boys would try to emulate their fathers. The Montreal *Gazette*, for example, wrote that, "Ordinary parents of ordinary boys, remembering their own youth, and the temptations boys are subjected to, sometimes by desire to imitate their elders, sometimes by a spirit of foolish bravado"[34] and smoke. The WCTU was concerned about this view of smoking as a rite of passage and wrote in its "Catéchisme de Tempérance," "Beaucoup de jeunes garçons commencent à fumer parce qu'ils auront l'air hommes...."[35] These attempts at coming of age were often doomed to failure because of the harsh effects

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[32]Rose Henderson in "Proceedings," p.43.

[33]"Gus. Francq tells some observations of Europe," *Le Monde Ouvrier*, 8 March 1919, p.3. My thanks to Eric Leroux for this reference. For more on Francq, see Leroux, *Gustave Francq: Figure Marquante du Syndicalisme et Précurseur de la FTQ*. (Montreal: VLB Éditeur, 2001).

[34]"A Legislative Mistake," Montreal *Gazette*, 23 February 1893, p.4.

[35]Société chrétienne de tempérance des dames de la province de Québec (WCTU), "Catéchisme de tempérance à l'usage des familles et des écoles de la province de Québec," CIHM 26045, p.13.

of smoking on the immature boy. In a sermon Reverend W.H. Warriner, a Congregationalist minister in Montreal, described the "first experiences of the smoker... the faintness, dizziness, nausea and vomiting."[36] The scenario of boys smoking their father's tobacco and getting sick was described in Marc Legrand's poem "Les Petits Fumeurs"published in the Montreal women's journal *Le Journal de Françoise*:

Au lieu d'apprendre leurs leçons
Fumaient quatre petits garéons,
Sur le bureau de leur papa,
Ils avaient trouvé du tabac

Chacun n'ayant pas de papier,
Avait découpé son cahier,
L'un se brûle avec un charoon,
Et dit: "Fumer, c'est vraiment bon!"

Le second prend un fier maintien,
Et dit: "Ma foi, ça va très bien!
Avec des larmes dans les yeux, L'autre dit: "c'est déicieux!"

Le plus petit, crachant, toussant,
Dit: "je suis un homme à présent!"
Le soir, ils se mirent au lit,
Grelottants et le front pâli.

On les soigna longtemps,
Ils redevinrent bien portants.
Ils furent sages désormais:
Ils ne fumènt plus jamais.[37]

According to these beliefs, smoking required a considerable amount of physical maturity. The strength of the tobacco supposedly acted as a natural guard against boys smoking

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[36]"Mr.Cook's Bill Praised," Montreal *Herald*, 27 February 1893, p.8.

[37]Marc Legrand, "Les Petits Fumeurs," *Le Journal de Françoise*, 18 May 1907, p.64.

until they were men. Barring this natural barrier, fathers were to decide if their boys had become men. Quebec MP and nationalist Armand Lavergne, for example, argued that, "You cannot take from the parents the right to give that training to their children which they consider best for them, and if a father should see that the smoking of cigarettes does not harm his son" the young man should be allowed to smoke.[38]

Medical understandings of tobacco also reinforced the belief that smoking was an exclusively male coming-of-age ritual. Indeed, there was a medical consensus on the dangers of boys smoking before their bodies were strong enough. Montreal doctors, some of the leading lights of the medical profession in the country, spoke out against boys smoking. Dr. William Osler, the internationally renowned pathologist who began his career at McGill, opposed smoking by youth.[39] Similarly Professor Foucher, an ophthalmologist at the Montreal campus of Laval University's Medical Faculty, observed that most child smokers "sont pâles, petits, étoilés, dyspeptiques et leur peau jaune terreuse reflète l'état misérable de leur santé." He concluded by writing that "j'ai ordonné à mon jeune collégien de s'abstenir de fumer quand bien même il croirait en obtenir de bons résultats."[40]

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[38]*Debates*, 23 March 1904, p.351-352. On Lavergne, see Réal Bélanger, *L'impossible défi: Albert Sévigny et les conservateurs fédéraux (1902-1918)* (Québec: Les Presses de l'Université Laval, 1983) and Hélène Pelletier-Baillargeon, *Olivar Asselin et son temps: Le militant* (Montreal: Fides, 1996).

[39] Michael Bliss, *William Osler: A Life in Medicine* (Toronto: University of Toronto Press, 1999), p.274.

[40]Professor Foucher, "Quelques remarques sur l'usage du tabac en rapport avec la muqueuse de la bouche et des voies respiratoires," *L'Union médicale du Canada*, March 1897, p.198. For references to Foucher, see Denis Goulet, *Histoire de la Faculté de*

Continuing to reinforce the cultural belief in smoking as a rite of passage to manhood, the medical consensus held that moderate smoking by adult men was safe. The issue was individual self-control. While most historiography has gone to great lengths to uncover whether or not doctors saw tobacco as a cause of diseases or a curative, most doctors considered adult men smoking safe, if done moderately.[41] Montreal medical journals published articles claiming tobacco to be both a curative and a cause of disease.[42] To be dangerous tobacco had to be abused and to be a cure it had to be smoked in moderation. Smoking was seen as helpful for victims of tuberculosis. A 1896 *L'Union médicale du Canada* article reported the findings of Dr. Jankau, a German pathologist, who argued that tobacco was useful to people in the early stages of tuberculosis. According to Jankau, while excessive consumption can burn the stomach, tobacco disinfects the mouth, "déprime les fonctions génitales," and acts as a sedative on the central nervous system. For this last reason Jankau maintained that smoking a pipe often

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Médecine de L'Université de Montréal, 1843-1993 (Montreal: VLB Éditeur, 1993), p.77.

[41]R.B. Walker, "Medical Aspects of Tobacco Smoking and the Anti-tobacco Movement in Britain in the Nineteenth Century," *Medical History*, 24, 1980, pp.391-402. There were a number of studies suggesting a link between tobacco and lung cancer before the end of the 1940s, but they were either poorly publicized or written off as being flawed. See Sir Richard Doll, "The First Reports on Smoking and Lung Cancer," in S. Lock *et al. Ashes to Ashes: The History of Smoking and Health.* (Amsterdam: Rodopi, 1998), pp.130-163; Christopher C. Booth, "Clinical Research," p.224 and David Cantor, "Cancer," p.557, both in W.F. Bynum and Roy Porter, eds. *Companion Encyclopedia of the History of Medicine*, (London: Routledge, 1993).

[42]I have looked at *l'Abeille Médicale*, 1879-1882; *le Gazette Médicale de Montréal,* 1888-1892; *l'Union médicale du Canada*, 1872-1930; *le Montréal Médicale*, 1901-1920; *Canada Medical and Surgical Journal*, 1872-1888; *Montreal Medical Journal,* 1901-1910.

prevents attacks of "asthme nerveux."[43] Tobacco was clearly helpful only if used in moderation.

Other articles enumerated the cases where tobacco was helpful and dangerous, all of them noting that moderate smoking was safe. In 1909 *L'Union médicale du Canada* republished an article entitled "Le Tabagisme" with an editorial commentary in the footnotes: "Voici une article bien fait, qui sera utile aux Canadiens, grands fumeurs." The article enumerated the numerous health problems associated with abuses of tobacco, from tobacco heart to "cancer des fumeurs," or lip cancer, to memory loss and abortion. Marc also listed a few examples where tobacco was helpful, especially in the areas of constipation and digestion: "s'il est fumé à petite dose."[44] He concluded by saying that "La question n'est donc pas absolument tranchée."[45] Professor Foucher discussed the effects of smoking on the respiratory pathways. Aware of "tout le mal qu'il peut produire," he did not want to approve of smoking, prescribe it as a medication and then watch his patient fall into "l'usage immodéré." On the other hand, he did not want to condemn it, an "habitude qu'il chérit," and fall into "l'exagération de l'abstention absolue." He thus sets out rules to healthy smoking: "L'effet irritant et toxique du tabac dépend de la manière dont on en fait usage.... C'est dire en d'autres termes qu'il faut fumer

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[43]"L'usage du tabac chez les malades," originally published in *American Medical Review*, Vol.I, No.4. Republished in *L'Union médicale du Canada*, May 1896, pp.336-337. On tobacco and tuberculosis, see also "Tobacco as an Antizymotic," *Canada Medical and Surgical Journal*, July 1884, p.767.

[44]Dr. Marc, "Le Tabagisme," *L'Union médicale du Canada*, 1909, pp.587-590.

[45]*Ibid.*, p.594.

modérément, lentement, un tabac faible en principes actifs."[46]

Some influential doctors downplayed the dangers of adult smoking while maintaining a doctrine of moderation. For example, in his monumental *Principles and Practice of Medicine* used to train generations of doctors, Osler dismissed tobacco heart, writing "Cardiac pain without evidence of arterio-sclerosis or valvular disease is not of much moment."[47] By the eighth edition of the same book, "tobacco heart" was not even listed.[48] Osler, a moderate smoker himself, claimed elsewhere that he did not get many cases of "tobacco heart" and that he had never heard of a fatal instance of it.[49] Osler maintained that the cigarette "in moderation ... soothes physical irritability and mental and moral strabismus."[50] During a cigarette prohibition debate in 1903, Dr. T.G. Roddick, McGill professor of Clinical Surgery, founder of the Canadian Medical Association and Member of Parliament, told the House of Commons that after a child is finished growing

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[46]Professor Foucher, "Quelques remarques...."

[47]Sir William Osler, *The Principles and Practice of Medicine: Designed for the Use of Practitioners and Students of Medicine*. Third edition, (D. Appleton and Company, 1898), p.764. This quotation does not appear in earlier editions, nor in many later editions. It was also used in pro-tobacco propaganda like the widely circulating Leonard K. Hirschberg, "The Truth About Tobacco," originally published in *Harper's Weekly*. Republished in *CCTJ*, March 1913, pp.43-45. Osler's smoking habits are explored most extensively in Bliss, *William Osler*, p.78, 94-95, 274-275.

[48]Sir William Osler, *The Principles and Practice of Medicine: Designed for the Use of Practitioners and Students of Medicine*. Eighth edition, (D. Appleton and Company, 1912).

[49]William Osler, "Ephemerides, 1895: IX Tobacco Angina," *Montreal Medical Journal*, Vol. XXIV, No.11, May 1896, p.879.

[50]*Ibid.*

"we cannot declare, as medical men, that very much harm follows...."[51] It is clear that most medical authorities concluded that moderate tobacco consumption by grown men was perfectly healthy.

This belief in moderation can be explored in greater detail by looking at doctors' case files. McGill Otolaryngologist (ear, nose and throat specialist) H.S. Birkett, for example, frequently saw patients with ailments caused by smoking though in my sample I found no woman admitting to Birkett that she smoked before the First World War.[52] Habitually he asked his patients how much they smoked and then often instructed them to "moderate" their tobacco consumption. It is from Birkett's assessments of how much tobacco was too much that we can deduce some idea of what moderation was. Birkett's instructions varied considerably from patient to patient to the point of contradiction, much depending on the condition of the individual patient. These varying assessments of excess and moderation were given for pipe, cigarette and cigar smokers. For example, most pipe-smoking patients who admitted to smoking one pound or more of tobacco a month were usually told to cut down or moderate their consumption.[53] For cigarette smokers, the

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[51]*Debates*, 1 April 1903, pp.832-834. For biographic information on Roddick, see Edward H. Bensley, *McGill Medical Luminaries*, (Montreal: Osler Library Studies in the History of Medicine, 1990), pp.35-37.

[52]For biographic information on Birkett, see "Herbert Stanley Birkett" Bensley, *McGill Medical Luminaries*, pp.63-65. My base sample of the Birkett casebooks was the books from 1892, 1895, 1900, 1905, 1910, 1915, 1920, 1925 and 1930. Fonds H.S Birkett, Osler Library, McGill University.

[53]Examples of this are Files No.4151, 8 May 1895; No.16363 13 March 1910; and No.16690 9 September 1910.

upper end of moderation was between 10 and 12 cigarettes a day.[54] Fewer cigar smokers are mentioned in the Birkett case files with five cigars being considered excessive.[55]

According to doctors, gender in particular played a role in assessing an individual's ability to smoke moderately and thus, a woman's ability to safely perform this ritual of masculinity. Sociologist Mariana Valverde has written that alcoholism, the disease on which doctors modeled their studies of other addictive substances like tobacco, was seen as a disease of the will and women were seen as being more susceptible to abuse because they had inherently less will-power.[56] The idea that women were biologically prone to excess has been well studied by historians looking at subjects that range from the medical history of hysteria to stereotypes of women shopping to women activists at the Commune in Paris.[57] Valverde adds that men of the lower classes, men of passion, writers, artists, and "empire builders" were also likely candidates to abuse because their passions escaped their wills.[58] And as with alcohol, doctors saw women as more susceptible to abusing

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[54]No.20865, 17 November 1914; No. 7289, 3 Feb. 1900, No.12659, 24 Oct. 1905.

[55]No.29781, 12 May 1930.

[56]Mariana Valverde, "'Slavery from within': the invention of alcoholism and the question of free will," *Social History*, vol.22, No.3 (October 1997), pp.251-268.

[57]See, for example Cynthia Wright, "'Feminine Trifles of Vast Importance': Writing Gender into the History of Consumption," in Franca Iacovetta and Mariana Valverde, eds. *Gender Conflicts: New Essays in Women's History* (Toronto: University of Toronto Press, 1992), pp.229-260; Ruth Harris, "Melodrama, Hysteria and Feminine Crimes of Passion in the Fin-de-Siecle," *History Workshop Journal* 25 (Spring 1988), pp.31-63; Joan Wallach Scott, *Only Paradoxes to Offer: French Feminists and the Rights of Man* (Cambridge: Harvard University Press, 1996).

[58]Mariana Valverde, "'Slavery from within.'"

tobacco because of their apparently weaker wills. The doctor of Montreal socialite Lady Williams-Taylor explained moderation to her as follows: "My private physician in London, who is the best there is, advised me to smoke - but in moderation. He said that if women would smoke three cigarettes a day, one after each meal, that 'nerves' as a disease would practically disappear." She concluded, however, that women are prone to excess, at which point smoking becomes dangerous.[59] This belief that women were likely to smoke excessively was shared by others outside of the medical profession. Lady Julia Drummond, for example, advised that women should perhaps avoid the habit because they were "often prone to excess."[60]

Smoking was also part of the rituals of doctors becoming "medical men" in both of Montreal's medical schools. These were bastions of male culture with women only admitted to the McGill Medical School in 1917, and the Université de Montréal in 1924.[61] In these buildings, medical students could smoke almost anywhere. At McGill limits were only put on smoking in 1907 after the Medical Faculty building burned.[62] At Université

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[59]"Favor Feminin Smoking," Montreal *Star*, 17 April 1914, p.1.

[60]*Ibid.*

[61]On women in the McGill Medical Faculty, see Margaret Gillet, *We Walked Very Warily: A History of Women at McGill* (Montreal: Eden Press Women's Publications, 1981), pp.280-303; Little has been written on female medical students being admitted to Université de Montréal. For a cursory mention, see Goulet, *Histoire de la Faculté de Médicine de L'Université de Montréal*, p.211, 261-263.

[62]"Montreal Notes," *CCTJ*, November 1910, p.25. The right to smoke in the dissecting room was put into question after the McGill Medical Faculty burned in 1907; students were still allowed to smoke in the faculty reading room. See McGill Faculty of Medicine Minutes, 5 October and 30 October 1907, p.228, p.230 and p.232, RG 38, McGill University Archives.

Laval à Montréal, which would become the Université de Montréal in 1920, the smoking regulations for the medical faculty were part of the gendering of space of the faculty. While smoking was explicitly banned in the dispensary and the waiting room of the maternity hospice, two places where women worked, in the "Réglements concernant l'amphithéâtre d'Anatomie et les salles de dissection" noise was forbidden, but smoking was not.[63] The epicentre of this male smoking space was the dissecting room. The McGill *University Gazette*, for example, jokingly compared the Anatomy room to a smoking-room where one could also dissect.[64] Generally, in anatomy classes students smoked to "disguise the odors of putrification."[65] At the McGill medical school John F. Todd complained, "I am spending about six hours a day in the dissecting room. My clothes, even my undershirt, (when I take it off at night, you can almost wring the odour from it) are so thoroughly permeated with the smell, that it is only on two days, Friday and Sunday, that I attempt to rid myself of it."[66] One article in a Montreal medical journal maintained that smoking " is so indulged in the dissecting room it is apt to persist after their studies" and the strong smell of tobacco smoke risked making women and children

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[63]"Constitutions et Règlements de l'Université Laval" Publiés par ordre du Conseil Universitaire, 4ième édition,(Québec: Des Presses à vapeur de Augustin Côté et cie, 1879), Les Archives de l'Université de Montréal, pp.60-63. The rules were the same for the Montreal campus.

[64]"Between the Lectures," *University Gazette*, 7 February 1888, p.85.

[65]*Canada Medical and Surgical Journal*, January 1880, p.282.

[66]John F. Todd to his mother, 8 Feb. 1897 in *John L. Todd Letters.*

who were sick even sicker and non-smoking "gentlemen" would thus avoid hiring them.[67]

According to contemporaries, the ritual of smoking while dissecting was one of the traditions which made the profession unsuitable for women. The McGill *University Gazette*, for example, commented on the University of Geneva that allowed women into the male sphere of medical school. Finding the idea ridiculous the paper painted the following picture: "It is not an uncommon sight for a Russian student (female) to be found working away in the "Anatomie" with a lighted cigarette in her mouth." These female students' respectability was then questioned in the article by noting that they were mainly from eastern Europe, were not respected in Geneva, and never amounted to much.[68] Part of the way this meaning was evoked was by having these female doctors undergo the same rites of passage as male doctors, though slightly feminized with the specification of the cigarette.

Among physically mature men, smoking created gender solidarities that bridged cultural boundaries. As anthropologist Marcel Mauss has argued, passing on gifts can be a "bond of alliance and commonality" when it may otherwise seem that none other appears.[69] For example, Montreal journalist Jules Fournier was offered a cigarette by Chief McCarthy, the police officer taking him to prison after being charged with publishing a defamatory article. This helped him to declare McCarthy polite on all counts, despite the

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[67]*Canada Medical and Surgical Journal*, January 1880, p.282.

[68]"College News," *University Gazette*, 7 February. 1888, p.85.

[69]Marcel Mauss, *The Gift: The form and reason for exchange in archaic societies* (originally published as *Essai sur le Don*, 1950) translated by W.D. Halls, (London: Routledge, 1990), p.13.

fact that McCarthy was in a conflicting relationship with Fournier.[70] While teaching at the École Normale, Charles Gill was touched at the end of the year when his third year students bought him a box of cigars and a package of choice tobacco, though he thought it was a bit expensive considering their meager bursaries.[71] Moving to the 1920s, male employees of the Bank of Montreal, as Clare Jennings has shown, received pipes as wedding presents while women received household items, reflecting and reinforcing the norms of separate spheres.[72]

III. The Public Sphere

Smoking also set the tone for high-minded discussion. Cultural journals and newspapers included columns entitled "en fumant" which focused on issues of civil society as their subjects. One such case was the "En Fumant" column of *Le Monde Illustré* which discussed, for example, the secret ballot in Canadian elections or "Le despotisme et la barbarie de L'autocrate de toutes les Russies."[73] Similarly, authors of fiction used

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[70]Jules Fournier, *Souvenir de Prison*, in Jacques Hébert, *Trois Jours en Prison* (Montreal: Club du Livre du Québec), p.73.

[71]Charles Gill to Louis-Joseph Doucet, 20 June 1911 in Hamel, ed. *Charles Gill*, p.31.

[72]Clare Jennings, "The Bank of Montreal Staff Magazine: Images of Work and Leisure," (Undergraduate paper, 101-364B, McGill University, 2000), p.11.

[73]"En Fumant" *Le Monde Illustré*, 12 July 1890. For other examples of this column, see 20 October 1888, p.198; 2 November 1889, p.214; and 14 June 1890, p.235. This trend was not unique to Montreal. See the series, "The Good-Night Pipe," *Trinity University Review*, 10:10, October 1897, pp.117-118. The series continues in the following months of the *Review*.

smoking to evoke the same high-minded masculinity. In one instance, Dr. Ernest Choquette, describes in a short story a group of doctors reuniting, coming from long distances, and discussing their first cases. Choquette sets the stage for this storytelling by depicting a post-dinner scene: "Sur une table, il y avait des bouteilles ouvertes de congnac, des facons de genière, des carafons de vin, des cigares, des cigarettes, des verres...."[74] Working class writers adopted this same high-minded smoking ethic. In the Montreal working class newspaper *The Echo*, one political economy columnist used an after-dinner scene around a rooming house kitchen table to discuss tariffs and working class consumption: "When the table had been cleared, the two young men sat over their tobacco, the captain, as before, smoking his cigar, the painter his pipe - and discussed the day's events." The author's argument then played out in their words.[75] In a more macabre sense, Montreal poet Emile Nelligan, anthropomorphized tobacco, making it his companion while questioning his very existence in the poem "Rondel à ma Pipe":

> Les pieds sur les chenets de fer
> Devant un bock, ma bonne pipe,
> Selon notre amical principe
> Rêvons à deux, ce soir d'hiver.
>
> Puisque le ciel me prend en grippe
> (N'ai-je pourant assez souffert?)
> Les pieds sur les chenets de fer
> Devant un bock, rêvons, ma pipe.
>
> Preste, la mort que j'anticipe
> Va me tirer de cet enfer

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[74]Dr. Ernest Choquette, "Premiers Cases" in *Carabinades* (Montreal: Déom Frères, Éditeurs, 1900), p.51.

[75]*The Echo*, 11 October 1890.

Pour celui du vieux Lucifer;
Soit! Nous fumerons chez ce type,
Les pieds sur les chenets de fer.[76]

Smoking sets the tone for Nelligan's contemplation of death. His move from what he saw as Hell on earth to Hell, is not done alone. It is worth noting that while both tobacco and beer have a physiological effect on the body, it was tobacco that Nelligan chose to use as his companion, not his mug of beer, underlining tobacco's particular cultural role of setting a tone for high-minded discussion.

Considering this leisurely ideal, the cigarette smoker was the target of significant condescension since cigarettes took such a short amount of time to smoke in comparison to pipes or cigars. Indeed, the cigarette smoker's masculinity was put into question. For example, a cartoon in the middle class Montreal weekly *Canadian Illustrated News*, entitled "*Ex Fumo Dare Lucem*" (figure 2, see p.46) presents two men, one a "Cigarette Lunatic," the other a "Cigarette Idiot," both of whom present opinions that the cartoonist finds to be absurd.[77] According to the cartoon, the cigarette is neither manly, nor does it give "an intellectual look." It promotes apathy and "makes one do nothing," clearly not the productive leisure of a cigar or pipe. Cigarette smoking was often linked to a youthful restlessness, not content to pass time pensively smoking a cigar or pipe. In William Douw Lighthall's *The Young Seigneur*, the narrator is part of a secret society called the "Centre-Seekers" in which he and his young friends discuss important topics. One of his friends,

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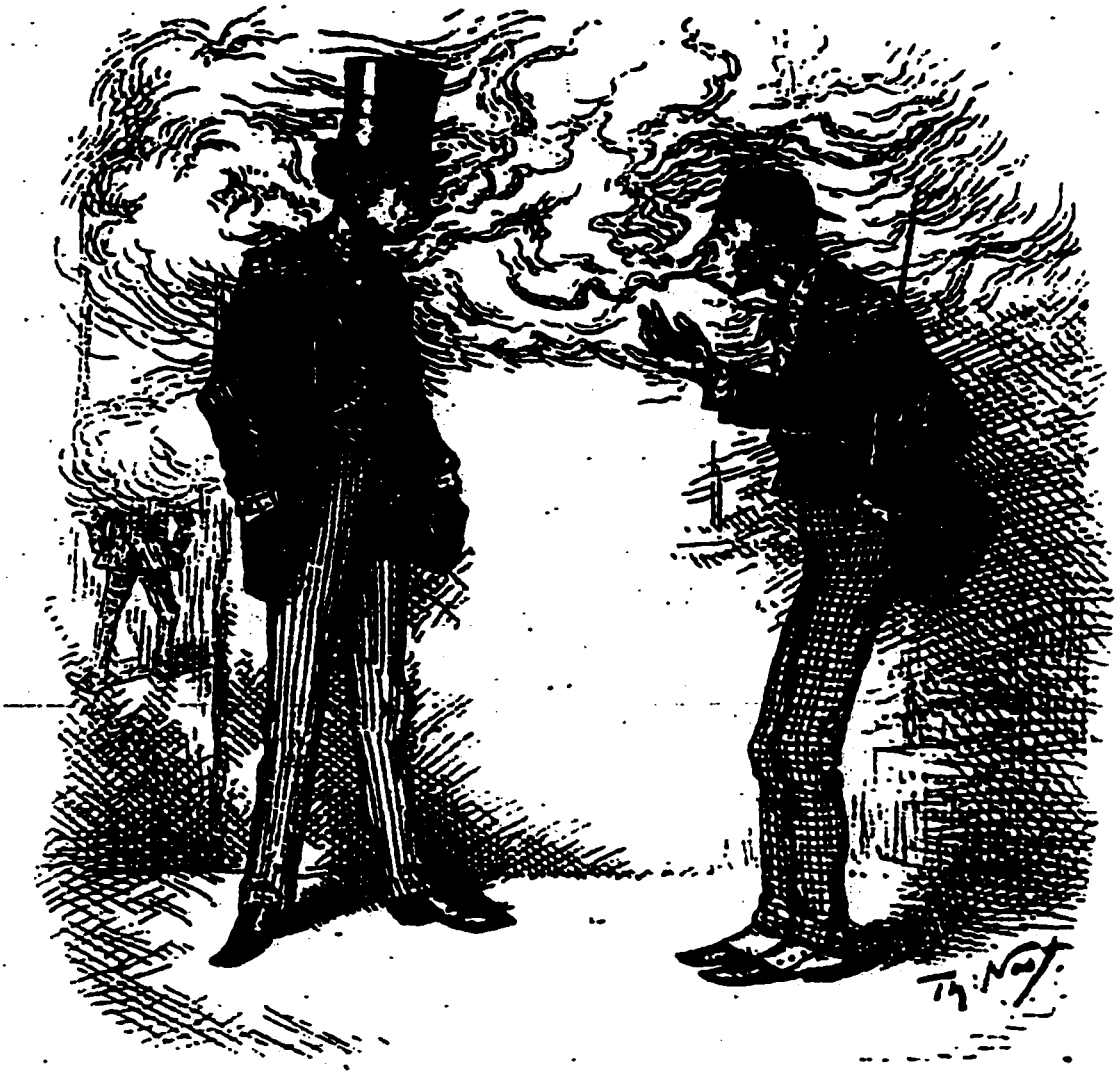
[76]"Rondel à ma Pipe," *Poèmes Choisis: Émile Nelligan* (Montreal: Fides, 1983), p.51.

[77]"*Ex fumo Dare Lucem,*" *Canadian Illustrated News*, 18 March 1882, p.176.



EX FUMO DARE LUCEM.

CIGARETTE LUNATIC.—"It not only gives one a manly air, but adds such an intellectual look."
CIGARETTE IDIOT.—"Yes, and makes one do nothing, and care for nothing; and one feels as if life was *all smoke*.

Figure 2 *Canadian Illustrated News* (1882).

described as a philistine, makes a youthful declaration that he enjoys himself most at his theatrical club where "we have the prettiest girls and chummiest fellows in town.... There's philosophy in it too, by jove! I've done lots of philosophy by the smoke of the cigarette."[78] Lighthall, a noted anti-modern, not only relates the cigarette to youth, but also to a lack of thoughtfulness - a criticism of the speed of cigarette smoking.

A cigarette smoker's masculinity was especially put into question if he rolled his own. Cultural sources suggest that the roll-your-own cigarette was linked to being a dandy. According to historian Leora Auslander, dandies:

> were men for whom living elegantly was essential. They dressed carefully, expensively, and distinctively. They furnished their apartments with like extravagance and attention. They also cultivated their bodies, disciplining their gestures, their gaits, and their stances. Some were heterosexual, some were homosexual. A few married, most did not... all acted as if they were men of leisure.[79]

Commentators used the image of rolling-their-own cigarettes as a criticism that dandies lacked substance, not willing to take on the responsibilities of breadwinning. Rolling a cigarette took a significant amount of time and suggested an interest in detail since hand-rolled cigarettes were already available on the market. This concern for detail would have been fine but it took such a short length of time to smoke a roll-your-own cigarette and seemed like an insignificant amount of pleasure for so much work. The cartoon, "The

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[78]William Douw Lighthall, *The Young Seigneur* (Montreal: Wm.Drysdale & Co., 1888), p.31. Donald A.Wright, "W.D. Lighthall: sometime Confederation poet, sometime urban reformer" (M.A. Thesis: McGill University, 1991).

[79]Leora Auslander, "The Gendering of Consumer Practices in Nineteenth Century France" in Victoria de Grazia, ed. *The Sex of Things: Gender and Consumption in Historical Perspective*. Berkeley, University of California Press, 1996. p.90.

Figure 3: "The Herculean Labours of a Cigarette Smoker" (*Canadian Illustrated News*, 1879).

Herculean Labours of a Cigarette Smoker" (figure 3, see p.48) from the *Canadian Illustrated News* demonstrates the case.[80] The cigarette smoker is portrayed as the dandy, dressed to extremes as well as sitting with severely disciplined posture. He also makes extraordinary gestures to roll the cigarette, falling into Auslander's definition of a dandy.

Another example, this time from a literary source, sets the social context of the smoking dandy. The roll-your-own cigarette was used to construct the dandified character of Gaston in Rodolphe Girard's short story, "Fin d'un Célibataire." Gaston is described as a "invulnérable célibataire," a "cynique et stoïque vieux garçon" who in the story turns thirty, has a nightmare about marriage, gets drunk and asks his girlfriend to marry him. Before his nightmare, however, Gaston's character is developed through his choice in furniture, "un divan aux prétentions orientale, ses membres longs et secs comme des queues de billard" which he flops down upon to take his fateful nap, and by his rolling and smoking of a cigarette.[81] He is a man of extreme leisure, with even his furniture resembling billiard cues, more image than real as his furniture only has "prétensions" to being oriental. Finally, he rolls and smokes a cigarette, doing a great deal of work for less payoff than a smoker would get from a pipe or cigar and falls asleep..

The amount of time a man could possibly smoke any kind of tobacco and devote to this sort of thoughtfulness was limited by his job. Working class historians of Montreal

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[80]"The Herculean Labours of a Cigarette Smoker," *Canadian Illustrated News*, 1 February 1879, p.80.

[81]Rodolphe Girard, "Fin d'un Célibataire" in *Mosaique* (Montreal: Deom Frères, 1902), pp.9-10.

have shown that as industrial capitalism took hold, the nature of work changed.[82] Among other changes, work moved from the workshop to the factory and workers lost a great deal of control over their time which was strictly regimented by factory hours. Previously, as historian Alain Corbin has pointed out in regards to France, "[t]he rhythm of work was easily adapted to a generous consumption of alcohol and tobacco."[83] By the end of the 1860s, Montreal cigar makers had lost their customary right to smoke on the job and in the late 1880s, factory rules of conduct presented by a "Leather Dresser" at the *Royal Commission on the Relations of Labor and Capital* prohibited smoking in the tannery.[84] While there were fire risks related to smoking on the job, this particular set of Factory Regulations had little to do with fire hazards. In fact, smoking was listed in the same line as singing and talking without permission, and thus was clearly framed as a question of work discipline. François Lainé, the witness at the Commission, maintained that he had seen these rules were enforced and workers were fined 25 cents for breaking them.[85] Some companies provided a small amount of time and space for workers to smoke. Smoking was not allowed in the Grand Trunk Railway Works in Point St. Charles, for example, where in the early 1890s at least 2000 were employed, but the company set up a

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[82]Bradbury's *Working Families* summarizes much of this literature for Montreal.

[83]Alain Corbin, *Time, Desire and Horror: Towards a History of the Senses* (Cambridge: Polity Press, 1995), pp.5-6.

[84]Jean Hamelin and Jacques Rouillard, *Répertoire des grèves dans la province de Québec au XIXe siècle* (Montreal: Presses de l'École des hautes études commerciales, 1970), p.21-22.

[85]"Rules of Establishment...." Royal Commission on the Relations of Labor and Capital (hereafter RCRLC), *Quebec Evidence*, pp.593-594.

room 150 feet by 50 feet, where men could eat and then, for 15 minutes at the end of their lunch hour, they could smoke.[86]

Spatially, smoking also demarcated the borders of a high-minded masculine public sphere. Separate spheres ideology situated women's place in the private sphere, the family home, where she was the nurturer. Yet even here a woman's power was limited and middle and upper class family homes were divided spatially into male and female spaces. The male areas, the library, study, billiards room and the smoking room were often decorated with things related to male public sphere activities, like books, maps, scientific equipment and weapons. These rooms were complete with the accessories with which a man would do business and would also have greater access to the outside world. According to architectural historian Annmarie Adams, the more upper class the family, the more separated the smoking room was from the rest of the space inhabited by the family.[87] In the home, smoking inscribed in space the high-minded tone of the male public sphere. The after-dinner smoke was among the most well-known negotiation of gendered space in the home involving smoking. Women retired to a drawing room or parlor while the men withdrew to a library or smoking room. In the extreme case of this ritual a smoking jacket and hat were worn to protect a man's clothes from smoke, so his wife would not smell it later. According to etiquette, this was not only a spatial move, but a change in topics of

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[86]Testimony of Frederick Wanklyn, Assistant Mechanical Superintendent of the Grand Trunk Railway, Royal Commission on the Liquor Traffic (hereafter RCLT), *Quebec Evidence*, p.742 and p.746.

[87]Annmarie Adams, *Architecture in the Family Way* (Montreal and Kingston: McGill-Queen's University Press, 1996), p.77-78.

conversation. Dinner conversation was to be light - no politics, business, or religion. After dinner, men and women segregated, and men could talk about these serious subjects and smoke.[88]

The ability to separate men and women and to create this kind of public sphere around the smoking room was highly dependent on economic prosperity. Journalist Robert de Roquebrune remembered in his memoirs that there was a smoking room in the house his family moved to on arriving in Montreal at the turn-of-the-century. The family, however, was only moderately well-to-do, as much of the entire family's leisure, regardless of gender, was spent in the smoking room. Eventually, his parents decided the house was too big and it would help economically if they moved. The family found an apartment on St. Denis street which had a smaller room that his father declared "La pièce la plus importante après la salle à manger" where the family gathered in the evenings and smoked, yet it did not have the status of a smoking room.[89]

Most Montreal homes did not have a smoking room or a drawing room where men could retire for a smoke. Geographers Jason Gilliland and Sherry Olson have calculated that the average dwelling in Montreal in 1901 had only four rooms – most likely two bedrooms, possibly a living room, depending on the size of the family, and a kitchen.[90] With no space set aside to smoke, middle class etiquette dictated that the home was

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[88]Visser, p.267 and p.281.

[89]Robert de Roquebrune, *Quartier Saint-Louis: Récit* (Montreal: Fides, 1966), p.42, pp.165-166.

[90]Jason Gilliland and Sherry Olson, "Claims on Housing Space in Nineteenth Century Montreal," *Urban History Review*, Volume 26, No.2, (March 1998), pp.3-16.

Figure 4: Following smoking etiquette.

supposed to be female space and non-smoking. Some advertisers played on this belief. In 1912 Wrigley's Spearmint Gum was advertised as a more appropriate odour and taste than a cigar if a man was going home to see his family (figure 4).[91] Tobacco companies also tried to sell their products by playing on the home as a female space. Fortier's ten cent cigar, the Chamberlain, was advertised to have an "Arome Parfumé," an attempt to curry favour with women.[92] Another tobacco advertisement alleged that if a man smoked Jacques Cartier tobacco " votre épouse ne s'objectera plus à votre pipe."[93] Some men tried to claim that letting them smoke at home would be helpful in fighting the more dangerous vice: liquor. Dr. Jacob Dubé, in a speech to the Montreal Dominion Alliance for the

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[91]Montreal *Herald*, 22 January 1912, p.11.

[92]28 November 1907, *Le Canada*, p.9.

[93]9 October 1907, *Le Canada*, p.1.

Suppression of Alcohol, addressed married women in the crowd making a plea for men to be given "the privilege of smoking in the house with his friends - and without fear of an aftermath of complaints that the smoke spoiled the curtains."[94] According to Dubé, more important than the curtains was where a man would go if he was not allowed to smoke in the house: a tavern. And if a man had to go to a tavern to have a smoke he would be exposed to more serious temptations, like alcohol or other unnamed vices.[95]

Other commentators portrayed the spatial rules around smoking as a choice between marriage and tobacco. They could either be married to a respectable woman or married to tobacco, and when asked to choose, some chose tobacco. Poets were particularly active in expressing this renunciation of what was a domesticized masculinity. They anthropomorphized tobacco, making it a replacement for woman.[96] The most famous work in this genre is Rudyard Kipling's "The Betrothen," a poem that complains of the demands of being married, and the simple pleasure of smoking, summing up with "And a woman is only a woman, but a good cigar is a Smoke."[97] Montreal journals reprinted similar poems, like the poem "My Love," a parody of the Robbie Burns poem, "My Luve is Like a Red Red Rose," published in Montreal *Saturday Night*:

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[94]"Let the Hubby Smoke," Montreal *Star*, 12 March 1910, p.27. Republished in *CCTJ*, April 1910, p.35.

[95]*Ibid.*

[96] On the sexualization of objects, see the essays in de Grazia, ed. *The Sex of Things*.

[97]Rudyard Kipling, "The Betrothen" in Wilfred Partington, ed. *Smoke Rings and Roundelays: Blendings from Prose and Verse since Raleigh's Time* (London: John Castle, 1924), pp.61-63.

My Love is like the red red rose
That breathes the sweet perfume
In my love all charms repose,
And I, those charms consume.

My love is no expensive wife,
Tho' very dear she be;
Three pence a day, upon my life,
Is all she costeth me.

Of flowers and jewels, bonnets and lace,
She never feels the need;
So flowers at her command I place,
Save, only one poor weed.

And yet not e'en the fairest girls
Can with my love compare;
Altho' she boasts no glossy curls,
Not e'en one scrap of hair.

Thrice daily after every meal,
I press her to my lips;
And then as sweet a kiss I steal,
As been from lily lips.

May I all other earthly loves
from my remembrance wipe;
While loving one poor piece of clay,
My beautiful my - pipe. (By C.D.)[98]

The *CCTJ* even further sexualized this relationship between a man and his pipe, noting the "long days and nights of constant and close companionship you and your friend the pipe become as near akin as man and wife, indeed, a great deal nearer than some couples in these days." The sexual theme of the article is then heightened and describes one encounter where "You pick the old pipe up some winter evening, and as you turn it over and around in your hand, preparing to filling [sic] it with the weed that brings the

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[98]C.D. "My Love," Montreal *Saturday Night*, 14 September 1895, p.4.

color to its cheeks."[99] While these examples show men choosing tobacco over domesticization, the very dichotomy of the choice underlines the interdiction of smoking in the private sphere.

According to liberal prescriptions of smoking, the ideal place to smoke was in a homosocial male environment. The *Canadian Illustrated News* expressed this ethic in an article promoting the growth of men's social clubs. These clubs allowed men to "meet together for their own improvement, or for the good of others, or to relax themselves from the cares and business of the day...and...smoke a friendly pipe."[100] Montreal tobacco companies frequently tapped into the broad link between high-minded leisure and smoking, often focusing on "back-to-nature" themes. Returning to nature, historians have told us, was an important theme of nineteenth century middle and working class cultures, seen as an important antidote to industrialized city life.[101] For the summer of 1899, for example, J. Hirsch and Son, Co. launched a brand of tobacco called "the angler" aimed at "the summer resort trade."[102] In 1910 S. Davis and Sons advertised their domestic brand "Perfection Cigar" by using two vacationing narratives: "With Song and Story an evening around the camp fire passes pleasantly, especially if there is a box of 'Davis Perfection' cigars"; and

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[99] "Apotheosis of the Pipe," *CCTJ,* August 1903, p.53.

[100] *Canadian Illustrated News*, 6 September 1873, p.146.

[101] See Patricia Jasen, *Wild Things: Nature, Culture, and Tourism in Ontario, 1790-1914* (Toronto: University of Toronto Press, 1995), pp.105-132. For the importance of this to working classes, see Suzanne Morton, *Ideal Surroundings: Gender and Domestic Life in a Working Class Suburb in the 1920s* (Toronto: University of Toronto Press, 1995), pp.127-128.

[102] "Montreal Correspondence," *CCTJ,* June 1899, pp.213-215.

then "It certainly makes an early fishing trip more enjoyable if you take along a goodly supply of this popular brand."[103] S. Davis and Sons also changed the packaging of their 25 cent cigar "La Mencita," putting them in metal canisters to appeal to tourists and campers.[104]

Smoking made visible the borders of this high-minded male public sphere that sometimes could otherwise be invisible. "Smokers," for example, were male-only social nights that were complete with other activities like music, speeches and drinks. Its link to tobacco was the only sign that it was male-only, unlike a ball. Smokers were frequent in Montreal, often held in festive seasons, like immediately prior to Christmas, and were commonly held by Francophone and Anglophone males from all classes. They were part of an associational life that made up the public sphere in Montreal. In December of 1900, for example, the bourgeois Montreal lacrosse club hosted a free smoker open to all – "a compliment to Lady Nicotine."[105] Similarly in December of 1907 *La Patrie* reported that numerous union leaders attended the Montreal bookbinders' union smoker held at St. Joseph Hall where there was music, singing and speeches.[106]

Smoking played a part in demarcating politics as male. The powerful Liberal

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[103]Montreal *Gazette*, 9 May 1910, p.4.

[104]"Montreal Correspondence," *CCTJ*, June 1899, pp.213-215.

[105]"My Lady Nicotine," *Gazette*, 1 December 1900, p.1. On the social makeup of the Montreal Lacrosse Club, see Alan Metcalfe, *Canada Learns to Play: The Emergence of Organized Sport, 1807-1914* (Toronto: McClelland and Stewart, 1987).

[106]"Le concert tabagie des relieurs: Discours, chant et musique," *La Patrie*, 9 December 1907, p.9.

senator L.-O. David, argued that women should not get the vote because the political sphere of public assemblies and political clubs was full of crude discussion: "dans une atmosphère viciée par les fumées du tabac et de l'alcool... Quel triste spectacle!" In David's opinion, this was no place for a woman.[107] Indeed, some women saw the political arena in a similar light and were not interested in putting their reputations into question or being harassed by entering the male public sphere of politics. Andrée Claudel, the women's columnist at the Liberal weekly *Le Pays*, recounted a conversation between five French-Canadian elite women who frequented a tearoom in the afternoons. The women proclaimed that they were not interested in the vote because it would mean entering polling stations where the men smoked: "Y songez-vous, ma chère, aller dans ces affreux 'polls' où les hommes fument et où on croise un tas de yeux, ah non! Merci."[108] In this case, men smoking and their uninviting gaze constructed the political sphere as an uncomfortable space for these women.

Unsurprisingly, male smoking also helped demarcate cigar stores as male space. The cigar store was a centre of male culture for all classes. At the turn of the century, industry insiders estimated that were 2,000 cigar stores in Montreal.[109] Tobacconists and cigar stores were often associated with other male oriented services like barbershops and

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[107]L.-O. David, "Le suffrage féminin," *Au soir de la vie* (Montreal: Librairie Beauchemin, 1924), p.55.

[108]"Perruches entre elles," *Le Pays*, 5 February 1910, p.2.

[109]Mortimer Davis, "Minutes," Royal Commission Re: The Tobacco Trade of Canada (RCTT), RG13, box 2317, file 349/1903, p.1174-1177.

billiards.[110] They also served as "fronts" for illegal male sporting culture activities like gambling and lotteries. At the turn of the century the Montreal police frequently raided cigar store gambling dens.[111] Indeed, cigar stores were particularly useful as fronts because few may have questioned men entering a cigar store as opposed to a less gendered space.[112]

These fronts were far from the high-minded ideal of smoking promoted by men who followed late nineteenth century codes of respectability in Montreal. The *CCTJ*, for example, condemned such places.[113] Numerous cigar store owners attempted to turn their stores into centres of high-minded male public spaces. Elite tobacconist A. Michaels, for example, put on a display of sketches of the Japanese land forces in action during the Russian-Japanese War, apparently attracting a crowd.[114] Another cigar store owner installed a New York Stock Exchange "ticker" to attract a business clientele.[115] Yet another in the business district on St. James Street offered "comfortable and well appointed lounging, smoking and writing rooms, together with the leading English,

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[110]A. Michaels, for example, reported that a Mr. Giroux kept a barbershop at the "rear" of his premisses, "Minutes," RCTT, p.128. Louis Fortier kept a billiards parlor in his "Eden Cigar Store." See *CCTJ*, January 1902, p.17.

[111]*CCTJ*, April 1903, p.14; *CCTJ*, April 1905, p.23.

[112]For cigar stores and gambling in a later period, see, Suzanne Morton, "A Man's City: Montreal and Male Space in the 1940s," in Myers *et al.*, *Power, Place and Identity*, pp.169-182.

[113]"Montreal Correspondence," *CCTJ*, September 1899, p.337; June 1906, p.17.

[114]"Attracts a great deal of Attention," *CCTJ*, May 1905, p.49.

[115]"Montreal Correspondence," *CCTJ*, October 1905, p.19.

Canadian and American magazines and periodicals."[116] Cigar stores were to be "breeding places for all sorts of arguments and controversies on all sorts of subjects...."[117] In fact, the *CCTJ* suggested that tobacconists open a smoking room at the back of their stores to let their patrons rest "for an odd half-hour."[118] The cigar store as a leisure space, however, was to be restricted on a class basis. "Naturellement," wrote *Liqueurs et Tabacs*, "il ne peut être question d'admettre les vagabonds dans un magasin bien dirigé...." Yet there was no problem in allowing "les gens de bonne société... se rencontrer quelques moments et converser des sujets qui les intéressent" while smoking a good cigar.[119]

Despite tobacconists' interest in creating a centre for the respectable male public, every Christmas they tried to convince women to buy gifts for their male relatives in their stores. The *CCTJ* suggested the tobacconist should offer to send a selection of the gentleman's cigars over to the house for her perusal, so she would not have to suffer the indignity of entering a cigar store.[120] But barring this, the *Journal* also gave advice on some of the ways tobacconists could encourage women to come to their stores. This advice gives insight into the factors, in addition to smoking, that made cigar stores unfriendly spaces for women. For example, the *Journal* suggested that cigar store

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[116]"Montreal Notes," *CCTJ*, November 1913, p.33.

[117]"Cigar Stores as Social Centres," *CCTJ*, January 1908, p.67.

[118]"Good Shopkeeping," *CCTJ*, June 1905, p.19.

[119]"Les Flaneurs Chez les Tabaconistes," *Liqueurs et Tabacs*, October 1904, p.42. Also on "loafers" see *CCTJ*, July 1903, p.65 and "Loafers Not Wanted," *CCTJ*, August 1907, p.65.

[120]"Pointers for Retailers," *CCTJ*, November 1902, p.629.

advertising should "[t]ry to convey assurance ... that the woman customer will be subject to absolutely no embarrassment or annoyance in entering the store...." The article continued that though it shouldn't be necessary to actually give these instructions, clerks were to be told not to stare, "nor [to perform] any actions whatever, to denote that the customer is an unusual one."[121] A few years later the same journal told a story of one tobacconist who had taken "pains" to receive the patronage of women buying gifts for men. Their advertisement sought to "make every lady who read the evening papers feel that it was perfectly proper and matter-of-fact that she should step in to the store and execute her commission."[122]

IV. Deference and Self-Control

Smoking was perfectly respectable in public places like smokers, medical schools, political events, and cigar stores - all places designated as homosocial male public space, and furthermore, smoking was one of "the mechanisms whereby that sphere was created and maintained as a masculine province."[123] Yet according to this etiquette of smoking, the male public sphere was not supposed to be an all-pervasive "smoking section." When women were present in public places where classes mixed, the etiquette of smoking was modified for social distinction. According to etiquette, streets, for example, were still considered male space and women who frequented them risked their dignity. Without

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[121]"Ladies Trade," *CCTJ*, December 1908, p.57.

[122]*CCTJ*, February 1911, p.55.

[123]Ryan, *Women in Public*, p.9.

careful attention to comportment and dress, a woman on the street could be misunderstood to be a "street walker" or prostitute.[124] Historian David Scobey has argued that the middle class flouted rules of gendered spheres to create gendered visions of order in the midst of industrial cities that they saw as hives of disorder. In these gentrified spaces men and women walked publicly, exerting intense self-control over every movement of their bodies.[125] Especially important, John Kassons adds, was not to draw attention to the internal workings of the body.[126] Thus, men chewing gum, eating and smoking were frowned upon in these rarefied mixed sex situations. Etiquette experts in Montreal denounced men smoking on streets when in the company of a woman. One guide told its readers that if you meet a woman in the street "on fera le sacrifice du cigare ou de la cigarette commencée que l'on jettera discrètement et sans ostentation."[127] Similarly, *La Presse*'s etiquette columnist, when asked if a young man could smoke a cigar while escorting a woman on the street, answered in no uncertain terms: "Non, ce ne serait pas poli."[128]

The power dynamics of this kind of public display gave genteel woman a theoretical veto over public smoking. In the most bourgeois of settings where there was

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[124]For a study of Montreal prostitution and female juvenile delinquency see Tamara Myers, "Criminal Women and Bad Girls: Regulation and Punishment in Montreal, 1890-1930" (Ph.D. Dissertation: McGill University, 1996).

[125]David Scobey, "Anatomy of the promenade."

[126]Kasson, *Rudeness and Civility*, pp.117-132.

[127]Sauvalle, *Mille Questions d'étiquette*, p.119.

[128]"Le Courrier de Colette," *La Presse*, 18 July 1914, p.7.

less interaction of classes, a man could ask for permission to smoke in a woman's presence. Madame Sauvalle considered it "presque superflu de dire qu'un homme n'allume jamais une cigarette devant des femmes sans leur demander la permission." He then went on to write that this would only be appropriate behaviour in the dining room of a hotel where there was no smoking room. There, a man could discretely ask the women or have an orderly ask the women present at the table if the men could smoke.[129] Permission was not always forthcoming. The *CCTJ* told the story of a "smart young lady" who arrived at a railway carriage that already had three or four men in it. One of them, "in the familiar style we know so well," took out a cigar and match box and asked, "I trust madam, that smoking is not disagreeable to you?" to which the woman responded: "Really, sir, (with the sweetest of smiles), I Can't [sic] tell, for as yet no gentleman has smoked in my presence."[130]

Not smoking while with a woman in public was nothing less than a performance of masculine respectability. This etiquette was acted out in the cartoon "Comment on arrive à prendre femme" (figure 5, p.64). The cartoon begins with the man at home with drinks on his table, enjoying a cigar. On a "promenade hygenique," which includes smoking a cigar, he sees a woman. He continues to smoke his cigar until he resolves to talk to her. When he decides to approach her, out of respect, the cigar disappears. Later we see his former life of cigars and drink put on the top shelf as he and the woman sit in a drawing

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[129]Sauvalle, *Mille Questions d'étiquette*, pp.117-8.

[130] "It Remains to be seen," *CCTJ,* August 1900, p.345.

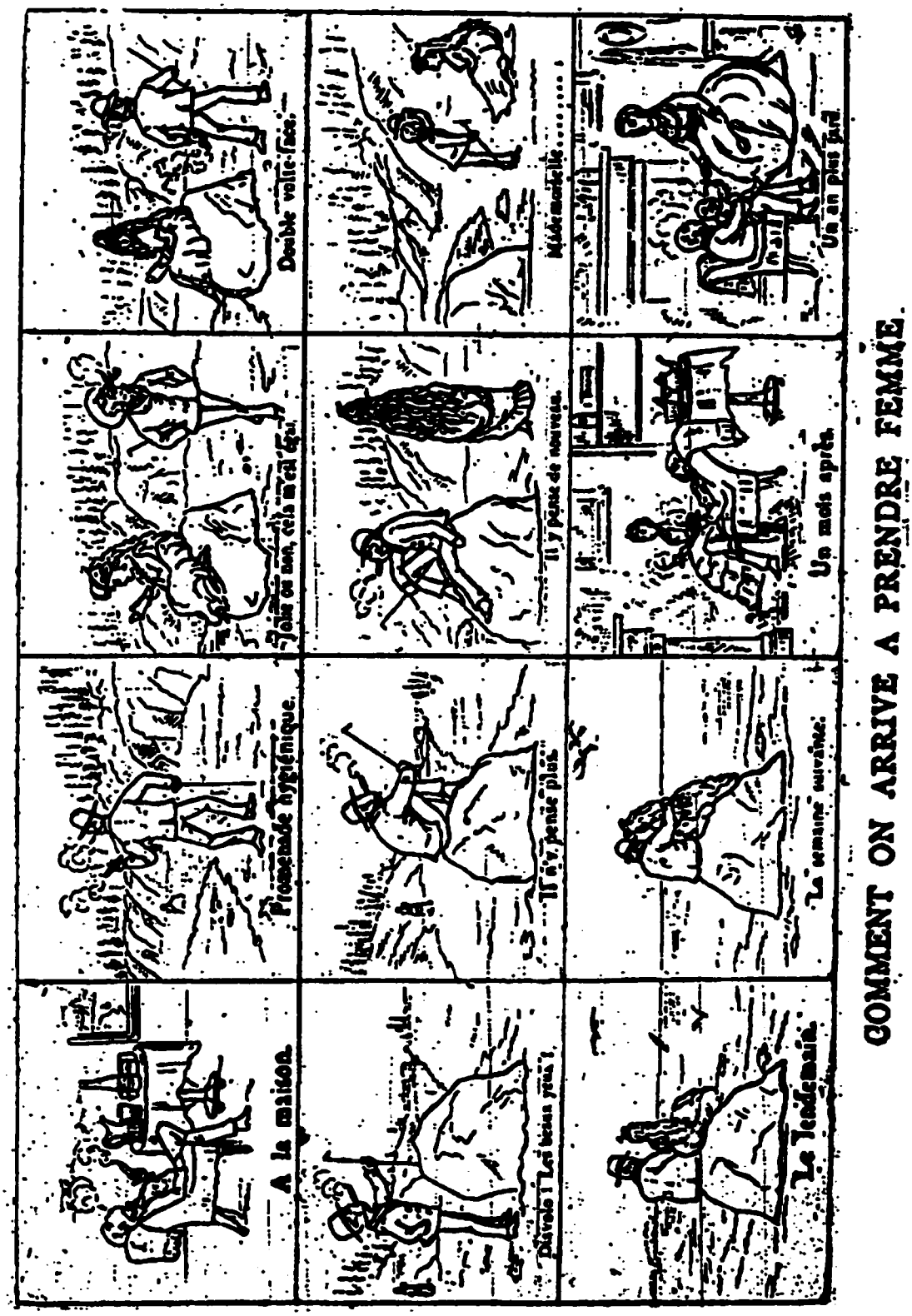

Figure 5: *Le Monde Illustré* (1886)

room. A year later a child enters the picture.[131] On one level, the cigar was a marker of the man's bachelorhood, and on another it was a demonstration of his gentility in throwing it away.

This vision of the respectable etiquette of smoking was often linked to class status. Such was clear in the outrage of Ruth Cameron, the author of the Montreal *Herald*'s "Evening Chit-Chat": "That a man should not smoke when walking with a woman on the street is a rule that I suppose most men know, even if, knowing the right, they still occasionally pursue the wrong." Indeed, this rule was being flouted even by those who, according to Cameron, should have known better:

> I stood in front of the finest hotel in this city the other day, and saw a man dressed in the extreme of fashion - tall silk hat and clothes of the very latest cut - hand a fine lady into a very magnificently appointed automobile with a very gracious and lordly manner, and then climb in and sit beside her chatting with her while a cigar tilted from the corner of his mouth.[132]

For Cameron, class was about more than money. It was about having the cultural capital of understanding proper gender conduct in public. Breaking down this image, she recognized this man as being "upper class" by being in front of the "finest" hotel, wearing the "extreme of fashion," getting into the automobile in a "lordly manner," and having a cigar in the mouth. The woman, according to this semiotic understanding of bourgeois heterosexuality, was a monument of self-control. She remained passive, and allowed herself to be objectified as she was "handed" into the automobile. Smoking the cigar on

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[131]"Comment on arrive à prendre femme," *Le Monde Illustré*, 21 August 1886, p.124.

[132]Ruth Cameron, "The Evening Chit-Chat," Montreal *Herald*, 7 June 1910, p.9.

the street while a woman was present was the only thing that did not fit the "class of man" within this vision of gender relations. Men were to exert self-control and not indulge in tobacco while with women in public.

These ideals of personal conduct and self-control while sexes mixed dictated that some public spaces were hopelessly vice-ridden. An example of this failure to live up to these highly gendered bourgeois codes of conduct was the tavern. Some establishments where men could drink and smoke based their respectability on their separation of the sexes. In 1893 the Lyceum Theatre, for example, had a separate section for men to smoke and drink without women and its manager claimed that this was part of why it was a respectable theatre.[133] All taverns were not so respectable. In Dr. Ernest Choquette's short story "Loulou" two individuals find their moral decline in the tavern. First, Robert Renault, a boy from the country comes to the city to go to medical school. After refusing several times his fellow students' invitation to go "out on the town," he finally gives in:

> Un soir cependant, dans l'amtosphère des cigarettes, du cognac brulé, du scotch, qui embaumait le salon de l'*Aurore*, il avait senti se fondre insensiblement dans le même nuage les restes flottants de ses scrupules; et il les avait suivis machinalement... les autres partis en caravane pour un chahut d'enfer.

To join the circle was to enter into the smoke, and once in, his scruples disappear like the smoke of the cigarettes. Yet the consequences of entering these taverns were more severe for a woman. The second person to morally fall was Loulou, a woman who is also out "on the town" with this group. Renault asks her why she did not leave the group, and she

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[133]William Walter Moore, RCLT, *Quebec Evidence*, p.532.

finishes his statement "pour redevenir honnête, n'est-ce pas?"[134] While the narrative did not include any mention of her drinking or smoking, the fact that she was in such an environment put her respectability into question. The mingling of the sexes here played an important role, as did the alcohol and tobacco smoke, in putting this woman's character into question.

One of the most controversial mingling of the sexes and classes in public at the turn of the century was on Montreal public transit. Indeed, the smoking controversies on Montreal tramways bring together the use of smoking as a border of a high-minded male public sphere, the material limitations to this etiquette, and its use as a performance of respectability. Tramways were exemplary, as Donald Davis and Barbara Lorenzkowski have argued, of male spaces where women were made to feel "as intruders whenever they ventured into the public 'male' sphere of travel and commerce."[135] Traditionally, to accommodate female travelers, smoking was only permitted in the last four rows on each car.[136] Yet this could barely have been seen as adequate to protect a woman's respectability. There was no wall between the smoking and non-smoking section, so this symbolic border floated into the rest of the tramway, bringing discomfort and leaving the strong suggestion on a woman's clothing that she too had been smoking.

The symbolic border between men and women on Montreal transit was altered on the first of December, 1901, when the Montreal Street Railway Company (MSR) banned

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[134]Ernest Choquette, "Loulou," *Carabinades*, p.154.

[135] Davis and Lorenzkowski, "A Platform for Gender Tensions," p.432.

[136]*Le Monde Illustré,* 1890-91, p.199.

smoking on the back four seats during the winter.[137] At the end of the nineteenth century women were increasingly entering into this public sphere, whether it was for shopping or a job in Montreal's downtown offices. For women to do this respectably, men would have to stop smoking on the tramways. Beginning in 1897 the Western Union of the Montreal WCTU began a campaign to end smoking on the MSR, seeing results in 1901.[138] The Dominion WCTU, probably inspired by what it saw as a victory in Montreal, passed a resolution in late 1902 that pitted women as non-smokers with rights to clean air and the space on the tramway against the offending male smokers. The resolution read:

> Whereas, women as well as men pay full fare on railway trains and street cars, boats, etc. and have a right to immunity from the poisonous atmosphere of tobacco, Resolved, That we demand consideration from all corporations who provide means of indulging this habit at the expense of discomfort to others.[139]

There were other reasons outside of the WCTU campaign for the change in MSR policy. Abolishing the smoking section was also a solution to crowding problems on the tramway. The smoking section blocked the entrance of "pay-as-you-enter" cars and passengers found it difficult to get on the tram. Many riders mistakenly allowed tram cars to pass them by as they had the impression that the tram was already full. In reality it was half

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[137]Miss Dougall, "Report of the Committee on Resolutions," *12th Annual Report of the Dominion WCTU*, (1901), p.58, FA 885 MU 8447.2, OA.

[138]Western Montreal WCTU 1894-1950 minute book, 11 November 1897, FA 885 MU 8450.4, OA.

[139]Dominion WCTU Reports, 1 November 1902. "Executive Committee" at the Toronto District Headquarters, p.63. Jessie B. Woodbury, "Report of Committee of Resolutions." FA 885 MU8398.10, OA.

empty and only crowded in the smoking section.[140] In fact, in 1913 when Montreal City Council banned smoking completely on Montreal tramways, it was in the hope of dealing with the overcrowding problem, not the rights of all riders to have fresh air, that was at issue.[141]

Despite the broader problems of crowding, opposition to the MSR policy and the later City Council ban on smoking reduced the question to one of female intrusions into the male sphere. Indeed, particularly masculine opposition to these new rules came quickly. In 1903, the tobacconists organized the "Association des commerçants de Vins et de liqueurs Licenciés de la Cité de Montreal" and sent several delegations to the MSR, but with little success.[142] They saw the issue as a female invasion of the male world. While arguing for new smoking cars to be constructed by the Tramway Company, their official organ *Liqueurs et Tabacs* took advantage of the fact that the word "Compagnie" in French is feminine, writing "tout en donnant satisfaction à la grande masse de la population masculine, elle améliorerait ses recettes."[143] Thus they created the double meaning that the company should improve its policy and that women keep within the private sphere and try to improve their cooking. Journalists also made the issue a question of women trespassing in the male sphere. In November of 1907, probably just after summer smoking period

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[140]"Dans les Tramways," *La Presse*, 20 November 1907, p.4.

[141]Minutes, Montreal City Council, 9 June 1913, p.107; 17 June 1913, p.414; 23 June 1913, p.610; AVM, 83-1-10-3.

[142]"Montreal Chat," *CCTJ*, November 1903, p.23.

[143]"Fumera-t-on dans les chars urbains?" *Liqueurs et Tabacs*, November 1903, p.38.

ended, a *La Presse* editorial lashed out at the anti-smoking laws as discriminating against men. The editorialist wrote that if men are supposed to stop smoking to end the crowding on tramways, women should not be allowed on with hats and that: "sont retenus par des épingles de plus d'un pied de long et celles qui portent leur parapluie comme un bébé en tenant les baleines" at the eye level of other passengers.[144] Indeed, this was gender conflict, men wielding burning tobacco and women with hair pins and umbrellas, and the spoils were the gendering of space on Montreal public transit.

Men argued that the time they spent on the tramways was crucial smoking time since work discipline and etiquette already limited the amount of time a man could devote to smoking. When smoking was banned in 1913 a renewed campaign with a new tobacconist organization, the Montreal Protective Retailers Association of Tobacco and Cigars, stepped up their efforts to allow smoking on public transit.[145] They circulated a petition, in French and English, opposing prohibition of smoking and asking either for special smoking cars like in Europe or "to take other practical means to permit passengers to smoke on the present cars."[146] The organization approached the Montreal Trades and Labor Council to support their initiative. When the Trades and Labor Council met on the question, members showed particular enthusiasm for the petition. Numerous speakers pointed out that "the working man" suffered most from the law, "as during the day time

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[144]"Dans les Tramway," *La Presse*, 20 November 1907, p.4.

[145]"News and Views of Labor World, Home and Abroad," Montreal *Star*, 21 February 1914, p.9.

[146]"Montreal Notes," *CCTJ*, March 1914, p.21.

the only opportunity he often had for a smoke was when travelling [sic] back and forward from his work." This was clearly playing on working men's limited leisure time as well as on rules of etiquette stipulating that respectable men did not smoke at home. Delegate Fontaine called for smoking trailers to be put on tramways "to allow us to have our smoke without it interfering with non-smokers." The motion passed with unanimity and the Labor Council sent three delegates of support instead of the one which had been requested by the Retailers Association.[147]

In the end the class alliance was impressive. The campaign concluded with a petition of 45,000 signatures presented to City Council by Alderman and soon to be Mayor Médéric Martin. The Montreal *Gazette* described the presentation of the massive petition as follows: "From behind a pile of petitions which littered his desk Ald. Martin arose in the City Council yesterday and made a plea for the smoking citizens of Montreal.... [He] called a messenger and had the pile of petitions conveyed to the more spacious desk of the city clerk." The clerk was then asked to send the requests to the MSR and to ask whether it would be possible to act on their proposals.[148] Yet after it was submitted, nothing seems to have changed and smoking was still prohibited by the MSR.

Despite the controversy that enveloped the MSR rules regarding smoking, there is

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[147]These delegates were J. Wall, Gustave Francq, and N. Fontaine. See "Labor Council to Support Petition for Car Smoking," Montreal *Herald*, 6 March 1914, p.3.

[148]"Smokers Want to Puff on Street Car," Montreal *Gazette*, 10 March 1914, p.5; Montreal City Council Minutes, 9 March 1914, AVM 2-2-29.

little evidence that they were followed.[149] Indeed, etiquette remained the key to separating smokers and non-smokers, allowing for the creation a male homosocial space on tramways. Smoking on tramways also offered a prime mixed-class opportunity for men to perform respectability and to defer to women in public. At the same time smoking was supposedly banned on Montreal Tramways, Louis D'Ornano, editor of the Montreal middle class weekly *L'Album Universel,* recounted what he called "a moral tale" describing the smoking section on the tramway. D'Ornano began his moral tale by describing the smoking section on the tramway as an inclusively male public sphere that he was not entirely comfortable with because of its mixed-class nature:

> Dans une promiscuité toute démocratique, la cigarette du jeune homme mêlait sa fumée à celle du havane d'un financier ou d'un bourgeois tandis que sur le tout planait le nuage épais et âcre de la pipe des travailleurs.

This male space rocked to a stop to pick up a group of women who tried to get to the traditional non-smoking section of the tramway. They pushed through the smoking section, but as one of them tried to pay, a large worker smoking an enormous pipe twisted it in his mouth and the burning tobacco fell on her fur coat. According to D'Ornano, this was "une lady" who was "assez maitresse de ses nerfs pour éviter une scène," thus conforming to norms of conduct for women in public. The worker on the other hand failed miserably at upholding these codes of public respectability. He could not control his appetites: " [Il] tirait des formidables bouffées de sa pipe" and it was these "appétits

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[149]Indeed, while tobacco was banned on the tramway, the Montreal Fire Department was still regularly called on to extinguish fires on trams caused by smoking, *Fire Register: Montreal Fire Department*, 1912 entries 253, 1157, 1832, 2103.

désordonnés" that forced him into his precarious existence, not his poor pay.[150] What is more, though the worker attempted to apologize to the woman, d'Ornano felt it was done with a bitterness and revolting cynicism that ultimately was rooted in his social class: "Cet homme: gauche, brutal, rustre au possible, le symbol de l'humanité pauvre, à la fois trop fière ou trop acerbe, pour se plier devant une créature fortunée." Indeed the worker failed to defer to the woman and the gesture showed that there would never be universal harmony while the poor had such contempt for their "social superiors." While the woman, according to d'Ornano, demonstrated her "breeding" through her impressive poise, not lashing out at the worker, he suggested that rather than use public transportation a woman wearing such an expensive coat might think of avoiding damaging it by using a private car. Clearly the message was that the public sphere was a dangerous place for respectable women and these sorts of problems would not exist if women did not try to enter male spaces. Finally, d'Ornano called on the "omnipotent" Montreal Tramway Company to resolve the situation by running smoking cars. The proposal would preserve male space to smoke, a woman's dignity, and would rely less on working class men to follow etiquette.

The liberal prescriptions that linked masculinity with smoking were at their height in the years before the First World War. They discouraged many women from smoking. Rural women in Quebec who had smoked in the first half of the nineteenth century were considered uncivilized like female smokers of other "uncivilized races" and women in Montreal who smoked risked being viewed as prostitutes. Even middle class women thought twice about admitting to smoking. To smoke for males was a ritual of transition

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[150]"Chonique: En Tramway," *L'Album Universel*, 2 February 1907, p.1353.

from boyhood to manhood and could bridge the gap between men of diverse cultural backgrounds. Smoking made visible the boundary of the male public sphere and played a role in defining respectable male behaviour. Respectable smoking invoked a leisurely and thoughtful state of mind in a homosocial environment. When women were present in this public sphere, to refrain from smoking became a mark of male gentility and self-control. Furthermore, both men and women could abstain from smoking and make public gestures of gentility, making respectability affordable for many working class people. Yet there were significant class barriers to following these structures of etiquette if a man decided to smoke. The amount of time he worked in an industrial workplace limited the time he could spend smoking in any kind of high-minded all-male atmosphere. What is more, the costly spatial demands of these prescriptive systems made it close to impossible for all but the most bourgeois to adhere to the ideals of smoking. Indeed, etiquette demonstrates one important avenue by which the ritual of smoking was used as a language, tied up in gender, class and ethnic relations and liberal notions of the individual in late nineteenth century Montreal.

Chapter Two

Bourgeois Connoisseurship and the Cigar

Etiquette prescribed many of the rules around smoking – who was to smoke; where they were to smoke; when, how much and in what spirit. But outside of taking a dim view of cigarettes, these rules said little about what was to be smoked, and among smokers this was an important question. For many, what a man smoked was an expression of how he saw himself and how others interpreted his identity. Tobacco selection in turn of the twentieth-century Montreal was extensive – diverse sizes and shapes of cigars originating around the world from Indonesia to Trois-Rivières; pipe tobacco in loose shag, leaf, or plugs using flavoured, non-flavoured or home grown tobacco; hand mixed or machine manufactured; cigarettes hand or machine rolled using tobacco from Turkey to South Carolina. Though all men were said to have the "right" to smoke, all tobaccos did not pass on the same level of social prestige to their smokers. Tobacco products were organized hierarchically and understanding this hierarchy was the foundation of connoisseurship. Connoisseurs were to be rational men – the antithesis of female shoppers who were portrayed as irrational and even hysterical.[1] Among men, hierarchies of taste distanced gentlemen from the poor and nouveaux riches and helped

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[1]On the creation of the "Straw Woman" consumer, see Jill Greenfield, Sean O'Donnell and Chris Reid, "Gender, Consumer Culture and the Middle-Class Male, 1918-1939," pp.183-197, and Christopher P. Hosgood, "Mrs.Pooter's Purchase: Lower-Middle-Class Consumerism and the Sales, 1870-1914," pp.146-163, both in Alan Kidd and David Nichols, eds. *Gender, Civic Culture and Consumerism: Middle-Class Identity in Britain, 1800-1940* (Manchester: Manchester University Press, 1999). For a Canadian example, see Cynthia Wright, "Feminine Trifles of Vast Importance."

differentiate the "civilized" from the "uncivilized" in the construction of racial ideologies.[2]

The cultural categories of tobacco connoisseurship were most clearly exemplified in the cigar. The cigar was a symbol of wealth and power and its smokers were criticized for their extravagance. The most expensive and most prized cigar was the Cuban. The St. James Club, one of Montreal's elite men's clubs, imported them specially to satisfy their members and the *CCTJ* declared: "The Havana cigar is admittedly the king of cigardom."[3] Cuban cigars were the most popular imported cigars, but their sales barely kept up with increases in Canada's population. Sales of their cheaper Canadian-made cousins, however, increased until the War, though they never reached the same level of popularity again.[4] According to connoisseurs, price was not a true mark of quality. Connoisseurs,

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[2]On connoisseurship as a male identity, see Leora Auslander, "The Gendering of Consumer Practices in Nineteenth-Century France," in de Grazia *et al.*, eds. *The Sex of Things*, pp.79-112; Frank Mort, *Cultures of Consumption: Masculinities and Social Space in Late Twentieth Century Britain* (London: Routledge, 1996); Hilton, *Smoking in British Popular Culture*, pp.17-59.

[3]*CCTJ*, February 1898, p.37. Quotation from *CCTJ*, March 1898, p.2.

[4]These statistics were kept in pounds and only occasionally in numbers of cigars. They do, however, reflect a more accurate amount of tobacco smoked in cigar form since cigars come in numerous sizes. In 1901 56,630 lbs. of cigars were imported from Cuba. This number is unusually low because of the fallout from the Spanish-American War. In 1897, for example, 69,317 lbs. were entered for home consumption. By 1911 this number had risen to 87,559 lbs. Consumption of Canadian-made cigars rose from 101,142,481 in 1891 (*CCTJ*, September 1900, p.379) to 141,096,889 in 1901 and 227,585,692 in 1911. This works out to 21 cigars per person in 1891, 26 per person in 1901 and 32 in 1911. These cigar numbers are taken from those published in the *CCTJ*, June 1912, p.48. The height of Canadian-made cigar consumption in Canada was 1913 with 294,772,933 cigars. There was a slight decline to 288,219,892 in 1914 when the country was plunged into depression just before the war. Throughout the 1920s Canadian-made cigar consumption hovered between a low of 168,097,587 in 1925 and a high of 270,049,761 in 1920. For statistics on cigar consumption in Canada between 1901 and 1931, see *Canada Yearbook*, (Ottawa, 1932), p.721.

manufacturers of Canadian-make cigars, their rollers and the state all had a clear idea of the values upon which this hierarchy was based. They pointed to the skilled labour of the cigar maker as well as the *terroir* of the tobacco as the cultural categories that accounted for the value of a cigar. These experts went further and attempted to explain what it was about skilled labour and *terroir* that made a superior cigar. A cigar's quality was derived from cultural visions of race and gender as well as the learned skill of the worker or the natural qualities of a country's soil and climate. In late nineteenth and early twentieth century Montreal these cultural categories were unstable because of the precariousness of Imperial rule in Cuba as well as the industrial transition and conflict around cigar making. An exploration of the values upon which cigars were judged in Montreal offers a useful case study of how hierarchy was built into the liberal ritual of smoking. It also proposes a genealogy of the cultural categories on which this hierarchy of taste was based, and their subsequent precariousness immediately before the First World War.

I. Class, Gender and Connoisseurship

The cigar in turn-of the-century Montreal was a symbol of masculine wealth and power. Cigar companies fostered this symbolism by naming their products after military heros, political leaders, and American industrialists. Among Montreal cigar manufacturer J.M. Fortier's brands, for example, were mythic heros like Richard I, Alexander III, and major American industrialists like "Vanderbilt."[5] One of the most popular cigars at the turn of the century was J. Hirsch, Sons and Co.'s the "Stonewall Jackson" and S. Davis

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[5]*Cigar Makers' Official Journal (CMOJ)*, March 1899, p.4.

and Sons frequently advertised their "Nobleman" cigar. Cartoons frequently criticized the misuse of wealth and power by portraying the rich as a fat pig who smoked cigars. In 1910, *La Presse* offered children a model from which they could learn how to draw the wealthy pig: the top hat, tie, and formal suit jacket along with the cigar completing the image of class (figure 1).[6] Similarly, later that year in *La Presse*'s humour section, the cigar was prominent in the iconography of the upper class man in "Contraste" to the poor shoeless man (figure 2, see p.79).[7] Finally, criticisms of wealth and power turned to American foreign policy in 1906 when *L'Album Universel* reprinted a cartoon of a fat, cigar-smoking American man crushing Cuba (figure 3, see p.79).[8] Pre-World War One literature in Montreal also linked the cigar to the abuse of power. For example, in Jules-Paul Tardivel's 1895 novel *Pour la Patrie* the villain, Montarval, sits and smokes a cigar, staring out the window as his father,

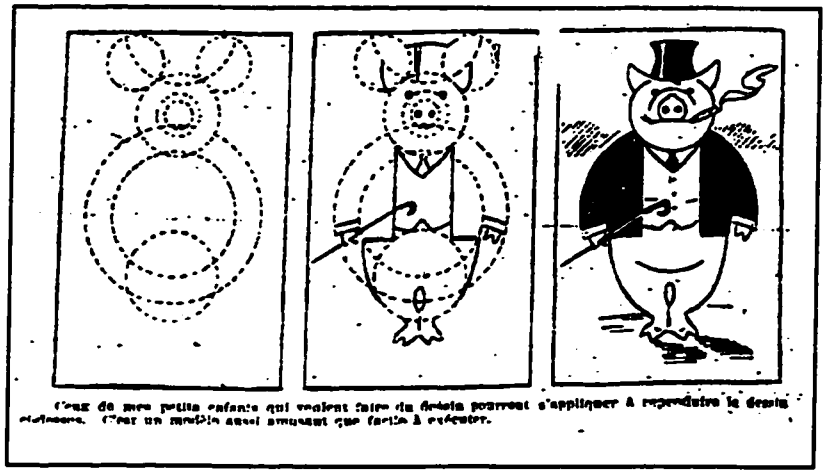

Figure 1: Drawing wealth, *La Presse* (1910)

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[6]"Pour Les Dessinateurs," *La Presse*, 25 June 1910, p.2.

[7]"Contraste," *La Presse*, 25 October 1910, p.2.

[8]*L'Album Universel*, 24 November 1906, p.1027.

Figure 2: Cigar as wealth, *La Presse* (1910)

Figure 3: Cigar as power, *l'Album Universel*, (1906)

close to death, tries and fails to reconcile with him.[9] Tardivel used the cigar to emphasize Montarval's selfishness – a man who uses this crucial time for his leisure rather than to care for his father. In Hector Berthelot's *Les Mystères de Montréal: Roman de Moeurs*, the character Cléophas wakes up from a night of drunkenness to the smell of "un cigare à l'arôme des plus délicats." The smoker is the Count of Bouctouche, who wants to hire Cléophas to help him swindle an inheritance to which he no longer has any claim.[10] The use of the cigar as a symbol of misused wealth was carried beyond literature to the pulpit. Rev. Herbert Symonds, the Vicar of Christ Church Cathedral in Montreal, criticized moral reformers who took aim at moving pictures. Noting that 90 per cent of movie-goers were working people, he compared them to those of his own class who organize bridge and poker games "with cigars and liquid refreshments." He continued ironically "we may incidentally deplore the frivolity of the masses who instead of saving something from their earnings spend it all at the movies."[11]

These images of power and wealth highlight the expense of a cigar and its association with the elite. Yet cigars ranged in price from those that sold at two for five

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[9]Jules-Paul Tardivel, *Pour la Patrie* (1895) translated (Toronto: University of Toronto Press, 1975), p.21.

[10]Hector Berthelot, *Les Mystères de Montréal: Roman de Moeurs* (Montreal: Imprimerie A.P. Pigeon, 1901), p.43. This story was originally published from 20 December 1879 to 31 July 1880 in *Le Vrai Canard*, reprinted in *Le Canard* 23 May 1896 to 18 February 1897 and then finally published as a book in 1901. For more information on Berthelot and the publishing history of *Les Mystères de Montréal*, see its entry in *Dictionnaire des oeuvres Littéraires du Québec: Tome Premier, des Origine à 1900*, sous la direction de Maurice Lemire, (Montreal: Fides, 1978), p.510-512.

[11]Herbert Symonds, *A Memoir* (Montreal: Renouf Publishing Co., 1921), p.182.

cents to more expensive ones that could cost a dollar each. Clearly most smokers could afford the cheapest cigars. What drew the association between cigars and elite smokers closer together was the ideology of connoisseurship. Like with wine and champagne, connoisseurs learned how to evaluate a cigar through an expensive process of testing and information gathering that amounted to a type of cultural class formation. Connoisseurship had much in common with the amateur ideology that drove sport in nineteenth century Montreal. Today, it is no mistake that in French the word *connaisseur* is used almost interchangeably with the word *amateur*. Historian Alan Metcalfe has written that amateurism dictated that sport was more than a game – it "was a vehicle for demonstrating that a person was a gentleman."[12] As such, sports were to be played according to the spirit of their rules rather than written rules. The spirit of the rules was never codified, giving "the system an exclusiveness that practically guaranteed that outsiders would be unable to gain access."[13] Like the disdain held for the codified rules of sport, price tags were not judged to be "true" arbiters of a cigar's quality. Indeed, some in the cigar industry maintained that in the best cigar stores, prices should not be put on boxes of cigars because true connoisseurs would know a good cigar.[14]

Cigar companies attempted to cast their customers as "men of taste" who would

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[12]Alan Metcalfe, *Canada Learns to Play*, p.120.

[13]*Ibid.*, p.120.

[14]*CCTJ* April 1901, p.183. This was part of a larger debate about price tickets that occurred in the grocery business as well. See Keith Walden, "Speaking Modern: Language, Culture, and Hegemony in Grocery Window Displays, 1887-1920," *Canadian Historical Review* 70,3 (September 1989), p.285-310.

recognize quality. As well as using the names of political, business and military leaders for their brands, cigar companies also marketed brands like "Verdi" and "Walter Scott" – images of quality in music and literature.[15] In contrast, failures in connoisseurship reflected poorly on the manhood of the smoker and their claim to being middle or upper class. A 1921 Montreal *Herald* article, for example, looked back at the rivalry between Sir William Van Horne and Sir Thomas Shaughnessy. Both were among the richest men in the country, but in the story, the question of who was socially superior was based on a measure of connoisseurship. According to the article, when Van Horne did not want to talk to reporters he would give them eight inch long black cigars made from Hudson Bay tobacco. The cigars were described as being "rank beyond description" and Van Horne attributed the project of growing the tobacco in the Hudson Bay area to Shaughnessy. When a connoisseur, the English author Sir Edwin Arnold, stayed with the Van Hornes, several reporters gathered to interview them.[16] Van Horne mischievously gave them all Hudson Bay cigars, and while they smoked "the apartment became uninhabitable by any one save a Siwash or an Esquiman," the journalist derogatorily equating strong odours to native peoples. Van Horne finally asked Arnold what he thought of the cigar and Arnold responded that it was "the rankest, reekingest, deadenest, most odiferous, and most generally outrageous cigar I ever encountered in all my travels." Van Horne countered that this was proof of Shaughnessy's poor taste: "I might have known it. Tom

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[15]*CMOJ*, March 1899, p.4.

[16]On Arnold, see Brooks Wright, *Interpreter of Buddhism to the West: Sir Edwin Arnold* (New York: Bookman Associates, 1957).

Shaughnessy likes 'em!"[17]

Connoisseurship was an acquired taste that shielded "men of culture" from the "nouveaux riches." A good example of this sort of condescension is found in William Henry Drummond's poem "How Bateese Came Home." The poem is a version of the parable "the Prodigal Son," that centres on the theme of French Canadians going to the United States to find their fortune. As part of Drummond's *Habitant Poems* it was enormously popular and frequently read publicly to great laugher.[18] The protagonist of the poem, Jean Bateese Trudeau, thinks himself too well-educated to stay in Quebec and emigrates to the United States to make his fortune. After a prosperous first summer and then a year of failure, Bateese is penniless and returns home hungry where he is, of course, met and welcomed by his father. In the winter between these two summers Trudeau returns to Quebec as John B. Waterhole, and the narrator, a French-Canadian man who speaks in broken English like many of the French Canadians in Drummond's *Habitant Poems*, is impressed by Waterhole's new sense of connoisseurship:

> Den we invite heem come wit' us, "hotel du Canadaw"
> W'ere he was treat mos' ev'ry tam, but can't tak' w'isky blanc,
> He say dat's leetle strong for man jus' come off Central Fall
> An' "tabac Canayen" bedamme! He won't smoke dat at all!
>
> But fancy drink lak "Collings John" de way he put it down
> Was long tam since I don't see dat - I t'ink he' goin' drown!-
> An' fine cigar cos' five cent each, an mak' on Trois-Rivières

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[17]"Shaughnessy Likes 'Em!" Montreal *Herald*, 27 October 1921, p.4.

[18]Arthur L. Phelps, "Introduction," in William Henry Drummond, *Habitant Poems* (Toronto: McClelland and Stewart limited, 1970), pp.7-16. See also, "Dr. Drummond in St. John," Montreal *Herald*, 11 April 1900, p.12.

L'enfant! He smoke beeg pile of dem - for monee he don't care!-[19]

The relationship between the "tabac canayen" and the five-cent cigar from Trois Rivières reveals much about Bateese and Drummond. Bateese fails as the connoisseur and speaks condescendingly to the narrator in saying that the "tabac canayen" is not worth smoking. Drummond in turn condescends to the habitant narrator, who is portrayed as a "typical" French Canadian, by allowing the narrator to be impressed by the five-cent cigars from Trois-Rivières. Indeed, a five-cent cigar from Trois-Rivières could not be a good cigar, according to the worldly standards of the bourgeois connoisseur.[20]

Connoisseurs of cigars believed that women could not join their ranks. The best example of the belief that cigar connoisseurship was an exclusively male ability was observable each Christmas in tobacco trade journals and newspapers. According to these reports, one of the few times that women shopped for cigars was just before Christmas when they bought presents for their husbands and brothers. Though women were stereotyped as consumers, the tobacconists did not believe that women could know how to buy a good cigar: "A woman who buys cigars for her husband is very much like a man who buys a hat or a dress for his wife."[21] The *CCTJ* abounded with jokes about the way in

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[19]William Henry Drummond, *The Habitant and other French Canadian Poems* (New York and London: G.P. Putnam's Sons, 1897), pp.24-33; Citation from pp. 28-29. For another example along this line see "Ottawa Jottings," *CCTJ*, September 1900, p.383. The journal discusses a beginner tobacconist who wanted 1000 clear Havana cigars but "doesn't know Havana from Rimouski," meaning this tobacconist could not recognize a good cigar.

[20]Arthur L. Phelps, "Introduction," in William Henry Drummond, *Habitant Poems* (Toronto: McClelland and Stewart limited, 1970), pp.7-16.

[21]*CCTJ*, January1913, p.13.

which women bought cigars. Underlying these jokes was a lack of respect for the way women consumed other goods. One such joke recounted a discussion between a husband and wife as to whether the "New Women" would smoke cigars. The husband maintains that if these women did smoke cigars, they would certainly die from smoking the cheapest bargain cigars, bought at $1.49 a box.[22] Similarly the *Journal* recounted a poem where a husband took desperate measures to deal with his Christmas cigars:

> He stood alone upon the bridge alone [sic], and the river flowed beneath;
> 'Now is my time,' he fiercely hissed, between his clenched teeth.
> A splash! The deed is done, and down there sinketh in the deep
> That Christmas box of 'nice' cigars, his wive had bought 'so cheap.'[23]

It was also believed that women bought cigars for the fancy cigar box, not for the quality of the cigar: "No man, unless he be very callow, will buy a highly ornamental box of cigars," wrote the *Journal*. Accordingly, the only reason the trade in these items continued was because women continued to buy them with an eye to a new jewelry box. Men apparently preferred a plain cedar box of "a brand with which he is acquainted."[24]

This ideology of connoisseurship stated that women could not possess the proper knowledge to buy quality tobacco, cigarettes, or cigars. Along the same logic, the *CCTJ* wrote that tobacconists were ill-advised to hire women to work in their stores because women could not possibly understand the cigar trade. The *Journal* maintained that "[women] have not, nor can they acquire, the knowledge necessary in advising the

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[22]*CCTJ*, February 1898, p.41, reprinted from *Tid Bits*.

[23]*CCTJ*, February 1899, p.69.

[24]"The Holiday Package and the Cigarette Insert," *CCTJ*, October 1912, p.11.

customer as to the merits of a cigar or brand of tobacco." This was not a problem for the connoisseur - "The man who knows exactly what he wants" - but for others who needed advice, a woman clerk would not do.[25] A subsequent article explained that the tobacconist at a hotel in Chicago had hired a female clerk and business went up. While she had a good knowledge of the trade, the real secret to the increased business was attributed to her "immaculate neatness" – a skill that women were seen to possess.[26]

II. Skilled Labour

According to cigar connoisseurs, women could not be connoisseurs because a significant portion of a cigar's value was derived from the male-dominated cigar rolling process. The most skilled cigar makers used few tools to roll a cigar from start to finish. They began by choosing, blending and shaping the filler tobacco into a "bunch" which was then rolled into a binder leaf. The last stage involved rolling the wrapper leaf around the bound filler. There can be little question of the link between skilled labour and the taste of a cigar. Patricia Cooper, the historian of American cigar makers, poses the relationship between skill and taste as a question of the cigar maker's ability to shape the filler:

> A wrong twist in the leaf or too many leaves crossed at one place created blockades for smoke and flavor which the experienced smoker could detect. All the taste had to reach the smoker and the "draw" had to be smooth and complete. A cigar packed too loosely allowed too much hot air to pass through too quickly, "like a chimney with too much draft." The smoke had to travel at just the right pace so that the smoker had only to puff and not pull on the cigar, but not too quickly so as to be harsh or

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[25] *CCTJ*, October 1900, p.429.

[26] *CCTJ*, November 1900, p.473.

burning.[27]

Increasingly, different grades of cigars were made with different work processes that were seen as undermining the craft. The cigars most vulnerable to de-skilling were the five-cent cigars. Five-cent cigars were nothing short of an institution and their price could not be raised so manufacturers introduced molds and groups of women or children to reduce costs. With mold and group work the speed of production could be increased and highly paid journeymen cigar makers replaced with poorly paid children and women.[28]

Using this kind of labour, however, meant that the theme of "skilled labour" was more difficult to use honestly in advertising. Companies who used skilled labour frequently mentioned it in their advertising, believing that the logic of a good cigar relied heavily on its labour. This was not just the rhetoric of pro-union cigar manufacturers. One of Montreal's most anti-union cigar manufacturers, S. Davis and Sons, frequently stressed the importance of their cigar makers in their advertisements (figure 4, see p.88).[29] Cigar manufacturers elsewhere went to more extreme measures to advertise the skill of

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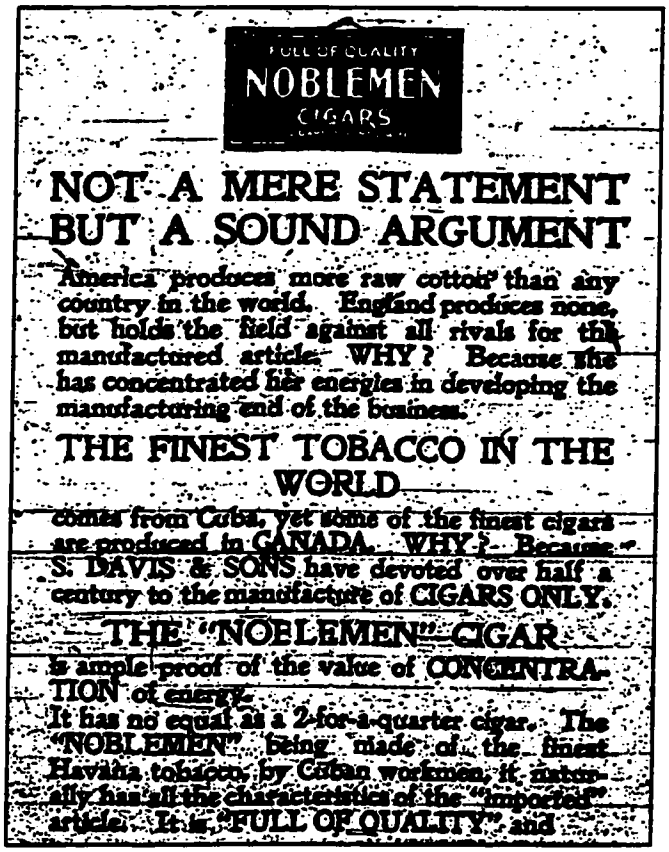
[27]Patricia Cooper, *Once a Cigar Maker: Men, Women, and Work Culture in American Cigar Factories* (Chicago: University of Illinois Press, 1987), p.53.

[28]*Ibid.*, chapter 2.

[29]Until the First World War, there were more strikes at S. Davis and Sons than at any other cigar factory in Montreal. I have tracked these, for the nineteenth century through Hamelin *et al. Répertoire des grèves dans la province de Québec au XIXe siècle.* (Montreal: Presses de l'École des hautes études commerciales,1970) and then for the twentieth century through the *Labour Gazette*. Some of them were quite bitter, and usually focused on the issue of pay reductions. More in-depth accounts can be found in the Industrial Disputes Files, RG 27 PAC. For a particularly hostile example, see Vol.303 T-2691 Strike No.119. Lasting from December 1913 to 15 August 1914, Davis emerged victorious and his correspondence in the file takes a strikingly patronizing tone towards his workers.

their cigar makers. One cigar manufacturer in Ottawa, with the consent of the Cigar Makers' International Union (CMIU), put cigar makers on display in their front window as they worked. Though the spectacle of these "human advertisements" was opposed by the two Montreal locals of the CMIU, they were overruled by the International head office. In fact, the Ottawa spectacle was not an isolated incident. Brenner Brothers, a cigar firm in London, Ontario, put Cuban cigar makers on display at the Western Fair and Keith Walden reports that Cuban cigar makers were put on display at the 1891 Toronto Industrial Exhibition. The practice underlines the cultural importance that skilled labour had in selling cigars.[30]


FULL OF QUALITY
NOBLEMEN
CIGARS

NOT A MERE STATEMENT
BUT A SOUND ARGUMENT

America produces more raw cotton than any country in the world. England produces none, but holds the field against all rivals for the manufactured article. WHY? Because she has concentrated her energies in developing the manufacturing end of the business.

THE FINEST TOBACCO IN THE WORLD

comes from Cuba, yet some of the finest cigars are produced in CANADA. WHY? Because S. DAVIS & SONS have devoted over half a century to the manufacture of CIGARS ONLY.

THE "NOBLEMEN" CIGAR

is ample proof of the value of CONCENTRATION of energy.

It has no equal as a 2-for-a-quarter cigar. The "NOBLEMEN" being made of the finest Havana tobacco, by Cuban workmen, it naturally has all the characteristics of the "imported" article. It is "FULL OF QUALITY" and

Figure 4: Advertising workmanship (*Gazette* 1910)

Cigar manufacturers were undermining the process by which these skills were learned, making it difficult to guarantee the quality of the cigar. The skill to make a good

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[30]"Proceedings of the 20th Session," *CMOJ*, September 1893; The company may have been Brown Bros. which is reported to have put cigar makers in their windows as "advertisements." See *CCTJ*, March 1898, p.10; "London Correspondence," *CCTJ*, p.441. Similarly, Keith Walden notes that, see *Becoming Modern in Toronto*, p.164.

cigar was supposed to be acquired during a three-year apprenticeship.[31] Apprenticeship agreements in the eighteenth and early nineteenth centuries set out a set of responsibilities between master and servant. In exchange for the apprentice's labour, he received little or no pay, but was to be fed, sheltered, clothed and taught a craft.[32] By the 1880s, as Bettina Bradbury has written, in the move from artisanal shop to industrial factory, this apprenticeship system had already broken down and many cigar makers who had completed their apprenticeship were not able to complete a full cigar. [33] Six months after finishing his apprenticeship, journeyman cigar maker Edmond Gauthier testified at the Royal Commission on the Relations of Labour and Capital that he could only roll a cigar by mold, not by hand, and then admitted that he did not know his trade.[34] Because the industry's reputation relied so heavily on skill, to the extent that the value of the product was partially dependant on it, this breakdown in the apprenticeship system was a crisis. One manufacturer, for example, writing anonymously in the *CCTJ* admitted that cigar manufacturers used the apprenticeship system to cut labour costs rather than to train competent cigar makers. In his view "a cigarmaker never learns the theory of cigar

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[31]For descriptions of the poor treatment of apprentices, see among others, the testimonies of Théophile Charron, pp.24-26; Achille Dabenais, pp.26-29, RCRLC, *Quebec Evidence* .

[32]Testimony of Alphonse Lafrance, RCRLC, *Quebec Evidence*, p.31.

[33]While many apprentices in other trades may not have been paid, cigar making apprentices were. See Testimony of Edmond Gauthier, RCRLC, *Quebec Evidence*, p.29. For apprenticeship in much of the North American industry, see Cooper, pp.48-49. Bradbury summarizes much of the literature on apprenticeship in her *Working Families.*

[34]Testimony of Edmond Gauthier, RCLRC, *Quebec Evidence*, p.29.

building...[and] we are turning out goods, that, if accepted by the public, cannot be altogether satisfactory to ourselves, nor representative of cigar perfection." His solution was to open cigar making schools.[35]

Another solution allowing the public to continue to have faith in the cultural value of skilled labour, this one presented by unionized cigar makers, was to regulate the number of apprentices in each factory so the system would not be abused. The CMIU attempted to do this through their "Blue Label." A cigar manufacturing company could use the union label if they agreed to a bill of prices per thousand cigars, hired only union cigar makers and packers in the factory and the use of apprentices limited. In 1900, for example, the Montreal unions attempted to regulate apprentices in four unionized cigar factories by going on strike to demand that only five apprentices be used in any Blue Label factory.[36] In exchange for accepting union demands, the union allowed the cigar manufacturer to put the Blue Label on his cigars, vouching both for their quality, and the conditions under which they were made.

The notions of skill represented in the Blue Label also reflected the racial and gender prejudice in the trade. Though cigar making was seemingly something that could be learned through apprenticeship, unionized cigar makers maintained that not everyone

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[35]"Apprenticeship Schools," *CCTJ*, December 1899, p.495.

[36]On the strike, which the CMIU won and which succeeded in forcing the resignation of the president of the Dominion Cigar Manufacturers Association, see "Montreal Correspondence," *CCTJ*, April 1900, p.147 as well as the listings in Hamelin. For the changes in apprentices allowed in unionized factories, see *La Presse*, 14 April 1900, p.25 and the testimony of Patrick J. Ryan, RCRLC, *Quebec Evidence*, p.36 and Cooper, *Once a Cigar Maker*, p.48-49.

could be a cigar maker. Male cigar makers argued that the skill of making a good cigar could not be learned properly by women or certain non-white men. Cooper has shown that skill in the cigar making trade was constructed on the basis of a "white male working class culture." And while gender exclusion was reflected in the use of the CMIU label as women were not made to feel welcome in the union and were actively discriminated against racism was even more overtly displayed. In fact, the label was adopted by the CMIU in 1880 and the fine print of a 1890 example printed in the Montreal union journal (figure 5), *The Echo*, read:

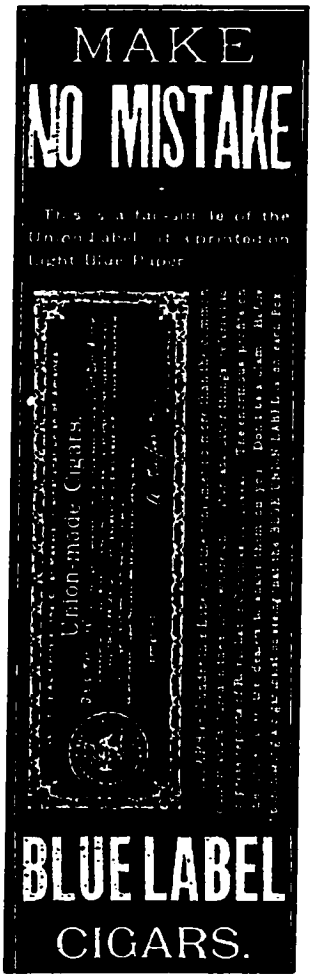

Figure 5

> This certifies that the Cigars contained in this box have made by a First-Class Workman, a member of the Cigar Makers' International Union of America, an organization opposed to inferior rateshop, COOLY, PRISON, or FILTHY TENEMENT-HOUSE WORKMANSHIP. Therefore we recommend these Cigars to all smokers throughout the world.[37]

By 1910 the label had changed, getting rid of references to "Coolies" and all words in the above sentence after "organization," replacing them with "devoted to the advancement of the MORAL, MATERIAL and INTELLECTUAL WELFARE OF THE

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[37]*The Echo*, 1 September 1890.

CRAFT."[38] Still, some claimed the history of racial exclusion as part of the Label's "noble history." The "Chronique Ouvrière" in *La Patrie* gave a brief history of the union label, tracing its origins to Gilded Age San Francisco cigar makers. As background, the article pointed to the Burlingame Treaty concluded with China on 28 July 1868. The treaty allowed Chinese immigrants to enter into the United States and by 1878, 4,000 were employed in the San Francisco cigar trade. At the same time, only 500 white cigar makers were employed. According to the article, by 1881 the situation had deteriorated, as there were only 179 white cigar makers and 8,500 Chinese cigar makers and the effects "du travail de ces jaunes" were disastrous since they worked for between 30 and 60 cents per day. Finally in 1874 a local of the cigar makers' union adopted a white label "pour distinguer les produits des ouvriers blancs des produits des ouvriers jaunes." In 1876 the movement became more widespread, and the Pacific Coast Cigar Makers Association was formed. Fifty cigar manufacturers who employed only white cigar makers joined in the association's label campaign. The use of the label then became general among cigar makers and other unionists who competed with "des jaunes" and non-unionists of any colour.[39] Henri Bourdon, the author of the article, could have distanced the earlier blatantly racist history of the label from that of the 1910 label (which was more ambiguous about race) but clearly he felt that the label's racism would not hurt, and may even help the Blue Label's promotion.

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[38]For an image of the new label see *La Patrie*, 16 May 1910, p.5 and Cooper, *Once a Cigar Maker*, p.106.

[39]Henri Bourdon, "Chronique Ouvrière," *La Patrie*, 15 June 1910, p.5.

Most of the label's promotion in Montreal called for class, rather than racial, solidarity. As an 1890 article in *The Echo* suggested: "All men having the interest of the working people at heart will ask for UNION MADE CIGARS."[40] The main promoters of union-made cigars were the Montreal locals of the CMIU and the Montreal Trades and Labor Federation (MTLF), where the CMIU was powerful. In the 1880s, the Montreal locals of the CMIU gained new life, though significant campaigns to promote the label did not begin until the 1890s.[41] The Montreal locals promoted Blue Label cigars in their newspapers, in meetings of their locals, through short animations, label exhibitions, during labour day parades, and through asking consumers to boycott other goods. In 1908 the secretary of the label committee of the MTLF, C.R. Salmon, spoke in support of union labels at early Montreal cinema halls like the Readoscope and the Duluthoscope. Almost like a precursor of a television commercial, while he spoke an animation of a union label, sometimes the cigar maker label and sometimes others, would appear on the screen. He would also speak during the intermissions.[42]

Another method by which union cigar makers promoted their label and the goods that wore it was through label exhibitions. The 1910 exhibition took place over two days, beginning on Saturday afternoon when factory work ended for many and continuing on

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[40]"BLUE LABEL CIGARS," *The Echo*, 7 June 1890.

[41]"L'Union Internationale des Cigariers," *Le Repos du Travailleur*, 1 September 1890, p.3.

[42]"Nouvelle Ouvrière," *La Presse*, 28 April 1908; on 3 May 1908 he showed two animations at the Vitoscope on Mount Royal Street, one for the laundry workers' label and the other for the cigar makers', see "Nouvelles Ouvrière," *La Presse*, 4 May 1908, p.11.

through Sunday afternoon. Despite the sweltering heat, the label exhibition attracted an estimated 1500 visitors.[43] Union labels were displayed prominently, companies who used union labour showed their goods, and speeches were given on the co-operative movement and the importance of the union movement. Union-made cigars were awarded as prizes during the exhibition's festivities. Cigar makers played an important part in the exhibition's organization. Benjamin Drolet, the president of the CMIU local 58, at that time the only local of the International in Montreal, was both a speaker and member of the organizing committee.[44]

In addition to promoting union label goods, the Label Committee also arranged for boycotts of non-union cigars. In January 1898, the MTLF came to an agreement with the Steve Brodie Theatrical Company that the Company would no longer advertise for what the MTLF called the "General Arthur scab cigar." The Company would receive 100 Blue Label Cigars as well as a union advertisement.[45] Later that same year a committee of the MTLF interviewed the lessee of St.Helen's Island Park, asking him to sell only union cigars.[46] Similarly Adolphe Gariepy, a Montrealer and the third Vice President of the CMIU, moved a successful motion at the MTLF in August 1907 to boycott the "Papa"

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[43]"Nouvelle Ouvrière," *La Presse*, 13 June 1910, p.13.

[44]*Ibid.* The *CMOJ* lists two Montreal locals of the CMIU, local 58 and local 226, until about 1901 when, during a break in the run of the *CMOJ* on microfilm, local 226 disappears.

[45]Montreal Trades and Labor Council, Minutebook, 20 Jan. 1898, p.15. Les Archives d'UQAM.

[46]*Ibid.*, 7 April 1898, p.28.

and "Romeo and Juliette" cigars because the factory at which they were being produced was on strike.[47] The Cigar Makers' Union also used national boycotts through the *CMOJ*. In 1899 after Montreal cigar manufacturer J.M. Fortier, "the largest scab manufacturer in Canada," had two labour journalists and five cigar makers arrested for libel, Local 58 called for a boycott of Fortier goods, listing all Fortier's brand names in the *CMOJ*.[48] How effective these tactics were is not clear. The *CMOJ* claimed that the public responded well to label promotions and boycotts. The *Journal* pointed to an example of one cigar manufacturer, Villeneuve and Co., that in 1899 returned to the union label after repudiating it three years earlier. Adolphe Gariepy claimed that the Company had come back to the union because its sales were down and the cigar manufacturer had been reduced from 125 hands to three. Immediately after union labour was engaged, according to Gariepy, the company hired 25 men, for him proof that the Label was popular.[49]

This promotion of the Blue Label as a symbol of the value of the cigar was done against a back drop of opposition in Parliament. In response to a number of cases of Blue Label counterfeiting in and around Montreal, as well as use of the Label without union permission, the CMIU attempted to register it as a trade mark.[50] In the past, the CMIU

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[47]*Ibid.*, 1 Aug. 1907, p.106.

[48]March 1899, *CMOJ*, p.4. The arrests and ensuing libel trial are described in Ian McKay, ed. *For a Working-Class Culture In Canada: A Selection of Colin McKay's Writings on Sociology and Political Economy, 1897-1939* (St. John's: Canadian Committee on Labour History, 1996), pp.xxii-xxvii, 43-47.

[49]*CMOJ*, June 1899, p.5.

[50]Cooper maintains that counterfeiting of the label was fairly common. *Once a Cigar Maker*, p.105. For a Toronto example, see, "The Label Sustained by the Courts,"

had successfully litigated to protect their claim on the label. There was growing concern within the union after defeats in US courts, and the CMIU concluded that their position would be stronger if the label was registered.[51] As it stood, however, the Trade Marks and Industrial Designs Act only allowed people or corporations to register trade marks and trade unions did not conform to either of these descriptions. The Dominion Trades and Labor Congress from 1897 to 1905 sought an amendment to the Trade Marks and Industrial Designs Act that would permit unions to register labels as trade marks and to prosecute counterfeiters.

Parliamentarians' anti-union beliefs doomed the amendment. In 1897, 1899 and 1901 it was defeated in the House while in 1898 and 1905 the amendment made it to the Senate where it was also defeated. The Bill had powerful enemies who were influential in Parliament. The Montreal secretary of the Canadian Manufacturers Association (CMA) expressed concerns about the bill to the Parliamentary Committee of the CMA and the CMA sent a delegation to lobby the Senate Committee on Banking and Commerce.[52] In fact, the CMA itself took credit for the Bill's failure at its 1901 convention and claimed to be instrumental in the Bill's demise in 1903.[53] In Parliament debate usually amounted to

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CMOJ, March 1891, p.10-11 and for cases in Montreal, see, "La Chronique Ouvrière," *La Patrie* 10 November 1907, p.3 and the same column, 16 May 1910, p.5.

[51]For a Canadian example, see "The Label Sustained by the Courts," *CMOJ*, March 1891, p.10-11. *Debates*, 22 April 1897, pp.1073-1074.

[52]Minutes of Parliamentary Committee 13 Apr. 1901. P.56. Vol.61, CMA Papers, MG 28 I230.

[53]Report of the CMA 30th Convention, *Industrial Canada*, CMA Papers, MG 28 I230 Vol.3, p.104; Probably Printed in *Industrial Canada* 19 Oct 1903 "Union Label,"

an attack on working peoples' right to organize as well as to accuse unions of corruption. Occasionally, however, they addressed the relationship between labour and value. Two positions on the bill became clear. First, on 10 May 1898, Senator James Dever, a Saint John merchant, rose in support of the bill. Declaring himself to be a "man of commerce," Dever argued that it was an issue of consumer democracy. If union goods were truly better than non-union goods, as he believed, they would be bought. If, on the other hand, the union products were inferior, the consumer would not buy them. Boycotts were of secondary importance because organized labour was not a large concern in Canada.[54]

The counter-argument was upheld consistently by the former Prime Minister and Leader of the Opposition in the Senate, Mackenzie Bowell. Bowell maintained that the bill was "vicious in principle" because the union label was not a voucher of quality. Instead, he argued, products are the outputs of companies not of workers. The label would then allow trade unions to put this mistaken principle into action through a boycott.[55] The *CCTJ* went further, maintaining that truly skilled cigar makers were consistently in demand in Canada and therefore would always be well paid. A union card, it continued, was not proof of "superior ability," but rather "too often the badge of arrogant incompetence" as those who did not have skill were the only cigar makers who needed a union. If the cigar maker wanted to improve his lot in the world, he would

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p.179.

[54]*Debates of the Senate*, 10 May 1898, p.795.

[55]*Debates of the Senate*, 29 April 1898, p.639.

practice at night and improve his skills.[56]

III. Cuba

In addition to skill, the second criteria for a good cigar, even one that was made in Canada, was that the tobacco had to be grown in Cuba. There were several theories as to why Cuban tobacco was superior. The 1910 *Encyclopaedia Britanica* explained that "The superiority of Cuban tobaccos in flavour and aroma, especially for cigar fillers, has long been recognized, but exactly to what conditions these qualities are due is not fully known." One theory argued that the "aroma and other good qualities" of Cuban tobacco were caused by bacteria and that it could actually be extracted from Cuban tobacco and put into poorer tobacco to increase its value.[57] The bacterial theory, however, was not universally accepted. The *CCTJ* mocked it, writing that it was authored by German scientists who would "try to change cabbage into Cuban."[58] Similarly, *Liqueurs et Tabacs* took the sarcastic position: "Donc, hâtons-nous, importons des microbes de Cuba et cultivons-les, acclimatons-les, ils nous havaniseront notre tabac, ces charmants microbes."[59]

A more accepted theory explaining the superior quality of Cuban tobacco posited that it was a question of "*terroir*." Like with grapes used to make champagne and French

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[56]"Unionism in the Cigar Trade," *CCTJ*, April 1901, pp.157-158.

[57]"Tobacco," *Encyclopaedia Britannica* Eleventh Edition, (New York: Encyclopaedia Britannica Company, 1910), pp.1036-1037.

[58]"Tobacco and Bacteria," *CCTJ*, August 1899, p.305.

[59]"Le Tabac et ses Délices," *Liqueurs et Tabacs*, April 1902, pp.32-34.

wine, it was the experience of the cultivator and their relationship to the soil and climate that determined the quality of the tobacco leaf.[60] The *Encyclopaedia* noted that very slight changes in climatic conditions could drastically affect the quality of the tobacco and that "ordinary meteorological records are of little use in determining the suitability or not of a region for a particular kind of leaf: this essential point must be determined by experience."[61] Articles in the *CCTJ* fell in line with this view that it was the long-established relationship between the knowledge of the farmer, the quality of the land and the climate that garnered Cuban tobacco its reputation. In 1901 the *Journal* told a story of two American men who bought a farm in the best tobacco growing region of Cuba, *Pinar del Rio.* They planted their tobacco fields, the plants came up, and in their opinion they were on their way to "a bumper" crop. Their neighbours, however, experienced in the ways of the soils and climate told them they were making mistakes, but the Americans did not heed the advice. When it came time to be examined for sale, their crumbling tobacco had no buyers. Next year, the *Journal* wrote, the Americans "will follow the advice of their neighbors." While Cuban growers "do not themselves know how to

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[60]Here I am following the work of historian Kolleen M. Guy who has argued that the concept of *terroir* was central to nineteenth century views of French geography, and equally important to the way champagne got its value. See her paper, "Rituals of Pleasure in the Land of Treasures: Wine Consumption and the Making of French Identity in the late Nineteenth Century,"presented at the "Food and Drink in Consumer Societies Conference" held at the Haglem Museum, Wilmington, Delaware, 12-13 November 1999. She draws on the work of nineteenth-century French Geographer Vidal de La Blanche. See Jean-Yves Guiomar, "Vidal de La Blanche's *Geography of France,*"in Pierre Nora, ed. *Realms of Memory: The Construction of the French Past* Vol.2, (New York: Columbia University Press, 1992), pp.187-209.

[61] *Encyclopaedia Britannica,* p.1036.

describe their ways of analyzing the exceptional qualities of soil, atmosphere and moisture which gave the Vuelta Abajo leaf its primacy," they did, however, know how to treat the leaf and get the best return.[62] The key was not only climate and soil conditions, but knowledge.

Tobacco experts believed that this intelligence could not be possessed by all people. Lines were drawn based on race. Canadian tobacco farmer and expert Louis V. Labelle's well-circulated 1898 pamphlet, "Traité de la culture et de l'Industrie du tabac," maintained that race was also crucial in understanding who could grow good tobacco. Using Mexico as an example, he explained that a country could have the natural advantages of climate and soil and still not produce good tobacco:

> Pendant de longues années, les tabacs Mexicains ont été considérés comme très inférieurs, parce que cette culture était laissée aux mains des *peones* ignorants, qui gaspillaient l'oeuvre de la nature ... par une uncurie et une ignorance incroyable.

According to Labelle's pamphlet, Mexican tobacco improved quickly once French colonists arrived and gave the crop the care it needed. This racial inability to grow tobacco "properly" existed in other places; Labelle listed Central America, most countries on the Gulf of Mexico, the Antilles, Brazil and Indonesia – in fact, in most countries, except Cuba and parts of the United States.[63]

The *Encyclopedia Britanica* was explicit about the racially specificity of who possessed "proper" knowledge about growing tobacco. For example, when it analyzed

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[62]"Havana News Items," *CCTJ*, August 1901, p.373.

[63]Louis V. Labelle, "Traité de la culture et de l'Industrie du tabac," (1898) p.13. CIHM 08362.

valuable tobacco grown on the Indonesian island of Sumatra that was used as a wrapper because of its colouring, the *Encyclopaedia* assessed its value partly on the quality of the island's soil and climate but "perhaps to an even greater degree to the care taken at every stage of its cultivation and preparation. The work is done by Chinese coolies under European - chiefly Dutch supervision."[64] Once again, intelligent management was equated with Europeans. The *Encyclopaedia* explained Cuban superiority by writing that even during the slave period, tobacco had been a "white man's" crop, "for it requires intelligent labour and intensive care."[65] In fact, tobacco in Cuba was far from being a "white man's crop." As Fernando Ortiz has shown, it had been a crop of native peoples before Europeans arrived and then of black slaves before Europeans began farming it.[66]

"Cuban" as a cultural category, even when built on these racial categories, was less stable than most commentators suggested. Numerous sources regularly claimed that Cuban tobacco had declined in quality. In January of 1898 the *CCTJ* reported that "the insurgents" in the Spanish-American War (1898) had destroyed 600,000 tobacco plants in the Pinar del Rio and Santa Clara districts and two months later the same journal reported that the quality of Cuban cigars had dropped.[67] Over ten years later the *Encyclopaedia Britanica* made similar claims that the decline had actually happened long before the

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[64]*Encyclopaedia Britannica,* p.1039.

[65]See "Cuba," *Encyclopaedia Britannica*, p.599.

[66]Fernando Ortiz, *Cuban Counterpoint: Tobacco and Sugar* (New York: Alfred A. Knopf, 1947).

[67]*CCTJ,* January 1898, p.4 and March 1898, p.3.Other reports of destruction of Cuban crops can be found in the *CCTJ,* March 1898, p.77; July 1898, p.181-182.

Spanish-American War. The *Encyclopaedia* asserted strongly that during the Ten Year War in Cuba (1868-1878) much of the best tobacco had been destroyed. The fields had then been replanted using Mexican and American seeds. And while there were considerable attempts to destroy this tobacco after the war, the *Encyclopaedia* claimed "'Cuban tobacco' does not mean to-day, as a commercial fact, what the words imply, for the original *Nicotiana Tabacum*, variety *havanensis*, can probably be found pure to-day only in the out-of-the-way corners of Pinar del Rio."[68]

While it was likely that the quality of Cuban tobacco fluctuated, the suggestion of tobacco being Cuban was more important than the quality of the tobacco. From 1897 to 1908 a Cuban cigar could be recognized by the Canadian revenue stamp that was on its box. All cigar and tobacco products were stamped by revenue officials, certifying that the excise had been paid and excise rates differed according to the origins of the product: a blue stamp for cigars from Cuba, Manilla or China; a black stamp for cigars that were made with Cuban tobacco in Canada; a pink stamp for cigars made from tobacco from other countries; and green for Canadian tobacco.[69] Many smokers and tobacco industry experts that influenced smokers' opinions interpreted these stamps as guarantors of quality rather than as simple excise categories. Early in 1905, for example, the *CCTJ* wrote an editorial noting that the government had actually developed the stamp system to protect

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[68]"Cuba," *Encyclopaedia Britannica*, p.599.

[69]"Uniform Revenue Stamps Meeting with Approval," Montreal *Herald*, 26 March 1908, p.11.

consumers from fraudulent tobacco.[70] Similarly, in 1908 when the different coloured stamps were abolished, the primary concern among Members of Parliament was that consumers would no longer have any idea of the quality of their tobacco. One tobacconist in Toronto even threatened to get the signatures of concerned smokers on a petition opposing the abolition of the stamp system.[71]

In the debates around the abolition of the coloured excise stamps, cigar manufacturers argued that "Cuban" was not a stable cultural category or sign of quality. One cigar manufacturer argued in the Montreal *Herald* that the label only defined the tax division through country of origin and there was no guarantee that all tobacco of that country was going to be of equal quality. The same manufacturer pointed out that there could be a difference of up to $14 between Cuban cigars all with the same stamp and weight, and cigar manufacturers themselves were known to go to Cuba to choose their own leaf. Others attacked cigars made in Canada with Cuban tobacco. J.M. Fortier claimed that the excise labels were not stamps of quality and the black stamp only meant that the finished cigars had been taxed at the rate of cigars made with Cuban leaf. These black stamp cigars may also have included Canadian tobacco. He noted that Inland Revenue had recorded 99,000 pounds of Canadian tobacco entering factories that only manufactured products with the black label.[72] What he was suggesting was that cigar

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[70]"Editorial," *CCTJ*, October 1905, pp.11-12.

[71]"Uniform Revenue Stamps Meeting with Approval," Montreal *Herald*, 26 March 1908, p.11.

[72]*Ibid.*

manufacturers were substituting cheaper tobacco for the more expensive Cuban and using the black label to maintain the appearance of being Cuban, even if it meant that they were taxed at a higher rate.

The appearance of being Cuban was a priority for cigar manufacturers. Not only was this achieved through manipulating the revenue stamps, but manufacturers also used advertising to attach their products to the myth of Cuban superiority. Through advertising they evoked a sense of "Cubanicity" that could be attached to any cigar to raise its value.[73] The *CCTJ* observed that what was important in a cigar was not its origins, but its perceived origins. The *Journal* remarked that it was "curious... that factories all over the world still stick to Spanish words and traditions in branding and labelling [sic] their output. If a Rhode Island cigar-maker wishes to say that his box is something really uncommonly fine he marks it 'Deliciosos.'" The advertising expert-come-semiotician continued by arguing that the Cubanicity of the cigar was also stressed through the colours of the ribbons that tied the cigars together - either red or yellow - the colours of the Spanish flag. Writing during the Spanish-American War, the author underscored the symbolic importance of the colours, quipping: "When Cuba has become Americanized, red, white and blue ribbons may make their appearance in cigar boxes, but that sign of the times has

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[73]My use of the word Cubanicity borrows from Roland Barthes' discussion of "Italianicity" in his "The Rhetoric of the Image" in *Image - Music - Text* (Fontana, 1977).

not yet been observed."[74] In Montreal L.O. Grothe's brand, the "Boston" is a good example (figure 6). The cigar ring (label around the cigar) was red, yellow and gold, the colours evoking the cigar's Cubanicity, even though it was made in Montreal and named after an American city.[75]

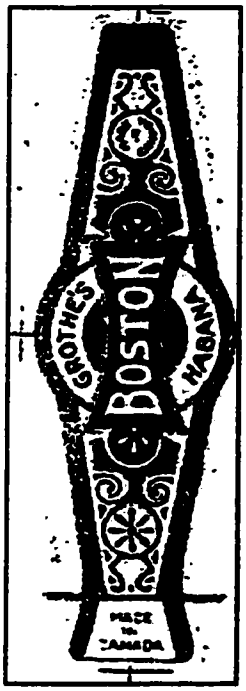

Figure 6

Few firms worked as hard to cater to the connoisseur's preference for Cubanicity as the Montreal firm Granda Hermanos y Cia.. The partnership between Frank Granda and Nathan Michaels opened on the first of July 1900 and continued until at least 1919.[76] The firm pioneered the production of the "authentic" Cuban cigar, made in Canada. [77] Michaels owned several cigar stores in Montreal and was from an important Montreal tobacco family. His father founded the Stonewall Jackson Cigar Factory, a longstanding concern in Montreal, and both his brothers owned cigar stores that dealt in expensive tobacco goods.[78] Along with of his brothers, Granda had learned the cigar making trade during their childhood in Cuba, working in New York and then Montreal.[79] And while both partners were experienced in the tobacco business, only the

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[74]"Brands, Labels, and Size Names," *CCTJ*, February, 1900, p.75.

[75]Rolph-Clark-Stone Ltd. Collection, McCord Museum Archives, .43.

[76]The firm disappears for *Lovell*'s business directory in 1919.

[77] *CCTJ*, August, 1901, p.372; and "In Memoriam," *CCTJ*, December 1906, p.17.

[78]"In Memoriam," *CCTJ*, December 1906, p.17.

[79]"Manufacture of Clear Havanas," *CCTJ*, March 1903, p.49.

Spanish proprietor's name was kept to capture the cachet of Cubanicity. In its first month Granda Hermanos y Cia. sold 23,100 cigars. Sales soon skyrocketed and in June 1901 it sold 172,575 cigars. The company then had to move to a larger factory as it had oversold by 350,000 cigars and needed new production space.[80]

Granda Hermanos y Cia. and the Canadian-made Cuban cigar industry that followed them resulted from three factors. First, it represented an attempt by manufacturers to profit from the higher tariffs on imported Cuban cigars imposed by the Laurier tariff of 1897. Second, the Spanish-American War resulted in the arrival in Montreal of a few Cuban and Spanish cigar makers fleeing hostility in the United States.[81] They immediately found work and when the War ended and Cuban tobacco leaf was once again widely available, the third factor came into play: the belief in Cuban tobacco's superiority. For Montreal cigar entrepreneurs to persuade the public that an authentic Cuban cigar could be made in Montreal, they appealed to the criteria for the best cigars set out by connoisseurs: the origins of the tobacco and skilled labour.

Granda Hermanos y Cia. trumpeted the authenticity of their Cuban tobacco by reporting regularly to the *CCTJ* about Frank Granda's buying trips to Cuba where he personally selected the leaf used in the firm's cigars.[82] By 1902 the Company added a stockholder in Cuba who acted as a resident buyer in order that "their leaf tobacco

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[80]*Ibid.*

[81]"Montreal Correspondence," *CCTJ*, May 1898, p.125.

[82]"Granda Hermanos Y Ca.'s [sic] New Factory," *CCTJ*, August 1901, p.373.

interests on that island consequently received the closest attention."[83] Granda Hermanos y Cia. also used advertising to pander to the male connoisseur's attempt to legitimize his consumption of tobacco through the knowledge of the process of making a cigar as well as by calming fears of industrial capitalist transformation processes. The company mounted displays in cigar store windows of "the leaf in all its phases, from the tobacco plant in bloom to the goods ready for rolling."[84] Indeed, according to Keith Walden, decoration of store windows was probably at its height in this era, a public education strategy to alleviate anxieties of changing work processes and products associated with industrial capitalism. The Granda Hermanos y Cia. sought to authenticate its cigars by displaying evidence that even though they were rolled in Montreal, the cigars were manufactured with the same Cuban tobacco and were of the same quality as those rolled in Cuba.[85]

While acquiring Cuban tobacco was more easily achieved after the Spanish-American War, skilled labour was far more complicated to find. Other Canadian companies like Granda Hermanos y Cia. insisted on having an all-Cuban or Spanish workforce to produce an authentic Cuban cigar. Here, the CMIU's claim that not all races were equally skilled returned to haunt them as it was believed that Cubans and Spaniards were superior to any other cigar makers in the world, including the French Canadian, Anglo-Saxons (largely English, Scottish and Irish) and Jewish membership of the Montreal

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[83]"Granda Hermanos y Ca. [sic]," *CCTJ*, February 1902, p.89.

[84]"Montreal Correspondence," *CCTJ*, 11 June 1901, p.273.

[85]Walden, "Speaking Modern."

locals of the CMIU.[86] For Montreal connoisseurs, the image of the Cuban cigar maker was of a tradesman with greater innate skill than a Canadian worker, yet it was never clear why the Cuban cigar maker was superior. The author of the column "Men and Things" in the Montreal *Herald* maintained that Cuban cigar makers "are far above the average workers in intelligence." He attributed this intelligence to the tradition of having a reader in the cigar making factory. Yet he maintained that the manipulation of the leaves was largely "mechanical."[87] A second connoisseur more intimately linked to the Canadian industry wrote in his exposé of the Cuban cigar industry that "[the] cigarmakers are the usual Bohemian lot" who seemed to come and go as they please in a liberty that was unheard of in Canadian cigar factories. This was an image of the artist, rather than of the factory worker, and while the artist may have had defaults, there was no question that "they are all expert cigar rollers." According to this informant, a further difference between the two workforces was that Cuban cigar makers were exclusively male, unlike in Canada, where women were employed in larger numbers.[88] Still, these explanations did not answer why Cubans were broadly regarded as the most skilled of cigar rollers and observers put forward no explanation. The fact that many believed that the best cigars came from Cuba probably lead to the racial stereotype that Cubans were biologically

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[86]The names of new members of both Montreal locals of the CMIU were published monthly in the *CMOJ*. I surveyed from 1881 to 1920. Though I understand it is not entirely an accurate indicator, I have roughly determined their ethnic origins through these names.

[87]"Men and Things," Montreal *Herald*, 16 May 1908, p.4.

[88]"Havana, Mecca of the Cigar World," *CCTJ*, April 1904, p.19.

superior cigar makers.

There is evidence that Cuban cigar makers in Montreal fancied themselves more skilled than Canadian cigar makers. The *CCTJ* reported in 1903 that during a strike at the Granda Hermanos y Cia., the Spanish and Cuban cigar makers attempted to have the Canadian union cigar makers fired "whose work they claim is inferior to their own."[89] Similarly, in 1910 *La Presse* reported that the Cuban workers at S. Davis and Sons went on strike because they refused to work with Canadians or Americans.[90] At issue was the method of rolling the cigar. The grade of cigars made by the Spanish and Cuban cigar makers in Montreal required special skills, a technique known as "Spanish Hand Work." It differed from "German Hand Work," the method of most CMIU cigar makers in Montreal, on three counts. First of all, it used "long filler" instead of the short filler in most ten cent cigars. The use of this longer filler allowed the skilled roller to forego the binder leaf. Finally, the Spanish method of packing the cigars differed from the German method in that the German method only split the cigars into three or four different colours while Spanish sorters could get up to 75 different shades out of one factory's cigars.[91]

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[89]"The Granda Hermanos Y Ca. Strike," *CCTJ*, March 1903, p.37.

[90]"Une Grève Sanglante," *La Presse*, 26 July 1910. See RG 27 Vol.298 T-2687 Strike No.3284 "Industrial Disputes File" PAC. There is a further issue that I do not have the sources to approach. The CMIU was in conflict with the Spanish Union of Cigarmakers in Tampa and some cigar makers brought this dispute to Montreal. See "Cigarmakers' Feud," Montreal *Star*, 11 May 1901.

[91]"The Granda Hermanos y Ca. [sic] Strike," *CCTJ*, March 1903. "La fin d'une Grève," *Liqueurs et Tabac*, March 1903, p.32. "Manufacture of Clear Havanas," *CCTJ*, March 1903, p.49. It is worth noting that in Cooper's brief mention of Spanish Hand Work, she defines it as only using Clear Havana leaf. See *Once a Cigar Maker*, p.50. I use the definition outlined in the *CCTJ* because there seems to be a difference in process

The typology of cigars was important for elite connoisseurs. Matthew Hilton has recently argued that the bourgeois connoisseur's choice of tobacco or cigar was a declaration of independence and individuality.[92] Similarly, sixty years earlier Cuban Historian Fernando Ortiz wrote that the particular size and shape of the cigar, the vitola, "is an outward manifestation of the *vitola* of the smoker."[93] The *CCTJ*, however, wrote little about the *vitola* and maintained that there was a woeful lack of interest in this terminology in the Montreal trade and among the city's smokers.[94] Granda Hermanos y Cia. sought to play on this sense of individualization and self-expression by offering an enormous number of "*vitola*." As well as images of tobacco in its raw state, Granda Hermanos y Cia. window displays exhibited all the cigars they made from the smallest, "the feminine" Senorita, to the largest, Grandas Selecto.[95]

Industry observers noted that Granda Hermanos y Cia.'s advertising was extensive in comparison with other cigar advertising and was the largest campaign of its time.[96] Not surprisingly it was based on being "equal to anything made on the Island of Cuba at a very

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beyond just a difference in leaf. The S. Davis and Sons brand "Nobleman" was advertised as being made by Cuban cigar makers with Cuban tobacco, but members of the CMIU maintained that it was German Hand Work. See *Gazette*, 4 May 1910, p.4 and letter from the executive of CMIU local 58 executive B.Drolet, A.Boivert, A. Gariepy 16 Dec. 1913, RG 27 Vol.303 T-2691, "Industrial Disputes File," Strike No.119, NAC.

[92]Hilton, *Smoking in British Popular Culture.*

[93]Fernando Ortiz, *Cuban Counterpoint*, p.43.

[94]"Ignorance of Cigar Names," *CCTJ*, January 1899, p.6.

[95]"Montreal Correspondence," *CCTJ*, 11 June 1901, p.273.

[96]"A Successful Year," *CCTJ*, January 1904, p.51 and *CCTJ*, February 1906, p.25.

much lower price than the imported." Typically, almost all of their brands had Spanish names.[97] To push their claim of authenticity even further, in April of 1902 the company announced that it would offer $500 to anyone who could tell the difference between one of their cigars and a high quality Cuban import. Several Montreal tobacconists took up the challenge. M.H. Parkinson and M. Hinfom, for example, were only able to tell the difference 44 per cent of the time.[98] The contest results as well as solicited commentaries from the contestants were then printed in the industry trade journals to promote the Granda Hermanos y Cia. brands among tobacconists.[99]

The Cuban and Spanish cigar makers clearly understood the importance of the claims of authenticity to the company's sales and corporate image. They had their own union, the "Federacion de las Uniones de Habano en los Estados Unidos y Canada," separate from the CMIU, and offered their own union label as a further claim to authenticity to manufacturers who would agree to their conditions.[100] Their strategies during strikes also reflected the cultural weight their labour held. In early 1903, shortly

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[97]*CCTJ*, August 1901, p.372. The only exception to the Spanish brand names was the "Rothschilds" which was also appropriate as a symbol of wealth.

[98]"Une haute prétention complètement justifiée," *Liqueurs et Tabacs*, May 1902, p.32.

[99]*CCTJ*, July 1902, p.355.

[100]*CCTJ*, March 1903, p.53. J. Granda of Montreal, Frank Granda's firm after he left the Granda Hermanos y Cia. adopted the label. S. Davis and Sons also used the label in 1903, see *CCTJ*, June 1903, p.41.

after Frank Granda left the company, the Cuban workers went on strike.[101] In a powerful move the Cubans circulated a memo to businesses and to the media claiming that Granda Hermanos y Cia. was using non-Cuban labour. The *CCTJ* called the episode "about the worst piece of labor history that has ever come under our notice." Furthermore, they told other Canadian cigar manufacturers that those dealing with Canadian cigar makers were sleeping in a veritable "bed of roses" compared with Spanish labour. Indeed, the claims were seen as a vicious attack on the reputation of the company, and in the final agreement, which apparently came quickly after the memo was circulated and published in the Montreal press, the Cuban cigar makers had to issue another circular to the local business community and the press denying their previous statements.[102] The episode highlights the cultural value of racialized labour in the construction of a cigar's value. Both business and labour believed that connoisseurs of cigars would demand not only authentic Cuban tobacco but authentic Cuban labour if these cigars were to be sold at a high price.

The Granda Hermanos y Cia. had attempted to respond to a bourgeois notion of taste that saw skilled labour and *terroir* as the criteria of a cigar's quality. To understand these qualities was the mark of class and gender-specific connoisseurship - a construction

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[101]Frank Granda was taken to court by Nathan Michaels in March of 1902 for beginning another cigar company with his brothers, something that was apparently outlawed in their partnership. On the court case see, "Montreal Chats," *CCTJ*, October 1902, p.551 and "Granda Hermanos Y Ca. [sic] Vs. Frank Granda," *CCTJ*, December 1902, p.131. When Frank left the company he quickly joined his brothers Jose and Domingo in the firm of J. Granda. They continued to compete with the Granda Hermanos y Cia. until 1920. At that point they are no longer listed in *Lovell*'s business directory.

[102]"The Granda Hermanos Y Ca. [sic] Strike," *CCTJ*, March 1903, p.37. See also, "La Fin d'une Grève," *Liqueurs et Tabacs*, March 1903, p.32.

of "men of taste" that legitimized male consumption and gave hierarchy to the liberal ritual of smoking. Yet this "rational" hierarchy of tobacco products was based on culturally constructed categories that depended on notions of race and gender as much as work process, soil or climate. The value skilled labour brought to a cigar was being undermined by industrial capitalism, personified in cigar manufacturers who used apprentices as cheap labour and others who asserted that products were not made by workers but by manufacturers. Similarly, it is not clear whether Cuban tobacco was of the same quality it had been before the Ten Years War and the Spanish-American War. Regardless of the quality or the authenticity of Cuban tobacco, the image of Cuban quality could be evoked through excise stamps, Spanish brand names, and the colours of the Spanish flag. It was these notions of skilled labour and the *terroir* of the tobacco – the structures of bourgeois connoisseurship – that elevated the cigar as a symbol wealth and to the height of prestige among tobacco products.

Chapter Three

Conflicts in Connoisseurship: Debasing *le Tabac Canadien*

This hierarchy of taste which created a social hierarchy of smokers was used to assess the quality of other tobacco products that were far more popular than the cigar. In late nineteenth-century Montreal, bourgeois connoisseurs most reviled French-Canadian homegrown pipe tobacco. Not everyone agreed with this assessment. Rural French Canadians had long grown tobacco for their own consumption as well as for sale on local markets. This tobacco, largely because of its accessory role in the habitant economy, often did not have a standard taste and was particularly strong. Still, rural French-Canadian smokers had grown accustomed to it and, along with the clay pipe, *le tabac canadien* held national symbolism. Arriving in late-nineteenth century Montreal, rural French-Canadian immigrants found that their national symbol had different meanings in the city. Their clay pipes had become a symbol of poverty and their tobacco, the smell of rural backwardness. Bourgeois connoisseurs gave the label of inferiority to *le tabac canadien* using the same cultural categories they had employed to judge the cigar. French-Canadian *terroir* in particular – "intelligent" labour, climate and soil was singled out as inappropriate for growing tobacco. In addition to bringing to light notions of taste that competed with those promoted by bourgeois connoisseurs, this chapter demonstrates how one system of meaning becomes culturally dominant over another. Indeed, the hierarchy of taste used by bourgeois connoisseurs for social distinction was promoted in two ways. First, to insult the smokers of *le tabac canadien* bourgeois connoisseurs not only claimed these smokers were uncivilized, they appealed to racial prejudices and linked habitants to Natives who

they claimed grew inferior tobacco. What is striking here is that these bourgeois connoisseurs were not only Anglophones – some French Canadians joined in the criticism of *le tabac canadian*. The key division amongst Francophones was not merely the question of whether French Canada was to have a rural or urban identity. Also at issue was the nature of French-Canadian agricultural practices and their integration into larger networks of capitalist exchange.[1] Indeed, the second way in which the tastes of bourgeois connoisseurs became hegemonic was through the tobacco industry itself. Sir William Macdonald, the monopolistic entrepreneur who dominated the pipe tobacco industry until the mid-1890s, shared in these dominant norms governing taste and used only imported tobacco in his products. His systems of distribution and production served as powerful mechanisms to promote Canada-wide tastes and to overwhelm local tastes like *le tabac canadien*.

I. Pipes and la Patrie

In Montreal three kinds of pipes dominated: the clay, the meerschaum and the briar.[2] Archaeologists have analysed these pipes in Montreal, primarily on a socio-economic level. They have concentrated on the clay pipe and have hypothesized that the

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[1]On city and country relations, see Keith Walden, *Becoming Modern in Toronto* and especially Raymond Williams, *The Country and the City* (London: Chatto and Windus, 1973).

[2]It is difficult to assess the popularity of different pipes but I conclude that these pipes were the most popular because of their frequent mention in my sources. Other pipes occasionally mentioned or found in archaeological digs were corncob pipes and china pipes.

clay pipe had long been the staple of Montreal smokers, though it was losing favour to the cigarette and the briar pipe at the turn of the twentieth century. This hypothesis is corroborated by the *CCTJ* which maintained that "The day of the clay pipe has gone, probably never to return."[3] While clay pipes may have been less popular, they still had significant class symbolism. They were cheap: *CCTJ* quoted them at one cent each in 1912, but they were also fragile.[4] Archaeologists have also asserted that the clay pipe remained in use in impoverished areas.[5] Literary evidence like the character of Roland in Rodolphe Girard's 1912 collection of short stories, *Contes de Chez Nous*, also links the clay pipe to poverty. Financially ruined, Roland pawns his "pipe d'aristocrates" that his sister gave him and is reduced to smoking a clay pipe.[6]

Clay pipes were partially judged by the length of their stems and questioned as to their healthiness. In 1882, the *Canadian Illustrated News*, in its column "The Family Physician" wrote of the dangers of boys smoking, especially with "dirty short pipes."[7] Dr. Foucher went into greater depth on the question of the length of the pipe stem in an article in *L'Union médicale du Canada*: "L'effet irritant de la chaleur diffère aussi dans chaque

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[3]*CCTJ*, June 1912, p.15.

[4]"Public Men Who Smoke," *CCTJ*, June 1903, p.93.

[5]I.C. Walker, "Nineteenth-Century Clay Pipes in Canada," *Ontario Archaeology*, No.16, 1971, pp.19-35; I.C. Walker, *Clay Tobacco-Pipes with Particular Reference to the Bristol Industry* (Ottawa: Parks Canada, 1977), pp354-360.

[6]Rodolphe Girard, *Contes de Chez Nous* (Montreal: 1912), pp.208-209.

[7]"A Chat about Tobacco by a Family Physician," *Canadian Illustrated News*, 28 October 1882, p.287.

cas selon que le tuyau est long, non conducteur de la chaleur, ou que la chaleur arrive directement, sans atténuation, à la surface des muqueuses."[8] This logic was rooted in the humoral theories of the Greek physician Galen and situated the pipe within questions of heat and bodily fluids.[9] As such Foucher also suggested the use of cigar and cigarette holders to avoid danger. Class underlies this medical issue as clay pipes with longer stems, the longest known as a church warden, were extremely fragile, and probably best for smoking at home rather than on a break from work or on the travels to and from work. The moral questionability of the short stemmed pipe can be seen in the weekly cartoon "La Débauche" in *La Presse* (figure 1).[10] The character La Débauche, who was dressed in the tradition of the *Habitant* was always up to some mischief, and even while not smoking, constantly had his short-stemmed clay pipe in his mouth. These ethnic clichés were not always appreciated. *Le Journal*

Figure 1: La Débauche (far left), *La Presse* (1914)

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[8]Professor Foucher, "Queques remarques...."

[9]Vivian Nutton, *Galen: problems and prospects* (London: Wellcome Institute for the History of Medicine, 1981).

[10]"En Roulant ma Boule," *La Presse*, 11 July 1914, p.8.

de Françoise, for example, reprinted a complaint in *Le Courrier de Montmagny* that "La Débauche" would give foreigners a bad impression of French Canadians.[11]

Matthew Hilton has posited that there may have been a great variety of clay pipes, and the same type of individualistic representation that occurred through the bourgeois choice in pipe may have also occurred among working class clay-pipe smokers.[12] Indeed, two clay pipes held at Montreal's McCord Museum, one with the fleur-de-lis and the thistle images moulded into the clay and another with a crown and anchor, seem to support Hilton's thesis. Yet considering their fragility, their current good condition and the fact that they found their way into the museum when the museum has only eight clay pipes in good condition suggests that these clay pipes were not the everyday pipes of Montreal or Quebec smokers.[13] Still, the clay pipe probably played a significant role in Montreal's working class cultural life. At least one Montreal union, for example, gave them out to their members at parties.[14] Considering the paucity of sources for working class notions of smoking, no conclusive answers can be offered here.

Among the more valuable pipes, the meerschaum was probably the most expensive and most esteemed among Montrealers (figure 2, see p.119).[15] Meerschaum pipes were

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[11]"Respect à notre langue," *Le Journal de Françoise*, 18 February 1905, p.647.

[12]Hilton, *Smoking in British Popular Culture*, p.65.

[13]McCord Museum, Thistle and Fleur de Lys pipe M 953.6.9 and the Crown and Anchor, M 953.6.9.

[14]"Nouvelles Ouvrières," *La Presse*, 15 January 1913, p.7.

[15]http://www.maddogcurios.com/pip/mp-8.jpg.

carved out of magnesium silicate imported from Greece and were usually equipped with an amber mouthpiece. Their status is clear from the prize list for the 1890 Montreal Labour Day Picnic. The winner of the 120 yard sack race received a meerschaum pipe, while the second place prize was an entire box of Sohmer Union Made cigars. Meerschaum pipes were also first prizes in the quarter mile running race, the shot put, and the one hundred-yard pipe race.[16] The value of the meerschaum is also affirmed in literary sources like Hector Berthelot's *Les Mystères de Montréal: Roman de Moeurs* where the rich Count Bouctouche, whom we met in the previous chapter smoking a good cigar, is also described as smoking a meerschaum pipe.[17] More popular than the meerschaum was the briar pipe. Indeed, numerous articles in the *CCTJ* claimed that the briar was almost universally smoked.[18] Made from the root of the thorny briar bush, they were more durable and expensive than the clay pipe but less costly than the meerschaum. They were also more respectable than the clay pipe with one of the most popular brands of tobacco in Canada being named after the briar pipe. The popularity of the briar pipe is also suggested

Figure 2: Meerschaum pipe.

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[16]*Ibid.*

[17]Hector Berthelot, *Les Mystères de Montréal: Roman de Moeurs* (Montréal: Imprimerie A.P. Pigeon, 1901) p.38. Originally serialized in *le Vrai Canard*, 20 Dec. 1879 - 31 July 1880, 1880-1881; then in *le Canard*, 1896-7.

[18]See, for example, "Pointers on How to Smoke a Pipe," *CCTJ*, November 1906, p.39 and "Editorial Notes," *CCTJ*, Feb 1908, p.13.

by the fact that one shape of the briar was named "The Canadian" (figure 3).[19]

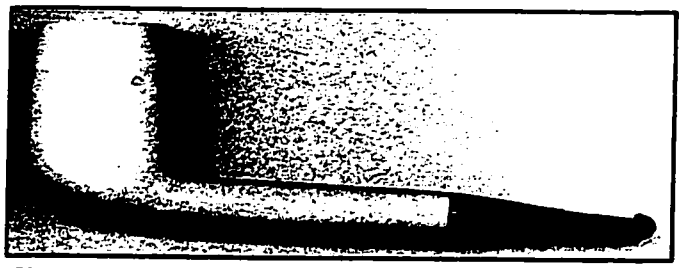

Figure 3: "Canadian" briar pipe.

The clay pipe remained powerfully symbolic. For example, Ontario businessman and Canadian Minister of Customs, William Paterson smoked a "common clay pipe" which was understood by the press to be a declaration of his popular roots and simple tastes.[20] The symbolism of the clay pipe had particular meaning in French Canada. In nineteenth century Quebec art, the clay pipe, along with *la ceinture flechée* and the *tuque* was an essential part of the visual construction of the habitant: Henri Julien's "Un Vieux de '37'," (figure 4) an image made famous in the 1970s by the FLQ, is the most

Figure 4

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[19]H. Paul Jeffers, *The Perfect Pipe: A Celebration of the Gentle Art of Pipe Smoking* (Short Hills, New Jersey: Burford Books Inc., 1998), p.66. This image is taken from http.//vtpipes.com/images/pipes/w12f.jpg.

[20]"Public Men who Smoke," *CCTJ*, June 1903, p.93; on Paterson, see Robert Craig Brown and Ramsay Cook, *Canada, 1896-1921: A Nation Transformed* (Toronto: McClelland and Stewart, 1974), p.10.

notorious example.[21] Similarly the picture of nationalist Henri Bourassa smoking a clay pipe was a means of associating Bourassa with the past and his French-Canadian roots (figure 5).[22] Indeed Bourassa was a noted smoker of the short clay with six in front of him at his *Le Devoir* desk.[23] His visual statement was understood by those who saw him smoking the clay pipe. Lionel Groulx, for example, saw the symbolism of Bourassa's pipe, commenting that he recalled seeing Bourassa in a Montreal presbytery smoking "la démocratique pipe de plâtre."[24] Another observer remembered Bourassa smoking his short clay pipe on a Rimouski stage in 1907 while waiting to give a speech. Though he was billed to speak after fellow nationalists Olivar Asselin and Armand Lavergne, Bourassa's nationalist oratory began with him smoking his pipe while the others spoke.[25]

Figure 5

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[21]*Henri Julien Album*, (Montreal: Beauchemin, 1916), p.186.

[22]Desmond Morton, *Marching to Armageddon: Canadians and the Great War, 1914-1919* (Toronto: Lester & Orpen Dennys,1989), p.102.

[23]Louis Robillard, "'Monsieur' Bourassa, solennel et familier," in *Hommage à Henri Bourassa* (Reproduced from a memorial edition of *Le Devoir*, 25 October 1952), p.142.

[24]Lionel Groulx, "Henri Bourassa ou le causeur prestigieux,"in *Hommage*, p.91.

[25]Ernest Bilodeau, "Cinquante années de souvenirs," in *Hommages*, p.158.

II. The taste of "*la patrie*" and *du vargeux*

Tobacco smoke was another declaration of class, ethnicity and nation. *Le tabac canadien* was symbolic of rural French Canada and in literature it was often presented as synonymous with the smell of "*la patrie.*" In 1897 William Henry Drummond wrote of two *voyageurs* coming home for Christmas:

> And while each backwoods troubadour is greeted with huzza
> Slowly the homely incense of "tabac Canayen"
> Rises and sheds its perfume like flowers of Araby
> O'er all the true-born loyal Enfants de la Patrie." [26]

Similarly, in his memoirs, journalist Robert de Rocquebrune recalled listening to his father tell the family's history while smoking *le tabac canadien*: "In my memory, these old family tales are somehow fragrant with the odour of the Canadian tobacco he smoked in his stubby clay pipe. The past seemed to float for an instant beneath the rafters before evaporating in a bluish haze."[27] Similarly, in 1905 *La Presse* assigned *le tabac canadian* a significant role in a feature on distinctly French-Canadian cultural practices. During the traditional "veillée d'hiver," French Canadians visited families and friends in the winter, jigging to the violin, playing cards, and flirting. During the evening when the women had retired to one room and, as *La Presse* reported "se content leurs peines et leurs joies," the men sat in another, smoking their pipes and discussing "des mérites et des qualités de leur

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[26]William Henry Drummond, *The Habitant and other French-Canadian Poems* (New York: Knickerbocket Press, 1897), pp.58-59.

[27]Robert de Rocquebrune, *Testament of My Childhood* (Translated by Felix Walter, University of Toronto Press, 1964, original publication, 1958, Fides), p.24.

tabac en faisant des expériences comparatives"[28] While the role of smoking in separating men and women was not distinct to rural French Canadians, the tobacco they smoked was.

Smoking *le tabac canadien* could be a declaration of allegiance to French Canada. An article by Léon Ledieu in *Le Monde Illustré* provides two such examples. Shortly after the 1891 elections Ledieu wrote that he overheard an habitant talking about the smoking habits of the minister elected in his constituency: "C'est bien de valeur ... j'ai vu notre ministre, je croyais que c'était du monde autrement que nous. Je l'ai vu fumer du tabac canadien." The habitant thought that his minister was the type to present himself as above the rest of the population, but seeing him smoking *le tabac canadien* made the country man reassess the character of the politician. The choice of smoking tobacco, while being a personal act by the politician, was taken as a public declaration that the politician was part of the habitant's community. For his part, the urban editor Ledieu used the story to associate himself with rural French Canadians, the same community as the farmer and the ideological home of the French-Canadian nation. Ledieu, to make sure that there was no misunderstanding, declared that *le tabac canadien* was not a mark of inferiority, noting that he himself smoked it - when it was of good quality.[29]

Most commentators believed that rural French Canadians preferred homegrown tobacco to other types of tobacco. In Louis-Joseph Doucet's collection of short stories *Contes du Vieux Temps: ça et là*, Doucet goes on a search for Quebec's rural past. He travels ten leagues from Montreal on the North Shore of the St. Lawrence River to find an

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[28]"Les Veillées," 28 January 1905, *La Presse*, p.1.

[29]Léon Ledieu, "Entre Nous," *Le Monde Illustré*, 4 April 1891, p.766.

informer, an old man who could recount French Canada's "true" past. To win over the old man, Doucet offered some tobacco that he had brought from the city. The old man politely said that Doucet should keep his tobacco because "il ne vaut pas le mien." Doucet's tobacco was, according to the old man "du vargeux."[30] There can be little doubt that *le tabac canadien* had a strong smell and flavour. *Le tabac canadien* was usually made up of a number of different kinds of leaf tobacco, particularly "Canelle," "Petit Rouge," and "Big Havana." These were all strong tobaccos with Canelle, for example, earning its name from the smell of its smoke which had the odour of burning cinnamon.[31] The use of tobacco here must be understood in the context of Doucet's narrative intent. He was evoking a very specific rural past. The farmer who smoked his own tobacco which was stronger tasting than industrially produced tobacco was key to evoking a heartier rural past.

Certainly there is a grain of anti-modernism in these examples, yet others less interested in evoking a romantic French-Canadian past also argued that men who acquired a taste for *le tabac canadien* preferred it.[32] Sir William Macdonald, the "Tobacco King of Canada," told the 1902 Royal Commission on the Tobacco Trade that "[t]hose who are accustomed to Canadian tobacco in this Province like it. They have been brought up upon

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[30]Louis-Joseph Doucet, "Coin Natal," in *Contes du Vieux Temps: ça et là*, (Montréal: J.G. Yon, Éditeur, 1911), pp. 75-77. "Vargeux" is an old French-Canadian word for "weak."

[31]Felix Charlan, "Tobacco Culture in Canada," *CCTJ*, June 1910, pp.31-33.

[32] Anti-modernism is outlined in T.J. Jackson Lears, *No Place of Grace: Antimodernism and the Transformation of American Culture* (New York: Pantheon Books, 1981). Also see Ian McKay, *The Quest of the Folk.*

it to a large extent, and it is satisfactory to them...."[33] Joseph Picard of the Rock City Tobacco Company maintained that in some districts of Quebec unrefined leaf tobacco was popular and that its consumption ate into the amount of industrially manufactured tobacco sold.[34] And in 1908 tobacco expert Louis V. Labelle told a somewhat shocked "Commons Commission on Canadian Tobacco Products" that many rural Quebec smokers preferred their *tabac canadien* to industrially produced tobacco, what a panel member termed as "good" tobacco.[35]

Analyzing how much French-Canadian homegrown tobacco was consumed in Quebec is difficult. Excise statistics, for example, included little Canadian tobacco because taxes were applied only when tobacco entered into factories, and Canadian tobacco rarely was used in factories. Occasionally certain gaps between the rough excise statistics and census statistics can tell part of the story. For example, in 1872, 55,000 pounds of Canadian tobacco was returned for excise in Quebec, whereas just the year before when the census was taken, 1,195,345 pounds were reported to have been grown. In the early 1870s the amount of Canadian tobacco that entered into factories in Quebec declined to a low of 10 pounds in 1875. This gap led Montreal tobacconist David H. Ferguson to complain in 1876 that only one-fourth to one-fifth of one per cent of all tobacco grown in

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[33]"The Tobacco Kings of Canada before the Tobacco Inquiry," *CCTJ*, December 1902, p.721.

[34]Evidence of Joseph Picard before the RCTT, pp.633-4.

[35]Louis V. Labelle, "Canadian Tobacco Products Before the Select Standing Committee on Agriculture and Colonization," 27 March 1908, Appendix 2, *House of Commons Journals*, Vol.1907-08, p.69.

Quebec, which he estimated to be between four and five million pounds, was taxed. Ferguson may have been exaggerating, considering he was calling for protection against this "homegrown" competition.[36] Indeed the 1881 Census reported 2,356,581 pounds of tobacco grown in Quebec, well under Ferguson's 4 to 5 million pounds.[37] Yet if tobacco farmers were not reporting their sales to excise officials, they may not have reported their entire crops to census officials either. Beyond the census and excise statistics, other contemporary observers remarked on the amount of homegrown tobacco that was being sold without being excised. In 1899 there was enough Canadian loose leaf tobacco on the market that the Dominion Cigar Manufacturers' Association called for the Canadian government to make it illegal.[38] J.M. Fortier told the Royal Commission on the Tobacco Trade that there were 4 million pounds of Canadian tobacco sold without paying duties on markets around Quebec.[39] As late as 1908, Felix Charlan, the head of the Tobacco Division of the Canadian Department of Agriculture told a Commons Committee on Agriculture and Colonization that he estimated 2.5 million pounds of Quebec tobacco

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[36]Evidence of David H. Ferguson to the *Select Committee on the Causes of the Present Depression*, 6 April 1876, p.254. These numbers of pounds of tobacco circulating, and the census numbers quoted hereafter, may have been "closer to the facts than the truth." We have no idea at which stage of the drying process this tobacco may have been. This would affect the weight of the tobacco, especially in comparison with dried cut tobacco. The larger point still can be made that massive amounts of tobacco were being sold "under the table."

[37]*Census of Canada*, 1881, p.241.

[38]"Sale of Leaf Tobacco," *CCTJ*, October 1899, p.377.

[39]Evidence of J.M. Fortier, RCTT, p.1429.

never entered excise and was sold directly to consumers in markets around the province.[40] Indeed, there is ample evidence that huge amounts of Canadian tobacco were being smoked without ever entering into factories or excise statistics.

Homegrown tobacco in Quebec developed out of the habitant tradition of growing small amounts for household consumption with accessory production being sold on local markets.[41] According to a pamphlet written by William Saunders, the Director of the Experimental Farms in Ottawa, Quebec farmers grew no more than a few acres of tobacco on their land.[42] One other tobacco improvement pamphleteer gave his instructions for both field and garden.[43] Tobacco cultivation began in mid-April when seeds were planted in a sheltered location.[44] The plants were then transplanted to a well-drained garden or field in early June. About two months after being transplanted, the tobacco plants were "topped" with the top leaves and flowers pinched off, the lower leaves became thicker and

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[40]Evidence of Felix Charlan before the Select Standing Committee on Agriculture and Colonization, "Canadian Tobacco Products," Appendix 2 of the *House of Commons Journals*, Vol.1907-08, p.5.

[41]For early nineteenth century Quebec, Allan Greer discusses the usefulness of the term "accessory production" in *Peasant, Lord, and Merchant: Rural Society in Three Quebec Parishes* (Toronto: University of Toronto Press, 1985), pp.204-5.

[42]Wm. Saunders, Director of Experimental Farms, Ottawa, 4 April 1898. Bulletin No.30 CIHM 26383, p.6.

[43]Dr. G. Laroque, "Culture et Préparation du tabac: à l'usage de l'amateur et du cultivateur de tabac en particulier; suivies des articles de la loi concernant la culture et la vente des tabac canadiens."(Lévis: Mercier & Cie, Imprimeurs-Libraires, 1881), pp.15-16.

[44]This discussion of tobacco cultivation is derived primarily from Dr. G. Laroque, "Culture et Préparation du tabac" as well as Jordan Goodman, *Tobacco in History: The Cultures of Dependence* (New York: Routledge, 1993), pp.171-2 and C. Mackenzie, *Sublime Tobacco*, (London: Chatto and Windus, 1957), pp.292-3.

heavier. A week to ten days later, small shoots appear in the axil (the point between the leaf and the stalk) of the plant. These shoots were "suckered" (removed) and the plants were harvested shortly thereafter. In Quebec, the leaves were dried by open-air curing (as opposed to fire cured or flue-cured which is the method by which present-day cigarette tobacco is cured) in an attic or barn.

If farmers had the time they transformed their tobacco into a number of consumable products. To make Canada Twist, Canadian Roll or plug, the tobacco was tightly twisted together and then compressed in a tobacco press. Much was also sold in bales without being transformed. Demand was high with one manufacturer, writing in the late 1870s, that the popularity of this tobacco left little raw Canadian tobacco for industrial manufacturers.[45] This was not surprising since the retail price on local markets in 1910 could be as high as 75¢ to $1 a pound.[46] Farmers could easily sell it through intermediaries: one farmer reported that farmers could sell "[in] their houses, to the people passing by who purchased tobacco, either agents or to the diverse companies that were sending agents, or to the traders."[47]

With heavy rural emigration, this smell and taste was increasingly transferred to Montreal. Between 1880 and 1890 Montreal posted its fastest rate of growth with large numbers of French Canadians relocating to the city for factory work.[48] Homegrown

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[45]A.D. Porcheron, "Tabac Canadien," (CIHM 12126), see Section III.

[46]F. Charlan, "Tobacco Culture in Canada," *CCTJ*, June 1910, p.31.

[47]Evidence of Joseph Alcides Dupuis, RCTT, p.968.

[48]Paul-André Linteau, *Histoire de Montréal depuis la Conféderation.*

French-Canadian tobacco was obtainable in Montreal, though it was probably not as widely available as the tobacco produced in Montreal factories. Some farmers brought their tobacco directly to the city. In 1907 Berri street tobacconists Guertin and Bouchard purchased a bail without inspecting their acquisition. After the farmer had left, without giving his name or address, Guertin and Bouchard unwrapped the tobacco to find a six pound brick in the middle.[49] A commission was told in 1876 that in Montreal loose-leaf Canadian tobacco was sold by small grocers through intermediaries. Every week during the winter, one Montreal firm was offered 150 barrels, 75 pounds each.[50] The use of intermediaries continued into the twentieth century. The Royal Commission on the Tobacco Trade outlined the activities of tobacco intermediary G.N. Gervais. He bought tobacco from farmers, sometimes transforming it slightly into cut tobacco, but also selling it as leaf. Gervais then hired Montreal tobacconist Philippe Roy to retail it in his store and to sell it door-to-door using a wagon that Gervais provided.[51] Both farmers and intermediaries sold Canadian tobacco at Montreal markets.[52] Montreal tobacco

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[49]*La Presse*, 12 December 1907, p.16.

[50]Special Commission on the Depression in Trade, evidence of David H. Ferguson, 6 April 1876, p.255.

[51]See evidence of Joseph Alcides Dupuis and Philippe Roy in the Royal Commission on the Tobacco Trade, p.969 and 1247. For other examples of Montreal tobacconists selling Canadian tobacco using wagons, see the evidence of tobacco merchant and manufacturer Napoleon Landry, p.1209.

[52]On the history of markets in Quebec see Yves Bergeron, "Le XIXe siècle et l'âge d'or des marchés publics au Québec," *Journal of Canadian Studies* 29,1 ((Spring, 1994), pp.11-36.

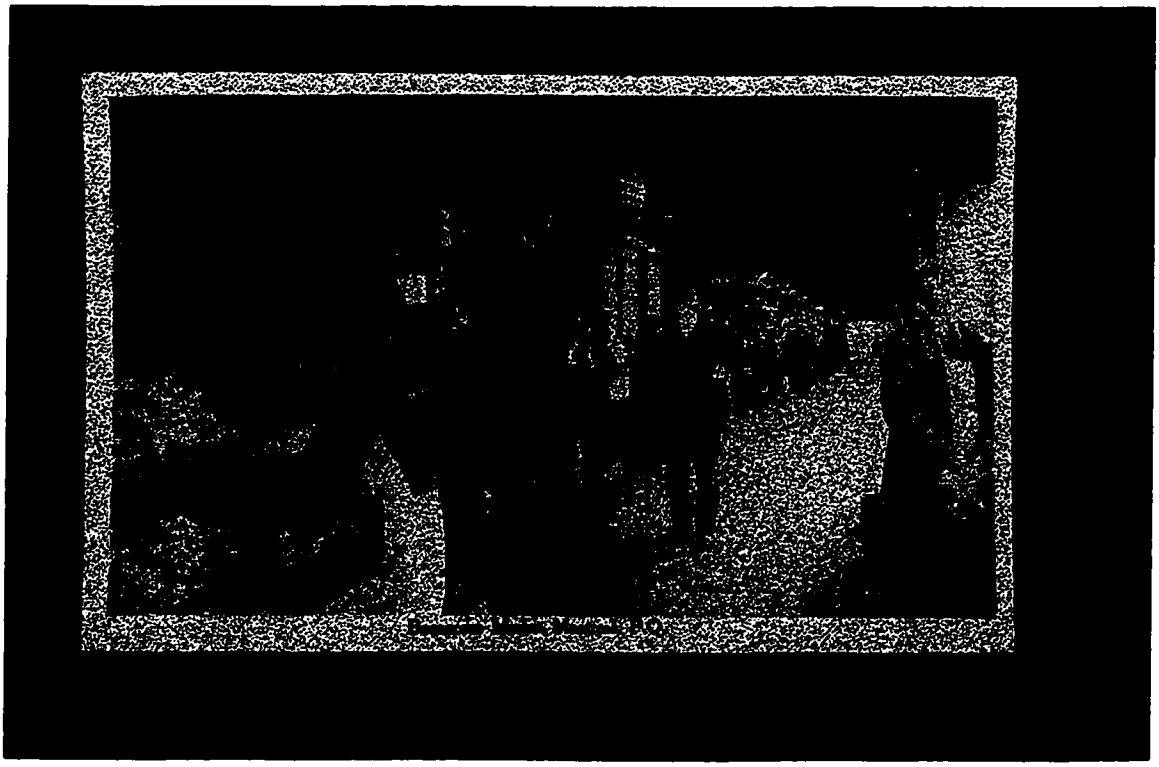

Figure 6: Selling *le tabac canadien* at Bonsecour Market.

manufacturer Jacob Goldstein submitted a plug of untaxed Canadian tobacco bought at Bonsecour Market as proof of the massive unregulated Canadian tobacco trade (figure6).[53]

As well as taste, price was another reason why many in Montreal may have smoked *le tabac canadien*. J.M. Fortier, for example, claimed Canadian tobacco was sold at 6 or 7¢ a pound in Beauseceour market, much lower than the 1910 prices quoted above. By comparison *Liqueurs et Tabacs* listed the wholesale prices of the ATCC's at 60¢ to $1.75 a pound.[54] Some Montrealers may have grown it themselves. Bettina

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[53]Testimony of J.M. Fortier, "Royal Commission on the Tobacco Trade," p.1430 and p.1432; Image from Collection Michel-Bazinet, BNQ, cote 25-7-b.

[54]*Liqueurs et Tabacs*, January 1902, p.46.

Bradbury has shown that some Montreal working-class families kept gardens: a small amount of tobacco may have been grown here in the same fashion as it had been grown in small gardens in the country.[55] In 1914 *La Presse* responded to a question on how "empecher les vers de manger les plants de choux et de tabac." While the author of the question may not have been from the city, *La Presse*'s readers were largely urban and the decision to publish the question suggests that there was interest in tobacco growing in Montreal.[56]

Indeed, smoking *le tabac canadien* became part of social events in Montreal in which rural emigrants participated. In January of 1913, *La Presse's* column "Nouvelles Ouvrières" covered a carpenter's union installation ceremony with unusual detail. The event promised a speech and a "fête intime et récréative" with "chants, musique, et autres distractions des plus agréables, y compris du bon tabac canadien." Two days later, *La Patrie* column reported that the event had been a great success with "les dévoués organisateurs distribuaient aux assistants pipes et tabac ainsi que des rafraichissements."[57]

III. Bourgeois Connoisseurship and *Le Tabac Canadien*

While smokers who had formed their taste in rural Quebec might have been happy with their *tabac canadien*, it was increasingly seen as odious by those outside the province. In 1891 the Federal Government replaced the tobacco which it usually supplied

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[55]Bradbury, *Working Families*, p.47.

[56]"Courrier de Collette," *La Presse*, 9 May 1914, p.7.

[57]"Nouvelles Ouvrières," *La Presse*, 13 and 15 January 1913, p.7.

to Northwest natives, a Macdonald tobacco that they considered "standard" and was made of foreign tobacco, with *le tabac canadien*. The natives rejected it and the unnamed company took a heavy reduction in their books. A government official later warned of the difficulty of providing Canadian tobacco to natives: "It is a fact beyond dispute that the Indians of the North West, when they purchase tobacco for themselves as a rule choose the highest grade sold by the Hudson Bay Company, and it would be a very difficult matter to get them to accept a grade inferior to the Department's standard."[58] Numerous members of Parliament from outside of Quebec also commented that Canadian tobacco's flavour "is sometimes repugnant to a smoker who is accustomed to using the imported tobacco."[59] Similarly, John Todd who frequently sent specially chosen tobacco home to his father in Ontario, recounted in a letter to his mother what he considered the disgusting smoking habits of rural French Canadians. Barnum and Bailey's circus had come to Montreal and every "Canuck paysan and paysanne too, who could scrape together the 'necessary' took in the circus." He zeroed in on one family "consisting of Papa, Maman, Bébé, two little girs [sic] and four boys, the eldest perhaps fifteen. Papa and the sons all smoked common, clay pipes, crammed full of vile smelling 'tabac rouge.'"[60]

Some Montrealers objected to *le tabac canadien*. This was especially true of sources close to the tobacco industry. The *CCTJ*, the Montreal-based industry's primary

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[58]Unnamed government official to M. Arahill and Co. 23 Nov. 1896. Laurier Papers, Reel 744, pp.9137-40.

[59]*Debates*, 24 Feb. 1905, pp.1682-3.

[60]John F. Todd to Rosanna Todd, 20 June 1895 in *John L. Todd Letters, 1876-1949*, p.62 also John F. Todd to Rosanna Todd, 6 February 1895, p.55.

trade journal, concerned over the subsidized flight of cigar factories to suburban Montreal maintained "if it keeps at the present rate it will be only a few years when a cigar factory within the borders of any city will be as scarce as sweet smelling tobacco in a habitant's pipe."[61] Perhaps the most surprising commentary on *le tabac canadien* came from *l'Album Universel* (figure 7, see p.134).[62] The cartoon clearly demonstrates that there was no unified French-Canadian opinion on *le tabac canadien*. It linked the strong smell of homegrown tobacco to the mores of a street person. Taken as a whole the cartoon "Une bonne pipe de tabac canadien" plays on respectability, with *le tabac canadien* giving the lounger the means of appropriating middle class public space.

Even the suggestion of using homegrown tobacco in cigars scandalized some. Though few were ever produced, in 1898 the *CCTJ* declared there was no " hope for the much maligned Canadian leaf cigar, which, with all its aromatic qualities, is still the peer of German filth."[63] On the other side of the capital and labour divide, William V. Todd of Hamilton, the third vice-president of the Cigar Makers' International Union (CMIU), believed that to use it in any union product would sully the good name of the union label. At the 1891 CMIU Congress he declared that "cigars made from Canadian leaf are the

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[61]*CCTJ,* February 1898, p.37.

[62]*L'Album Universel*, 20 October 1906, p.845.

[63]According to the *CCTJ* in 1898 Canadian leaf cigars made up only 1% of production August 1898, p.214. Quotation is from *CCTJ*, March 1898, p.3.

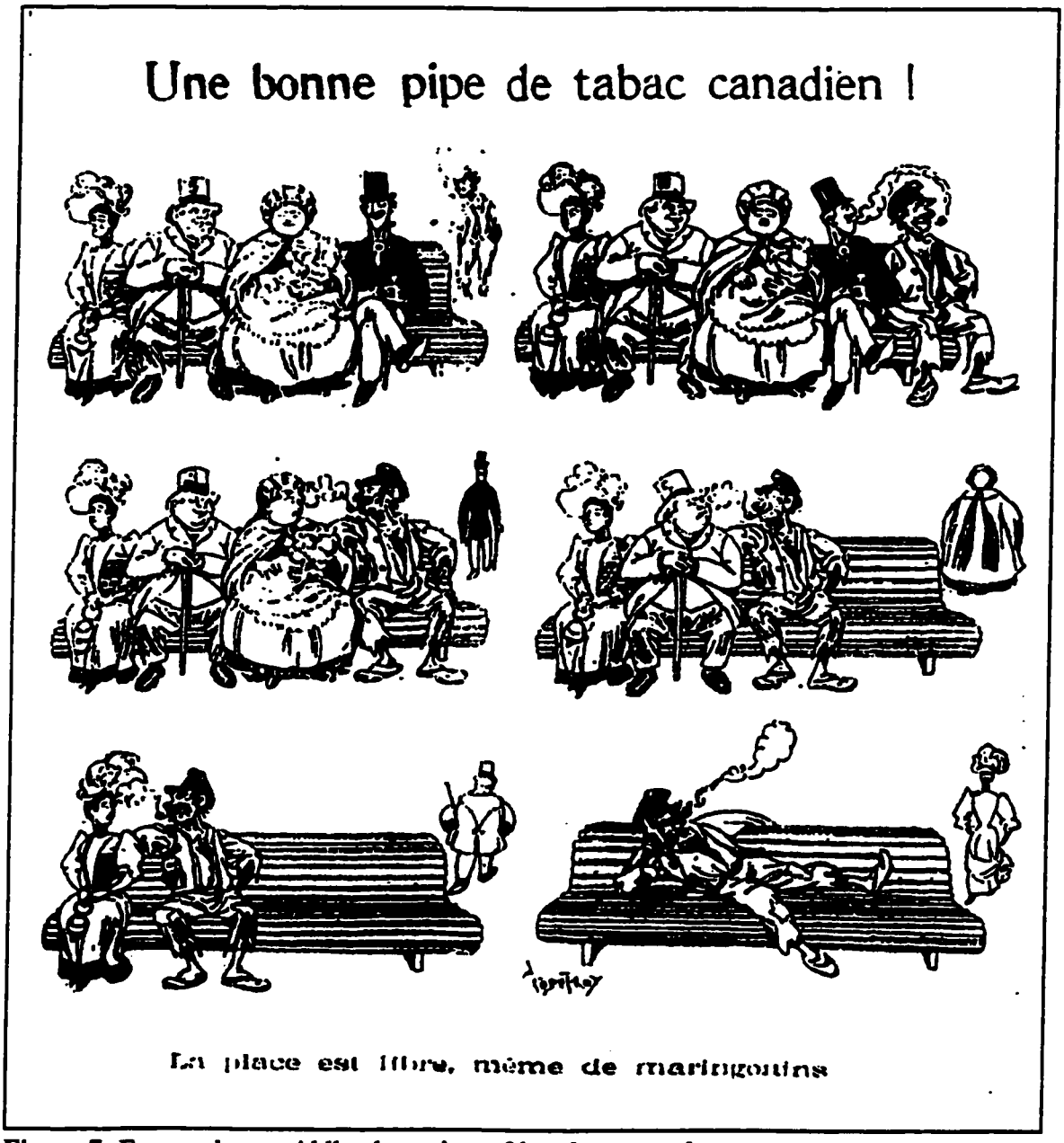

Figure 7: Francophone middle class view of *le tabac canadien.*

vilest of the vile" and asked that the union label be denied to any cigar made from Canadian tobacco.[64] Similarly, in 1912 the Montreal daily *La Patrie* wrote "Canadian tobacco cannot rank with that of Havana in the manufacture of cigars" though because of its strength these cigars could play a role in knocking out cholera germs.[65]

Critics of *le tabac canadien* placed Canadian homegrown tobacco in the same structures of bourgeois connoisseurship that exalted Cuban cigars. The only way in which French-Canadian tobacco was judged differently than cigars was on the question of skilled labour used to roll a cigar. Indeed the skill necessary to cut and mix tobacco did not weigh heavily into the evaluation of Canadian homegrown pipe tobacco. Matthew Hilton has found that the British connoisseurs' sought individualization through having their own personal mix of tobaccos to express their personality. In such a case, mixing required significant skill. While there is little evidence of demand for special tobacco mixtures in Montreal, advertisements suggest that the most elite smokers sought them out. A 1907 advertisement for E.A. Gerth's smoking tobacco announced that it was made with imported tobacco "evenly blended, [with] precision and care."[66] The fact that Gerth, an elite tobacconist who sold expensive goods, offered the product and chose to advertise it in the Montreal *Gazette* suggests that it was an elite good.[67] A number of hypotheses are

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[64] *CMOJ*, Oct 1891, p.9.

[65]"One Good Use for Tobacco," *CCTJ*, November 1912, p.51.

[66]Montreal *Gazette*, 2 September 1907, p.3.

[67]For other advertisements of Gerth's tobacco products, see Montreal *Gazette*, 5 September 1907, p.5 and 16 September 1907, p.5.

possible to explain why tobacco workers were unsuccessful in asserting the value of their labour in the product they made if we extrapolate from what we know about the cigar makers' experience. For cigar makers, the question of skill was central to their claims, as well as the advertising of their bosses. De-skilling of cigar makers would be more successful after 1920.[68] The de-skilling of tobacco workers and mechanization of smoking tobacco production happened long before the period under study in this thesis, and the tobacco workers' label could make fewer claims of their importance to the tobacco making process than the cigar makers.[69] On top of questions of skill, when unionized tobacco workers tried to promote their label, they were probably hamstrung by notions of the legitimacy of women in the workforce. By the 1890s the Montreal industry had a workforce of largely women and children and claims that buying union-made tobacco (if unions had taken an interest in unionizing them) was promoting breadwinning employment even if some of the workers, regardless of age or gender were the breadwinners of their families, would probably have been ignored.[70] Regardless, by the 1890s, skilled labour was less essential to the connoisseur's notion of good pipe tobacco.

More important than the skill of tobacco mixing and cutting was the question of *terroir*. To grow good pipe tobacco took "intelligent" labour as well as an acceptable climate and reasonably good land. In this framework, all precursors to Anglo-Saxon

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[68]Cooper, *Once a Cigar Maker*.

[69]Goodman, *Tobacco in History*, pp.228-229.

[70]Lewis, "Productive and Spatial Strategies in the Montreal Tobacco Industry, 1850-1918."; Stuart Kaufman, *Challenge and Change: The History of the Tobacco Workers International Union* (Chicago: University of Illinois Press, 1987).

industrial farming of tobacco were considered "uncivilized." Louis Lewis, for example, a tobacco buyer from New York who would soon set up a cigar factory in Montreal, reinterpreted the history of the plant in the *CCTJ*. He wrote that "When first discovered by the Spanish and Portuguese the plant was small, and in flavor [sic] 'poor and weak, and of a biting taste.'" It was "cultivated... in the rude manner common to uncivilized races...." Progress, he maintained, was slow for the next 300 years, but in the last 50 years "its cultivation has been reduced to almost an exact science, and the quality of the leaf is in a great measure within the growers of the plant."[71]

In this version of tobacco history, the habitant and his product were associated with pre-industrial and unscientific techniques. In short, *le tabac canadien* represented an outdated mode of production. One example of this reconstruction of tobacco history came from Felix Charlan, the Federal Government's tobacco expert from France. After explaining that tobacco probably originated in Central America, it was given as a gift to the European discoverers of North American by the natives. Tobacco was then "Revived by the Europeans who conquered the country step by step, it was only at a comparatively recent date, hardly more remote than half a century, that tobacco culture became really worth its name." He continued by noting that between the time Europeans arrived and industry began "part of the population, especially in Lower Canada (Quebec), gradually acquired the habit of using the indigenous plant, consumed in a rudimentary form ... and

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[71]Louis Lewis, "The World's Tobacco Crops: A Description of the types of leaf grown all over the world," *CCTJ*, October 1898, p.359. The first reference I have found to Lewis moving to Montreal is *CCTJ*, November 1903, p.23.

unfermented."[72] Habitant tobacco was put into the same category as that grown by natives and according to Charlan, was of inferior quality. Resonance of this distaste for native tobaccos can be found in numerous silences in the sources I have surveyed. Native peoples were absent from tobacco advertising in Montreal, and the trade journal of the Montreal tobacco industry never spoke of the "Cigar Store Indian" even though they frequently discussed the decoration of cigar stores.

More specifically, French-Canadian homegrown tobacco was criticized for three reasons. First, it was criticized for not being a pure breed of tobacco.[73] This was linked to a broader trend in agricultural improvement for greater crop production. Indeed, historian E.A. Heaman has noted that habitant livestock was criticized by improvers in the last half of the nineteenth century for being of mixed pedigree.[74] Second, others believed the distinct smell and taste was due to homegrown tobacco's unsystematic drying and curing process. Within the habitant economy, tobacco was an accessory crop with its production schedules being set by the weather, the seasons and the work schedules of other crops - not by the needs of the tobacco crop itself. This meant that the tobacco could be harvested too early or too late for best results. It could also mean that any preparation that had taken place before the sale to consumers could occur before drying and curing had finished. Thus, when Montreal tobacco manufacturer A.D. Porcheron wrote his

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[72]F. Charlan, "Tobacco Culture in Canada," *CCTJ*, May 1910, p.31.

[73]Felix Charlan, "Tobacco Culture in Canada," *CCTJ*, June 1910, pp.31-33.

[74]E.A. Heaman, *The Inglorious Arts of Peace: Exhibitions in Canadian Society during the Nineteenth Century* (Toronto: University of Toronto Press, 1999), p.40.

tobacco improvement manual he counselled that unlike the dominant practice used since tobacco began to be grown in Quebec, tobacco was not to be rolled until March, when the leaves " auraient ainsi le temps de se débarrasser de leur odeur de *vert* et de prendre l'arôme qui leur convient."[75] Louis-V. Labelle maintained that it was because of the curing methods of the habitant that "On y trouve aisément l'une des raisons de l'odeur souvent nauséabonde du tabac canadien"[76] The costs of some of the suggestions being made in later improvement pamphlets were probably prohibitive. Some of these pamphlets called for the construction of entire new curing barns with the pamphlets even giving plans for the barns. If the tobacco was only being grown in small amounts as accessory production, this kind of investment did not make sense, especially as there was already a market for their product as it was. Thus, radical changes in the curing and drying process were not likely to happen unless the place of tobacco in the habitant farm economy changed significantly.

While it is important to note that these criticisms of the drying and curing of tobacco and its impure breeding came from agricultural experts, who, all the same, were probably connoisseurs of tobacco, other consumers also had an idea of Canadian tobacco's "odeur de vert." Indeed, it was inadvertently institutionalized within the system of excise stamps. As discussed in the last chapter, the 1897 Inland Revenue Act dictated that each box of tobacco products had to have one of four different coloured stamps signifying the

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[75]Porcheron, "Tabac Canadien," Section I.

[76]Louis V. Labelle, "Traité de la culture et de l'Industrie du tabac," CIHM 08362. St-Jacques, 11 April 1898, p.106.

tobacco's rate of excise: in an unfortunate coincidence, the stamp used for Canadian tobacco was green.[77] Sticking Canadian tobacco with the green label brought to mind images of poorly-dried, green-tipped tobacco that would smell strongly when burned. The *CCTJ* even claimed that it had become a joke in comic opera, musical comedy and vaudeville houses. Typically, the routine went:

> Pat - I want a smoke - the worst sort.
> Mike - Why don't you smoke "Canada Green" then? It's the worst sort I ever tried.[78]

The debasing of Canadian homegrown tobacco was done in comparison to the imported tobacco that was used by tobacco manufacturers. Once again Canadian excise statistics provide only a partial picture of how much industrial tobacco was consumed in Montreal or Canada since the government only took production statistics city by city rather than consumption statistics and since Montreal provided most of the industrial tobacco to the rest of the country, these statistics only give a national picture. A second problem exists in the fact that statistics on smoking and chewing tobacco were included together. Still, these numbers are useful as general indications of imported tobacco consumed. In 1895, 10,083,400 pounds of tobacco were excised in this category. The amount of tobacco processed in factories increased to 10,538,183 pounds in 1900; 13,246,843 in 1905; 17,647,982 in 1910 and 21,694,110 pounds in 1913 on the eve of the First World War. As with Canadian tobacco and tobacco grown for cigars, Canadian experts once again pointed to the "intelligent" labour, climate and soil as being the

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[77]Montreal *Herald*, 26 March 1908, p.11.

[78]*CCTJ*, December 1909, p.23.

foundations of pipe tobacco's value. Quality pipe tobacco came from Virginia, Kentucky and Missouri. Voicing this structure of connoisseurship, Louis Labelle maintained "Ce qui fait réellement la supériorité des produits Américains au point de vue de l'industrie, c'est l'intelligence et les soins apportés à leur culture et à leur préparation pour le marché."[79]

These values by which connoisseurs rejected *le tabac canadien*, shaped the pipe tobacco most Canadians smoked through Sir William Macdonald's control of the tobacco industry. Macdonald had established a foothold in the tobacco market during the American Civil War and, by the 1880s, was considered the largest pipe tobacco manufacturer in Canada – his primary brand being "Brier" pipe tobacco.[80] Macdonald controlled the market by price setting through the Dominion Wholesale Grocers Guild.[81] Competitors like Tuckett's of Hamilton and two Montreal firms, Paegels and Ferguson and Porcheron, had difficulties competing with him since he regularly undercut his competitors, at times selling at a loss. He did this in 1893 and 1895 and shortly thereafter

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[79]Labelle, p.13.

[80]See the testimonies of Montreal tobacconists Mary Pelletier, pp.1323-1324; Joseph Lacoste, p.1326; and the evidence of Montreal's Albert Hebert, one of the largest wholesale grocers in Canada, p.1404. The questioners at the Royal Commission also believed Macdonald was the largest tobacco company in Canada. See the question of Goldstein, p.1126. In the M.B. Davis testimony and the question of Fleming, pp.1179-80, in the W.C. Macdonald testimony, RCTT. Fleming's belief is particularly important as he may have seen the Federal Government's "Blue Books" which kept track of excise for individual companies. I have never been able to local the Blue Books. Besides the Royal Commission evidence, see the opinion of an unnamed government official who claimed that Macdonald tobacco was the "industry standard" in M. Arahill and Co. 23 Nov. 1896, Laurier Papers, Reel 744, pp.9137-40.

[81]This was part of a broader trend in Canadian business to stop price cutting. See Michael Bliss, *A Living Profit: Studies in the Social History of Canadian Business, 1883-1911* (Toronto: McClelland and Stewart, 1974), pp.33-54.

bought two of his Montreal competitors.[82] Macdonald firmly believed that tobacco's value was established through the relationship between the "intelligent" labour of the farmer, the climate and the soil: *terroir*. In 1902 he explained that he would not use Canadian tobacco in his products noting that he briefly experimented with it in 1860 but consumers, especially in the western Canadian trade, did not like it.[83] Macdonald, while being a non-smoker, firmly believed that the climate and soil in Canada were not appropriate for growing tobacco, telling the Royal Commission "They cannot change the climate or the soil of the country - you cannot grow oranges here, and you cannot grow figs."[84]

Macdonald based his ideas about what made good tobacco on a belief in *terroir* which required "intelligent" labour, a good climate and good soil. In his view, and those of many French-Canadian and English-Canadian bourgeois connoisseurs, all three of these elements had to be present if tobacco was to be judged of good quality. Quebec *terroir* was found lacking. Montrealers who adhered to this structure of connoisseurship "read" smokers of *le tabac canadien* as rude or backwards. What is more, the clay pipe that was smoked by rural Quebeckers was also seen as dirty and unrespectable. The fact that both cost very little served to reduce the diversity of cultural and social meanings of French-Canadian smoking habits to one of poverty. This criteria for weighing the quality of

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[82]Testimony of Hormidas Laporte, p.1260; Testimony of W.C. Macdonald, p.1196, RCTT.

[83]"The Tobacco Kings of Canada before the Tobacco Inquiry," *CCTJ*, December 1902, p.719.

[84]*Ibid*, p.721. Stanley Brice Frost and Robert H. Michel, "Sir William Christopher Macdonald," in *Dictionary of Canadian Biography*, Volume XIV (Toronto: University of Toronto Press, 1998), pp.689-694.

tobacco was culture-specific. Indeed, many rural French Canadians preferred the strong taste of *le tabac canadien* and many who had recently immigrated to Montreal may have seen it as a national symbol - a link to their rural past and part of turn-of-the-twentieth-century French-Canadian national identity.

Chapter Four

Unmaking Manly Smokes

Thus far I have argued that the years immediately before the First World War were the height of the association between masculinities and smoking. Respectable smoking was a ritual of, and set the boundary to, the liberal public sphere. Men were supposed to purchase their tobacco and to smoke with self-control and rationality – two fundamental principles of nineteenth-century liberal citizenship. Women, according to notions of smoking etiquette and tobacco connoisseurship, were biologically incapable of either. What is more, bourgeois connoisseurs created hierarchies of tobacco products along the lines of their beliefs regarding race and gender. French Canadians, whose smoking tastes were formed in rural Quebec, did not agree with this hierarchy of value. Still, it was legitimized by the condescension of connoisseurs and by the power of tobacco entrepreneurs. This hierarchy of smoking products provided the basis for a social hierarchy of smokers. In sum, these dominant notions of respectable smoking reflected and perpetuated beliefs of inclusion, exclusion and hierarchy which set the boundaries of the late nineteenth-century liberal public sphere.

Within this context, the Quebec WCTU organized the province's first legislative and educational campaigns against smoking. Between 1892 and 1914, the WCTU played a prominent role in having thirteen anti-smoking motions presented to the Quebec and Canadian legislatures. These motions ranged from proposals for age restrictions on smoking tobacco to calls for prohibition of the cigarette. But the WCTU's success in having anti-smoking motions must be considered in light of the fact that only one law

resulted and it was considered a defeat by most WCTU supporters. The WCTU anti-smoking campaigns were particularly unsuccessful in Quebec. In the 1890s, when the targets of WCTU legislative efforts were provincial governments, Quebec was one of only two provinces (the other was Manitoba) which did not legislate age restrictions for smokers. In 1914, the Quebec WCTU was the only provincial union to pull out of the Dominion WCTU tobacco prohibition campaign. What is more, support for these Dominion and provincial anti-smoking campaigns was particularly weak in Montreal.

Despite these failures, the WCTU anti-smoking campaigns provide insights into at least three kinds of questions. First, it is a useful case study of women's public activities and the difficulties which faced women who sought to influence formal politics before enfranchisement. Indeed, if, like Mary P. Ryan, we consider formal political representation in the nineteenth century a ritual of increasingly class-inclusive male power, then the WCTU was challenging fundamental assumptions underlying that ritual of male power. Discursively, WCTU members anchored their public campaign in the private sphere, taking on the role of mothers concerned about what doctors considered to be the degenerative effect of smoking on boys.[1] More concretely social gospel-inspired churches provided women an important platform for personally participating in the public sphere. Secondly, the weakness of the Montreal WCTU's legislative anti-smoking campaigns serves to highlight some of the more controversial aspects of the social gospel before the First World War. The WCTU's anti-smoking position originated in a particularly

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[1]Mary Ryan, "Gender and Public Access: Women's Politics in Nineteenth-Century America," in Calhoun, ed. *Habermas and the Public Sphere*, pp.259-288.

gendered vision of social gospel Protestantism concerned over national racial degeneration.[2] Because of the WCTU's proposed infringement on individual rights – in the case of age restrictions the rights of parents and, in the case of prohibition, of smokers and commerce – its call for the state to play a role in the moral formation of individuals was far more controversial than suggested by much of Canadian social gospel historiography.[3] Third, in Montreal, a minority of people thought the state should play this role. While some have asserted that French Canadian opposition was the root of the failure of the anti-smoking movement, I want to go beyond race-based explanations in order to better explain French Canadian antagonism to this WCTU cause.[4] Culture is key here. French Canadians opposed anti-smoking measures because of their Roman Catholic religion and the French language, providing an insurmountable obstacle for the WCTU. Still, in Montreal, the weakness of the anti-smoking movement was the result of more than just the opposition of French Canadians. In particular, Protestant denominations that were less influenced by the social gospel also opposed prohibition measures. In sum, by looking at the WCTU and its opponents, this chapter explores the unique and contradictory liberal alliance between cultural groups in Montreal and the extent to which the liberal order

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[2]Richard Allen, *The Social Passion: Religion and Social Reform in Canada, 1914-28* (Toronto: University of Toronto Press, 1971), especially chapter one.

[3]Neil Semple, *The Lord's Dominion: The History of Canadian Methodism* (Montreal: McGill-Queen's University Press, 1996) and Sharon Cook, *"Through Sunshine and Shadow": The Women's Christian Temperance Union, Evangelicalism, and Reform in Ontario* (Montreal: McGill-Queen's University Press, 1995).

[4]Ruth Dupré, *"To Smoke or Not to Smoke: that was the Question": the Fight over the Prohibition of Cigarettes at the turn of the century* (Montreal: Cahier de recherche, École des Hautes Études Commerciales, 1997).

shifted due to collectivist demands for a new relationship between the liberal individual (man) and the state.

I. Opposing Tobacco

The WCTU's concern over smoking was part of a larger concern over national physical and mental degeneration.[5] For example, smoking was seen as endangering the nation's military ability by hindering the physical development of boys. The WCTU's "Catéchisme de Tempérance" cited a German law which forbade the sale of tobacco to minors (under16 years old) because smoking stunted growth and the development of German youth into strong soldiers.[6] In the House of Commons, Robert Holmes quoted a British Parliamentarian who alleged that the defeat of the Spanish in the Spanish American War and the French in the Franco-Prussian War "was easily traceable to the habit of cigarette smoking."[7] Another MP quoted an American doctor who claimed that three times as many recruits to the army during Spanish-American War were rejected because they lacked "the vitality necessary to make a good soldier," than in the Civil War, with the cause apparently being the cigarette.[8]

Another WCTU pamphlet, "Testimony Concerning the 'Cigarette'," argued that

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[5]Hilton has made this argument for Britain. See *Smoking in British popular Culture*, pp.162-175.

[6]"Catéchisme de Tempérance,"pp.13-14.

[7]*Debates*, 1 April 1903, p.827.

[8]*Debates*, 23 March 1904, p.338.

smoking put the country's businesses at a disadvantage. It cited American businessmen who would not hire employees who smoked cigarettes and Montreal MLA Michael Hutchinson who observed, "The boy who smokes Cigarettes [sic] is handicapped when seeking a situation. He must take second place every time; and rightly so."[9] Thus, the nation's business would also be condemned to second place in a competitive market. Liberal ideals of self-control were front and centre in the mind of Montreal MP, Robert Bickerdike when he noted that "we are all agreed that the boy who is addicted to the cigarette habit cannot succeed in this country."[10]

According to the WCTU, smoking also contributed to the moral degeneration of the race and nation. Smoking played a part in the construction of male delinquency as the WCTU claimed that smoking led to boys stealing tobacco or stealing money to buy tobacco.[11] Rev. Elson I. Rexford of the High School of Montreal wrote that any group that worked "to discourage the use of tobacco by our boys is entitled to receive the active support of all who are interested in the development of good Canadian Citizenship."[12] Occasionally this was expressed in terms of race. The Montreal *Witness*, for example, editorialized, "How infinitely more should the country sacrifice a luxury which is degenerating our race!"[13]

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[9]"Testimony Concerning the 'Cigarette'," p.11.

[10]*Debates*, 1 April 1903, pp.820-821.

[11]WCTU, "Catéchisme de Tempérance," p.16.

[12]*Ibid.*, p.4.

[13]Montreal *Witness*, 28 March 1903, p.4.

While the language of the WCTU and its supporters was often secular, the social gospel urge to create Heaven on Earth was the force that propelled them to organize and oppose smoking.[14] Indeed, in terms of the total Protestant population in Montreal, a disproportionate portion of the WCTU's membership came from the Presbyterian, Methodist and smaller social gospel-influenced churches. One of the few existing Montreal WCTU membership lists broke down the 1888 membership by church: Presbyterians made up 44.8 per cent; Methodists, 24.9 per cent; Congregationalists, 9.6 per cent; and Baptists, 4.3 per cent. Anglicans, less influenced by the social gospel, made up 12.7 per cent of the membership. In comparison, the 1891 Census enumerated Montreal's Protestant population at 45 per cent Anglican, 34 per cent Presbyterian, 15.6 per cent Methodist, 3.5 per cent Baptist and 2 per cent Congregationalist.[15]

The WCTU's criticisms of smoking were part of a female strand of the social gospel belief that stressed the role of women in reforming and protecting Canadian society. A key element of this reform agenda was altering male pastimes.[16] Indeed, WCTU literature frequently went beyond questions of children smoking to call for a reform of activities seen as masculine. In its "Catéchisme de Tempérance," written to be read in schools and homes, the Montreal WCTU asserted that smoking was a waste of money and

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[14]The same motivations help explain the popularity of eugenics among social gospelers. See Angus McLaren, *Our Own Master Race: Eugenics in Canada, 1885-1945* (Toronto: McClelland and Stewart, 1990).

[15]Montreal WCTU, *Annual Report* (1888), p.19, Rare Book Room, McGill University Canada, *Census*, 1891, pp.312-313, p.204.

[16]Cook, *"Through Sunshine and Shadow"*, p.6, pp.75-133.

that it was especially harmful to the poor as it took bread off their tables.[17] The pamphlet maintained that smoking led men to drink and to enter vice-filled areas.[18] As I showed in the first chapter, the WCTU also campaigned against men smoking on tramways as unfair male control of space. And, as the outrage of tramway smokers demonstrated, attacking smoking was not taken well by men. The Quebec narcotics division superintendent remembered that in her first three years in the position, she had learned "to walk softly, act thoughtfully...[and be] 'Wise as serpents and harmless as doves,' if any real good is to be accomplished."[19] Furthermore, she reported to her Dominion counterpart that many members "hesitate in coming out openly on this question for fear of annoying some one."[20]

Morally reforming men and protecting the future of the nation would begin by focusing on preventing boys from smoking. As I have shown, doctors were unanimous in their belief that smoking was dangerous for boys, and the WCTU claimed that it was their duty as mothers to protect boys from tobacco. Yet even WCTU members seemed to be failing in this quest to prevent boys participating in this dangerous rite of passage to manhood.[21] Their frustration is summed up in WCTU activist Annie L. Jack's poem "A

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[17] "Catéchisme de tempérance," p.13.

[18] *Ibid.*

[19] Metcalfe, "Report of the Department of Narcotics," *12th Annual Report,* Quebec WCTU, 1895, p.65. All subsequent Quebec WCTU reports from FA 885 MU 8447, OA.

[20] Sara Rowell Wright, "Report of Department of Narcotics: Quebec," *8th Report of the Dominion WCTU,* 1895, p.87. All subsequent Dominion WCTU reports from , FA 885 MU 8398, OA.

[21] Cook, *"Through Sunshine and Shadow,"* p.84.

Lesson Learned":

My boy learned to smoke,
Who taught him the filthy act?
And who will own at the judgement day
In the teaching they took a part;
I tried to keep him pure
And clean as boy should be,
But in the world he fell so low
And nothing can comfort me.

Is that the babe I've kissed?
O vile polluted breath,
And tainted blood with the poison weed,
That leads to a slow, sure death.
My bonnie, sweet-mouthed boy,
Tobacco stained to-day,
We need more strength in this hour of need.[22]

The WCTU promoted the use of the state to compensate for this failure on the part of parents. This use of the state differentiated believers in the social gospel from the Evangelical Protestantism and revivalism that had developed in North America since the 1830s. Christians who adhered to early Evangelical Protestantism saw the relationship between God and the individual as supreme.[23] In order for individuals to stop smoking, they had only to ask Christ for help and they would lose their desire to smoke.[24] The extent to which Christian denominations supported WCTU anti-smoking motions varied

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[22]Annie L. Jack, "A Lesson Learned," *8th Annual Report,* Quebec WCTU, 1891, p.39.

[23]Neil Semple, *The Lord's Dominion: The History of Canadian Methodism,* p.138.

[24]H.T. Crossley, *Practical Talks on Important Themes* (Montreal: William Briggs Publishing, 1895), pp.194-200. For more on Crossley see Semple, *The Lord's Dominion,* pp. 219-220.

according to how far these motions went in limiting individual freedoms. In the hope of saving the nation, social gospel-influenced denominations were not only willing to limit the right of parents to govern their children, they were also willing to prohibit the sale of cigarettes to adults.

The Methodist church was the denomination most willing to take up the entire WCTU anti-smoking agenda. Not only did their Sunday Schools encouraged their pupils to take the "Triple Pledge" against smoking, drinking and swearing, their churches held an annual "Cigarette Sunday." This was marked across Canada, with special lessons on the evils of smoking delivered to children. In 1892, the Montreal Methodist Conference was the first citywide church to pass an anti-smoking motion.[25] The Methodists would continue to champion WCTU anti-smoking motions when these proposals moved from age restriction on smoking to the prohibition of the cigarette. The Presbyterians showed similar support. The Montreal *Presbyterian Recorder* published anti-tobacco articles that coincided with the Quebec WCTU's first tobacco age restriction campaigns and the church officially opposed smoking in 1908.[26] In 1912, a Presbyterian and a Methodist minister accompanied the WCTU delegation that met Prime Minister Borden, calling for

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[25]*Sunday School Banner*, March 1904, p.iii. *Minutes of the Proceedings of the Fifth Session of the Montreal Annual Conference of the Methodist Church* (Montreal: William Briggs Publisher, 1892), United Church Collection, ANQ-M p.84.

[26] "Dr. Richardson on Tobacco," *The Presbyterian Recorder*, December 1892, p.330. "Digest of Minutes," Thirty-Fourth Session of the Synod of Montreal and Ottawa, p.23. 11-0-001-03-06-001B-01, ANQ-M.

the prohibition of the cigarette.[27]

While these sources betray an elite bias, WCTU supporters could also be found among the working class. They expressed disapproval in the "fire and brimstone" language historians have found to be typical of turn of the century working-class revivalist groups like the Salvation Army.[28] T.C. Vickers, a worker with the CPR in Montreal, wrote Prime Minister Laurier in 1907, disappointed that Laurier had not introduced tobacco prohibition legislation. Vickers invoked the God-given collective right to fresh air. "[You] cannot walk the streets to Breathe the Beautiful fresh aire [sic] that a Loving God has made for us," he complained. "But some Dirty Smoker thinks he has a Perfect right to Polute [sic] it." Vickers encouraged Laurier to convert, "to come over on the Clean side." For Vickers, it was not a matter of Laurier or his own opinion on tobacco, but the Lord's, and this, he told Laurier, was written in the book of Revelations chapter IX, verses 17 to 19:[29]

> And thus I saw the horses in the vision, and them that sat on them, having breastplates of fire, and of jacinth, and brimstone: and the heads of the horses were as the heads of lions; and out of their mouths issued fire and smoke and brimstone.
>
> By these three was the third part of men killed, by the fire, and by the smoke, and by the brimstone, which issued out of their mouths.

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[27]"To Prohibit Cigarettes," Montreal *Weekly Witness*, 20 February 1912, p.3.

[28]Lynne Marks, *Revivals and Roller Rinks: Religion, Leisure, and Identity in Late-Nineteenth-Century Small-Town Ontario* (Toronto: University of Toronto Press, 1996), p.157.

[29]T.C. Vickers to Wilfrid Laurier, 6 March 1907, Laurier Papers, PAC, C-845, pp.121093-7.

> For their power is in their mouth, and in their tails: for their tails were like unto serpents, and had heads, with them they do hurt.

These opponents of smoking were linked by a shared commitment to the social gospel. This worked in their favour in places where social gospel denominations made up a large percentage of the population. Indeed, in 1894, the Dominion WCTU reported that in Quebec the Eastern Township Unions, where social gospel Protestants were more numerous, were taking the lead in the province's anti-tobacco campaign.[30] Montreal, however, was not fertile soil for the WCTU. In 1891, denominations heavily influenced by the social gospel made up only 13.1 per cent of the population and this number was declining as the percentage of Roman Catholics rose.[31]

II. Opposing Prohibition

In Montreal, important newspapers opposed regulating the age of smokers, arguing that it was a case of the state usurping the rights of parent. The Montreal *Gazette*, for example, argued that the state could not fulfill the responsibilities of a parent: "The chances are that the bill will not catch the boy. Attempts to substitute the statute book for the parental rod have not hitherto been terribly successful."[32] Later it linked banning children from theatres, invoking curfew laws and anti-cigarette laws with the belief that "an attempt to do by statute what can only be effectively done by home influence, by a

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[30] *7th Annual Report of the Dominion WCTU*, 1894, p.76.

[31] Canada, *Census*, 1891, 1901, 1911.

[32] Montreal *Gazette*, 20 February 1893, p.4.

father's or a mother's precept and advice."[33] *Le Canada*, the Montreal Liberal party daily, editorialized in 1907 that "we must leave to parental authority, exercised directly or delegated to the professors and school masters, the responsibility of taking measures to eradicate a vice which does not interest society but the individual."[34] *La Patrie* invoked the parents' rights over their children: "Les gens ont le droit d'être libres en cette matière et pour la répression chez les enfants, c'est aux parents qu'il appartient de l'exercer."[35]

The dominant Christian churches in the city were also reticent about the state being used to police individual morality. On the surface, the Anglican church, the largest Protestant denomination in Montreal (10.8 per cent of population in 1891) and the Roman Catholic church, the largest religious group in the city (73.2 per cent of the population in 1891), held similar positions on tobacco. The Anglicans gave limited support to the WCTU campaign against boys smoking, but opposed prohibition.[36] In 1899, when a motion opposing children smoking went to the floor of the Montreal Anglican Archdiocese sessional meeting there was great controversy. Dr. D.L. Davidson[37], an Anglican with Methodist origins, declared that "no man had a right to foul God's fresh

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[33]"Children and Theatres," Montreal *Gazette* 3 April 1903, p.4.

[34]"The Cigarette," translated in the *CCTJ* May 1907, p.17, from *Le Canada*, 12 March 1907.

[35]"Contre les cigarettes," *La Patrie*, 4 December 1907, p.4.

[36]Canada, *Census*, 1891, pp.312-313.

[37]J.I. Cooper. *The Blessed Communion: the Origins and History of the Diocese of Montreal, 1760-1960* (Montreal: Archives' Committee of the Diocese of Montreal, 1960) pp.118-119.

pure air with tobacco smoke"[38] before making the following motion:

> That this Synod deplores the rapid extension and abuse of tobacco and cigarette smoking amongst all classes of the community and in particular amongst the Clergy of the Church, and amongst the young; and should express the hope that all members of the Church, Clerical, and Lay, may, by example and precept, do what they can to restrain the growing evil.[39]

Perhaps purposefully, the resolution avoided any suggestion that the state take on the role of a parent. Some openly mused about the influence of the social gospel within the Anglican Church. Dean Johnston of Montreal, for example, recounted that when he came to Canada in 1859, out of 70 clergymen in the Synod, only 12 did not smoke. The same, he said, was true in 1899, yet there seemed to be "a remarkable setting-in" against smoking and even more so against intemperance. There was a growing "recognition on the part of the clergy that an indulgence in smoking and drinking was detrimental to the progress of Christian work."[40] In contrast to the followers of denominations heavily influenced by the social gospel, many Anglicans would not support the prohibition of any tobacco product. Layman Mr. A.G.B. Chilton maintained that smoking only fouled "God's Fresh pure air" in as much as onions did. Furthermore, Rev. Mr. Clayton, a clergyman from Bolton, did not believe "that the person who occasionally indulged in a glass of wine or a quiet smoke was cursed by the d----l and was on the road to h—l. He strongly discountenanced the abuse of liquor or tobacco, but did not believe that either

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[38]Montreal *Star*, 18 January 1899, p.7.

[39]*40th Annual Session of the Synod of the Diocese of Montreal*, 17 January 1899, pp.34-35.

[40]Montreal *Star*, 18 January 1899, p.7.

were harmful if indulged in moderation."[41] J.I. Cooper, historian of the Anglican Church in the diocese of Montreal, has examined the diocese's attitudes to prohibition, finding that "Officially, Anglicanism did not go beyond enjoining moderation and insisting on individual responsibility...."[42]

Roman Catholics occasionally spoke out against children smoking, putting it in terms of racial degeneration. In 1887, for example, *Le Monde Illustré* gave a prize for the best essay on the "Influence pernicieuse du tabac sur l'avenir des races." Among the judges of the eighteen entries were Abbé Marcoux, the Vice-Rector of Laval University, and writer Raphael Bellemar.[43] In 1892, the Archbishop of Quebec, Cardinal Elzéar-Alexandre Taschereau, supported the Quebec WCTU's call for a ban on children smoking. Many other prominent Roman Catholics added their voices to the age restriction campaign. Conservative Premier L.-O. Taillon quoted from a journal of hygiene during debate over a 1893 bill to limit smoking by boys, noting that tobacco was harmful to all and thus especially to boys. Later, the future Liberal premier F.-G. Marchand supported prohibiting children from buying cigarettes, saying "that cigarette smoking led to the degeneration of the race."[44]

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[41]Montreal *Star*, 18 January 1899, p.7. For another affirmation in a more popular source that smoking was not considered a sin, see, "Etiquette," Montreal *Family Herald and Weekly Star*, 5 February 1895, p.6.

[42]Cooper, *The Blessed Communion*, p.125.

[43]X.Y.Z., "L'Influence Pernicieuse du Tabac," *Le Monde Illustré*, 31 December 1887, p.275; *Le Monde Illustré*, 21 January 1888, p.293.

[44]Montreal *Gazette*, 21 November 1895, p.1.

Adult smoking, however, was never defined as a vice. In Montreal, for example, while the Roman Catholic Church was concerned about morality and especially children becoming "le réceptacle de tous les vices," lists of vices in the Diocese of Montreal's official declarations included blasphemy, debauchery, going to cabarets and drunkenness - but never smoking.[45] Strikingly, Roman Catholic priests and temperance organizations in Montreal confined themselves to concerns over alcohol abuse and occasionally gambling, but never smoking.[46] Significantly, from 1905 to 1910 the most powerful temperance movement in Montreal, La Ligue antialcoolique, never expanded its interests to tobacco, and even its position on alcohol was for moderation not prohibition. What is more, while campaigning for the "suppression" of alcohol, the Ligue sought to limit liquor licenses not call for prohition.[47] As with alcohol, it was only the abuse of tobacco that was a sin and as such, tobacco consumption fell within a conception of liberty to consume all things that God put on the Earth.[48] Several Roman Catholic leaders opposed prohibition of alcohol

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[45]See *Les Mandements: Lettres Pastorales, circulaires et autres Documents publiés dans le Diocèse de Montreal* (Montreal: Arbour et Laperle) from 1890 to 1914. For examples of list of "Vices" see *Tome 11*, "Lettre Pastorale de Nos Seigneurs les archevêques et évêques des Provinces ecclésiastiques de Québec, de Montréal et Ottawa: Dangers des Mauvaises Compagnies," p.662. Les Archives de l'Archevêché de Montréal.

[46]See numerous letters in the dossier "Campagnes de Tempérance par les évêques de Montréal: Correspondance Générale, 1882-1906." For other Catholic temperance organizations in Montreal see the "Nouveau Manuel de la Ligue du Coeur de Jesus" in the dossier on the "Ligue du Sacre-Coeur (fédération des), 1905-1924" and "Société de Tempérance de l'église St.Pierre" in the dossier entitled "Société de Tempérance et de charité établies dans le diocèse de Montréal." These dossiers at Les Archives de l'Archevêché de Montréal.

[47]Hamelin and Gagnon, pp.175-230.

[48]*Ibid.*, p.19.

on these grounds. In 1898, canon P.-J. Saucier from Rimouski, for example, opposed prohibition because "Une loi de prohibition serait un attentat à la liberté naturelle puisqu'elle interdirait l'usage licite, en soi, d'une bien que Dieu a créé."[49] In 1925, two French Canadian doctors echoed Saucier's argument in an article on the possible health hazards of tobacco, saying that man had the "liberté dans l'usage des biens créés pour l'homme! L'usage très modéré du tabac est à peu près indifférent."[50]

While both Anglicans and Roman Catholics opposed prohibition as an incursion on their rights, they arrived at this position along different paths. For many Anglicans, whether the question was prohibition of alcohol or tobacco or the excesses of capitalism, individual rights stood as a bulwark against "Romish" despotism. In the late nineteenth century these beliefs lined up against social gospel beliefs in improving the collective moral environment. And while there were several social gospel advocates within the Montreal Anglican Church, proponents of individual responsibility and rights remained in control.[51]

In contrast to the Anglican position, the Roman Catholic use of individual rights to oppose the prohibition of tobacco was part of the Catholic response to what it saw as increasing materialism. The opinions of *La Patrie* editor J.I Tarte illustrate this position. Tarte, a non-smoking Montreal MP, a leader of the Dominion Alliance for the Suppression of Alcohol and devout Roman Catholic contended that because moderate smoking and

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[49]*Ibid.*, p.198.

[50]Pierre Fontanel, "Pour et contre le tabac," *L'École sociale populaire*, vol.133-134, (1925), p.23.

[51]For a recounting of the two positions by a Montreal church leader see Symonds, *A Memoir*.

drinking were not health problems, prohibition was inappropriate. Furthermore he contended, "Prohibition has not been very popular with us in Quebec... [not] because we drink more than the people of other provinces, but because we believe in freedom."[52] Tarte's position as a leader of a temperance movement at the same time as he opposed prohibition may seem contradictory. In fact, it made sense within late-nineteenth-century Roman Catholic doctrine on the relationship between the Church, the state and the moral formation of the individual. The Roman Catholic Church opposed state interference in the moral formation of individuals. In the second half of the nineteenth century, as a challenge to increasingly popular secular and materialist views of the relationship between humanity and the world, Pope Leo XIII released a series of Encyclicals to reassert God and the Church's role in these relations. Historians Jean Hamelin and Nicole Gagnon have shown that the Pope appropriated the language of the French Revolution, speaking broadly in terms of rights and liberties as well as the equality of individuals before God. This equality before God never implied social or material equality between individuals. Rather, freedom was the capacity to do right. Clerical authority was essential to this notion of liberty because it was the clergy that *taught* the individual how to make decisions.[53] At the heart of the Roman Catholic position was the belief that, through prohibition, the state was

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[52]Elva Desmarchais to Archbishop Paul Bruchési, 15 March 1907. WCTU Dossier, Les Archives de l'Archevêché de Montréal; "Joseph-Isreal Tarte," *Dictionary of Canadian Biography*, XII, pp.1013-1020; "Lois Prohibitives," *La Patrie*, 18 October 1907, p.4. *Debates*, 1 April 1903, p.842.

[53]Jean Hamelin and Nicole Gagnon, *Histoire du catholicisme québécois: Le XXe siècle, tome 1, 1898-1940* (Montreal: Boréal Express, 1984), pp. 18-19.

denying the Church its role in building morally strong, self-governing individuals who would be able to enter a world where the state would not be the individual's only moral guide. *La Patrie*, for example, argued that to restrict personal freedoms was only acceptable in the worst scenarios, and neither the abuse of alcohol nor tobacco was in this category of problems. What was worse, prohibition would deprive the individual of "les fruits qu'assurerait une réforme inspirée par la modération et susceptible de rallier mieux l'appui de toute les bonnes volontés."[54]

The fact that the Anglican and Roman Catholic churches – the two largest churches in Montreal – did not view tobacco as a danger suggests that the WCTU's first task was to raise awareness. Here, Montreal's particular linguistic duality worked against the organization. Indeed, while the WCTU did have a small French division, I have only found one WCTU anti-smoking pamphlet in French, and most of their proselytizing was done in English. Much more pervasive were Francophone newspaper editorials, such as those quoted above, which opposed both age restriction on smokers as well as prohibition. Educational programs had to be a priority for WCTU members as well. J. MacL. Metcalfe, the Quebec Narcotics Superintendent, reported in 1894 that after sending a letter to WCTU members with the opinions of nine "leading physicians and scientists as to the evil effects resulting from the use of tobacco," she had many replies that they had never given the subject much thought.[55] Again, in 1895, she complained that it was still

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[54]"Lois Prohibitives," *La Patrie*, 18 October 1907, p.4.

[55]J. MacL. Metcalfe, "Report of the Superintendent of Narcotics," *11th Annual Report*, Quebec WCTU, 1894, pp.79-80.

difficult to find workers because the department was "anything but a popular one," with some active WCTU members opposing its work and members remaining silent. Until at least 1899, the Montreal Central Union never had a Narcotic Superintendent and this may have contributed to the Quebec WCTU's inability to muster support for a cigarette prohibition petition in 1902.[56] The executive of the Montreal WCTU worried that "[numerous] cities in Ontario have obtained more signatures than the whole of Quebec."[57]

Despite not having a Narcotics Superintendent, the WCTU sponsored educational events opposing tobacco. By 1896, the WCTU's educational campaign in Montreal included anti-smoking lectures by physicians and WCTU members and the distribution of anti-smoking literature.[58] Over the next eighteen years, the various Montreal WCTU locals set up Anti-Cigarette and Anti-Tobacco Leagues in conjunction with local Methodist churches. Unlike their American counterparts, aimed at adults, the Montreal leagues were organized primarily for boys.[59] Among the earliest was the Westmount Anti-Cigarette Club which by 1897 had forty members, about twelve of whom attended the Club's bi-monthly meetings.[60] By 1905, there were three more Anti-Cigarette Leagues in

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[56]*Annual Reports,* Montreal WCTU, 1884-1899.

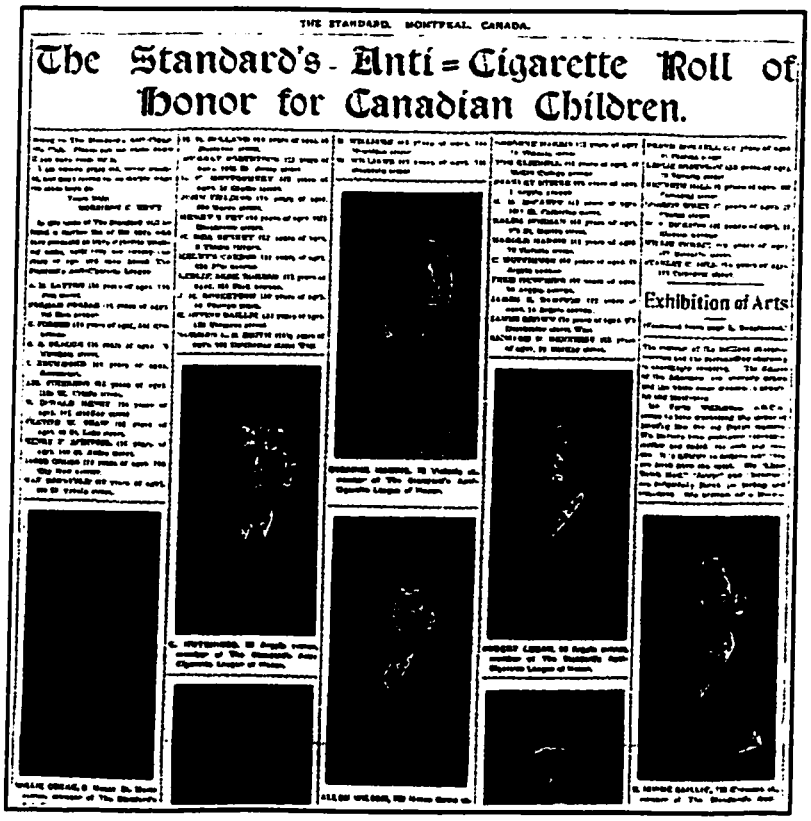
[57]"Executive,"1 Dec. 1902, Montreal WCTU Minute Book, 1902-06.

[58]*13th Annual Report,* Quebec WTCU, 1896, p.65.

[59]Cassandra Tate, "The American Anti-Cigarette Movement: 1880-1930." For an Ontario example of an American style anti-tobacco league, see, Richard Hobbs, "The Anti-Tobacco League," *Christian Guardian*, 2 August 1911.

[60]15th *Annual Report,* Quebec WCTU, 1897, p.75.

Montreal, one with the Western Union, and two large leagues numbering 350 members established by the Fairmount Union. The latter organized picnics and winter socials "to hold the boys together and ... [to give] new zeal" as well as get the interest of their parents.[61] Children who took "The Pledge" against smoking and joined the League had their pictures published as part of the Montreal *Standard*'s Anti-Cigarette Campaign (figure 1).[62]



THE STANDARD, MONTREAL, CANADA.

The Standard's Anti-Cigarette Roll of Honor for Canadian Children.

Exhibition of Arts

Figure 1

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[61] *22nd Annual Report,* Quebec WCTU, 1904-1905, p.78-79; *24th Annual Report,* Quebec WCTU, 1906-1907, p.66.

[62] "The Standard's Anti-Cigarette Roll of Honor for Canadian Children," Montreal *Standard*, 30 March 1907, p.6.

III. Legislative Campaigns

The Quebec WCTU's campaign to use the state to stop smoking began in 1892, and between 1893 and 1895 they had four bills presented to the Quebec legislature. Each of the bills would have made it illegal for children under 15 to smoke "[in] any public street, road highway, or building" under the penalty of a $2 fine. Moreover, no adult could sell tobacco to anyone under 18 without a written request from a parent or guardian.[63] These bills were part of a broader movement. In 1890, New Brunswick became the first Canadian province to set an age of majority for smokers.[64] A year later, British Columbia passed a law prohibiting minors from buying or being given tobacco and in the spring of 1892, both Nova Scotia and Ontario followed.[65] Similar proposals were considered in at least eight American state legislatures.[66]

The Quebec WCTU would never have the legislative success of its sister associations across Canada. I have shown the hostility of the two largest religious congregations in Montreal – the Roman Catholics and the Anglicans – to controls on youth smoking, the demographic weakness in the city of the supporters of the social

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[63]Montreal *Gazette,* 20 February 1893, p.3.

[64]"A Bill intituled [sic] an Act to Prohibit the Sale of Cigarettes to minors," *Journals of the House of Assembly of New Brunswick,* 1 April 1890, p.60.

[65]"An Act to Prohibit the Sale or Gift of Tobacco to Minors in Certain Cases,"*Journals of the Legislative Assembly of the Province of British Columbia,* 20 April 1891; "Minor's Protection Act," *Journals of the House of Assembly of Nova Scotia,* 25 March 1892; "An Act Respecting the Use of Tobacco by Minors," *Journals of the House of Assembly of Ontario,* 29 February 1892, p.47.

[66]Tate, "The American Anti-Cigarette Movement," p.133.

gospel, the opposition of important newspapers like the *Gazette* and *La Patrie* to state intervention, and the lack of enthusiasm for the project inside WCTU itself. Within this environment, the Quebec WCTU began its provincial campaign for age restriction legislation. The provincial campaigns demonstrated numerous ways women influenced the male public sphere. Indeed, in preparing the campaign Quebec WCTU president Mary Sanderson corresponded with the Quebec and Montreal Presbyteries, the Protestant Ministerial and Methodist Ministerial Associations of Montreal, the Royal Templars and Good Templars, and each MLA asking for their support.[67] Narcotics Superintendent J. MacL. Metcalfe wrote WCTU county presidents across Quebec, urging them to lobby their MLAs and each MLA was sent a pamphlet that detailed the harmful effects of tobacco.[68] The bill made it through the Legislative Assembly, but died on the order paper in the Legislative Council.[69] Further efforts to legislate age restrictions failed to pass through the Legislative Assembly, convincing the Quebec WCTU of the futility of securing such legislation in the province.[70] It petitioned twice more after the turn of the century,

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[67]J. MacL. Metcalfe, "Report of the Superintendent of Narcotics," *10th Annual Report*, Quebec WCTU, 1892-93, p.65.

[68]Her letters to County Presidents of the WCTU produced limited effect. Out of eighty letters, she received only thirteen replies, with six of these writing that they were too busy with other WCTU business. Metcalfe, "Report," 1892-93, p.65.

[69]Montreal *Witness*, 27 February 1893, p.6; Montreal *Gazette*, 27 February 1893, p.4.

[70]*Journeaux de l'Assemblée Nationale*, for the second attempt see 10, 16 and 21 November 1893; for the third attempt see 27 and 29 November 1894; and the final bill, see 8 and 20 November 1895. Montreal *Gazette*, 21 November 1895, p.1. *14th Annual Report*, Quebec WCTU, 1897, p.54.

but by 1907 it was opposing all attempts by the Dominion WCTU to move the fight back to the provincial level.[71]

While the WCTU faced legislative failures in provinces like Quebec and Manitoba, elsewhere it succeeded in passing age restriction laws. Yet in these provinces the laws were ineffective and tougher measures were deemed necessary. MPs from Ontario and Nova Scotia, for example, claimed that anti-smoking laws in their provinces were dead letters.[72] Deciding that age restriction legislation had proven "worthless," in 1899 the Dominion WCTU turned its attention to obtaining federal legislation that prohibited the manufacture, importation and sale of cigarettes to all Canadians, a restriction of trade that fell under federal jurisdiction.[73] For the good of the country, it was argued, adult men would have to give up cigarettes. The Montreal *Witness* compared the prohibition of cigarettes to the banning of margarine. Margarine was banned "for the sake of commerce" even though, as a cheap butter substitute, it would have nourished the "poor man."[74] M.K. Richardson called on MPs to cast aside "that bugbear of interference with personal

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[71]Anti-smoking lobbying on the provincial level in Quebec was limited to two petitions: the first on 28 February 1902 to forbid tobacco sales to anyone under 18, submitted by "Mary E. Sanderson and others"; a similar petition was sent on 12 May 1905 by the Quebec WCTU; For the Quebec WCTU's opposition to moving the cigarette prohibition campaign to the provincial level see their *24th Annual Report*, Quebec WCTU, 1907, pp.12-13.

[72]*Debates*, 1 April 1903, p.830.

[73]Cover letter to pamphlet "Testimony Concerning the 'Cigarette'," Annie O. Rutherford, Annie M. Bascom and Jennie Waters to MPs, 25 April 1903.

[74]Montreal *Witness*, 28 March 1903, p.4.

liberty." Was self-sacrifice not, he asked, the most admired quality of the individual?[75]

In addition to pushing for prohibition rather than restrictions, the federal campaign differed from provincial campaigns by focusing on prohibition of the cigarette rather than all tobacco products. The problem with singling out the cigarette in the 1890s was that few people smoked them. By the turn of the century, however, there was statistical evidence that cigarette smoking was on the rise. The WCTU, for example, quoted excise statistics showing a boom in cigarette sales from 76,000,000 in 1898 to 134,000,000 in 1902.[76] Cigarettes, the WCTU argued, were more dangerous than other tobaccos because the tobacco in cigarettes was milder than that used in cigars and smoked in pipes. The cigarette, the Dominion WCTU executive wrote to the *Witness*, "whets without satisfying the appetite" and is therefore more addictive. As well, the letter continued, cigarette smoke was more likely to be inhaled with its poisonous nicotine drawn "into the infinitely delicate lung tissues...."[77] The focus on the cigarette had a strategic advantage. Supporters of the WCTU claimed that the prohibition motion was harmless to adult men since they would most certainly smoke other forms of tobacco. Reminding the House that there were other forms of tobacco that an individual could smoke, W.S. Maclaren noted, "if gentlemen cannot forego the pleasure of smoking cigarettes for the purpose of helping the boys of this country, I am mistaken in the calibre of the men who occupy seats in this

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[75] *Debates*, 23 March 1904, p.344.

[76] WCTU, "Testimony Concerning the 'Cigarette'," back cover.

[77] "The Cigarette Evil," Montreal *Witness*, 26 March 1903, p.12.

House."[78]

When the cigarette prohibition petition came before the House in April 1903, WCTU representatives were in the gallery to watch over the MPs.[79] Despite their lobbying, the WCTU was still an outsider to this political process, with none of its members in Parliament and no suffrage rights for women. This gender inequality was pointed out by Mortimer Davis who wrote the Minister of Fisheries reminding him of his long support for the Liberal Party and of the large number of male voters who would be upset if cigarettes were outlawed. According to Davis, 36,000 merchants and wholesalers opposed the bill, and their tobacco shops were a "rendez-vous, really, for store-keeper's customers, to hang around the store and discuss politics, etc., with their friends."[80]

During debates on smoking over the next five years anti-prohibitionists in Parliament argued that prohibition was a female invasion of the male sphere of politics, an affront on individual (male) liberty and a vicious attack on male leisure activities. Some members attacked the bills as being evidence of women interfering in affairs that they did not understand. E.B. Osler, a Toronto MP rebuffed, "my lady friends who are so interested in this matter," by stating that "there is more evil wrought among the youth of this country, by bad cooking than by the use of tobacco...." Instead of lobbying, women

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[78]*Debates*, 23 March 1904, pp.339-40. Others continued on the theme of only outlawing cigarettes, not all tobacco products. See *Debates*, 1 April 1903, pp.830-831, and *Debates*, 16 March 1908, p.5103.

[79]"Anti-Cigarette Motion Adopted," Montreal *Gazette*, 2 April 1903, p.7.

[80]M.B. Davis to R. Préfontaine, Laurier Papers, C-802, p.75090-7509. The letter is undated but its positioning in the Laurier Papers suggests it was written in 1903.

should start teaching cooking courses to girls.[81] Prime Minister Laurier, in a more diplomatic tone, echoed Osler by suggesting that the women of the WCTU would be better off educating, thus not questioning male freedoms by pushing for prohibition legislation.[82]

Between 1903 and 1908, the WCTU succeeded in guiding four cigarette prohibition resolutions into Parliament, yet with the exception of one, all died "procedural deaths."[83] The watershed moment for the WCTU and its supporters came in 1908 when the Laurier government derailed the cigarette prohibition movement. After another bill was introduced calling for the prohibition of the importation, sale, and manufacture of cigarettes, on 16 March, 1908, A.H. Clarke of South Essex, part of Ontario's tobacco belt, turned the tables on the WCTU and proposed an amendment to the bill.[84] Instead of cigarette prohibition, Clarke called for changes in the Criminal Code to stop minors from smoking all types of tobacco.[85] With the support of Laurier and other ministers, the bill which restricted anyone under the age of 16 from buying tobacco or smoking in public passed with a vote of 61 to 51.[86]

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[81]*Debates*, 23 March 1904, p.354.

[82]*Ibid.*, p.363.

[83]This legislative path is summed up in *Debates*, 16 March 1908, pp.5088-5091.

[84]On the Ontario tobacco belt, see Lyal Tait, *Tobacco in Canada*, (Canada: T.H. Best Printing Company, 1968) pp.59-72.

[85]*Debates*, 16 March 1908, p.5123.

[86]"Anti-Cigarette Bill," Montreal *Star*, 16 July 1908, p.4; *Debates*, 16 March 1908, pp.5133-5134.

Taken at face value, the law seems like a victory for the WCTU. Yet this assessment must be questioned since some of the strongest supporters of cigarette prohibition, Robert Bickerdike, for example, voted against the bill. What is more, we should remember that the WCTU itself had abandoned their campaigns for age restrictions because they had found these to be hollow victories. Put in the context of the Montreal (and not coincidentally Canadian) liberal order, the law was a symbolic entry of the state into a domain previous considered the sole "jurisdiction" of parents. This was an acceptable compromise since there was some support, as I have shown, among Roman Catholics and Anglicans. It was certainly more acceptable than prohibition as it did not put the smoker's rights into question, but more importantly, it did not extinguish the right of the free exchange of commodities. The 1908 compromise demonstrated the hierarchy of rights, commercial over parental, within the Canadian liberal order.

That the victory of collective social reform over individual rights was symbolic rather than real became clear with the enforcement of this law. Though WCTU's supporters voted against the bill, the WCTU gave the new measures a period of grace to see if it would be enforced any better than the provincial acts of the 1890s. While the WCTU were still active in anti-smoking educational campaigns and continued to call for prohibition of the cigarette, the Act gave them a new focus: agitating for enforcement of the age restriction law. Three of their significant activities were giving copies of the law to tobacco dealers, making sure they understood the law's provisions and lobbying the police

for its enforcement.[87]

In Montreal, "the Act to Restrain the use of tobacco by the young" was sporadically enforced. In the first year there was only one conviction. The following year, there were 133 convictions. But in 1911, convictions dropped to four.[88] If a child was caught with cigarettes, the offender was brought before a judge of the Recorder's Court, or, after 1912, a judge of the newly-created Juvenile Court. The culprit was usually reprimanded and a promise extracted not to smoke anymore. The judge then pushed the accused to reveal the origin of the cigarettes. If the source was divulged, the judge looked for another witness to corroborate the evidence. Only after having corroboration would the judge proceed with a prosecution of the dealer.[89] By February 1912, it was not clear if officers were actually enforcing the law. Alderman Drummond had to go as far as to ask council if there was a law to restrain children from buying cigarettes in Montreal. The question wove its way though several levels of city officials and had to go to the Chief Lawyer of the City who affirmed that indeed there was a law and all that was necessary for its enforcement were orders to enforce it from the Chief of Police.[90] In 1912 convictions rose to 25 and in 1913 dropped to 22. In 1914, after the Juvenile Court hired two special

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[87]Daisy Cross, "Anti-Narcotics," *26th Annual Report*, Quebec WCTU, 1908-1909, p.64; Sophia Black, "Anti-Narcotics," *27th Annual Report*, Quebec WCTU, 1909-1910, p.63.

[88]Recorder's Court *Reports*, 1909-1911, AVM.

[89]"Minutes," Commons' Commission of Cigarettes, p.23.

[90]*Minutes*, City Council, Montreal, 26 February 1912, p.51; *Procès verbal*, Bureau des Commissaires, 23 March 1912, p.19, AVM.

officers, the count rose dramatically to 82.

The difficulties of convicting tobacconists pushed the police to use entrapment to gather evidence.[91] Yet the consequences of entrapment could be far from the intentions of those looking for better enforcement of the law. Tobacconist James Stephen sold cigarettes to an 11-year-old boy only to be promptly charged with selling tobacco to a minor by a special officer. Realizing that the boy and the police officer were making the rounds of all local tobacconists, Stephen called his cousin, also a tobacconist, alerting him to the coming visitors. When the boy attempted to buy cigarettes at the cousin's tobacco store he "was subject to a hearty thrashing" before the officer could intervene.[92]

By 1914, perhaps with hopes of finding a more sympathetic ear with the Conservative Party in power, the Dominion WCTU again prepared for a campaign to prohibit the cigarette. During preparations, the Quebec WCTU fell out of line with the Dominion cigarette prohibition efforts. President Mary Sanderson asserted that anti-smoking legislation "had been, in her opinion, practically useless" and the provincial Narcotics Superintendent argued that the tobacco prohibition campaign had received so many "turn downs" from the government that it would be better to spend their time, energy and money on educational campaigns.[93] The Quebec pullout was symptomatic of

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[91]At the Commons Commission on the Cigarette F.X. Choquet denied using entrapment only to be contradicted by Owen Dawson. See "Proceedings," p.23 and p.45.

[92]*CCTJ*, November 1913, p.37.

[93]Florence E. Woodley, "Quebec will not be Found Wanting," *Canadian White Ribbon Bulletin*, April 1914, p.59.

the reticence of Quebeckers to using the state to intrude on individual rights. Sharon Anne Cook, in her study of the Ontario WCTU during the same period, argues the WCTU was divided between supporters of progressive evangelism most obvious in the federal and provincial hierarchies of the WCTU who subscribed to Social Gospel beliefs of collective cleansing of society, and a more traditional evangelicalism of local unions which saw "salvation as being personal and experiential, rather than societal...."[94] One of the dividing lines between the two positions was an interest in using the state in projects of moral regulation. In the case of the Quebec WCTU cigarette prohibition campaign the two positions seem clear, with the only difference from Cook's framework being that the provincial hierarchy took the traditional position, a position which was more easily reconciled with liberal notions of freedom of the individual.

The Dominion WCTU's cigarette prohibition campaign continued, in spite of the provincial union's absence. But instead of letting the question go to a vote, the Conservative government diverted the issue to a Commons' Commission on the Cigarette that was to look into amending the 1908 age restrictions or to suggest other ways the "Evils Arising From the Use of Cigarettes" could be prevented.[95] The Commission heard testimony from Montreal, Toronto and Ottawa "experts" on boys smoking. Yet – no WCTU members were considered experts. Instead, officials linked to juvenile courts and reformatories as well as insane asylums gave testimony, six out of ten of them from Montreal. These reformers were interested in making tobacco age restrictions more

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[94]Cook, *"Through Sunshine and Shadow,"* p.13.

[95]"Proceedings," p.2.

effective rather than invoking prohibition. The Commission submitted two reports without making any recommendations for change, claiming that they had heard much theory but little empirical data.[96] In June 1914, the Parliamentary session ended and the committee took leave and never resumed its work, concerns over tobacco eclipsed by the First World War.

Indeed, the social gospel and the WCTU were not successful in their efforts to label smoking a "vice." After lengthy legislative and educational campaigns, the WCTU could not convince Parliament that the cigarette was so dangerous to the country that it would have to be prohibited. The age restriction law they succeeded in passing was not enforced and would be forgotten until the 1980s.[97] Part of the WCTU failure to pass stronger legislation may have been due to the fact that they had no members in Parliament. Indeed, with the support of social gospel-influenced churches they had not only pushed their cause into the male public sphere of formal politics, they had also attacked an almost exclusively male habit, and in Parliament MPs expressed nothing short of anger for these women. In the end, the Montreal and Quebec WCTU was worn down by this legislative fight to stop smoking, preferring to retreat to education campaigns and Bible studies.

There were, however, other significant obstacles to the WCTU's collective social reform in Montreal. The dominance of Christian denominations that were less influenced

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[96]*Ibid.*, p.6.

[97]Robert Cunningham, *Smoke and Mirrors: The Canadian Tobacco War* (Ottawa: International Development Research Centre, 1996), p.35.

by the social gospel as well as the fact that most of WCTU activism was done in English made the movement weak. The Anglican Church for the most part did not see tobacco as a vice, and regardless, was not won over to the collectivist spirit that defined the social gospel. For them, the individual was still paramount in deciding one's own moral future. The Roman Catholic Church, on the other hand, came to a similar position regarding the individual, but from a radically different theological route. As part of a response to growing materialism and secularism, the Church reasserted itself in the everyday lives of Roman Catholics by appropriating a language of individualism that did not imply equality of individuals on the earth, but equality before God. The moral will of the individual was to be formed through Church instruction, and freedom was the individual's right to make morally sound decisions. To impose state regulation of smoking was to deny the individual's right to make a moral decision as well as to limit the Church's role in Quebec society. The combination of the demographic weakness in Montreal of the most important promoters of the WCTU, their unilingual nature, and the rejection, to a great extent, by the Roman Catholic and Anglican Churches of state involvement in moral training of individuals, meant that dominant notions about smoking being a sign of respectable and mature masculinity were less challenged by the WCTU in Quebec and Montreal than elsewhere in Canada. What is more, the Montreal WCTU anti-smoking campaigns provides insights into the alliances, compromises and hierarchies of rights within the Canadian liberal order.

Chapter Five

Mass Consumption and Undermining Liberal Prescriptions of Smoking

At the same time the WCTU was trying to discredit them, these liberal prescriptions of respectable smoking were being undermined more successfully by the new values brought on by the production increases of industrial capitalism - values associated with mass consumption. Keith Walden has argued that industrial capitalism changed "not just the economic system and human relationships within it but also fundamental categories of cultural meaning."[1] Products that were costly became inexpensive with little explanation. Similarly, the values upon which a product's prices were based disappeared into the lights, colours and spectacle of mass advertising. There was little "natural" about these transformations, nor did they go unquestioned. Indeed, the new cultural categories around industrially-produced products became hegemonic through a process that saw both conflict and consent. Uncovering the ways in which consent was shaped is key to understanding the popular acceptance of not only the new industrial order, but also changes in popular liberalism.[2]

Tobacco underwent this industrial transformation of meaning in early twentieth-century Montreal. This chapter follows this transformation using two case studies. First, Canadian tobacco, so reviled by bourgeois connoisseurs, was increasingly used for pipe

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[1]Walden, "Speaking Modern," p.303.

[2]T.J. Jackson Lears, "The Concept of Cultural Hegemony: Problems and Possibilities," *American Historical Review* 90 (1985), pp.567-593. Outside of Walden's work, for examples of the use of hegemony and cultural selection in Canada, see Robert Storey, "Unionization Versus Corporate Welfare: The 'Dofasco Way'," *Labour/Le Travailleur*, 12 (Fall 1983), pp.7-42 as well as Ian McKay's *The Quest of the Folk.*

tobacco made by the ATCC with its smokers escaping social stigma. The federal government and the ATCC played the primary roles in altering Canadian tobacco's symbolism through tariff changes, "improvement" schemes in the fields and mass advertising. Canadian tobacco could escape its stereotype because the farming practices of the habitant could be modified and local tastes standardized, providing farmers were given the right incentives.

A second example of this process of legitimation is the mass-produced cigarette. As the WCTU had noticed, Montrealers were beginning to smoke more cigarettes. In fact, the cigarette had a long history in Montreal. Before mass production, they were advertised using the same appeals to elite values as cigars and, to a point, pipe tobacco. These hand-rolled cigarettes were not popular, and until mass-produced cigarettes were marketed in the late 1880s, the cigarette played only a marginal role in Montreal culture. Even so, it was not until the late 1920s that the cigarette surpassed the pipe in popularity. The ATCC used business structures and advertisements to capture the Canadian cigarette market. The ATCC's consignment system demonstrated that business structures could overcome their competitors' advertising. The company's own advertisements sought to overturn etiquette and did not appeal to the hierarchies of taste set out by bourgeois connoisseurs. Instead they aimed to create a mass market that included women and youth. Still, the ATCC's dominance in the market did not mean it was able to control fully the ways in which these new cigarettes were understood. These case studies underscore the new categories of culture surrounding tobacco under industrial capitalism – standardization, low price, vigour and ungendered and less elitist consumption – which

ultimately transformed the nineteenth-century liberal ritual of smoking. The old liberal categories of culture that defined tobacco as "good" or "bad," the speed and spirit in which it should be smoked and who could respectably smoke were all put into question. Finally, within the process of inventing, shaping and accepting these new cultural categories, business and government played a key role as did popular resistance by smokers and others to the legitimation of the new industrial order.

I. The Return and Transformation of *Le Tabac Canadien*

At the turn of the twentieth century a sustained effort was made to revive Canadian tobacco as an industrial commodity. From 1897, the Federal Government played an important role in promoting Canadian tobacco through its tariff policy. As part of the second National Policy, the Laurier government applied high tariffs to foreign tobacco affording significant protection to Canadian tobacco.[3] The results were quickly seen. Canadian tobacco entering excise for pipe tobacco alone rose from 474,205 pounds in 1896 to 690,141.5 pounds in 1897 and 1,949,429 pounds in 1898, affording the government significant revenues.[4] Cultivation expanded in both Quebec and Ontario.[5]

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[3]The specific revision to the Inland Revenue Act to change these excise duties was an amendment to Victoria 60-61 Chapter 19, No. 13 (m) and (n). This amendment put an excise tax of 14 cents per pound on stemmed foreign leaf tobacco and 10 cents per pound on non-stemmed.

[4]See "Comparative Statement of Manufactures" *Sessional Papers*, for these years.

[5]Lyal Tait reports that tobacco growth in Quebec between 1898 and 1910 moved from 5,800,000 to 10,000,000 pounds. In Ontario, expansion into tobacco was even more impressive, moving from 399,870 to 7,000,000 pound in the same years. See his *Tobacco in Canada*, pp.73-74.

The Federal Government also tried to change the tobacco itself. In 1905 it hired French tobacco specialist Felix Charlan to study and make Canadian tobacco more abundant and palatable for manufacturers. Charlan set up the Tobacco Division of the Department of Agriculture to provide information and guidance to tobacco growers. Experiments were done on the fermentation of pipe and cigar tobacco as well as testing which varieties of tobacco gave the highest yield in Canadian climates. In 1909, three experimental stations were set up, one in Essex County in Ontario and the two others in Quebec. These experimental farms were to act as examples of new farming and curing methods and seed distributors.[6] Clearly, some farmers were eager to profit from the government protection of their crop by growing their tobacco to the government standards. The *Association des Planteurs de tabac du district de Joliette*, for example, wrote Laurier asking for more instruction in drying and preparing tobacco for industrial purposes.[7]

The tariff encouraged manufacturers to use Canadian tobacco in their products. Not all tobacco manufacturers, however, were willing to use Canadian tobacco even if they could accrue significant profits from its sale. Most significant here was Sir William Macdonald who held to his beliefs that good tobacco could not be grown in Canada. Indeed, at the Royal Commission on the Tobacco Trade he complained bitterly about the Laurier Tariff but did not see switching to Canadian tobacco as an option. Nor did he see

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[6]F. Charlan, "Tobacco Culture in Canada," *CCTJ*, June 1910, p.35.

[7]L'Association des Planteurs de tabac du district de Joliette to Laurier, 22 January 1908, Laurier Papers, microfilm C857, p.134615, NAC.

it as competition, since he thought it was inferior.[8] This kind of resistance to new trends in business was typical of Macdonald. He was a conservative entrepreneur who was not interested in the ways in which doing business had changed at the end of the century. Examples are abundant of his conservatism: he only installed a telephone and elevator at his office in 1910; he never transformed his firm into a bureaucratic hierarchy like many business in the late nineteenth century, nor did he spend much on sales staff; his firm never manufactured cigarettes in his lifetime; he also did not advertise.[9] Macdonald was also extremely tenacious in his beliefs, even if they put his reputation at risk. When a court case resulted from the death of two girls in a fire that destroyed his tobacco factory, he appealed decision after decision until his application was eventually refused by the Judicial Committee of the Privy Council of England and he was forced to pay $1,999 in damages to the parents of the girls. This indeed was a paltry sum, especially for a man who, by February of 1898, was reported to have given $2,653,000 to McGill alone and if he had payed it earlier, he could have prevented the bad publicity that resulted from the court

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[8]"The Tobacco Kings of Canada before the Tobacco Inquiry," *CCTJ*, December 1902, pp.719-721.

[9]"Sir W. Macdonald At Last Has 'Phone and Elevator'," Montreal *Herald*, 2 May 1910, p.3. While I can not definitively say that Macdonald never advertised, I have extensively surveyed the Montreal popular press between 1895 and 1914 and have not found any Macdonald tobacco advertising. After Macdonald died in 1917, his company began producing cigarettes. On Macdonald, see E.M.D., "The House of Macdonald: The Unique History of a Great Canadian Enterpise," *Saturday Night*, Financial Section, 20 January 1923; "Canada's Tobacco King," *CCTJ*, July 1913, p.39. Frost and Michel, "Sir William Christopher Macdonald, " in *Dictionary of Canadian Biography*, Volume XIV , pp.689-694. On the standards of business at the turn of the twentieth century, see Alfred D. Chandler, *The Visible Hand: The Managerial Revolution in American Business.* (Cambridge, Massachusetts: Harvard University Press, 1977).

case.[10] His conviction that smokers valued his products because of the *terroir* of foreign tobacco, qualities that he claimed could not be replicated in Canada, would be tested by the promotion of and changes in Canadian tobacco.

In contrast to Macdonald were the ATCC's efforts to modify and promote Canadian tobacco. Like the federal government, the ATCC sought to instruct farmers on how to grow tobacco that could more readily be sold on the market for industrial use. The Company hired experts to visit and instruct tobacco farmers. It also set up model farm exhibitions at fairs and provided fertilizer for farmers. The ATCC and government were probably successful in changing tobacco farming in Canada and the taste of its tobacco. If farmers followed their instructions, an industrial style of tobacco was clearly being grown. Success is reflected in the cultivation statistics quoted above. Quebec City's Joseph Picard of the Rock City Tobacco Company, whose company sold Canadian leaf tobacco to those who appreciated its strong taste, commented on the change in taste of this new industrial Canadian tobacco, now even being used in cigarettes: "Par un subterfuge commercial, elle [ATCC] pousse les cigarettes de tabac étranger en arrière de leurs cigarettes de tabac canadien où elle le peut, dans le but de distraire autant que possible le goût acquis au tabac canadien."[11]

With the increase in growth of industrial Canadian tobacco, the ATCC sought to control supply. They discouraged others from entering into competition with them for raw

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[10]"Montreal Correspondence," *CCTJ*, February 1899, p.51; "Montreal Correspondence," *CCTJ*, July 1899, p.253

[11]Letter from Joseph Picard to Laurier, 20 March 1908, Laurier Papers, microfilm C860, p.138050, NAC.

leaf. When the Federal Government attempted to open up a Belgian market for Canadian tobacco the ATCC sent a delegation to the Government to oppose the efforts in Belgium.[12] Unlike in the U.S. where the American Tobacco Company vertically integrated, setting up its United Cigar Store chain, the ATCC attempted to control supply through a system of exclusive wholesaling and retailing contracts it began to use with Canadian tobacco in 1901. According to the contracts, the retailer would get a rebate of five cents per pound of tobacco if the retailer did not sell any other Canadian tobacco products than those offered by the ATCC. The contract system did not include imported smoking tobacco, thus not treading on the toes of Macdonald, who had, as one Quebec City tobacco manufacturer quipped: "means to defend himself."[13] This shut other manufacturers out of the market. Charles Lavoie, an organizer for the Tobacco Workers International Union described in 1904 what he mistakenly saw as a question of advertising but actually was the effect of the contract on the availability of union-made smoking tobacco brands: "I find here, that by the effective advertisement of the American Tobacco Trust, they have also succeeded in keeping our union-labelled tobacco from being on sale in this city."[14]

Tobacco farmers feared that the dominance of the ATCC would allow the company to set the price of tobacco. Some maintained that the ATCC boycotted their

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[12]Evidence of Albert Octave Dugas, RCTT, p.983. Dugas was a lawyer and MP for Montcalm.

[13]Evidence of Joseph Picard, RCTT, p.618.

[14] Lavoie quoted in Kaufman, *Challenge and Change*, pp.34-35.

tobacco because the farmers refused to sell exclusively to the company. Tobacco farmer Joseph Alcides Dupuis told the Royal Commission on the Tobacco Trade that in 1900 the ATCC called a meeting of tobacco farmers from Montcalm County and asked them to sell exclusively to the company. When the farmers refused, Dupuis maintained that the company suddenly bought very little tobacco from Montcalm Country growers.[15] Tobacco farmers feared that if the ATCC had a monopoly over all Canadian tobacco sales, they would only have one company to sell to and thus the ATCC would control prices.[16] As it was, many farmers felt the ATCC and its subsidiary had too much control over the price of tobacco. When the Federal Government finally moved to question the legality of the contract system, numerous farmers wrote Laurier in support of the move. Pierre Denis, a general store owner in St. Césaire, Québec, wrote on behalf of farmers from his community to thank the Prime Minister saying that the price of Canadian tobacco had increased by 25 to 30 per cent through the threat of government action. He gave the example of one farmer who received ten cents per pound of tobacco rather than seven.[17]

In order to take advantage of this cheap Canadian tobacco, businesses had to find a way to overcome homegrown tobacco's bad reputation among consumers, though they also had an interest in maintaining that this improved commodity was a "work in progress." In contrast to the articles they published denigrating Canadian tobacco during

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[15]Evidence of Joseph Alcides Dupuis, RCTT, p.991.

[16]D.B. McTavish, "Report of the Commissioner", *Sessional Papers*, 1903, no.62, pp.7-8.

[17]Pierre Denis, 6 July 1903 Laurier Collection, microfilm C802, p.74811, NAC. For numerous other letters of support see pp.83932-84001 of the same microfilm.

the same period, in 1899 the *CCTJ* tried to promote Canadian tobacco on nationalist grounds, instructing wholesale and retail tobacconists to display their patriotism and speak out in favour of Canadian tobacco to their customers: "It is only paying our just debt to the land of our birth and livelihood" and Canadian tobacco had advanced significantly in the previous year (see Chapter 3). These contradictory opinions would at once keep prices low for raw leaf and leave room for an appeal to consumers.

Both government and numerous businessmen sought to redefine the nature of Canadian tobacco. They stressed that Ontario was now growing better tobacco while Quebec produced "backwards tobacco." Thus, *le tabac canadien* was stigmatized rather than Canadian tobacco. "The 'tabac' of the habitant certainly deserved the odium that clung to it for many years," the *CCTJ* explained in 1899,"but the leaf now harvested by our Western farmers is as far removed from this weed as is silk from sackcloth."[18] Similarly Bernard G. Meyer, of the American tobacco dealers Meyer and Mendelsohn, targeted French-Canadian farmers, saying: "The great obstacle is the lack of intelligent method on the part of the farmers. These are almost entirely French-Canadians who have no conception of the proper handling of tobacco."[19] Charlan, for his part maintained that the tobacco farmers of Essex County in Ontario were "more enlightened or better advised ... and were carrying the tobacco industry for manufacturing purposes" whereas Quebec farmers both planted their tobacco too far apart making the leaves too light and harvested

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[18]"Canadian Tobacco," *CCTJ*, August 1899, p.289.

[19]"As Others See Us," *CCTJ*, November 1908, pp.44-45.

their tobacco too late allowing for frost damage.[20] In fact, these claims that Ontario was the heartland of tobacco cultivation were somewhat premature. It was not until the 1921 *Census* that Ontario overwhelmingly surpassed Quebec in tobacco cultivation, especially considering Quebec farmers may have been under-reporting the amount of tobacco they grew.[21]

ATCC advertising campaigns also attempted to promote Canadian tobacco. In 1902, Mortimer Davis told the Royal Commission on the Tobacco Trade that his company intended to create a demand for Canadian leaf tobacco. Through advertisements they intended on "educating him [the smoker] up to that."[22] At the same Commission, the head of the ATTC's advertising division, O.S. Perrault, testified that the ATTC's pipe tobacco division had spent $250,579 on advertising in the previous four years.[23] Later in 1908 Davis maintained that his companies were largely responsible for the new-found acceptance of Canadian tobacco, partially made possible through a million dollars of advertising.[24] Advertising, for the ATCC, was a way of erasing any reference to the origins of the tobacco used in certain products. Indeed, there were no references to the

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[20]F. Charlan, "Dominion Department of Agriculture: The Tobacco Division Organized, 1905," *CCTJ*, July 1910, p.23.

[21]While it is true that the 1911 Census reported that 1.5 million more pounds of tobacco was grown in Ontario than Quebec, by 1921, Quebec was outpaced by 6 million pounds. *Census of Canada*, 1921, Vol. V, p.445.

[22]"The Tobacco Kings of Canada Before the Tobacco Inquiry," *CCTJ*, December 1902, p.705.

[23]"The Tobacco Inquiry," *CCTJ*, December 1902, p.657.

[24]"Growing High-Class Tobacco in Canada," *CCTJ*, December 1908, p.27.

origins of the tobacco used in the ATCC's brands (figure 1).[25] Rather, the ATCC's main pipe tobacco brand "Empire" was advertised by its mass appeal, using the slogan "Its sale is big" as well as by setting their brand in opposition to the strong taste of *le tabac canadien*, claiming that Empire "Does not bite the tongue."[26]

Figure 1

The ATCC and federal government efforts to change and promote Canadian tobacco were successful. Industrial Canadian tobacco, backed by ATCC advertising, was not only escaping the stigma of *le tabac canadien*, it was seriously reducing Macdonald's sales. One Montreal wholesale tobacconist, Heliedore Fortier, the brother of the tobacco manufacturer J.M. Fortier, claimed that Macdonald's market share was half of what it had been before the tariff.[27] Indeed, the tariff hit Macdonald hard and his tobaccos were more expensive than ATCC brands, partially because of the tariff and

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[25]I surveyed *La Presse* and the *Montreal Star*, the two largest circulating newspapers in Montreal, looking at each Saturday for the years 1895, 1900, 1905, 1910 and 1914. I also did less systematic surveys of other Montreal newspapers like the *Gazette*, *La Patrie*, *Le Canada*, and the *Herald* for the years 1903, 1907, 1908, and 1914.

[26]Montreal *Star*, 14 July 1900, p.1.

[27]Evidence of Heliedore Fortier, RCTT, p.1416.

partially because he offered more profit for those who pushed his products.[28] By the time of the War, Macdonald had been dethroned as "Tobacco King of Canada." In November of 1917 the pipe-smoking preferences of Canadian troops at Shorncliffe, England were polled and Macdonald's plug tobacco came in third behind ATCC's "Imperial Mixture" and "Old Chum," with Old Chum containing Canadian tobacco.[29]

II. The Cigarette in Montreal

In contrast to the reinvention of Canadian tobacco, successfully marketing mass-produced cigarettes presented a whole different set of challenges for government, and especially business. Cigarettes had long been professionally rolled in Montreal. At least one high-end Montreal tobacconist, J. Rattray, had rolled them since the 1870s, not long after they had been introduced into northern Europe and the U.S, where they were smoked by the urban elites of both countries.[30] And even after mass-production technologies had succeeded in making cheaper cigarettes, hand-rolled cigarettes made with imported tobacco continued to be sold to the section of the population who had more money to

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[28]Evidence of George E. Forbes, RCTT, p.1270.

[29]Letter of Colonel commanding Canadian Troops at Shorncliffe, 18 November 1917, RG 9 III B1, Vol. 3263. File 5-32-42 (vol.1), PAC.

[30]Evidence of Mortimer B. Davis, RCTT, p.1061. One indicator that Rattray was one of the most elite cigar store owners and tobacco manufacturers was that he was one of the leading importers of Havana cigars. In October of 1902, for example, only E.A. Gerth imported as many Havana cigars into Canada as Rattray. See "Importations de la Havane," *Liqueurs et Tabacs*, December 1902, p.40. For a concise history of cigarettes, see Cassandra Tate, *Cigarette Wars* (New York: Oxford University Press, 1999), pp.12-13.

spend and valued imported tobacco and skilled labour as a sign of class distinction. Prices on these cigarettes ranged from the ATCC Yildiz cigarettes which were marketed "aux vrais amateurs de cigarettes" at 10 for 25¢[31] to the 5¢ Egyptian cigarettes sold in CPR dining cars at the turn of the century[32] to the "Smokerettes" which sold at 10¢.[33]

Cigarette manufacturers attempted to make these cigarettes more masculine than roll-your-owns by marketing them to appeal to the values of bourgeois connoisseurship built around cigars. Advertisements for these products trumpeted the fact that their tobacco was foreign and that the cigarettes were rolled by skilled workers. For cigarettes the most popular sort of tobacco was Turkish, used to make "Egyptian" cigarettes, yet Virginian was also used and advertised, as were mixtures of the two.[34] For many cigarette companies, the fact that skilled workmen rolled their cigarettes was important to their marketing. The ATCC, for example, advertised that the tobacco in their "Mogul" brand of cigarette received "as much attention as is given to a delicate infant."[35] This probably was an ATCC pitch to legitimize the female labour that may have been used on this cigarette. The advertising around Egyptian cigarettes, which were some times called

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[31]"Yldiz [sic] Magnum," *Liqueurs et Tabacs*, August 1902, p.28.

[32]Photograph 22570, Category 31, CPR Archives.

[33]Montreal *Gazette*, 14 October 1907, p.2.

[34]For Virginian cigarettes, see ad for Smokerets, Montreal *Gazette*, 14 October 1907, p.2. For Turkish tobacco cigarettes, see advertisement for "Tuckett's Special Turkish Cigarettes" Montreal *Gazette*, 9 June 1910, p.13. For a mixture of the two, see Benson and Hedges advertisement, *Gazette*, 12 September 1907, p.7.

[35]Montreal *Gazette*, 7 October 1907, p.4.

"Oriental cigarettes," invoked images of Imperial dominance. These were not images of battles or brute force but images of leisure or more vague imagery associated with the near East (Figure 2).[36] Their visions of domination are close to Edward Said's discussions of orientalism where having knowledge of those who were dominated was important to the process and depth of domination.[37] Knowing the quality of products in the Empire, in this case tobacco, and having the power to take them, was part of this Imperial domination.

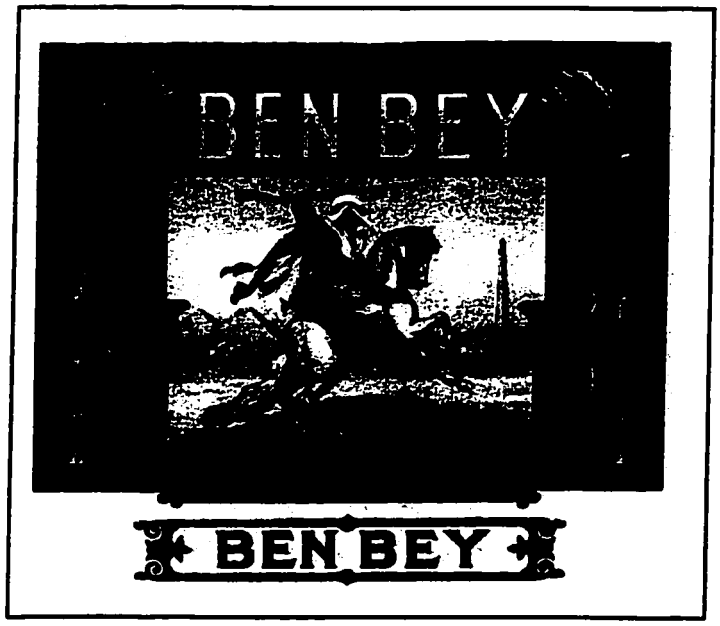

Figure 2

Like with other tobacco products, the values by which these cigarettes were supposed to be weighed, essentially skilled labour and origins of the tobacco, were supposed to be only understandable to men. Any other reason for buying cigarettes was not legitimate. To this end, the *CCTJ* wrote that another "feminine influence" in the tobacco trade was the cigarette insert. These inserts, little pieces of silk ribbon, enamelled buttons, engraved pictures or pieces of embossed leather were inside the wrapper of a

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[36]Rolph-Clark-Stone Ltd. Collection, M999.70.2.10, McCord Archives.

[37]Edward Said, *Orientalism* (New York: Vintage Books, 1978).

package of mechanically-rolled cigarettes. According to the *Journal*, they were popular with women and children who pressured men to buy certain brands with inserts in order that a woman could use them for decorating sofa pillows, wall panels and hatbands. Men would never buy cigarettes just for the insert. They bought a brand for its quality -- their ability to know a good brand being a mark of their masculinity.[38]

Despite the fact that many cigarette manufacturers tried to make cigarettes more masculine through appeals to values of bourgeois connoisseurship, cigarettes (as I showed in Chapter 1) were still associated with the sexually ambiguous dandy and thus never entirely lived up to the masculine ideals of the cigar and the pipe. Physically they were smaller than cigars and named to emphasize their appearance. In Europe they had been associated with women since Bizet's opera *Carmen* and were picked up by society women and "New Women" in England who were defying women's traditional gender roles.[39] Montreal newspapers ran stories about these women and their "foreign ways."[40] Montreal women were picking up on the association between untraditional gender roles and the cigarette. One author of a letter to Colette in *La Presse*'s etiquette column after asking "En deuil depuis sept mois d'une petite soeur, pourrais-je porter du blanc à l'été?" signed

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[38]"The Holiday Package and the Cigarette Insert," *CCTJ*, October 1912, p.11.

[39]On *Carmen*, see Richard Klein, *Cigarettes are Sublime* (Durham: Duke University Press, 1993). On New Women in Canada see Gwendolyn Davies, "The Literary 'New Woman' and Social Activism in Maritime Literature, 1880-1920," in Guildford and Morton, 233-250.

[40]"Strange ways in other lands," Montreal *Star*, 7 October 1905, p.23.

the pen name, "UNE QUI AIME LA CIGARETTE."[41] Here this woman used the cigarette to signify the fashionable lifestyle she led as well as her interest in defying tradition. In a similar vein, the fashion critic for the women's journal, *Le Journal de Françoise* used the pen name "Cigarette" suggesting that her role was to introduce new fashions to the *Journal's* readers.[42]

The ATCC picked up on this association between untraditional gender roles and the cigarette and began a short-lived advertising campaign building on these images. From mid-1905 the Company advertised their "Diva" cigarette on the back page of *la Journal de Françoise*. Divas were Egyptian cigarettes with filtered corks to make the smoke less harsh. They were described as "mignonne" and apparently made with especially pure tobacco for women.[43] The campaign was an anomaly among the company's other advertising strategies because it devoted a comparatively large amount of text to argue its product's case. It suggests that selling cigarettes to women was particularly difficult.

The advertisements for Diva cigarettes outlined what "kind of woman" smoked cigarettes and appealed to the image of the "Grand Dame" or titled women. An early narrative told of the former Princess of Wales, Alexandra, who would smoke cigarettes at intimate receptions with the women of the court. It concluded by saying that Diva

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[41]"Le Courrier de Colette," *La Presse*, 30 May 1914, p.9.

[42]For examples, see columns by Cigarette "Mode et Modes," *La Journal de Françoise*, March 1902, p.12 and "Notes sur la Mode," 1 February 1908, p.329.

[43]*Ibid*, 1 July 1905, p.107.

cigarettes "sont les favorites de nos mondaines canadiennes."[44] Another in the series referred to the great ladies of Spain, and then "tickled the cultural fancy" of upper class Canadian women by writing "En écoutant 'Carmen', l'opéra-comique de Bizet, beaucoup d'entre nous ont subi le sortilège qui émane de l'héroïne dont 'les rouges lèvres laissent échapper des volute de fumée blanche'."[45]

A second theme that this advertising campaign drew on was that of the "New Woman." The advertisements made cigarettes part of progress of modern life. One ad entitled, "La jeune fille moderne," trumpeted how much better life was for the young woman of today than women of the previous generation. It was better to play sports like golf or curling than to sit around and gossip and it was better to have a cigarette than to have a nervous breakdown.[46] Playing golf and smoking cigarettes improved marriage compared to the previous centuries as women had become companions to men and there was a greater community of interests.[47] Another ad entitled "Pour ma Dame," was narrated by a husband explaining why his wife was allowed to smoke: "La femme moderne a donné les preuves de sa capacité et s'est ouverte maintes carrières où, jusqu'ici, l'homme s'était seul engagé; elle a, par conséquent, conquis le droit à certains privilège jusque-là

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[44]*Ibid.*, 20 May 1905, p.57.

[45]*Ibid.*, 3 June 1905, p.72.

[46]*Ibid.*, 1 July 1905, p.102.

[47]*Ibid.*, 21 October 1905, p.217.

réservés au sexe laid."[48] Just as both men and women could kiss, so could they smoke.[49] "Les jeunes filles canadiennes," the campaign told readers "s'énorgueillissent à juste titre d'être tout à fait 'up to date' c'est-à-dire 'vingtième siècle'."[50]

These Egyptian cigarettes, like those marketed to men, were consumer products that sought distinction for individual smokers. And distinction went beyond Egyptian cigarettes. According to the Montreal *Star*, some Montreal society women smoked "Lady size" cigarettes and upper-end tobacconists claimed that some of these "titled citizenesses" were having special monograms put on their smokes.[51] Similarly the *CCTJ* reported that special gold tipped cigarettes were being given out to women.[52] The object was becoming personalized, and manufacturers attempted to make the cigarette what they thought was more feminine. Yet despite these appeals to society women, Egyptian cigarettes played only a small part in the market and the new mass-produced cigarette brands came to dominate.[53] The Diva campaign only ran until October 1905 and then nothing more was heard of the brand. Three years later the ATCC once again began advertising in *La Journal de Françoise* yet this time they would take a fundamentally different approach to

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[48]*Ibid.*, 1 July 1905, p.107.

[49]*Ibid.*, 17 June 1905, p.91.

[50]*Ibid.*, 14 August 1905, p.155.

[51]"Favor Feminin Smoking, if Not Done to Excess," Montreal *Star*, 17 April 1914, p.1.

[52] "Giving Cigarettes Away," *CCTJ* February 1914, p.33.

[53]Robert D. Lewis, "Productive and Spatial Strategies in the Montreal Tobacco Industry, 1850-1918,"; Mortimer Davis testimony in the RCTT, mentions Rattray as a minor manufacturer who had produced tobacco for 25-30 years, p.1054/925

attract female smokers. Rather than appealing to stereotypes of turn-of-the-century women smokers, the ATCC advertised their most popular brand, Sweet Caporal, using the slogan "fumées universellement."[54] The contrast between the two approaches is striking. While the Diva campaign clearly was about social distinction, the Sweet Caporal campaign sought to include women in a marketing campaign that targeted a mass market, undifferentiated by gender.

III. Cigarettes and Mass Consumption

The new mass-produced cigarettes were manufactured using the mass production technology of the Bonsack cigarette machine. Invented in 1881, the Bonsack cigarette machine revolutionized the cigarette industry. Briefly, the Bonsack Machine drastically reduced the individual production cost of each cigarette. Instead of individual workers rolling cigarettes, tobacco was fed into the Bonsack increasing the speed of production, and though more money had to be sunk into production equipment, costs were reduced to one sixth of pre-Bonsack cigarette production days.[55] Hand-rolling had been slow and costly. The *CCTJ* offered an estimation of the saving resulting from the new technology:

> When cigarettes were made by hand a smart girl could manipulate six pounds of tobacco in a ten-hour day, and roll 2,000 cigarettes. Then came the invention of the cigarette-making machine, which a single operative manages with ease. In a day it makes 200,000 cigarettes, thus saving the wages of ninety-nine girls - a sum of very nearly $15,000.[56]

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[54]*La Journal de Françoise*, 20 June 1908, back cover.

[55]Chandler, *The Visible Hand*, pp.249-250.

[56]"Cigarette Making," *CCTJ*, April 1906, p.45.

Indeed, like mass-produced pipe tobacco, the cigarette was inexpensive, part of the reduction in costs resulting from the de-skilling of cigarette rollers and the introduction of the Bonsack. In 1895, two of the D. Ritchie and Co.'s most popular brands, Majestic and Athlete Cigarettes, sold twenty for 15 cents.[57] By 1914 prices had only slightly risen with the ATCC's leading brands, Derby and Sweet Caporals selling six for 5¢ and ten for 10¢, respectively. Numerous dealers broke open packages offering them for a penny a piece.[58] The low price of the mass-produced cigarette meant that unlike with the cigar or Egyptian cigarette, few could be excluded from smoking cigarettes because of their price. There were impressive increases in cigarette sales before the First World War due to the rising sales of these cheap mass-produced cigarettes. In 1895, at the formation of the ATCC, 85,994,000 cigarettes were manufactured in Canada. By 1903 that amount had more than doubled to 176,435,240 and would double again five years later, to 384,591,744 cigarettes.[59] By the First World War 1,166,023,170 cigarettes were manufactured in Canada.[60] This works out to a jump from 23 cigarettes per person in 1901 to 81 cigarettes per person at the time of the next Census in 1911.[61]

Much of the increase in popularity of the cigarette came from changing

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[57]Montreal *Star*, 13 April 1895, p.16.

[58]Dawson, "Proceedings," p.24.

[59]*CCTJ*, June 1908, p.11.

[60]The pre-War 1914 statistic comes from *CCTJ*, July 1914, p.10.

[61]For cigarette statistics from 1901 to 1931, see table 15, "Quantities of Spirits, Malt and Tobacco Taken Out of Bond for Consumption, fiscal years ended 1901-1931," *Canada Yearbook*, 1932, (Ottawa, 1932), p.721.

associations between speed and masculinity in industrial Montreal. The fact that cigarettes were quick to smoke was becoming an attraction rather than a sign of unmanliness as it had been through the eyes of bourgeois connoisseurs. Historian Stephen Kern has argued that between 1890 and 1918 the desire for speed in transportation and industry spilled over into a greater desire for speed in leisure as evidenced in music and film.[62] The speed of smoking the cigarette appealed to this same desire. The cigarette industry promoted cigarettes capitalizing on the short amount of time it took to smoke a cigarette in comparison to other forms of tobacco. An editorial note in the *CCTJ*, for example, pointed out to its readers: "The cigarette is such a convenient form of smoking that it commends itself alike to old and young, and specially so to those people who may not have the time or the inclination for the longer smoke of a cigar or pipe."[63] Similarly, Bernard Baron, a British cigarette manufacturer, declared in the pages of the *CCTJ* that the cigarette was more appropriate for workers who only had time to take short breaks:

> This is an industrial age. Working persons often come out for a few moments. They do not have time to smoke a pipe, but they can always have a few whiffs of a cigarette. In the case of a pipe they have to fill it and it cannot be extinguished in the same way that the lighted end of a cigarette can be snipped off.[64]

Finally, the ATCC advertised the convenience and time saving properties of the cigarette: "Did you ever have trouble in cleaning and getting your pipe going? You can avoid all

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[62]Stephen Kern, *The Culture of Time and Space, 1880-1918* (Cambridge: Harvard University Press, 1983). For a fascinating example of changing conceptions of speed in Canada see Walden, *Becoming Modern*, pp.3-7.

[63]"Editorial Notes," *CCTJ*, February 1908, p.13.

[64]Bernard Baron, "Cigarette Age Coming," *Ibid.*, January 1914, p.39.

that trouble, with SWEET CAPORAL CIGARETTES."[65]

And while bourgeois connoisseurs had portrayed cigarette smokers as unmanly because of the short time it took to smoke a cigarette, the cigarette was also read as a sign of youthful vigour. *La Presse*'s cartoon "Son Idéal"(figure 3), for example, portrayed the kinds of men a woman could expect to marry as they aged. The first man, clearly younger than the rest, as well as having numerous other qualities, smokes a cigarette and is a far

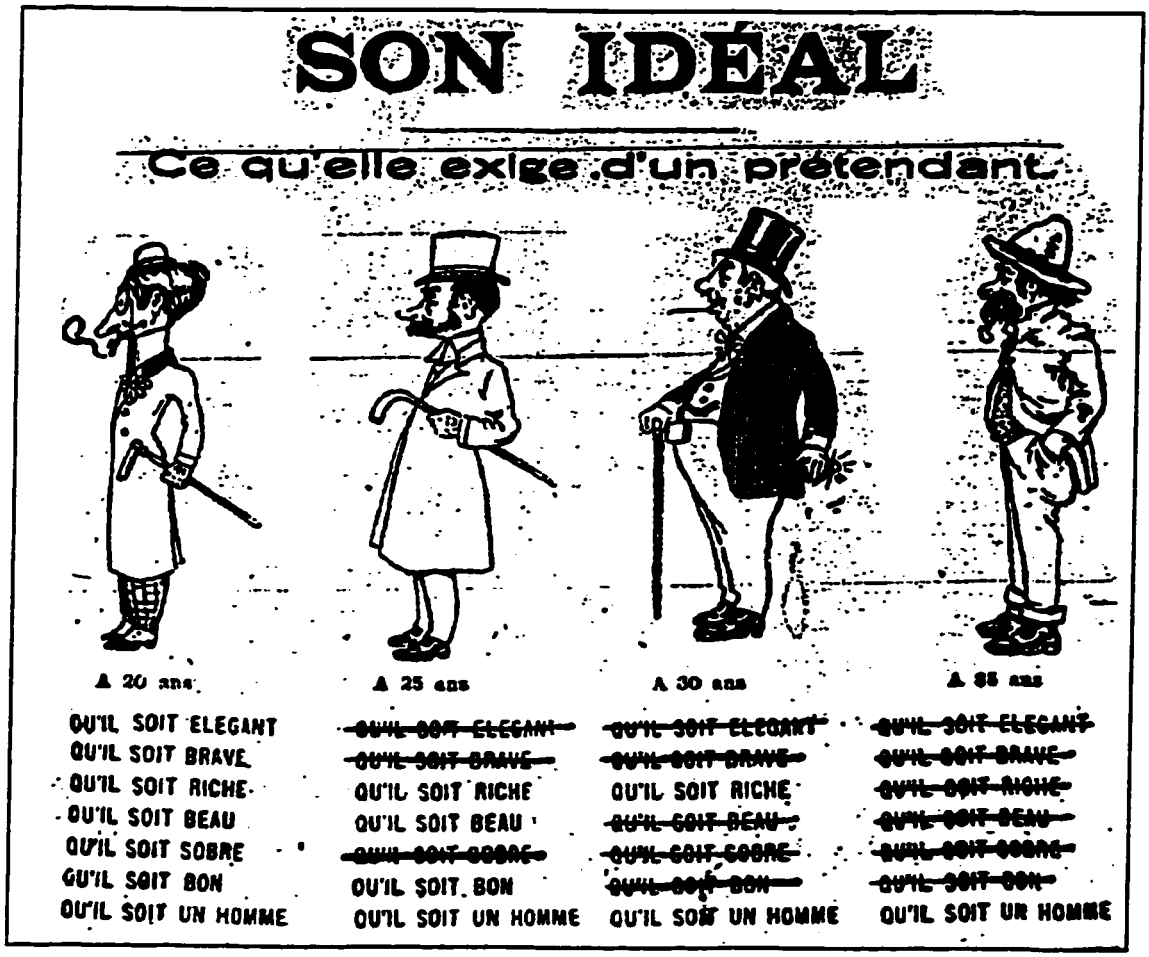

Figure 3

[65]Montreal *Herald*, 31 May 1910, p.11.

cry from the last man who has, according to the cartoon, few worthwhile qualities, and smokes an old clay pipe.[66] The cigarette was part of the way readers were to ascertain that the man was young. This cultural image was partially a reflection of the youthful "demographic" who took up smoking. During the First World War, when the Canadian soldiers at Bramshott, England were surveyed to find out their needs in tobacco, 60 per cent were cigarette smokers, 5 to 8 per cent were abstainers, and the rest were pipe smokers.[67]

The cigarette was often used to build an image of youthful male sexuality. In Dr. Ernest Choquette's collection of short stories different tobaccos appear at different times. In one story, after dinner, a group of older doctors discussed their first cases, while smoking cigars. This contrasts the use of cigarettes in another of his short stories centred around two students at Laval University. The narrator described a friend of his at school who was studying to become a notary. The friend's most significant weakness that distracted him from his studies was for women. In one scene, the two students stand together looking out on the city from Dufferin Terrace, "la cigarette aux lèvres," watching the boats in the harbour, the carts in the streets, children playing in courtyards, and most importantly, a beautiful Irish woman, that with whom both of the students fall in love.[68] The *CCTJ* also recognized that cigarettes were used sybolically in the theatre to construct youthful male sexuality. The *Journal* compared the use of the cigar and the cigarette in

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[66]"Son Idéal," *La Presse*, 26 November 1910, comic section, p.1.

[67]Letter 17 November 1917, PAC, RG 9 III B1 Vol.3263, file 5-32-42 (Vol.1).

[68]Choquette, "Un Chanceux" in *Carabinades*, pp.94-5.

theatrical productions: "Does 'Diamond Dicy' on the stage, ever fail to light a cigarette as he bargains for the carrying off of fair Angelica, or is the heavy villain in the novel ever known to omit the important details of 'flicking the ash from his cigar' preparatory to plunging into his most abysmal depth of wickedness."[69]

The tobacco industry promoted this depiction of the cigarette as the preference of youth. For example, the ATCC marketed a "Sweet Sixteen" brand of cigarette. The *CCTJ* reprinted an article from *Harper's Weekly* in August of 1898 calling for tolerance towards cigarette smokers on the part of older smokers who preferred the pipe or cigar.[70] Similarly, in 1905 the *Journal* maintained that the cigarette was looked upon as a "toy" by cigar and pipe smokers and cigarette smokers were "generally young."[71] And in 1913, they suggested that cigar stores begin offering juke boxes and soda fountains to attract the younger cigarette smoker who might bring his "best girl for a modest quencher."[72] It is also worth noting the use of the word "sweet" in both the Sweet Caporal and the Sweet Sixteen pointed to the weaker tasting tobacco used in the cigarette in comparison to the cigar or pipe tobaccos, especially *le tabac canadien*. Indeed this tobacco was probably easier to begin smoking.

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[69]"Apotheosis of the Pipe," *CCTJ* August 1903, p.53.

[70]"Evolution in Smoking," *Ibid.*, August 1898, p.233.

[71]"Origin of Smoking," *Ibid.*, April 1904, p.43.

[72]"The Soda Fountain and the Cigar Store," *Ibid.*, March 1913, p.11.

IV. Controlling the Cigarette

Yet it was not the entire industry that could attempt to legitimize the mass-produced cigarette. The ATCC was the only company whose advertisements could have any significant effect on consumers, especially around questions of sales. This power came from the ATCC's cigarette consignment system that effectively protected the ATCC brands from competition and made its advertising the most important in the industry. Pioneered among cigarette manufacturers by the ATCC's parent company in the U.S., these contracts gave the retailer or wholesaler a six per cent profit if they sold only ATCC brands. If the consignee chose to break the agreement, their profit would drop to two per cent. The consignment agreements not only shut out competition, they also gave the ATCC the right to determine the number of cigarettes and the brands that retailers and wholesalers would be offered. Consignees were then to make reports to the company as to the amount of cigarettes and which brands were sold. They could not resell to other retailers or wholesalers unless permission was given by the ATCC. This effectively allowed the ATCC to monitor its sales and make better decisions as to which brands to promote. The ATCC also reserved the right to stop consigning goods with the retailer or to pay the retailer only two per cent for consignment if the contract was broken.[73]

What is more, these consignment agreements were policed by company officials. If tobacconists were seen retailing cigarettes of other companies they would lose their six per cent reduction and even their ATCC cigarettes. Montreal tobacconist Phillipe Roy, for

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[73]The Consignment Agreement conditions were published in the "Report of the Commissioner" of the RCTT, *Sessional Papers*, no.62, 1903, pp.4-6.

example, could no longer get ATCC cigarettes after he was spotted by an ATCC representative displaying a competitor's cigarettes that he had bought at an auction.[74] ATCC officials were able to enforce the agreements through a number on each package of cigarettes that could be linked to the consignee who originally bought them. If the cigarettes were resold by a retailer to another retailer outside of the agreement, the original retailer could be held responsible.[75] Bernard Goldstein, formerly the owner of the American Cigarette Company, himself was blacklisted by the ATCC when the numbers on the bottom of a cigarette package that he had sold to Charles Gratton, a wholesaler who did not have an agreement with the ATCC, were tracked by a company representative.[76] According to one witness at the Royal Commission on the Tobacco Trade, Montreal's largest wholesalers all were under contract with the ATCC. This forced other Montreal cigarette companies to establish their own wholesalers, something that could be expensive and drive up prices.[77]

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[74]Evidence of Phillipe Roy, RCTT, p.1246.

[75]Evidence of Davis, p.1126; see also the evidence of Peter N. Menard whose job it was to check the numbers on the back of cigarette packages in *Queen v. the American Tobacco Company* exhibit A-38 in RCTT, P.1708.See also testimony of Bernard Goldstein in *Theo Hamel v. Mortimer B. Davis et al, sur accusation de conspiration pour restreindre le commerce (Art.216-520)* p.9 in RCTT, p.1786.

[76]Bernard Goldstein evidence, 18 January 1897, *Ibid.*, p.1632. See also testimony of Alphonse Brazeau in the same court case who was told by an agent of the ATCC, Louis Samenhoff, that he could not sell to Goldstein, p.1660.

[77]Evidence of O.W. Legault, p.1222. He lists the "cream of the cream" of wholesale companies as Hudon Hebert, Charles Lacaille & Co., A. Robitaille & Co.; Hudon, Arsoli; Lockerby Bros; Laporte Martin & Co. Evidence of C. De Cazil, p.1239.Testimony of J.B. Courtois, p.1317. Evidence of Jacob Goldstein, p.1368, RCTT.

The best example of the effect of the consignment system on the market was an attempt by the cigar manufacturer J.M. Fortier to branch out into cigarettes. In 1894 he began producing the cigarette brands Creme de la Creme, Parisian, Royal, Lafayette, and Imperial and business looked promising.[78] In July of 1895 he sold 390,000 cigarettes; in August, 536,000; and, peeking in October 1895, at 787,500. Then the ATCC contract went into place and sales declined to 302,500 in November and 391,000 in December of 1895.[79] By 1898, Fortier's own brother Heliedore, did not sell J.M.Fortier's cigarettes because he had signed the consignment agreement.[80] Fortier did not take this sitting down. Both in 1896 and 1897 he took the ATCC to court for conspiracy of trade and in 1902 successfully petitioned the Federal Government to call a Royal Commission inquiring into an "alleged tobacco combine." In fact, the Royal Commission found that Fortier was right and trade was being inhibited, but the Commission decided that it did not have the power to end the contract system. That would have to be done by Parliament, though Parliament never went further than to threaten to end the consignment system.[81]

The consignment system closed the door on many of the ATCC's competitors, and made the ATCC's cigarette advertising the most important advertising discourse on

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[78]Testimony of J.M. Fortier, RCTT, p.1341

[79]*Ibid.*, p.1340.

[80]Testimony of Heliedore Fortier, p.1422

[81]Michael Bliss has shown that the Canadian government was far more permissive towards monopolies than the American. See his *A Living Profit: Studies in the Social History of Canadian Business, 1883-1911* (Toronto: McClelland and Stewart Ltd., 1974), pp.33-54.

cigarettes in Montreal and the rest of Canada during the period. Indeed, competitors who may have sought to use advertising discourses that differed from that of the ATTC found that they did not work, not because they held little resonance with smokers, but because there was no way for a smoker to follow through on these consuming desires. Joseph Picard, for example, complained to the Royal Commission on the Tobacco Trade that the contract system severely restricted the measures he could take to sell his goods, singling out the uselessness of advertising: "advertising has no effect .... We would create a demand for our goods from the merchants, but we could not get our goods into the hands of the consumer."[82] Numerous Montreal tobacconists told the Royal Commission that there was demand for brands other than those produced by the ATCC, but the financial benefits of the consignment contract made them not worth filling. Abraham Michaels, brother of Granda y Hermanos owner Nathan Michaeals, an elite tobacconist at the corner of McGill and Notre Dame Streets got requests every day for Hamilton cigarette manufacturer George Tuckett's brand, "Karnac." Karnacs were sold at the same price and in direct competition with the ATCC's Sweet Caporal cigarettes, but because of Michaels' consignment agreement with ATCC, he could not sell them.[83]

The ATCC, for its part, poured enormous energies and money into advertising. The Company did most of its own advertising, rather than leaving it to tobacconists, Large businesses, as Keith Walden has written, "[bypassed] a conservative or uncooperative merchant to create popular awareness of products and to bring consumer pressure to

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[82]Testimony of Joseph Picard, RCTT, p.613.

[83]Testimony of Abraham Michaels, RCTT, p.1274.

bear."[84] Between October of 1895 and October of 1902 the ATCC spent $267,961.49 on cigarette advertising.[85] The Company advertised in newspapers, cigar store windows, fairs, trade shows and electric billboard signs. For the most part the ATCC advertisements combined repetition of an image with other novelties both to attract attention and to make the cigarette more ordinary. The Company consistently used the same image for its primary brand, Sweet Caporal - a woman dressed as a soldier, advertising in most newspapers from the most popular, *La Presse* and the Montreal *Star*, to the Liberal, *Le Canada* to the nationalist, somewhat anti-Semitic *Le Nationaliste*.[86] These were also the same advertisements that ran in *La Journal de Françoise*.

While newspaper advertising was important, it was only one way the ATTC promoted their brands. The Company had a booth at numerous fairs where it gave out free samples of their popular brands. One example of this was the "Grocers' Show" in Montreal where the ATCC gave out Sweet Caporal cigarettes as well as its more expensive Egyptian brands "Yildiz Magnums" and "Murad," bringing great crowds.[87] The booth also had other attractions. Keith Walden has argued that the searching urban gaze of the turn-of-the-century consumer was also interested in people, and race and

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[84]Walden, *Becoming Modern*, p.126.

[85]Evidence of O.S. Perrault, RCTT, p.1085.

[86]For examples of Sweet Caporal advertising, see *Le Nationaliste*, 14 April 1912, p.1; 27 *La Patrie*, May 1910, p.4; *Le Pays*, 6 August 1910; *Le Canada*, 27 May 1910, p.9; *La Presse*, 16 August 1910; Montreal *Star*, 10 October 1910, p.6.

[87]"Grocers' Show is in Full Swing," Montreal *Herald*, 21 April 1908, p.5.

gender often played important roles in attracting the white male middle class eye .[88] The ATCC used this fascination with race in particular by hiring an African-Canadian man, who the industry called a "mascot," named "Professor Brown." Frequently Professor Brown appeared at trade and industrial exhibitions becoming part of the ATCC delegation if not part of the display itself to heighten the excitement at the ATCC booth.[89] Professor Brown, who the *CCTJ* called "dusky, but dignified," also was seen around Montreal in 1901 driving an automobile promoting ATCC brands.[90] Both Professor Brown and the automobile would probably have attracted attention in turn-of-the-century Montreal.[91]

The use of the automobile illustrates the ATCC's proclivity to use new technologies to attract the attention of potential smokers. The ATCC were particularly effective at using electric signs in high traffic areas. For example, by 1902 there was an electric sign proclaiming "Smoke Sweet Caporal Cigarettes" at the corner of St.Catherine and St.Lawrence.[92] And in 1913 there was an electric sign advertising Sweet Caporal cigarettes at the corner of St.James and Windsor Streets, certainly a busy spot being near

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[88]Walden, *Becoming Modern*, pp.119-166.

[89]*CCTJ*, Janurary 1899, p.37. In 1914, as a reward for his 25 years of service as "Mascot" of the Imperial Tobacco Company, Professor Brown was given a trip to Europe. On the use of "exotic bodies" to attract crowds see, Walden, *Becoming Modern*, p.157.

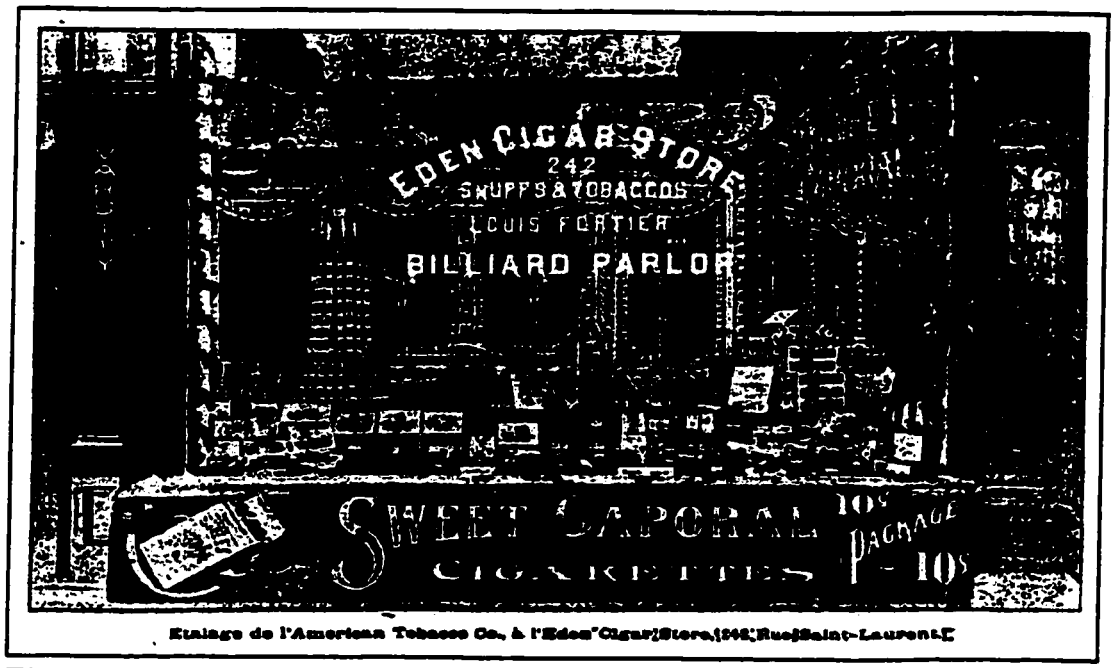
[90] "Montreal Correspondence," *CCTJ*, July 1901, p.319. He also visited the Toronto Exhibition, see *CCTJ*, September 1901, p.449.

[91]On the scarcity of cars in Montreal at the turn of the century, see Denis Veilleux, "La motorisation, ou, 'La rançon du progrès': tramways, véhicules-moteurs et circulation," (Ph.D. Dissertation: McGill University, 1998).

[92]*Liqueurs et Tabac*, January 1902, p.28.

both the Canadian Pacific Railway (CPR) terminus and Windsor Station.[93] Even when not using electricity, the ATCC chose places that guaranteed a captive audience. The company put up a huge Sweet Caporal sign in neighbouring Pointe Claire next to the CPR and Grand Trunk Railway tracks, difficult for passengers to miss.[94]

Another major venue for ATCC advertising, and their Sweet Caporal and Murad brands in particular, was in window displays. The ATCC hired artists to decorate several of their clients' window displays around Montreal. These displays did much to legitimize the changes in marketing and products that came with industrial capitalism, and in the case of the cigarette, sought to make them more acceptable as ways to smoke tobacco. Take for example Louis Fortier's Eden Cigar Store display on St. Laurent Boulevard (figure 4).



Etalage de l'American Tobacco Co. à l'Eden Cigar Store, 242, Rue Saint-Laurent.

Figure 4

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[93]*CCTJ*, June 1913, p.41.

[94]*Ibid*, April 1909, p.53.

In the centre of the display, what immediately attracts the eye, is a boat decorated with Sweet Caporal cigarette boxes arriving in a port that is constructed with Sweet Caporal cigarette boxes. The "Sweet Caporal Girl" can also be seen in the background. The repetition of the logo was important in making newer products more familiar as well as invoking the theme of abundance. The boat coming into port also underlines that the cigarette can be part of traditional commercial and Imperial activities, particularly of Britain. Finally, the artist has foregrounded the Sweet Caporal advertising with cigar boxes, cigars not being sold by the ATCC, linking traditional smoking habits with the new cigarette.[95]

The ATCC also promoted their cigarettes and other products like pipe and chewing tobacco through "premiums" included in each package. Smokers collected coupons or "tags" and then turning them in to ATCC premium department in Montreal for particularly manly rewards like wristwatches, guns and tents.[96] The popularity of the "Coupon Habit" was reflected through its penetration into popular culture demonstrated by a cartoon from the Montreal *Star* (figure 5, see p.208). The cartoon gives little explanation of the coupon scheme, suggesting that it would be broadly understood.[97] The launch of the ATCC Premiums department was made into a popular spectacle with the ATCC putting on display the smallest man in the world. The same attention to display of

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[95]"Une Flanerie du Soir," *Liqueurs et Tabacs*, January 1902, p.42. My description here follows the line of argument set out in Keith Walden, "Speaking Modern."

[96]For a tag prize list, see *CCTJ*, August 1901, pp.175-390. It took 1200 tags to get a gun.

[97]"Coupon Habit," Montreal *Star*, 8 October 1910, p.18.

"exotic" human bodies, as the ATCC had used with Professor Brown, once again attracted an enormous crowd.[98]

Figure 5: The Coupon Habit.

Perhaps the cigarette's most controversial promotion was its cigarette card campaign. In 1904 the National Council of Women's committee on objectionable printed material appealed to the Mayor of Montreal to prohibit the display of cigarette pictures "offensive to public morals."[99] Indeed, during the Select Committee on the Cigarette in 1914, Owen Dawson claimed that these picture cards entice little boys to buy cigarettes, and what was worse, when asked if they were lewd, he responded, "More or less, and suggestive."[100]

These ATCC promotions and advertising made it increasingly costly for retailers to not enter into the consignment agreements. According to Montreal tobacconists, the barrage of ATCC advertising moved smokers to buy ATCC brands. In 1896, Emmanuel Balasco, for example, maintained that ATCC brands were "The best known brands... in the

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[98]*CCTJ*, April 1904, p.65.

[99]"National Council of Women," Montreal *Witness*, 26 September 1904, p.2.

[100]Dawson, p.50.

world" and because of this, if one had a tobacco store, it was necessary to keep their brands.[101] Similarly, Montreal tobacconists William L. Ross and Theotime Valiquette both felt they had to keep ATCC cigarettes because there was such a demand.[102]

V. Mass Consumption Contested

While ATCC advertising was powerful in the business world, it was not fully successful in assuring the public of the mass-produced cigarette's virtues. Cigarettes were singled out in the late nineteenth and early twentieth century as being more dangerous than other forms of tobacco. In Canada and the US these rumours were also rampant and date back at least to the 1870s.[103] US government tests exonerated the cigarette of these charges in 1892. In Canada the Ministry of Inland Revenue cleared the cigarette of these accusations in 1908.[104] Historian Ian Tyrrell has sought to explain similar allegations against the cigarette in Australia by arguing, with little proof, that these rumours were

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[101]Emanuel Belasco testimony, ATCC charged with conspiracy, 18 December 1896, p.1707.

[102]Testimonies of William L. Ross and Theotime Valiquette, ATCC charged with conspiracy, 18 December 1896.

[103]C. Cassandra Tate, *The American Anti-Cigarette Movement, 1880-1930* (Ph.D. Dissertation: University of Washington, 1995, pp.61-64. In the thesis, Tate goes as far as to say that these kinds of excesses were typical of the American anti-smoking movement and eventually were part of its demise.

[104]"Cigarette Trial Progresses," *CCTJ*, May 1914, p.9. The testimony is also reported in Ottawa *Citizen*, 21 April 1914, p.1 and *La Patrie*, 9 May 1914, p.4. "Notes and Comments," Montreal *Herald*,10 April 1908. p.4.

evidence of the effectiveness of that country's anti-cigarette movement.[105]

In Canada, and particularly in Montreal, Tyrrell's explanation holds little water. Certainly it is true that these rumours did appear from time to time in temperance literature circulating in the city. For example, the WCTU's "Catéchisme de Tempérance" wrote that cigarettes contained "L'opium, la fève de tonca qui contient un poison mortel... le rhum et plusieurs autres drogues nuisibles."[106] In her "President's Address" at the 1892 Quebec WCTU annual meeting, Quebec WCTU president Mary Sanderson linked these allegations to fears about foreigners. In trying to motivate her membership to campaign against the cigarette she invoked "The fearful condition of 40,000,000 of Chinese, who are slaves to the opium pipe, with its attendant evils," as being "surely sufficient to alarm us as to the probable consequences of the use of the deadly cigarette, which is said to contain opium...."[107] Indeed, according to historian Cassandra Tate, before World War I cigarettes were linked to American prejudices about foreigners. Such may have been the case in Canada. With early cigarettes known as "Egyptian" or "Oriental" cigarettes, made with Turkish tobacco, and the advertising using images of the near East, it is not a difficult logical leap to make to link views of "dirty foreigners" and stereotypes of the "oriental" to tobacco and opium habits.

In 1903, the WCTU changed their position on additives in cigarettes in response to

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[105]Tyrrell, *Dangerous Enemies: tobacco and its opponents in Australia* (Sydney: University of New South Wales Press, 1999), pp.130-131.

[106]"Catéchisme de Tempérance," p.15.

[107]Mary Sanderson, "President's Address," *9th Annual Report,* Quebec WCTU, October 1892, pp.30-31.

an ATCC attempt to deny the validity of these rumours. During the WCTU's 1903 anti-cigarette campaign, tobacco companies ran two page advertisements with the results of medical studies that vindicated the cigarette from charges of impurity, making the not-too-subtle argument that cigarettes were not harmful.[108] Appeals to purity of products were not terribly out of the ordinary for advertising at the time, though it was an early example of medical discourse being used by cigarette companies.[109] The ATCC frequently used the quote, "La forme la plus pure sous laquelle le tabac peut être fumé" from the British medical journal, *The Lancet* to advertise its Sweet Caporal Cigarettes.[110] The WCTU was outraged by the claim of the cigarette's healthiness and the women wrote the Montreal *Witness* to argue that additives were not the reason they were opposing the cigarette. Dominion WCTU President Annie O. Rutherford, Corresponding Secretary Annie M. Bascom and Dominion Anti-Narcotics Superintendent Jennie Waters wrote:

> Be it understood here and now that the Dominion Woman's Christian Temperance Union is bringing no charge of adulteration against the cigarette. They are not basing their complaint upon the make-up of cigarette wrappers, or the kind of flavorings or tinctures used in their manufacture. Their quarrel is with the cigarette as a cigarette.

As I showed last chapter, the WCTU leadership believed the dangers of the cigarette were the moral and physical effects of inhaling cigarette smoke rather than the result of

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[108]These two-page advertisements were not run in Montreal newspapers.

[109]On concerns of purity and adulteration in advertising, see Walden, "Speaking Modern." Purity was also an issue in discourses on race and sex during this era. See Mariana Valverde, *The Age of Light Soap and Water: Moral Reform in English Canada, 1885-1925* (Toronto: McClelland and Stewart, 1991).

[110]*La Presse*, 3 September 1910, p.8.

additives.[111]

Tyrrell's conclusion is even less tenable considering the widespread nature of these rumours. In 1914 *La Patrie* ran an editorial maintaining that there was a popular consensus on the fact that cigarettes contained additives: "Quant aux cigarettes, on s'accorde généralement à les condamner sous prétexte qu'elles contiennent de la morphine, ou de l'opium, ou d'autres substances narcotiques," yet the editorial did not promote cigarette prohibition.[112] The allegations were also spread by physicians. In 1920 Romeo R. Boucher, in a *Union médicale du Canada* article reported that cigarettes contain "arsenic, de la crésote, de l'opium, du salpêtre, du 'tonca flavoring' des traces de rhum et de nombreuses autres matières." He considered these substances, in combination with the nicotine, harmful, but when consumed moderately there was less of a danger.[113] The popular nature of these rumours suggests that they were less the result of the early claims of the WCTU, and more about the new popularity of the cigarette and its addictive nature. Considering the WCTU's marginal status among francophones, the fact that these examples are all in French also suggests that the rumours were not the result of WCTU success, but broader concerns about the visible effects of the cigarette.

Indeed, there were other allegations against the cigarette that suggest the root of the rumours was a popular response to the cigarette's new industrial qualities. Owen

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[111]For the letter, see the Montreal *Witness*, 26 March 1903, p.12; and for a similar editorial, see Montreal *Witness*, 28 March 1903, p.4.

[112]"Cinemas et cigarettes," *La Patrie*, 9 May 1914, p.4.

[113]Romeo B. Boucher, "Intoxication chronique par le tabac," *L'Union médicale du Canada*, March 1920, p.134.

Dawson, the Clerk of the Montreal Juvenile Court told the Select Committee on Cigarettes that he believed the cheaper kinds of cigarettes were made of "guttersnipes" or the leftover tobacco in used cigarettes. John Bradford of the Montreal YMCA told Dawson that he had seen boys in the US get paid fifty cents to collect cigarette butts from the streets. The butts would then be ground up and put into cheap cigarettes.[114] Even some of the American innovators behind the Bonsack cigarette machine that revolutionized cigarette production worried that hand rolling was so important to the cigarette consumer that there would be a strong reaction to machine-made cigarettes.[115] If the cigarette was suddenly cheaper than it had been, what was the reason? No information was given on packages nor in advertising explaining why this might have been. Indeed, by not appealing to bourgeois values of connoisseurship, the ATCC left its cigarettes open to questions.

Both the cigarette and Canadian tobacco went through significant changes in symbolic association in the years immediately before the First World War. The Canadian government and the ATCC promoted the industrial transformation of Canadian tobacco in the fields and tobacco farmers found a ready, but monopolized market. They did what bourgeois connoisseurs said could not be done: they erased the stigma of smoking Canadian tobacco. But in the end, this was not the same *tabac canadien* that was traditionally smoked by the habitant. It had been standardized and had lost its regional

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[114]Proceedings," p.50.

[115]P.G. Porter, "Origins of the American Tobacco Company," *Business History Review* (Spring 1969), pp.59-76.

distinctiveness. In the case of the cigarette, industrial transformations in the factory had immense consequences how they would be understood. Like cigars, and to an lesser extent pipe tobacco, the cigarette had been judged within the cultural categories of bourgeois connoisseurs, yet their diminutive size, their association with European women and the length of time it took to smoke the cigarette made them less manly. What is more, some women who smoked in Montreal used the habit to distinguish themselves, to make statements opposing traditional beliefs that limited women's public role in society-- beliefs that ascribed women a passive role in sexual relations. With the application of Bonsack cigarette machine cigarettes became less expensive. Their cultural symbolism of being somewhat less than manly was transformed by new beliefs about speed and masculinity into positive symbolism of youthful masculine vigour. At the head of the transformation in Canadian tobacco and the cigarette, shaping cultural meanings, was the ATTC using its consignment system and its advertisements to legitimate the new categories of culture upon which both these products were based. And despite the power and financial resources of the forces promoting these values, the legitimation of these new cultural values was still contested in the pre-War era.

Conclusion

The construction of the late nineteenth-century liberal individual, submits Ian McKay, "was not the work of an idle week to 'normalize' the laws of liberal political economy and society."[1] It clearly was not a question of pointing to all living human beings as self-evident political subjects. Nor was it only a victory of politicians and businessmen over a group of priests and their followers. It was a complex and contested process in which people internalized notions of inclusion, exclusion and hierarchy that shaped how they saw themselves and others. This dissertation has argued that from 1888 until the First World War dominant prescription around smoking, like few other consumption rituals, were part of this process of legitimation. In the face of tobacco's addictive nature, individuals were to perform liberal ideals of self-control and rationality through rituals of smoking in a broadly defined public sphere. And even though these prescriptions were being supplanted by an emergent code of smoking conduct, these new rules were also symbolic statements of how individuals wished to transform the liberal order, not destroy it.

As with the nineteenth-century liberal "individual," gender, class and race played key, but often different, roles in the way the smoker was constructed. Masculine identities were to be formed around ideals of self-control and culturally specific rationality. Male smokers dramatized these values through their purchasing and smoking of tobacco. Smoking set the tone and boundaries of the male public sphere where high-minded

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[1]McKay, "The Liberal Order Framework," p.630.

communication was idealized. Women, according to nineteenth-century liberal prescriptions, were biologically incapable of either. Nor could they enter into this sphere without putting their reputations into jeopardy. Class no longer excluded a man from smoking, but it did present him with material barriers to achieving the ideals of self-control and rationality. He may not have been able to afford to make a rational purchase of a tobacco which bourgeois connoisseurs constructed as superior. He also may not have had the time to leisurely smoke in a homosocial environment, an act which was interpreted as a failure in self-control. Because these codes of conduct were not universal, smokers from other cultures when in Montreal broke local rules and saw the consequences in how their character was understood. Indeed, transgressing local prescriptions around smoking served in the construction of gender and race-based notions of incivility. Both material failures to perform liberal values and transgressions rooted in cultural difference served to justify the subordination and domination of entire races and classes.

In the past, too often the Roman Catholic church has been presented in opposition to the liberal order.[2] Yet clearly, by the turn of the century, there were moments of alliance, if indeed these were, in fact, two separate entities. Indeed, Fernande Roy has argued that French-Canadian businessmen were both liberal and Roman Catholic – they simply believed that the Church did not have a direct role in managing the economy or the state.[3] On the question of the prohibition of the cigarette, individual and property rights

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[2]Brian Young discusses the historical caricature of French Canadians in his *Politics of Codification: The Lower Canadian Civil Code of 1866* (Montreal: McGill-Queen's University Press, 1994), pp.173-190.

[3]Roy, *Progrès, Harmonie, Liberté*, pp.260-268.

were at stake for liberals, but for the Church its role in the moral formation of individuals was being put into question. Their interests converged in opposition to social-gospel collectivism. The 1908 age restriction legislation was a symbolic victory for the WCTU, winning a place for the state in shaping the moral decisions of children. The 1908 law was not a case of the state extinguishing rights, like prohibition was. Rather the state could play an auxiliary role in parenting and Roman Catholics were less opposed to such a notion considering they were also concerned over the degenerative effects of children smoking on the future of their "race." In Montreal, the local representatives of the state, particularly the police, had little interest in playing this role, underlining the point that the construction of the liberal order was a slow process and declarations in a legislature did not ensure local action.[4]

It is also important to note that while these liberal prescriptions were dominant, others used smoking symbolically to different ends. French-Canadian men smoked *le tabac canadien* to make declarations of allegiance to a particularly rural French-Canadian nation. Prostitutes and dandies used smoking to create feminine and masculine identities outside of dominant norms. "New Women" also challenge the masculine exclusivity of smoking rituals, symbolically calling for an expansion of the liberal order to include women as "individuals."

These prescriptions, particularly norms of taste, shaped the nature of industrialization in Montreal and the encroachment of capitalism in the countryside. The

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[4]This point is made for an earlier period in Alan Greer and Ian Radforth, eds. *Colonial Leviathan: State Formation in Mid-Nineteenth-Century Canada* (Toronto: University of Toronto Press, 1992).

cigar industry stands as a case in point. Because male skilled labour was valued in a cigar, only men were hired to roll the most valuable cigars. The importance of male skilled labour also shaped labour-capital relations. Unions had greater strength in dealing with cigar manufacturers because their skill was valued by consumers. An entire industry producing Canadian-made Cuban cigars was born because Cuban tobacco and Spanish workmanship was valued by consumers. Furthermore, notions of standardization of taste embraced by the ATCC transformed the methods by which tobacco was grown in rural Quebec. Conversely, industrial technology and culture profoundly effected smoking rituals. Cigarettes became cheaper and, what is more, they became more widely accepted as industrialization transformed notions of speed, thus undermining the ideal of the leisurely, self-controlled smoke.

Establishing smoking codes of conduct as dominant as well as introducing a new set of norms of taste and etiquette were processes characterized both by conflict and consent. Legitimization processes involved many actors – businesses, organized labour, federal governments of different political stripes, etiquette columnists, farmers, churches, doctors, cartoonists, novelists, painters, and poets – all played parts. Clearly, tobacco companies did not entirely control the symbolism of smoking as was demonstrated by not only those who chose not to follow liberal prescriptions, but also by the anxieties about additives in mass-produced cigarette.

Yet it is important to recognize that this was no consumer democracy, where rituals of resistance were staged through an infinity of smoking rituals. Some groups had more power than others to establish their norms of taste and etiquette as dominant.

Tobacco manufacturers, in particular, held great power on issues of taste. For the most part, they controlled what tobacco was available to smokers. When Sir William Macdonald monopolized the tobacco industry through his agreement with the Dominion Wholesale Grocer's Guild, he did more than push his competitors out of business – he popularized bourgeois hierarchies of taste by refusing to use Canadian tobacco in his products. Mortimer Davis and the ATCC took a larger role in the production of meaning of smoking that went beyond nineteenth-century liberal prescriptions. Not only did the ATCC use enormous amounts of advertising to try to stabilize and control the meaning of their products, their consignment system made their competitors' advertisements almost useless. Indeed, the ATCC monopolized the production of cigarettes and, among cigarette manufacturers, it also dominated the production of meaning of these new mass-produced products. The federal government also played a pivotal role in changing the taste of Canadian tobacco through its tariffs and agricultural education programs. It also quietly supported the ATTC's monopoly over cigarettes and Canadian tobacco because it refused to rule against the ATCC hold on the industry. Indeed, tobacco manufacturers and the state had powerful methods to structure the purchasing choices smokers could make.

While they could go to great lengths to structure the specific hierarchies of taste, successful tobacco businesses followed and promoted cultural currents already flowing through Montreal society. Pre-First World War Montreal saw the height of particularly liberal prescriptions that structured the ritual of smoking, and, in turn, served to normalize the exclusion of women from the definition of the liberal individual and to justify the subordination of the poor and cultural minorities. Yet despite its dominance, the cultural

logic of the nineteenth-century liberal order was beginning to be undermined. While the more significant evidence of the cultural transformation of the liberal order would arrive later in Montreal with, for example, the appearance of women more publicly in the workforce, the popularity of the cigarette and the expansion of mass consumption more generally, the origins of this transformation date to the pre-First World War era.

To conclude, we can harken back to the 1905 advertisement placed by the ATCC in *La Journal de Françoise*. According to nineteenth-century liberal prescriptions, the advertisement was in bad taste, promoting feminine smoking, yet three years previous the fashion editor of the *Journal* began signing her name "cigarette." Clearly, for some the cigarette signified change, not just in fashion, but towards all that was modern. The fact that this was an advertisement is fitting as this transformation in the liberal order had little to do with revolution. It was a transformation in hegemonic language. The advertisement's slogan, "fumées universellement" highlights the point. It shed the language of social distinction that previously had dominated tobacco advertisements. Published in a women's journal, it trumpets a new universality – an ungendered and less class-contemptuous vision of the liberal individual. Yet the cigarette, in the end, was a low cost consumer good, and though women and the working class began to be targeted as consumers, the gender, class and racial inequalities of society were undisturbed. Instead, this new language of rule would obscure these inequalities, and in the case of cigarettes, the health consequences of this shallow democratization added to the effects of social pacification sought through mass consumer capitalism more generally.

Bibliographic Note

Included in this bibliography are sources that I have used in the thesis. It does not include the numerous sources I have consulted that did not bear fruit. This is especially true of the literary sources I surveyed. My methodology with these sources began with literary works, like newspapers, which had the largest circulations. I established this through consulting Maurice Lemire (Dir) *Dictionnaire des oeuvres littéraires du Québec*, Volumes I and II and André Beaulieu and Jean Hamelin, *La Presse Québécoise*, Volume I to IV. I added to this as many works as possible by female authors since women were vastly under represented within the original group of popular authors.

I also want to acknowledge the importance of the increasing number of databases that allowed me to find many of these sources. Indexes that would have taken months to peruse became accessible in minutes. I realize that these must be used with care and they do not assure that all relevant sources within that record group were consulted. Yet neither did the paper indexes upon which these databases were based. Regardless, my principle methodology, as I discussed in the introduction, did not rely upon these database findings.

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