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MINIREVIEW

Comparison of Packaging Strategy in Retroviruses and Pararetroviruses

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Reverse transcription is not solely a retroviral mechanism. Animal hepadnaviruses, plant caulimoviruses, and badnaviruses have a RNA intermediate which is reverse transcribed into double-stranded DNA genome. Based on this fact, these three groups of viruses have been regrouped under the name of pararetroviruses. Although each one has developed its own strategy to assure an efficient packaging of their genome, it is clear that they have adopted a strategy where encapsidation prepares for initiation of reverse transcription. This is discussed in this review. © 1995 Academic Press, Inc.

GENOME ORGANIZATION OF RETROVIRUSES AND PARARETROVIRUSES

The Retroviridae family is constituted of three groups: oncoviruses, spumaviruses, and lentiviruses. Retroviruses are very diversified as they multiply in birds, mammals (including humans), and reptiles. They are capable of provoking cancers as well as asymptomatic infections. They are enveloped icosahedral viruses with a diameter of 100–140 nm. Their genome is a single-stranded, 8- to 11-kb linear RNA, capped, polyadenylated, and of positive sense. Despite their diversity, retroviruses present a genomic organization with a certain uniformity with the *gag, pol,* and *env* genes, respectively, coding for the structural proteins of the virion, the viral replication enzymes, and the envelope glycoproteins. While the *env* gene is translated from a spliced mRNA, the *gag* and *pol* genes are translated from the genomic RNA. The expression of *pol* requires the synthesis of a Gag-Pol polyprotein either by read-through of a termination codon or by ribosomal frameshifting in the −1 direction (Jacks *et al.,* 1988), Gag-Pol precursor representing 5–10% of Gag product translation. This precursor is then incorporated into the virion and processed into the essential viral enzymes: the aspartic proteinase, the reverse transcriptase/RNase H, and the integrase, in addition to Gag products.

Hepadnaviruses belong to a group of hepatotropic DNA viruses (Blum *et al.,* 1989), which includes hepatitis B virus (HBV) and the hepatitis viruses of the woodchuck, ground squirrel, Peking duck (DHBV), and heron. The hepatitis B virion consists of an enveloped nucleocapsid of 40 nm diameter. The envelope is made of the viral surface glycoproteins and the capsid is arranged with icosahedral symmetry ($T = 3$ and $T = 4$, 180 and 240 protein subunits; Crowther *et al.,* 1994). The genome is a small (about 3.2 kbp), circular, partly double-stranded DNA molecule with a single-stranded DNA region of variable length, ranging from 50 to 100% of the complementary strand length. The genome of the hepadnaviruses possesses three genes: the C gene (encoding the core protein), the P gene (the P protein), and the S gene (the glycoproteins of the viral envelope). The genome of the mammalian hepadnaviruses as the HBV contains a fourth gene, the X gene, which is absent in the genome of the avian hepadnaviruses.

Caulimoviruses constitute a group of DNA plant viruses which infect dicotyledonous hosts. The most studied virus among the caulimoviruses is the cauliflower mosaic virus (CaMV) which principally infects members of the Cruciferae (Pfeiffer and Mesnard, 1995). The CaMV is an icosahedral virus (420 subunits arranged with $T = 7$; Cheng *et al.,* 1992) with a diameter of 50 nm. The genome is a double-stranded, circular, and relaxed DNA of about 8 kbp. It contains single-stranded interruptions the number of which varies according to the studied strain. The CaMV genome encodes six polypeptides. While the genes I and II encode proteins specific for plant viruses, the genes III and IV code for the structural proteins of the virion and the gene V for the polymerase which is processed in the aspartic proteinase and the reverse transcriptase. Last, caulimoviruses have no envelope but within the infected cells the viral particles are embedded in the viroplasms. The main constituant of these cytoplasmic inclusion bodies is the viral protein

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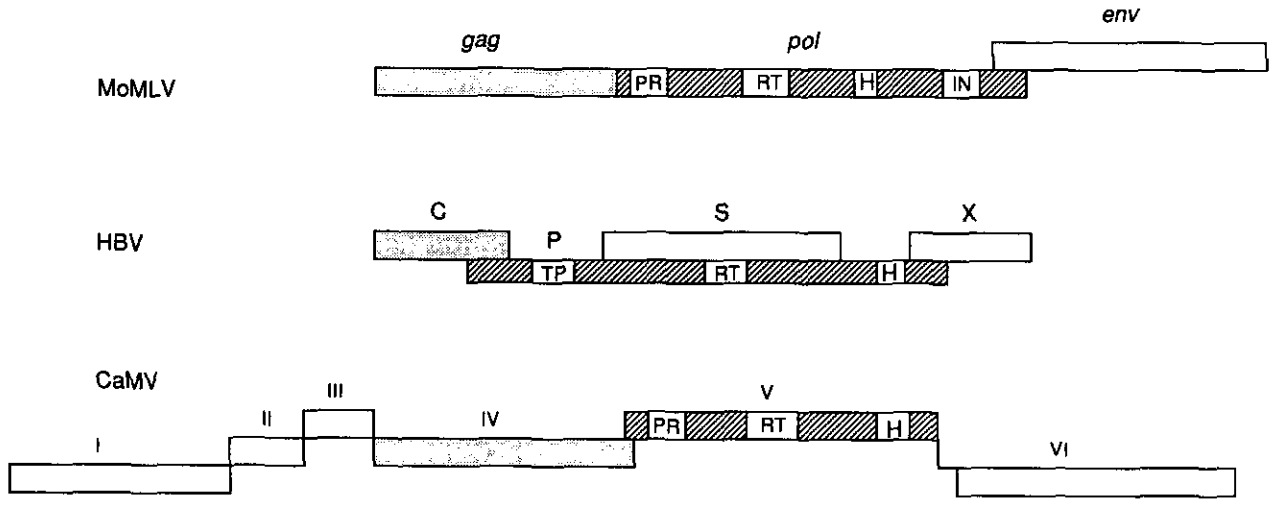

FIG. 1. Comparison of the genomes of a retrovirus (Moloney murine leukemia virus or MoMLV), a hepadnavirus (hepatitis B virus or HBV), and a caulimovirus (cauliflower mosaic virus or CaMV). H, RNase H; IN, integrase; PR, aspartic proteinase; RT, reverse transcriptase; TP, primer protein domain in analogy to the DNA terminal proteins of adenoviruses and bacteriophage ϕ29 (Salas, 1991).

encoded by the gene VI which is involved in the virus–host interaction.

Although the genome of the pararetroviruses is a circular double-stranded DNA, the distribution of the genetic information in the genome is similar to that of the retroviruses (see Fig. 1). The hepadnavirus counterparts of *gag* and *pol* genes are, respectively, the C and P genes; for the caulimoviruses, it is the genes IV and V. However, contrary to retroviral Pol, translation of HBV and CaMV polymerases is not dependent upon that of the capsid protein (Chang *et al.*, 1989; Schlicht *et al.*, 1989a; Schultze *et al.*, 1990; Wurch *et al.*, 1991). Comparison of polymerases encoded by retroviruses, hepadnaviruses, and caulimoviruses indicates: (i) the presence of common domains for reverse transcriptase and RNase H activities, (ii) the absence of an aspartic proteinase domain in the hepadnaviral P protein, (iii) and the absence of an integrase domain in the pararetroviral polymerases. Moreover, the hepadnaviral P protein contains a specific N-terminal domain also called primer protein (Wang and Seeger, 1992; Weber *et al.*, 1994), which is involved in the initiation of the pregenomic RNA reverse transcription. On the other hand, the primer necessary to initiate the caulimoviral reverse transcription is a $tRNA^{Met}$, the caulimoviruses having in common with the retroviruses the use of a tRNA to start the synthesis of the cDNA. Among plant viruses, the bacilliform badnaviruses belong also to the pararetrovirus group. Their double-stranded DNA genome contains an open reading frame encoding a polyprotein which is processed to yield the capsid protein, the aspartic proteinase, the reverse transcriptase/RNase H (Laco and Beachy, 1994). As nothing is known about packaging of their genome, the case of the badnaviruses is not discussed in this review.

PACKAGING OF THE RETROVIRAL AND PARARETROVIRAL GENOMES

The Gag proteins are assembled into an immature capsid by two different pathways depending on the retrovirus. In B- and D-type retroviruses, the immature capsid is preassembled within the cytoplasm and then transported to the cell membrane where the viral particle buds. In C-type retrovirus, the Gag precursor is transported to the cytoplasmic face of the plasma membrane and the immature capsid is formed concomitantly with the budding process. In both cases, as the virion buds from the cell, the Gag and Gag-Pol proteins contained in these immature particles are cleaved by the viral proteinase. Proteolytic processing of the Gag precursor generates three main structural proteins: the matrix protein (MA), the capsid protein (CA), and the nucleocapsid protein (NC). They are always linked in this order but are often interspersed by additional domains of unknown function. Likewise, the position of each of the three major structural proteins is the same in all retrovirus particles. The MA protein forms the membrane associated protein layer whereas the CA protein is involved in the structure of the viral core. The core is organized as a cone-shaped or an isometric body, and it contains two copies of genomic retroviral RNA which are coated by the NC protein. The reverse transcriptase/RNase H, the integrase, and the tRNA primer of reverse transcription are also found within the core.

During retrovirus assembly, two identical genomic RNA molecules are incorporated into the virion by a highly efficient and selective process. The viral RNA contains at its 5′ untranslated region a sequence element, termed ψ, that identifies the viral genome to be incorporated into the particle (Mann *et al.*, 1983; Watanabe and Temin, 1983; Katz *et al.*, 1986; Lever *et al.*, 1989). In many

cases ψ is not sufficient and other signals are implicated in the encapsidation process, suggesting that more than one sequence may be recognized during packaging (Linial and Miller, 1990). In most retroviruses, ψ is coincident with the dimer linkage site, a sequence that mediates stable and noncovalent intermolecular linkage of two identical RNA monomers (Darlix *et al.*, 1990; Awang and Sen, 1993). However, the possible functional relationship between RNA dimerization and packaging has not been demonstrated yet. RNA dimerization initiates early in the course of encapsidation and the RNA dimer undergoes additional maturation or stabilization event subsequent to virion release from the cell and processing of the Gag precursor (Fu and Rein, 1993; Fu *et al.*, 1994).

The NC domain of the Gag precursor is involved in the selection of the viral RNA from the pool of cellular RNAs and in its encapsidation into the virion by specific interaction with the viral RNA (Dupraz and Spahr, 1992). The NC protein is highly basic and characterized by the presence of one or two Cys-X_2-Cys-X_4-His-X_4-Cys motifs (Covey, 1986), called the cysteine–histidine box, which is involved in a zinc finger-like structure. The basic residues and the zinc fingers cooperate to interact with the genomic RNA (De Rocquigny *et al.*, 1992; Dorfman *et al.*, 1993). For spumaviruses, the Gag polyprotein does not contain any cysteine–histidine box but a strongly basic domain which most likely binds the viral RNA. After budding and proteolytic processing of the Gag polyprotein, the NC protein is likely involved in conversion of the RNA dimer from immature to mature form (Fu and Rein, 1993) and binds to the genomic RNA to form a chromatin-like ribonucleoprotein complex. While the functional significance of NC is well established, the role of others Gag domains in RNA packaging is unclear. It has been proposed that the MA domain could directly (Katoh *et al.*, 1991) or indirectly (Chazal *et al.*, 1994) influence RNA packaging. Beside encapsidation, the NC protein also (i) plays an essential role during retroviral replication (see the next paragraph), (ii) is able to bind double-stranded DNA *in vitro* (Lapadat-Tapolsky *et al.*, 1993), and (iii) is involved in the Gag polyprotein multimerization (Franke *et al.*, 1994; Carrière *et al.*, 1995).

The retroviral mechanism of nucleocapsid assembly differs strikingly from that of hepadnavirus. Whereas the retroviral genomic RNA packaging can occur in the absence of the Pol protein, in hepadnaviral packaging encapsidation of polymerase and genomic RNA are tightly coupled (Bartenschlager *et al.*, 1990; Hirsch *et al.*, 1990). Hepadnaviral assembly is initiated by the direct binding of the P protein to the ϵ sequence (Bartenschlager and Schaller, 1992), which is located near the 5′-end of the pregenomic RNA (Junker-Niepman *et al.*, 1990; Pollack and Ganem, 1993). For encapsidation of the DHBV genomic RNA, two regions have been characterized: a sequence similar to HBV ϵ and one nonadjacent region apparently not involved in packaging of the HBV genomic RNA (Calvert and Summers, 1994). The RNA-P protein complex could then be stabilized by the core protein and addition of further core protein subunits produces a particule capable of initiating reverse transcription for synthesizing the genomic double-stranded DNA. However, there is to date no information on the specific binding of the core protein to the P protein or a RNA–protein complex.

The HBV core protein consists of two domains: a domain from the N-terminus to about amino acid position 144, which directs the assembly of the capsid, and a C-terminal domain of about 40 amino acids, which binds nucleic acid through a protamine region (Gallina *et al.*, 1989; Birnbaum and Nassal, 1990). This region has no cysteine–histidine boxes but contains three overlapping SPRR motifs also found in some proline-rich DNA binding proteins. The C-terminal domain of the core protein is initially involved in packaging the RNA; subsequently during genome replication, it is likely involved in condensation of the genomic DNA into a more compact form to have an efficient encapsidation mechanism (Hatton *et al.*, 1992; Nassal, 1992). The influence of the C-terminal domain of the DHBV core protein has also been analyzed (Schlicht *et al.*, 1989b; Yu and Summers, 1991). Although the C-terminus of the DHBV polypeptide is relatively different, it is also required for production of virions containing matured viral genome.

On the other hand the exact mechanism of genome packaging in caulimovirus remain still unclear. As for the retroviral Gag polyprotein, the CaMV capsid precursor is also processed by the viral aspartic proteinase (Torruella *et al.*, 1989). After processing, only the central domain of the protein is found associated with the virion. The acidic N- and C-terminal portions are not detected within the viral particle (Martinez-Izquierdo and Hohn, 1987; Kirchherr *et al.*, 1988). The different steps of this processing remain unclear for the moment. The central domain contains a basic region flanking a Cys-X_2-Cys-X_4-His-X_4-Cys motif (Covey, 1986), which is essential for infectivity and could be involved in packaging of viral RNA (Scholthof *et al.*, 1993). In addition to the capsid protein, another polypeptide coded by the gene III has been characterized within the viral particle (Giband *et al.*, 1986; Dautel *et al.*, 1994). This protein is a non-sequence-specific DNA binding protein (Mesnard *et al.*, 1990) that acts through a C-terminal domain at the level of a proline-rich motif (Mougeot *et al.*, 1993), which is found in DNA-binding proteins such as histones. In a model in which caulimovirus replication and assembly are coupled (see the next paragraph), the capsid protein would control the reverse transcription step, whereas the gene III product would participate in packaging of the newly synthesized double-stranded DNA as suggested for the C-terminal domain of the hedpanaviral core protein.

RNA PACKAGING AND REVERSE TRANSCRIPTION

Although retrovirus and pararetrovirus particles contain viral genomes with different aspects, we propose

that RNA packaging could be considered as a common step to initiate reverse transcription. For hepadnaviruses, it is well known that reverse transcription takes place in the viral particle (Summers and Mason, 1982) and recent studies suggest that the ϵ sequence is not only involved in RNA packaging but also serves as template for the initiation of viral DNA synthesis (Wang and Seeger, 1993; Tavis *et al.*, 1994). The hairpin structure in the ϵ sequence is essential to both functions since all mutations in the ϵ stem-loop that block the interaction between genomic RNA and P protein affect both RNA packaging and reverse transcription (Pollack and Ganem, 1994; Fallows and Goff, 1995). From these results, it is obvious that encapsidation and initiation of reverse transcription are coupled events. After its formation, the RNA–P protein complex could be recognized by C protein subunits to form the viral particle. In this model, the reverse transcriptase is encapsidated in the viral particle owing to a ϵ–P protein interaction.

Although retroviral RNA is reverse transcribed into double-stranded DNA within the cytoplasm of infected cells, recently it has been claimed that reverse transcription could be initiated also in virion-derived particles (Zhang *et al.*, 1995). However, such a mechanism differs from the analogous event in hepadnaviral reverse transcription where RNA packaging and initiation of DNA synthesis is mediated by the P protein. In contrast to hepadnaviral encapsidation, packaging of retrovirus is controled by the NC domain of the Gag polyprotein as already mentioned. In addition, the NC protein also plays an essential role during retroviral replication by increasing RNA dimerization, by facilitating the binding of the tRNA primer to the primer binding site and by enhancing the strand transfer during reverse transcription (Allain *et al.*, 1994). The retroviral polymerase is incorporated into the capsid through interactions between the Gag domain of the Gag-Pol precursor and other Gag subunits.

Concerning caulimoviruses, there is to date no direct demonstration that the CaMV capsid would check the initiation of reverse transcription. However, different results suggest the involvement of a ribonucleocapsid in the caulimovirus reverse transcription: (i) the CaMV coat protein binds to viral RNA (Fuetterer and Hohn, 1987), (ii) virion-like particles containing putative replication intermediates sediment with CaMV virions on sucrose density gradients (Marsh *et al.*, 1985), and (iii) particles which band on CsCl gradients at a slightly lower density than mature virions are immunoprecipitated with anti-CaMV coat protein and contain minus-strand DNA hybridized to RNA (Marsh and Guilfoyle, 1987). Based on these results, we suggest that the reaction which leads to the priming for reverse transcription employs RNA sequences present in or next to a RNA-packaging signal which could be specifically recognized by the zinc finger domain of the capsid protein. Following its interaction with RNA, the capsid protein itself could facilitate the binding of the tRNA primer to PBS as described for retroviruses, and stimulate the initiation of the DNA synthesis. The caulimoviral reverse transcriptase would be trapped in the viroplasm where encapsidation would occur (Mesnard and Lebeurier, 1991). To form the viral particle, the RNA–capsid protein complex could be recognized by assembling capsid protein subunits via noncovalent interactions. Such interactions could be direct or mediated by the viroplasmic protein. In conclusion, we propose that reverse transcription does not occur before packaging (no free DNA is directly packaged into virions) but replication and assembly are coupled as described for hepadnaviruses.

It is not so surprising that RNA packaging could be considered as a necessary event for reverse transcription. This step allows (i) the separation of the pregenomic RNA from the pool of cellular RNAs, (ii) the protection of the RNA from cellular RNases during reverse transcription, (iii) the concentration of all the molecules involved in reverse transcription and thus to stimulate the different interactions between the template, the primer, and the enzymes, and finally (iv) to improve yield of viral reverse transcription.

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