# Retroviral Replication

Secondary article

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Retroviruses are enveloped animal RNA viruses that replicate via a DNA intermediate which is integrated into host cell chromosomal DNA. Expression of viral RNA and proteins from the DNA intermediate utilizes the normal transcription and translation machinery of the host.

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## Introduction

The retroviruses (the taxonomic family *Retroviridae*) comprise isolates of vertebrates, but elements with hallmarks of retroviruses are also found elsewhere, e.g. in insects. Retroviruses exemplify a variety of specialized interactions with their hosts, including induction of many kinds of diseases under laboratory conditions as well as in domestic and wild animals. In contrast to this variation in biological properties, all retroviruses share the same basic genomic organization and a mode of replication by which the ribonucleic acid (RNA) genome of viral particles is reverse transcribed into a deoxyribonucleic acid (DNA) form, the provirus, in the infected cell. Virus particles have a diameter of about 100 nm; a lipid envelope surrounds a core of structural and catalytic proteins, including the enzymes reverse transcriptase (RT) that copies the RNA genome into double-stranded DNA and integrase (IN) that catalyses integration of the double-stranded viral DNA into host chromosomal DNA (**Figure 1a**). During the past few decades retroviruses have received more scientific attention than any other group of animal viruses. While basic genome organization and replication mechanisms were defined in model viruses such as *Rous sarcoma virus* and *Moloney murine leukemia virus*, today the most intensively studied retrovirus is the human pathogen, the *Human immunodeficiency virus type 1* (HIV-1).

## Genome Structure

Retroviruses are plus-strand RNA viruses, i.e. the single-stranded RNA genome in virions represents the coding strand corresponding to messenger RNA (mRNA) (**Figure 1b**). Two copies of this plus-strand RNA are found in the virions (**Figure 1a**). Similar to a typical eukaryotic mRNA, the retroviral RNA genome harbours an m7G5′ppp5′Gm cap group at its 5′ end and a poly(A) tail at its 3′ end. The retroviral genome is organized with internally located coding regions, flanked by 5′ and 3′ untranslated regions (including U5 and U3) which function in viral replication. The RNA genome is 7000–10 000 nucleotides long and includes a short repeated (R) sequence found at both termini. The double-stranded DNA genome of the provirus is longer than the RNA genome because of additional terminally repeated sequences (long terminal repeats (LTRs = U3-R-U5)) generated during reverse transcription (**Figure 1b**). The R region of the RNA genome is involved in strand transfer during reverse transcription, while the LTR of the DNA harbours signals for integration of the provirus into host DNA and its transcription into viral RNA. The primer binding site (PBS) in the 5′ part of the genome and the polypurine tract (PPT) in the 3′ part play key roles in reverse transcription. The Ψ-region in the 5′ part of the genome contains signals important for dimerization and selective incorporation of viral RNA into new virions.

All retroviruses contain *gag*, *pol* and *env* open reading frames (ORFs). *gag* (group specific antigen) encodes a polyprotein which is eventually cleaved to yield core proteins of the virus particle. Three *gag*-encoded proteins found in all retroviruses are the matrix protein (MA), the capsid protein (CA) and the nucleoprotein (NC). In some retroviruses, the Gag polyprotein is cleaved to form one or two additional viral core proteins. MA has been found to be the Gag protein in closest association with the membrane, and genetic and structural studies show that CA forms the core shell, the major internal structure of the virion. NC is a basic protein known to have RNA binding properties. Enzymes needed for viral replication, RT and IN, are encoded by *pol*. The retroviral protease (PR) is encoded in different types of retroviruses in either the 3′ part of the *gag* ORF, the 5′ part of the *pol* ORF or by an ORF between *gag* and *pol*. All three enzymes are synthesized as part of polyproteins including Gag and incorporated into particles before maturation by proteolytic cleavage. *env* encodes proteins of the viral envelope, the surface glycoprotein (SU) and the transmembrane (glyco) protein (TM), both made as part of one precursor glycoprotein.

Retroviruses with only *gag*-, *pol*- and *env*-encoded proteins are called simple retroviruses. However, additional proteins with other roles in viral replication or in virus–host interactions are found in some virus genera (such as *Spumavirus* and *Lentivirus*), collectively referred to as complex retroviruses (Cullen, 1992). The lentivirus

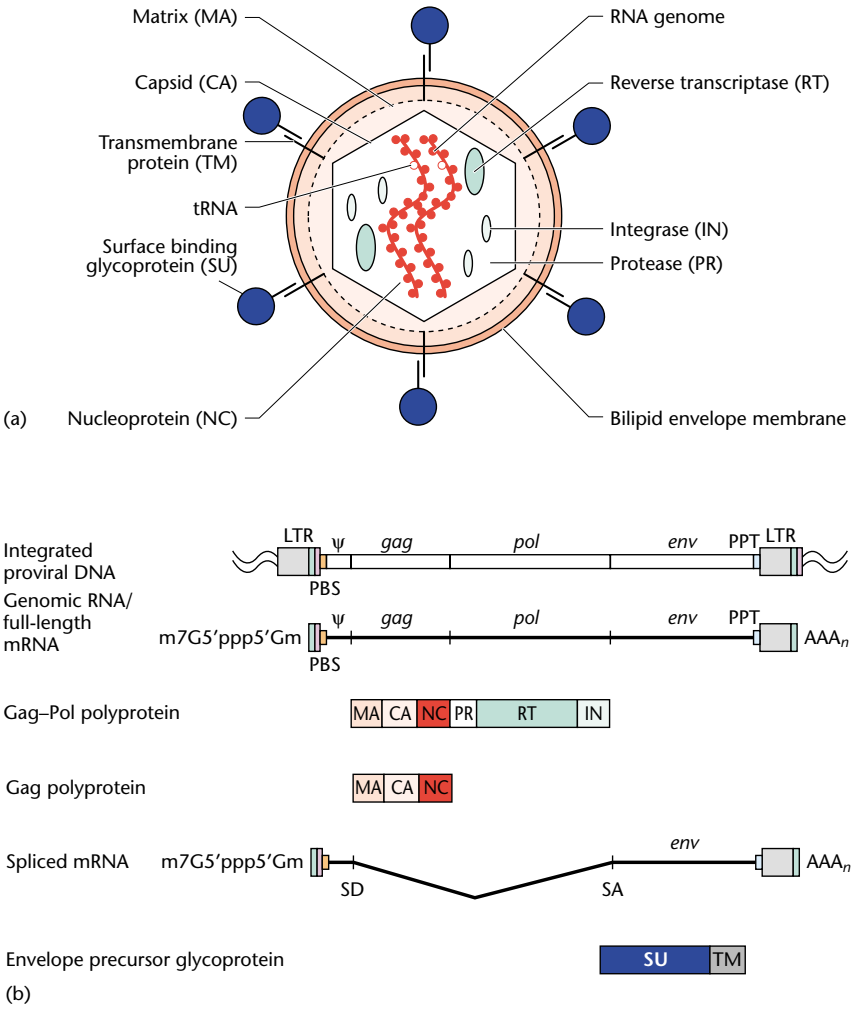


**Figure 1** Retroviral particle and genome structure. (a) Retrovirus particle showing the approximate location of its components using the standardized two-letter nomenclature for retroviral proteins. (b) Genome organization and gene expression pattern of a simple retrovirus, showing the structure of an integrated provirus linked to flanking host cellular DNA at the termini of its LTR sequences (U3-R-U5) and the full-length RNA that serves as genomic RNA and as mRNA for translation of the *gag* and *pol* ORFs into polyproteins. *env* mRNA is generated by splicing and encodes an Env precursor glycoprotein. LTR, long terminal repeat (U3-R-U5 for proviral DNA, derived from R-U5 downstream of 5′ cap and U3-R upstream of 3′ poly(A) in genome RNA); PBS, primer binding site; Ψ, packaging signal; PPT, polypurine tract; SD, splice donor site; SA, splice acceptor site.

HIV-1, for example, encodes six accessory proteins and has gene expression capabilities to generate additional variants of these.

The genus *Spumavirus* (Linial, 1999) has recently been found to lack many of the hallmarks of retroviral replication summarized here. For example, spumaviruses differ from other retroviruses in their morphogenesis pathway and in the functional organization of *gag*. They do not synthesize a Gag–Pol polyprotein but the *pol* gene product is made on a distinct spliced mRNA species. Infectious spumavirus particles appear to harbour DNA rather than RNA genomes, which, along with some of the other characteristics given above, provides some resemblance to *Hepadnaviridae*, another family of RT-containing viruses.

## Viral Entry

Retroviral entry (**Figure 2**) resembles that of other enveloped viruses, as studied in detail for influenza virus. The key steps are: (1) attachment of the virus particle to a specific receptor on the surface of a target cell; and (2) viral envelope:cell membrane fusion followed by internalization of the core of the particle into the cytoplasm. Identification of retrovirus receptors is currently an active field of

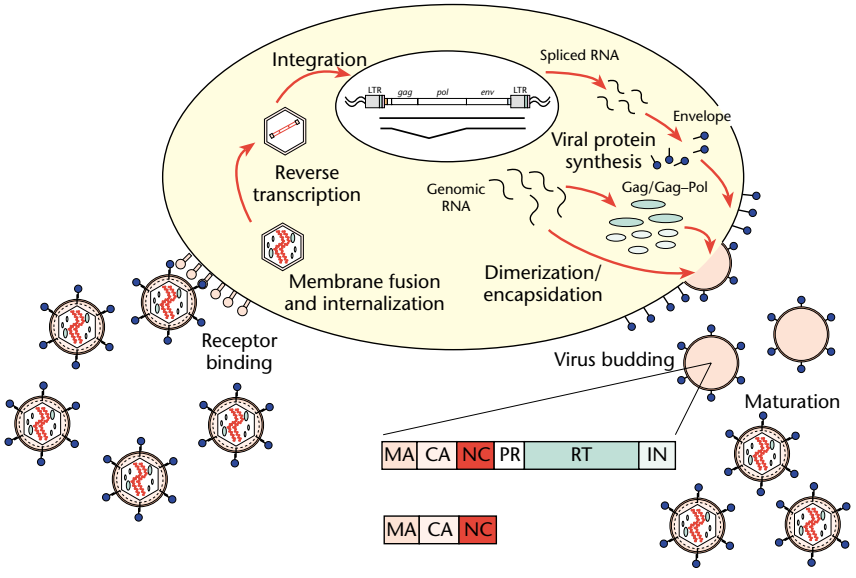


**Figure 2** Replication cycle of a simple retrovirus. The flow of the early part of the replication cycle goes from receptor binding and internalization at the left through reverse transcription to integration of the proviral DNA. The late part of the replication cycle proceeds from the provirus through transcription and processing and translation of viral RNA to assembly and release of viral particles. Maturation of the released particles involves cleavage of viral polyproteins by PR (protease).

research. Receptors are cell membrane proteins serving normal host functions of no apparent relevance to viral infection. The first receptor identified was that of HIV-1, which is CD4, a protein with a single transmembrane region and a large extracellular region. It is found on T-helper lymphocytes where it plays a role in signalling interaction with antigen-presenting cells. Later studies have shown that, in addition to CD4, HIV-1 infection depends upon the presence of coreceptors, which have been identified as various members of chemokine receptor families. Most of the retrovirus receptors known today have a predicted topology with minimal extracellular and intracellular domains and many transmembrane regions. Among these are the mouse ecotropic receptor, which has 14 putative transmembrane regions and functions as a transporter of basic amino acids (Albritton *et al*., 1989). Other receptors have been shown to function as phosphate transporters.

The species and tissue distribution of retrovirus receptors (and coreceptors) plays major roles in determining retroviral host range and tissue specificity. The receptor interacts with the SU protein on the viral envelope (Fass *et al*., 1997). Notably, closely related viruses may differ in receptor utilization. In this scenario, genetic interaction and phenotypic mixing by incorporation of heterologous envelope proteins into virions may serve to bypass host range barriers to infection.

Internalization of the core of the virion into the cell requires fusion of the membrane surrounding the virus particle and a membrane of the cell. Among retroviruses, fusion may take place directly at the outer cell membrane or at an endosomal membrane following endocytosis, where the latter fusion process takes place under relatively acidic conditions. The processes leading to fusion are poorly understood; however, a stretch of hydrophobic amino acids at the N-terminus of TM is believed to function as a fusion peptide invading the target membrane. In the overall process receptor-binding may be followed by a conformational change of viral Env proteins that brings the fusogenic domain of TM in close contact with the target membrane.

## Reverse Transcription and Integration

Retrovirus-specific enzymes (RT and IN) needed to complete the early part of the replication cycle leading to provirus integration are present in the virion and internalized in the target cell as part of the core particle. Hence, provirus establishment is independent of virus-specific gene expression in the target cell. The presence of RT in viral particles was crucial for its discovery in 1970 (Baltimore, 1970; Temin and Mitzutani, 1970) and for the subsequent unravelling of basic steps of reverse transcription of a retroviral genome. The net result of reverse transcription is the generation of a double-stranded DNA copy of the viral RNA; the DNA strand complementary to viral RNA is called the minus strand (or first strand), the other DNA strand the plus strand (or second strand). RT also harbours an essential ribonuclease H (RNAase H) activity that specifically cleaves RNA in RNA–DNA hybrids.

While detailed structural information on RT is available (Jacobo-Molina *et al*., 1993), the overall spatial organization of the nucleic acid–protein complex, in which reverse transcription takes place, is unknown. Known in some detail are the DNA synthesis steps of this complex process, which requires two consecutive strand-transfer reactions during minus-strand and plus-strand synthesis (**Figure 3a**). As with other DNA polymerases, RT requires a primer annealed to the template for initiation of DNA synthesis. The primer for minus-strand DNA synthesis is a producer cell-derived transfer RNA (tRNA) of which the 18 3′-terminal nucleotides are base paired to the PBS near the 5′ end of the RNA. After extension of the primer to the 5′ end of the template RNA, DNA synthesis is continued at the 3′ end of the viral RNA. This template shift from the 5′ to the 3′ end of the viral RNA (the first 'jump') is facilitated by the RNase H activity of RT and the terminally repeated R region sequences, and allows formation of a minus-strand DNA extending through the PBS. During minus-strand DNA synthesis, the RNA template is being digested by RNase H. A specific piece of the RNA template, the polypurine tract (PPT), is resistant to RNase H digestion and serves as a primer for the initiation of plus-strand DNA synthesis directed by the minus-strand DNA template. The PPT primer is extended through the minus-strand DNA and further into the 3′ part of the tRNA primer until RT stops at a modified nucleotide at the 19th position from the 3′ end of the tRNA. This DNA copy of 3′ sequences of tRNA is complementary to the minus-strand DNA copy of the PBS sequences of the viral RNA. This complementarity facilitates the plus-strand template shift (the second 'jump'), whereafter the minus-strands and plus-strands may be extended, resulting in a double-stranded linear DNA copy of the genomic RNA. Due to the initiation of synthesis of both strands at nonterminal positions, the resulting DNA copy is longer than the RNA

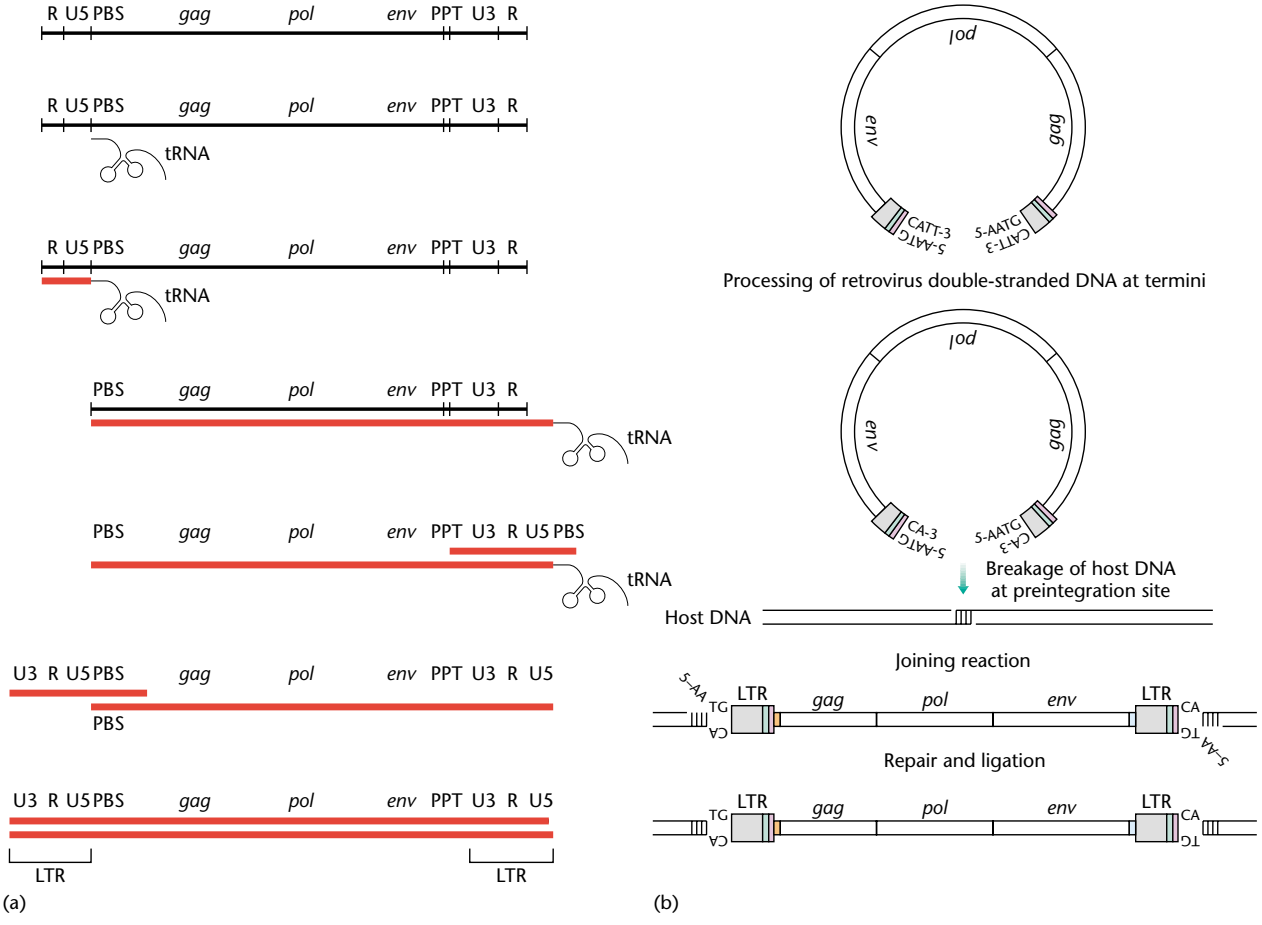


**Figure 3** Reverse transcription and integration processes. (a) Reverse transcription. Outline of the reverse transcriptase (RT)-catalysed steps leading from single-stranded genomic RNA (top; black line) to double-stranded proviral DNA (bottom; red line). (b) Integration. The viral DNA (top) is the product of the completed reverse transcription process of (a). Shown are the integrase (IN)-mediated cleavage and religation steps leading to joining of proviral and host DNA. Subsequent repair and ligation are carried out by host factors. Note the loss of two terminal nucleotides of the viral DNA and the generation of a short repeat of host sequences of the integration site.

genome at both ends. The LTR sequences harbour, in addition to the R region, U3 sequences from the 3′ end and U5 sequences from the 5′ end of the RNA genome.

This general scheme of reverse transcription is basically valid for all retroviruses; variations include the specific tRNA primer selected for initiation of minus-strand synthesis and the length of the U3, R and U5 regions. The exact order of some steps, such as the completion of minus-strand synthesis and the initiation of plus-strand synthesis, is unknown. Moreover, in some viruses such as HIV-1, plus-strand synthesis can be discontinuous as a result of additional priming from internal PPT sequences of the viral genome. The nucleic acid-binding NC protein is believed to play a role in the reverse transcription process by facilitating strand-dissociation and strand-reassociation steps. The retrovirus particle is diploid; however, reverse transcription seems to result in only one provirus. While the minus-strand transfer may occur at the 3′ end of either the same or the other RNA strand, the plus-strand transfer is intramolecular.

RT has no proofreading activity, and the rate of nucleotide misincorporation, in the range of one error per $10^4$–$10^5$ nucleotides incorporated, is higher than for cellular DNA polymerases. In addition to the template shifts in terminal regions needed for the overall process, RT also exhibits frequent template shifting at nonterminal positions. In a viral particle harbouring two nonidentical RNAs, such events may lead to generation of viral recombinants. Together, RT properties of allowing frequent mutation and recombination events contribute to retroviral diversification and evolution.

After the reverse transcription of the viral genome inside the viral core in the cytoplasm has been completed, the complex needs access to chromosomal DNA of the nucleus to allow the joining of proviral DNA and host DNA. For some retroviruses, such as HIV-1, viral proteins harbour specific determinants that direct the complex to a nuclear import pathway, giving access to the nucleus in nondividing cells. For other viruses, integration is dependent upon cell division, presumably because disintegration of the nuclear envelope during the cell cycle is needed. A complex containing IN and the viral DNA mediates integration of the provirus (**Figure 2**). Additional viral proteins and some not yet fully characterized host factors may also participate in this process. The structure of IN is known in detail (Dyda *et al*., 1994). This enzyme joins viral and cellular DNA by a concerted cleavage–religation reaction in which specific inverted repeat sequences at the viral termini are recognized and cleaved by the enzyme (**Figure 3b**). The cleavage takes place two nucleotides from the 3′ end of each strand. Host DNA is cleaved without any obvious sequence preference by a staggered cut introducing 5′ overhangs of 4–6 nucleotides, depending on the viral genus. The 3′ ends of the cleaved proviral DNA and the 5′ ends of host DNA at the cleavage site are joined by religation. After trimming of overhangs, gap-filling and ligation of the two other strands by cellular enzymes, the result is an integrated provirus, colinear with the DNA product of reverse transcription, except that two nucleotides have been lost from both termini. The flanking host DNA sequence is characterized by a 4–6-bp duplication relative to the preintegration site. An integrated provirus is stably maintained and duplicated during chromosomal DNA replication similar to host cell DNA. Importantly, such proviruses may be vertically transmitted through the germline of a species and may accumulate debilitating mutations over time. A current estimate is that such endogenous retroviral DNA constitutes about 1% of the human genome. In some cases an endogenous provirus at a specific integration site has even been maintained through evolutionary points of species divergence and may serve as a phylogenetic marker (Medstrand and Mager, 1998).

Proviral insertion is not site-specific with respect to the position in host DNA, and an inserted provirus may therefore affect a host gene by a loss-of-function or a gain-of-function mutation. Gain-of-function mutations in somatic cells have been intensively studied in animal models, where proviral insertional activation of critical genes, such as oncogenes, play an important role for retrovirus-induced development of cancer.

## Transcription and Translation

In the case of simple retroviruses, the transcription and translation steps leading to formation of retrovirus-specific RNA and proteins require only the host cell machinery used for expression of nonviral genes (**Figure 2**). The provirus is transcribed by RNA polymerase II of the host cell. Transcription is initiated at the U3-R border of the upstream LTR (termed the 5′ LTR) and terminates beyond the poly(A) addition site at the R-U5 border of the downstream LTR (3′ LTR). Major DNA elements directly and indirectly recognized by the RNA polymerase II transcription machinery are located in the LTR. These are sometimes classified as: (1) promoter elements serving as binding sites for the transcription complex at and immediately upstream of the initiation site; and (2) enhancer elements further upstream in U3 serving as binding sites for cell type-specific or ubiquitous transcription factors that facilitate transcription initiation. Poly(A) addition at the specific site that defines the 3′ end of the viral genome is controlled by a poly(A) signal similar to that of host mRNAs. This poly(A) signal, variably located in the RNA of the R or U3 region, functions through recognition by host protein factor(s). There is no evidence in favour of specific proviral signals for termination of retroviral transcription; presumably transcription may proceed through the provirus and into flanking host DNA, prior to cleavage and poly(A) addition. The presence of important regulatory signals in terminally redundant regions raises the question of a possible functional

distinction between identical signals found in the 5′ LTR and the 3′ LTR. In an only partially understood manner, such distinction may be achieved by cooperation among RNA or DNA elements within and outside the repeat regions; hence, the provirus seems to function as one regulatory unit causing transcriptional initiation in the 5′ LTR and polyadenylation in the 3′ LTR. In addition to the effect of viral regulatory signals, the level and stability of expression of a provirus is also influenced by poorly understood chromosomal position effects at the site of integration. Exceptions to this overall scheme of retrovirus transcription are the roles played by virus-encoded transcriptional transactivators of complex retroviruses, which may operate by enhancing transcription factor binding to DNA or by binding to RNA and stimulating RNA polymerase processivity. Retroviruses also provide a few examples of specialized gene expression, exploiting alternative transcriptional initiation sites within the provirus.

The nuclear transcription products eventually end up in the cytoplasm in the form of full-length genomes, as well as one or more subgenomic spliced mRNAs. Retroviral RNA contains splice donor and splice acceptor signals that interact with the cellular splicing machinery. Simple retroviruses harbour one splice donor and one splice acceptor site (**Figure 1**) used in formation of *env* mRNA. The intron encompasses most of the *gag* and *pol* ORFs and for most viruses also part of the 5′ untranslated sequences including the packaging signal Ψ. Complex retroviruses produce additional mRNAs controlled by several splice donor and acceptor sites. In these cases, the pattern of viral RNAs in the cytoplasm may be regulated in a temporal manner by a viral protein, Rev in the case of HIV-1. With the exception of this additional utilization of a viral protein by complex retroviruses, processing and nuclear export exploit pathways used for host cell mRNAs. However, splicing is only partial, and a fraction of retroviral transcripts remains unspliced and is exported to the cytoplasm in an unspliced, genome-length form. Specific RNA elements (constitutive transport elements) required for nuclear export of unspliced RNA have been identified in some retroviruses (Pasquinelli *et al*., 1997).

The proteins encoded by the *gag* and *pol* ORFs are synthesized in the cytoplasm on an mRNA equivalent to full-length genome RNA, while spliced *env* mRNA is translated on rough endoplasmatic reticulum-bound ribosomes (**Figure 1**). Retroviral translation exploits the cellular translation machinery; however, details of how the retroviral mRNAs interacts with this machinery are unknown. In particular, some aspects of *gag–pol* mRNA translation are complex. Among these is the role played by the long 5′ untranslated sequence preceding the *gag* start codon. For some viruses evidence has been obtained that this long leader may favour internal ribosome entry as a mechanism distinct from the normal cap-directed mechanism of start codon identification by ribosomal scanning of the 5′ leader RNA. Another complex feature is the suppression of translational termination at the *gag–pol* border found for almost all retroviruses. This translation regulation mechanism leads to the production of Gag polyprotein and Gag–Pol polyproteins in balanced ratios of about 20 Gag products to one Gag–Pol product. Among retroviruses, two distinct mechanisms of suppression of translational termination at the *gag–pol* transition have been described: readthrough from some murine retroviruses and frameshift for most other retroviruses. By readthrough suppression, a specific amino acid is incorporated in the Gag–Pol polyprotein at the position of a stop codon separating two ORFs, i.e. a specific aminoacyl- tRNA is attached to a ribosome at the stop codon in a manner compatible with protein synthesis. By frameshift suppression, a ribosome undergoes a reading frame shift (mostly to the minus 1 position) in a 'slippage' region upstream of the *gag* stop codon, and decoding of the mRNA may proceed past the stop codon in the altered reading frame. These two mechanisms of translational termination suppression both require specific RNA elements around the stop codon position. Common to these RNA elements is the ability to adopt secondary stem–loop or pseudoknot structures just downstream of the stop codon.

The *env* translation product harbours a hydrophobic N-terminal signal peptide that initiates transmembrane synthesis and is subsequently cleaved off. The Env precursor glycoprotein remains membrane-anchored by the transmembrane domain of TM; it becomes *N*-glycosylated in the lumen of the endoplasmatic reticulum and the Golgi apparatus and is cleaved at the SU–TM border by a host endoprotease during transit of the Golgi complex, before being positioned at the cell surface with SU linked to the extramembranal domain of TM by disulfide bridges, or for some viruses, including HIV-1 and other *Lentivirus* members, by noncovalent interactions only.

## Assembly and Release

All retroviruses are released from the cell membrane by a budding mechanism in which the bilipid-layer cell membrane wraps around the virus core. Early electron microscopy of retrovirus-producing cells revealed two distinct patterns of assembly before budding. Most retrovirus genera assemble according to the C-type pattern at the cell membrane, while the B-type and D-type patterns are characterized by capsid assembly in the cytoplasm and later association with the cell membrane. Virion maturation after release from the producer cell (**Figure 2**) is a feature characteristic of retroviruses. Maturation involves major changes in particle morphology leading to a more condensed core, as evidenced by electron microscopy (Yeager *et al*., 1998). The core structure is somewhat

pleomorphic and of different appearance for various groups of viruses.

Key components assembled into a retroviral particle are Gag polyproteins, Gag–Pol polyproteins, full-length viral RNA and host cell tRNA. Additional host-derived factors found selectively incorporated into the particle may also have functional importance. C-type viruses assemble at the inner cell membrane, presumably in regions harbouring membrane-spanning retroviral TM proteins with extracellularly associated SU proteins. The major player in particle formation is the Gag polyprotein, since particles are formed in mutants defective in *pol* or in *env*. The N-terminus of Gag (MA after cleavage) is modified for most viruses by addition of a hydrophobic myristate chain, which is important for its association with the cytoplasmic side of the plasma membrane. The CA protein domain seems to be important for capsid formation, most likely via a self-assembly domain, while the NC domain is required for inclusion of RNA into viral particles. For some retroviruses RT has specific tRNA affinity and may play a role for selective incorporation of tRNAs into the particle.

Genomic viral RNA is selectively incorporated into particles relative to spliced viral RNAs and host RNAs. Sequences important for encapsidation are mostly located in the 5′ part of the RNA upstream of *gag*. This region is termed the packaging signal (Ψ, psi for packaging signal). For most viruses, part of the packaging signal is absent from spliced *env* mRNA. This region of the 5′ RNA is characterized by stem–loop structures; for some viruses specific stem–loop structures have been found to be important for packaging. Structures in this region also play a role in formation of dimers of the two RNA genomes in the particle.

After release, the Gag and Gag–Pol proteins are cleaved into their mature forms by the viral protease (PR) which is part of Gag or Gag–Pol polyproteins in the particle. Retroviral proteases are aspartic proteinases. Detailed information on PR structure and catalytic mechanisms is available and has been important for development of drugs targeting the HIV-1 protease. Notably, the active form of PR is a dimer. It is not known how the timing of PR action during particle release and maturation is regulated. However, concentration-dependent dimerization in the particle may contribute to its activation. A detailed model of PR regulation would also have to take into account that PR is incorporated in particles as part of a precursor protein with reduced enzymatic activity.

Cleavage of Gag and Gag–Pol polyproteins is required for infectivity. For some viruses, maturation also requires PR cleavage of the C-terminal tail of TM extending into the particle, a cleavage reaction serving to activate the fusion properties of TM. Maturation of a viral particle after detachment from the cell may thus be one of the mechanisms that prevent viral replication prior to release and reduces reinfection of a producer cell.

## References


Albritton LM, Tseng L, Scadden D and Cunningham JM (1989) A putative murine ecotropic retrovirus receptor gene encodes a multiple membrane-spanning protein and confers susceptibility to virus infection. *Cell* **57**: 659–666.

Baltimore D (1970) RNA-dependent DNA polymerase in virions of RNA tumor viruses. *Nature* **226**: 1209–1211.

Cullen BR (1992) Mechanisms of action of regulatory proteins encoded by complex retroviruses. *Microbiological Reviews* **56**: 375–394.

Dyda F, Hickman AB, Jenkins TM *et al*. (1994) Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. *Science* **266**: 1981–1986.

Fass D, Davey RA, Hamson CA *et al*. (1997) Structure of a murine leukemia virus receptor-binding glycoprotein at 2.0 angstrom resolution. *Science* **277**: 1662–1666.

Jacobo-Molina A, Ding J, Nanni RG *et al*. (1993) Crystal structure of human immunodeficiency type 1 reverse transcriptase complexed with double stranded DNA at 3.0 Å resolution shows bent DNA. *Proceedings of the National Academy of Sciences of the USA* **90**: 6320–6324.

Linial ML (1999) Foamy viruses are unconventional retroviruses. *Journal of Virology* **73**: 1747–1755.

Medstrand P and Mager DL (1998) Human-specific integrations of the HERV-K endogenous retrovirus family. *Journal of Virology* **72**: 9782–9787.

Pasquinelli AE, Ernst RK, Lund E *et al*. (1997) The constitutive transport element (CTE) of *Mason–Pfizer monkey virus* (MPMV) accesses a cellular mRNA export pathway. *EMBO Journal* **16**: 7500–7510.

Temin HM and Mitzutani S (1970) RNA-directed DNA polymerase in virions of *Rous sarcoma virus*. *Nature* **226**: 1211–1213.

Yeager M, Wilson-Kubalek EM, Weiner SG, Brown PO and Rein A (1998) Supramolecular organization of immature and mature murine leukemia virus revealed by electron cryomicroscopy: implications for retroviral assembly mechanisms. *Proceedings of the National Academy of Sciences of the USA* **95**: 7299–7304.


## Further Reading


Arts EJ and Le Grice SFJ (1998) Interaction of retroviral reverse transcriptase with template-primer duplexes during replication. *Progress in Nucleic Acid Research and Molecular Biology* **58**: 339–393.

Chen ISY, Koprowski H, Srinivasan A and Vogt PK (eds) (1995) *Transacting Functions of Human Retroviruses*. Berlin: Springer.

Coffin JM, Hughes SH and Varmus HE (eds) (1997) *Retroviruses*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Fields BN, Knipe DM, Howley PM *et al*. (eds) (1996) *Fields Virology*, 3rd edn. Philadelphia: Lippincott-Raven.

Kräusslich HG (ed.) (1996) *Morphogenesis and Maturation of Retroviruses*. Berlin: Springer.

Patience C, Wilkinson DA and Weiss RA (1997) Our retroviral heritage. *Trends in Genetics* **13**: 116–120.

Skalka AM and Goff SP (eds) (1993) *Reverse Transcriptase*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

van Lohuizen M and Berns A (1990) Tumorigenesis by slow-transforming retroviruses: an update. *Biochimica Biophysica Acta* **1032**: 213–235.

Wlodawer A and Erickson JW (1993) Structure-based inhibitors of HIV-1 protease. *Annual Review of Biochemistry* **62**: 543–585.