History of retroviruses

The first identified retrovirus was the causative agent of leukemia in chickens (avian leukosis virus; ALV) by Ellermann and Bang in 1908.

In 1911, Peyton Rous reported the transmission of sarcomas in chickens through cell-free filtrates and subsequently it was named the Rous sarcoma virus (RSV).

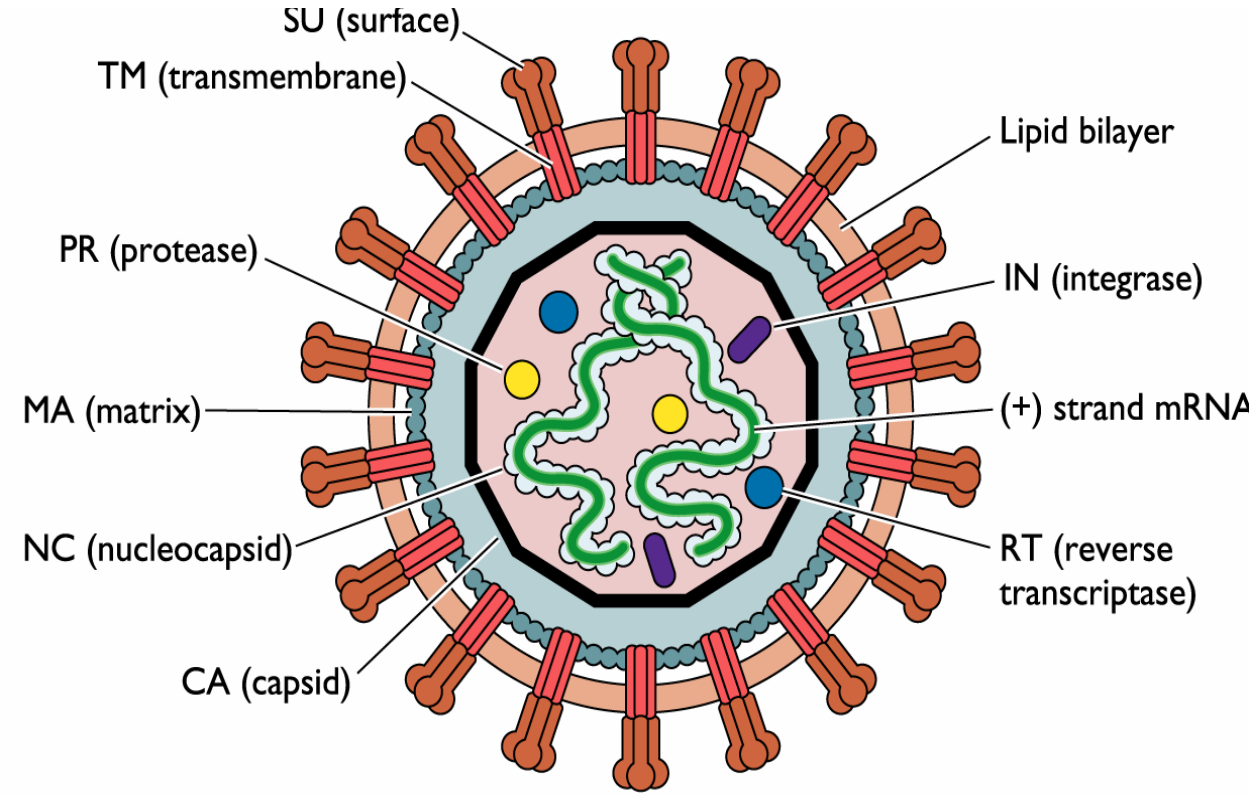
The first and only known human retrovirus that causes malignancy, Human Tlymphotropic virus type 1 (HTLV-1), was discovered and described in 1980.

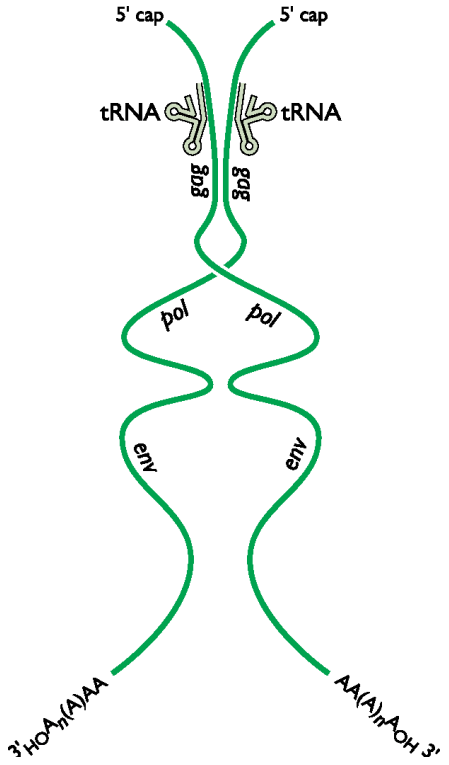
Three years later, Montagnier and co-workers isolated a virus from the lymph nodes of patients with acquired immunodeficiency syndrome (AIDS) and in 1984, the link between HIV-1 and AIDS was established by Gallo and colleagues.

TABLE 47.1 Retrovirus Genera

Name	Examples	Morphology
Alpharetrovirus	Avian leukosis virus (ALV) Rous sarcoma virus	C type
Betaretrovirus	Mouse mammary tumor virus (MMTV) Mason-Pfizer monkey virus (M-PMV) Jaagsiekte sheep retrovirus	B, D type
Gammaretrovirus	Murine leukemia viruses (MuLV) Feline leukemia virus (FeLV) Gibbon ape leukemia virus (GaLV) Reticuloendotheliosis virus (RevT)	C type
Deltaretrovirus	Human T-lymphotropic virus type 1, 2 Bovine leukemia virus (BLV) Simian T-lymphotropic virus type 1, 2, 3	Rod-shaped core
Epsilonretrovirus	Walleye dermal sarcoma virus Walleye epidermal hyperplasia virus 1	–
Lentivirus	Human immunodeficiency virus type 1 Human immunodeficiency virus type 2 Simian immunodeficiency virus (SIV) Equine infectious anemia virus (EIAV) Feline immunodeficiency virus (FIV) Caprine arthritis encephalitis virus (CAEV) Visna/maedi virus	Rod/Cone-shaped cores
Spumavirus	Human foamy virus	Immature

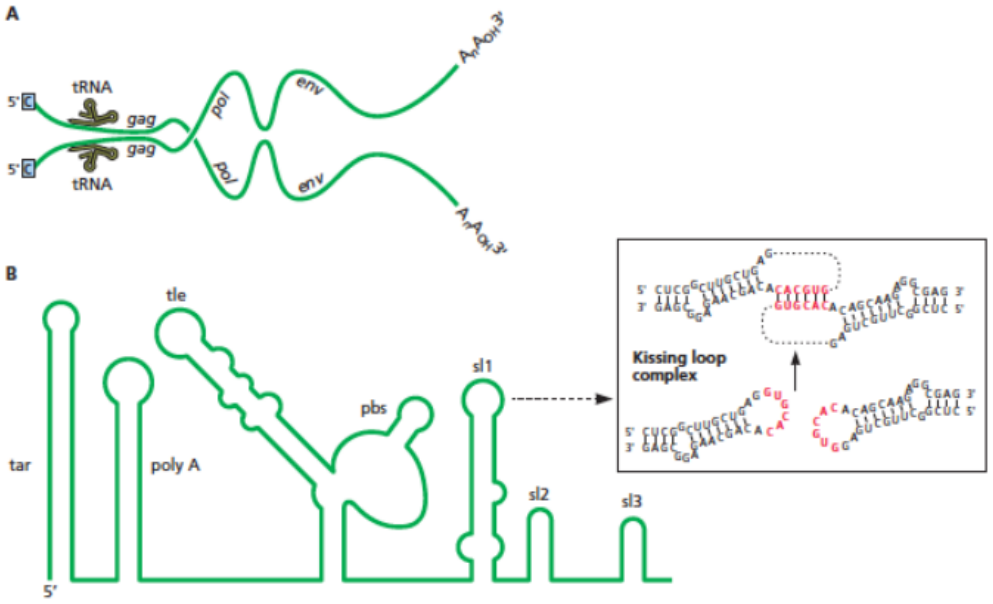
Retroviridae





Retroviridae

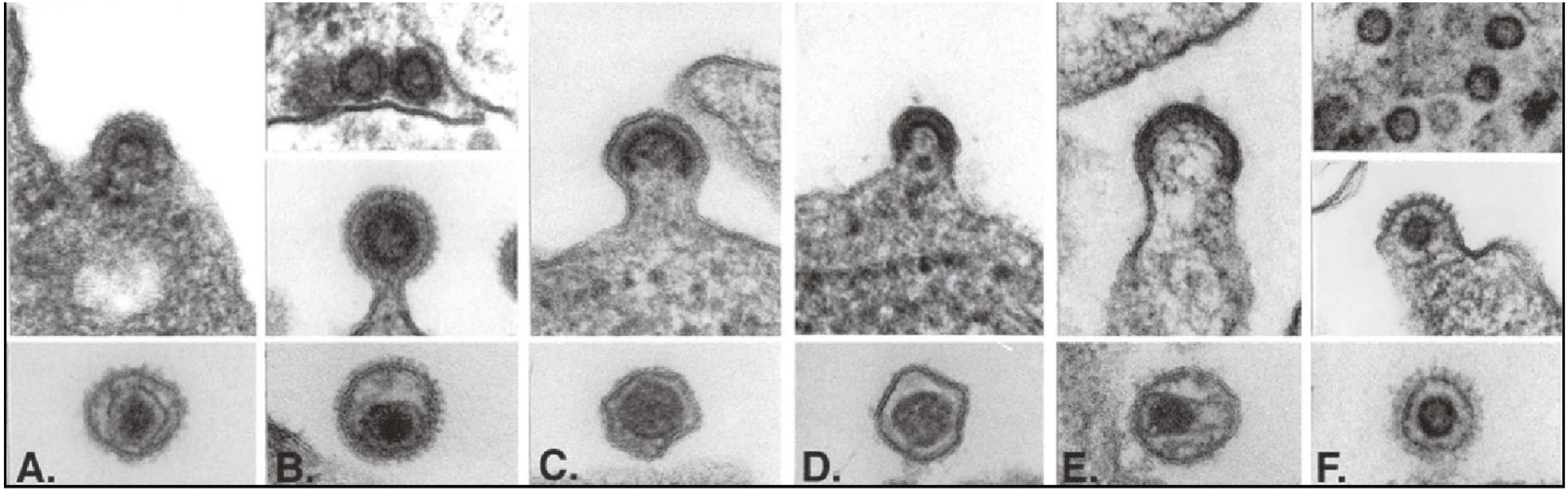
The diploid retroviral genome and a dimerization domain



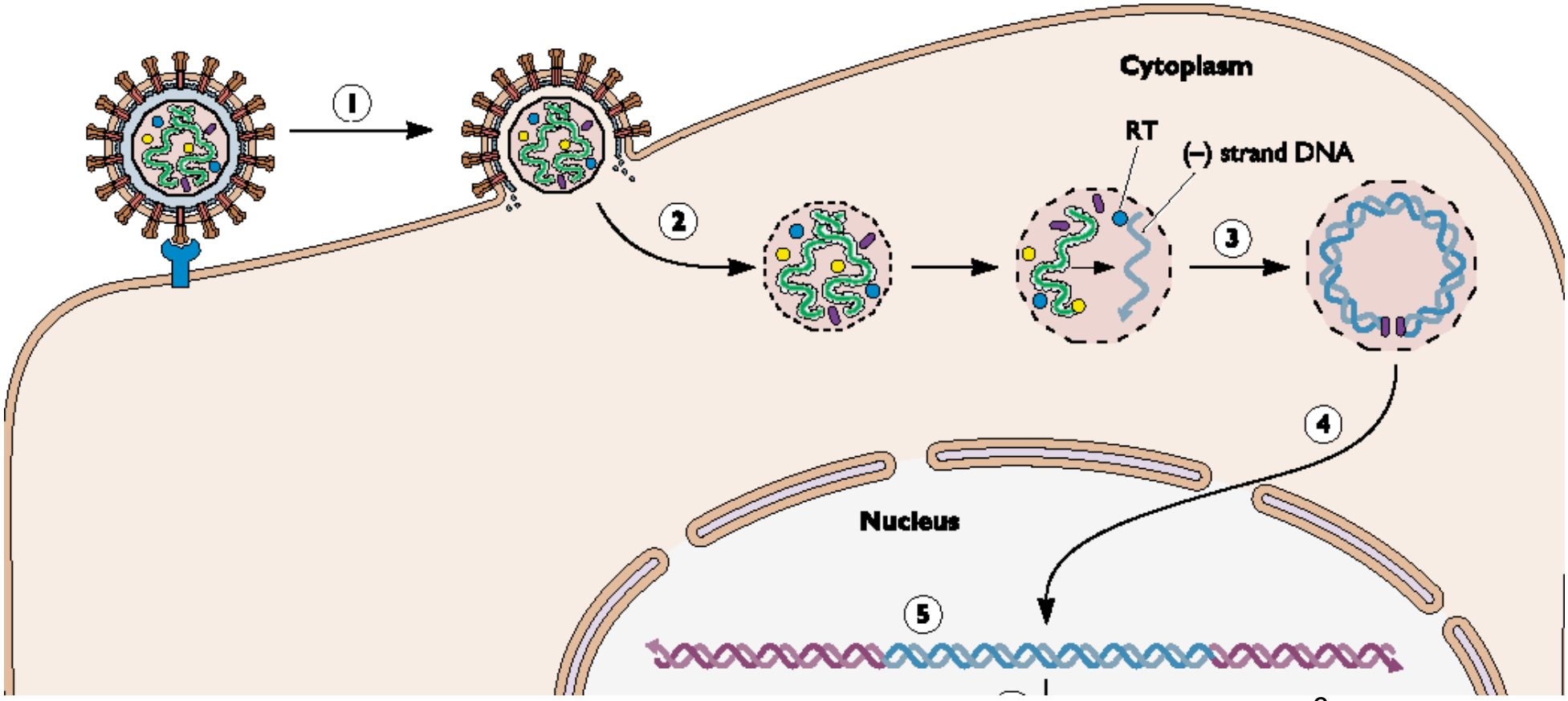
(A) The diploid retroviral genome. Points of contact represent multiple short regions of complementary base pairing. (B) Structural elements in the 5' end of genomic RNA comprise distinct stem-loop structures. In the human immunodeficiency virus RNA, these elements include the Tat-binding site (tar), a poly(A) stem-loop, and a section that resembles a tRNA anticodon loop called the tle (for tRNA-like element). The adjacent primer-binding site (pbs), comprising a sequence complementary to the 3' end of the tRNA primer, is followed by a stem-loop structure, sl1, that initiates genome dimerization by hybridizing with sl1 in a second viral RNA molecule to form a "kissing loop," as illustrated in the box. The sl1, sl2, and sl3 elements are required for efficient viral RNA packaging.

Retroviridae

Retroviruses are spherical in shape measuring approximately 80–120 nm in diameter, comprising of 2000–5000 molecules of a structural protein called **Group-specific antigen** (**Gag**) and 14 trimers of **envelope glycoprotein** spikes (**Env**).



Classe VI: Retroviridae



Retroviridae

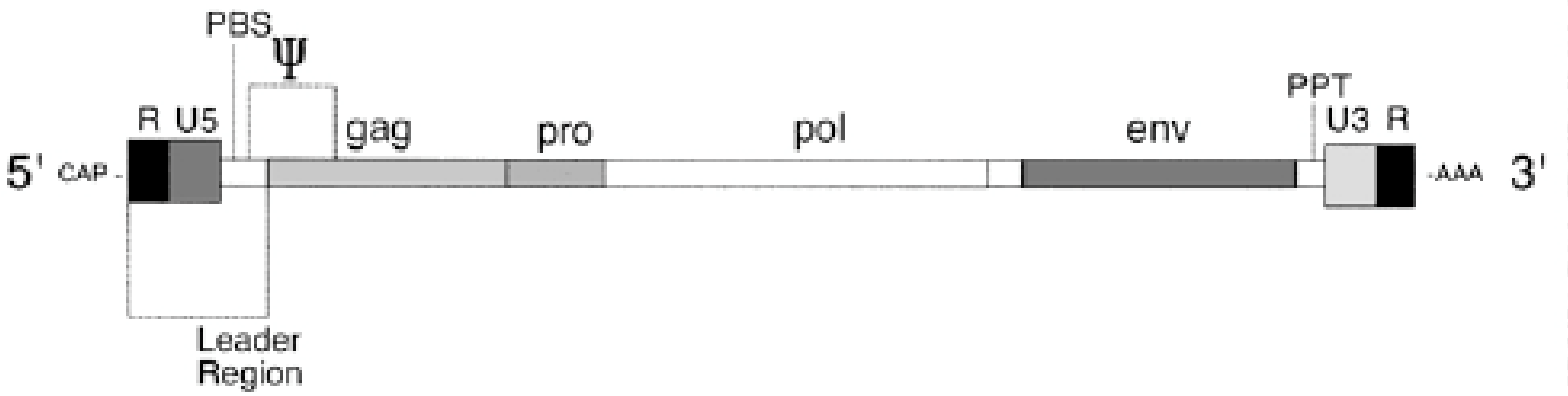
For all retroviruses, reverse transcription of an RNA genome produces a double-stranded DNA with long terminal repeats (LTRs).
The dsDNA is then integrated into the host cell genome, resulting in formation of a DNA provirus. The provirus serves as a template for synthesis of the viral genome and mRNAs by cellular RNA polymerase II.
Virions of members of the subfamily *Orthoretrovirinae* carry two copies of the RNA genome.

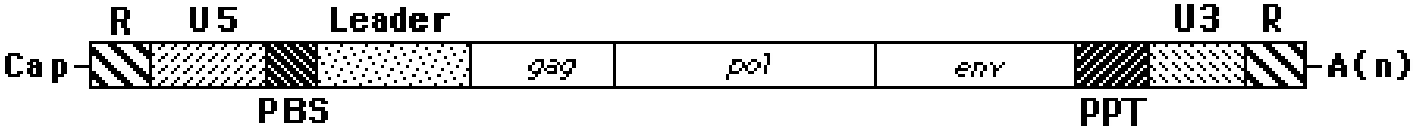
Infectious viruses have four main genes coding for the virion proteins in the order: 5′-*gag*-*pro*-*pol*-*env*-3′. Some retroviruses contain genes encoding non-structural proteins important for the regulation of gene expression and virus replication.

Others carry cell-derived sequences that are important in carcinogenesis (oncogenes). These cellular sequences are inserted either into a complete retrovirus genome (some strains of the alpharetrovirus Rous sarcoma virus) or in the form of substitutions for deleted viral sequences (all other transforming alpharetroviruses and gammaretroviruses). Such deletions render the virus replication-defective and dependent on non-transforming helper viruses for production of infectious progeny. In many cases the cell-derived sequences are expressed as a fused gene with a viral structural gene that is then translated into one chimeric protein (e.g. Gag-Onc protein).

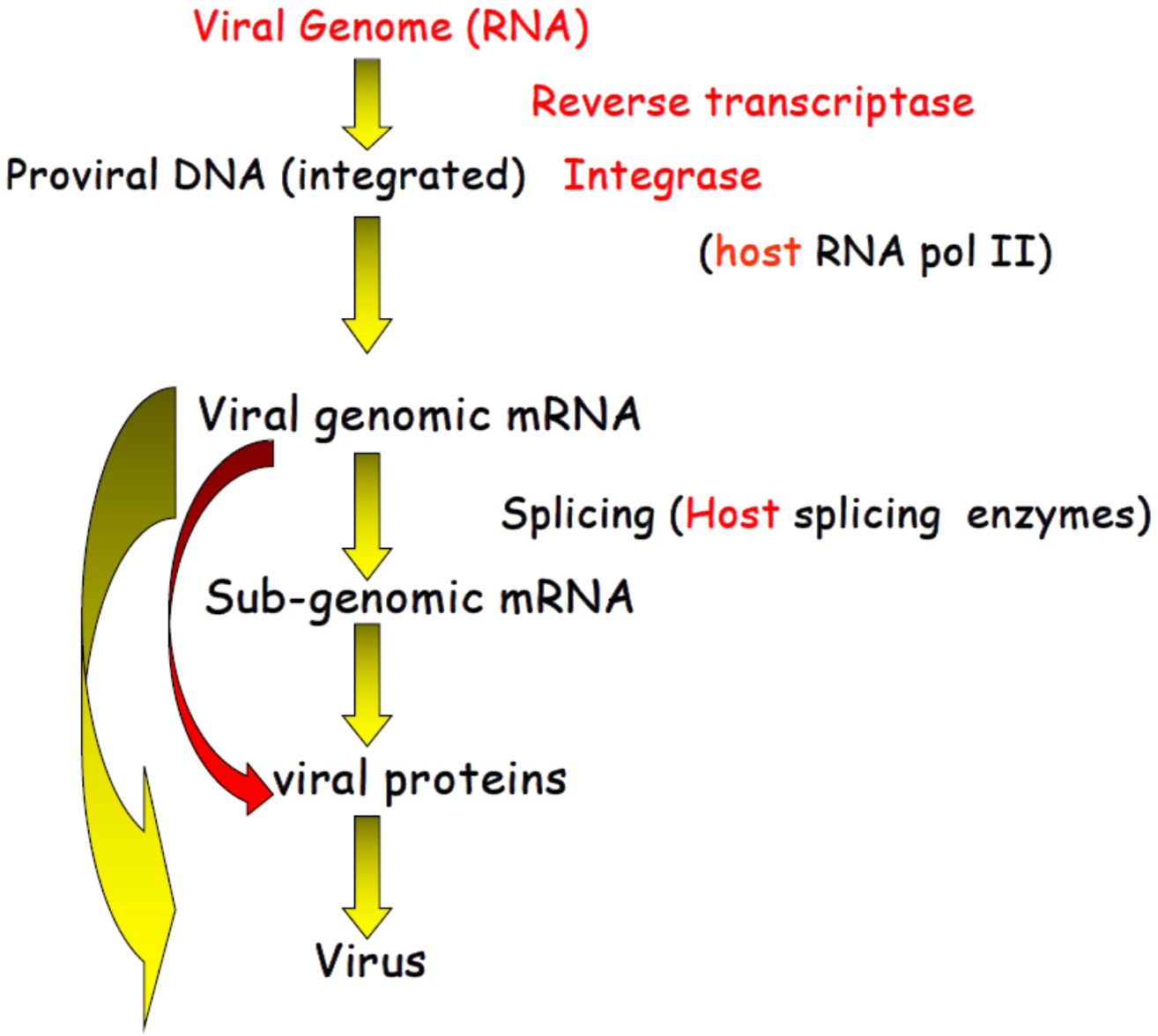
Genome of retroviruses

R - Direct repeats at the 5’ and 3’ termini. They are very important during retrotranscription. They contains the polyadenilation signal.
U5 - Non coding, unique sequence at the 5’ end. Important site for the integration process of the proviral DNA
PBS – Primer binding site, 18nt long sequence, anneals to the 3’ end of the cellular tRNA
Leader (90-500nt, downstream PBS, not shown), non coding, contains the SD and psi site
PPT (c.a. 10nt) serves as the primer for the synthesis of the DNA (+) strand
U3 (200-1200nt) Non coding, unique sequence at the 3’ end containing enhancer and promoter elements.





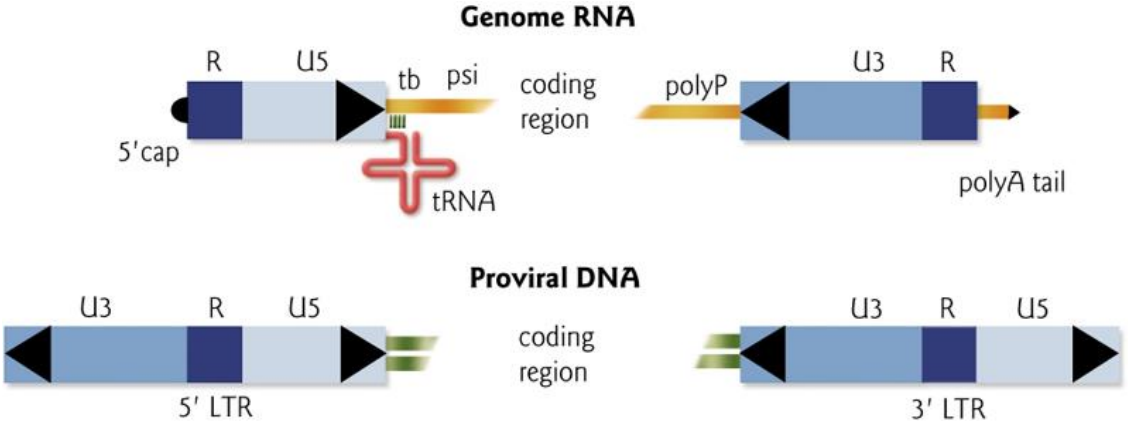
Life cycle of retroviruses



Reverse transcription

Reverse transcriptase
DNA polimerasi RNA-dipendente
DNA polimerasi DNA-dipendente
Attività elicasica
RNAasi H: degrada RNA quando presente sottoforma di ibrido RNA-DNA

Il DNA a doppio filamento che si forma attraverso questa reazione è noto come DNA provirale o provirus e risulta essere più lungo del vRNA poiché ha una copia in più delle regioni U3 e U5. Quindi nel DNA provirale, ad ogni estremità, c'è una ripetizioni diretta della sequenza U3-R-U5. Questa sequenza è nota come LTR (Long Terminal Repeat)



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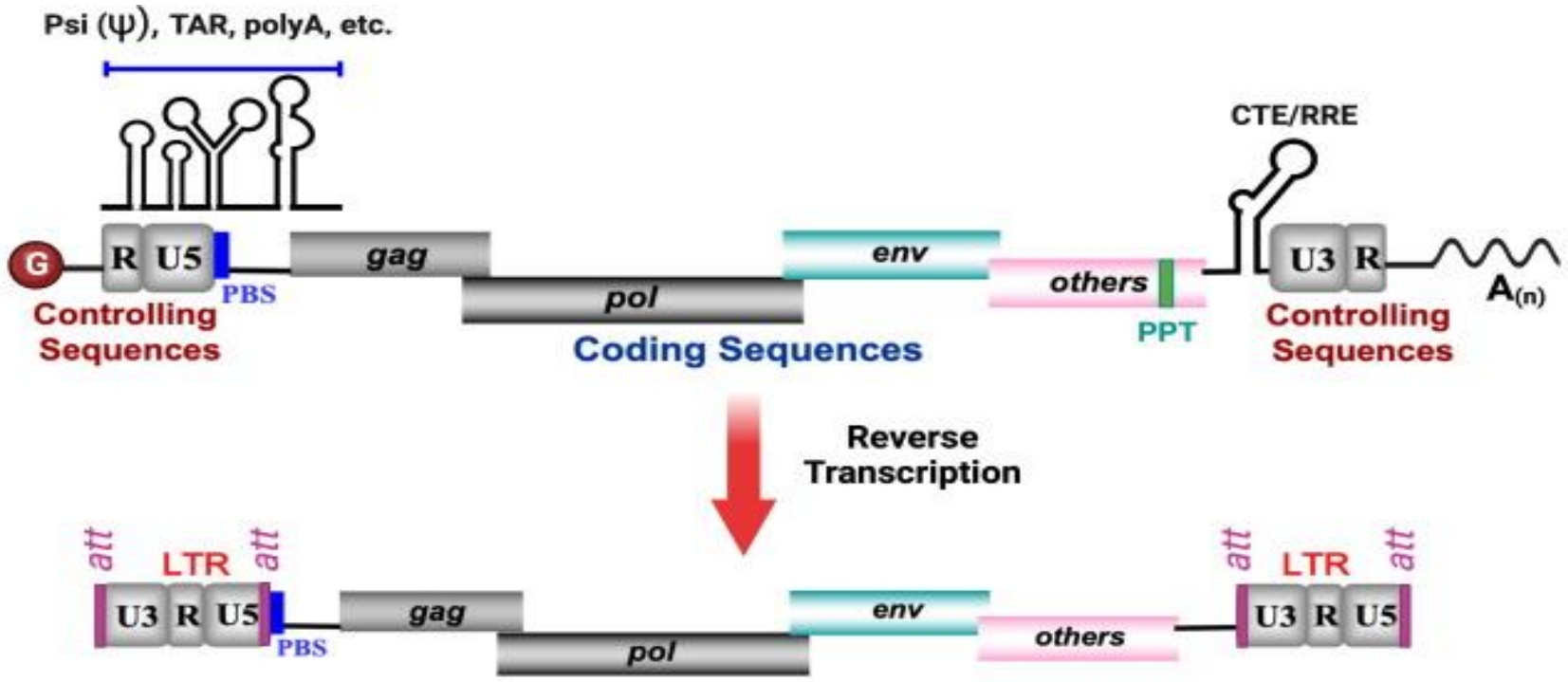
Genome and provirus



Cis-acting or Controlling Elements

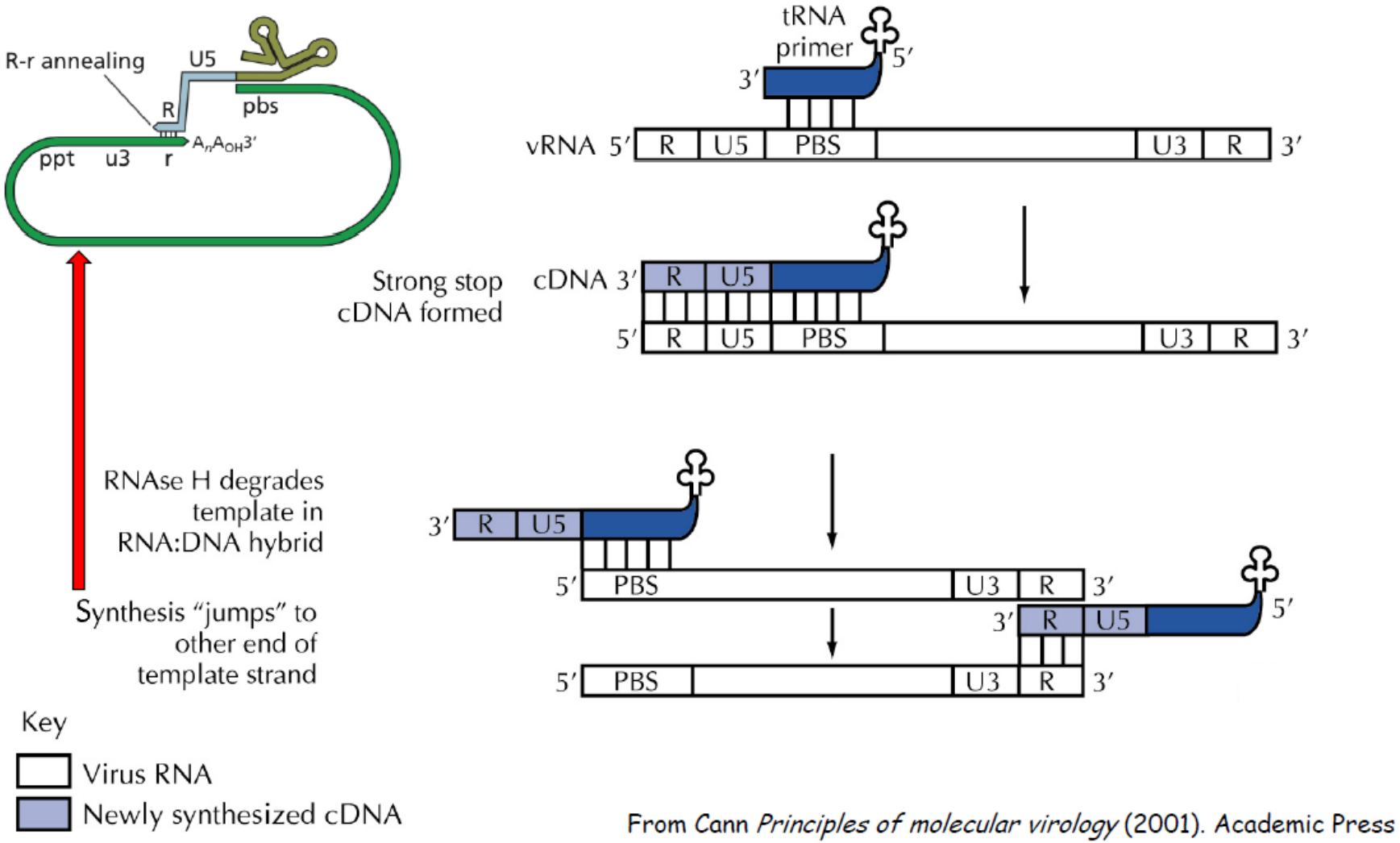
- non-coding sequences
- required for virus replication
- present at both ends of the viral genome
- include: LTRs, PBS, ψ, PPT, TAR, CTE or RRE, *att* sites, etc.

Trans-acting or Coding Sequences:

- code for viral structural, enzymatic, and regulatory proteins
- found between the controlling regions
- can be provided in *trans* for virus replication

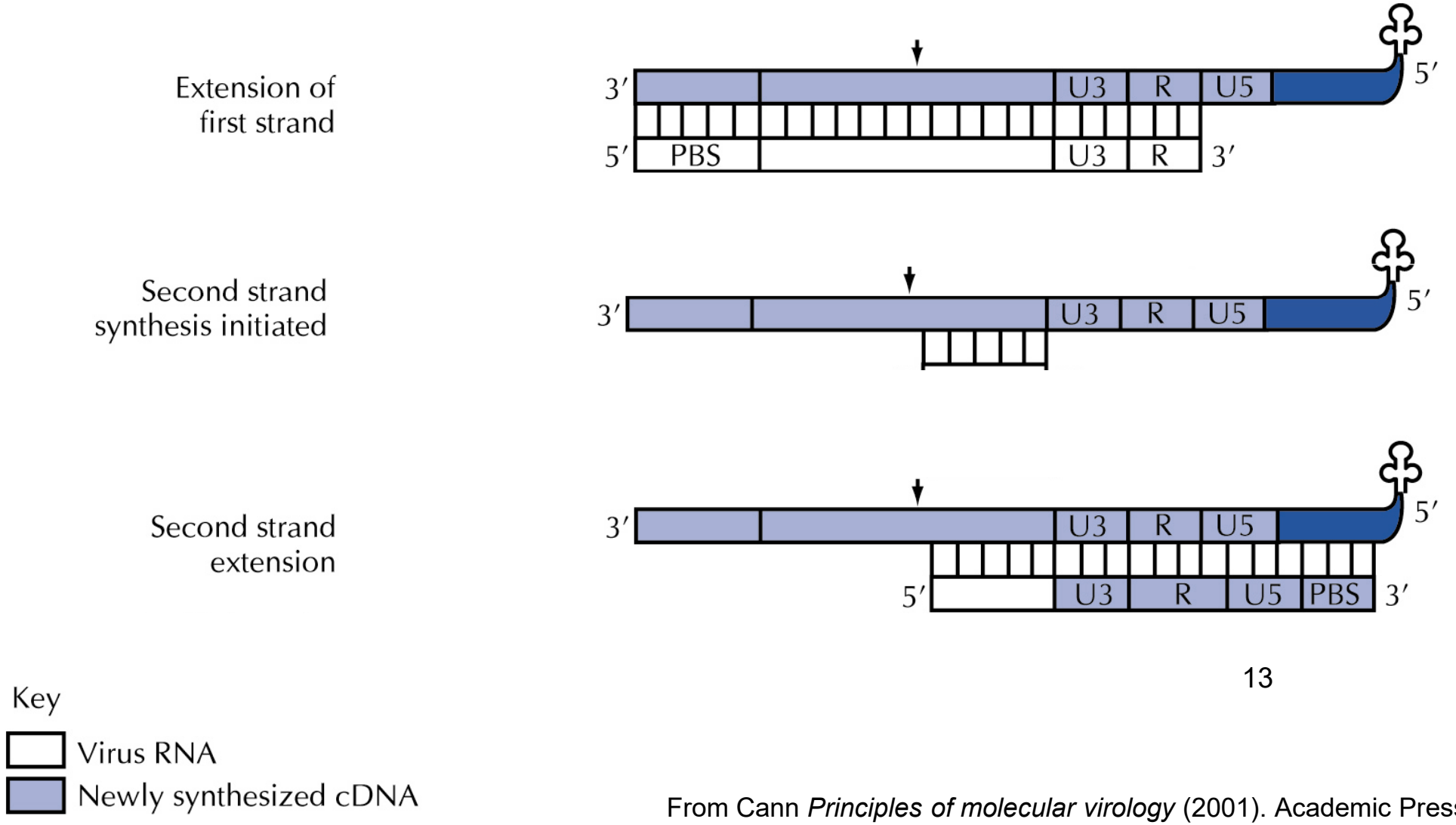
R, repeat region at the 5′ and 3′ ends; U5 and U3, the 5′ and 3′ unique sequences; PBS, the primer binding site; Ψ, the packaging signal; PPT, the polypurine tract which is the site of initiation of plus strand DNA synthesis; $A_{(n)}$, the polyA sequence; TAR, the trans-activation response element; CTE, the constitutive transport element; RRE, the Rev responsive element; *att*, the attachment sites used for integration of the viral genome. Only the two *att* sites at the outer ends are used for this purpose.

Reverse transcription (1)



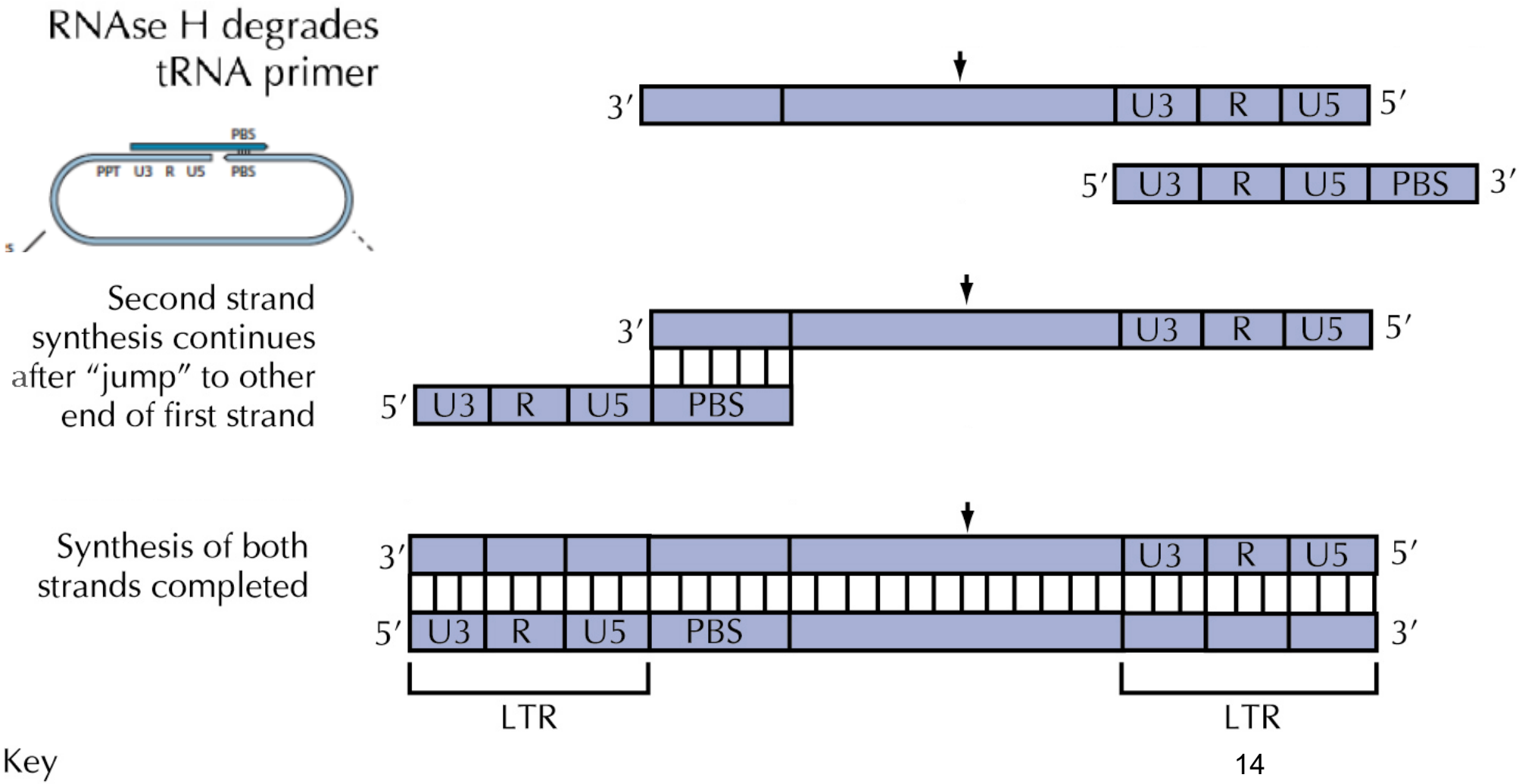
From Cann *Principles of molecular virology* (2001). Academic Press

Reverse transcription (2)



From Cann *Principles of molecular virology* (2001). Academic Press

Reverse transcription (3)



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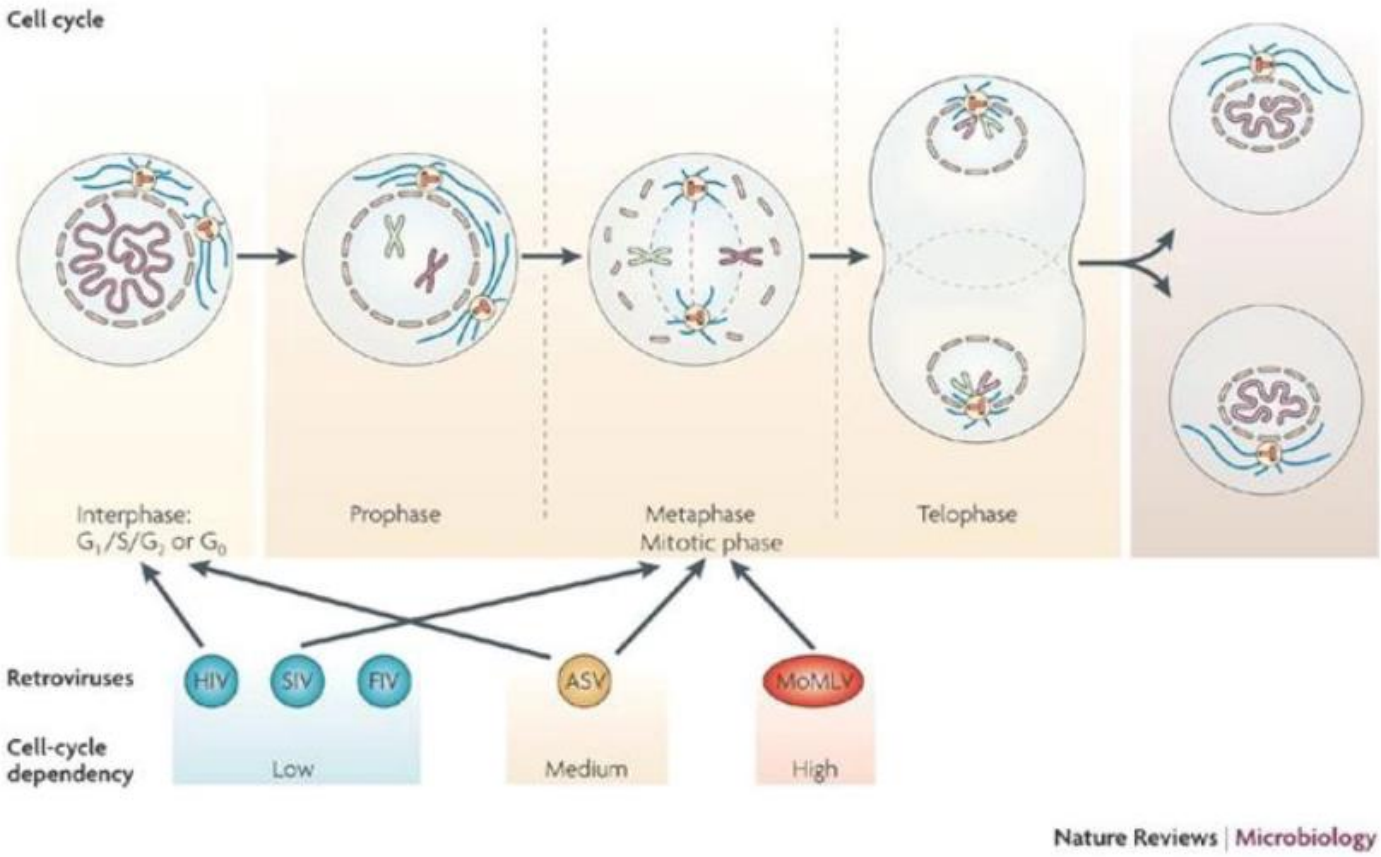
Proviral DNA nuclear entry

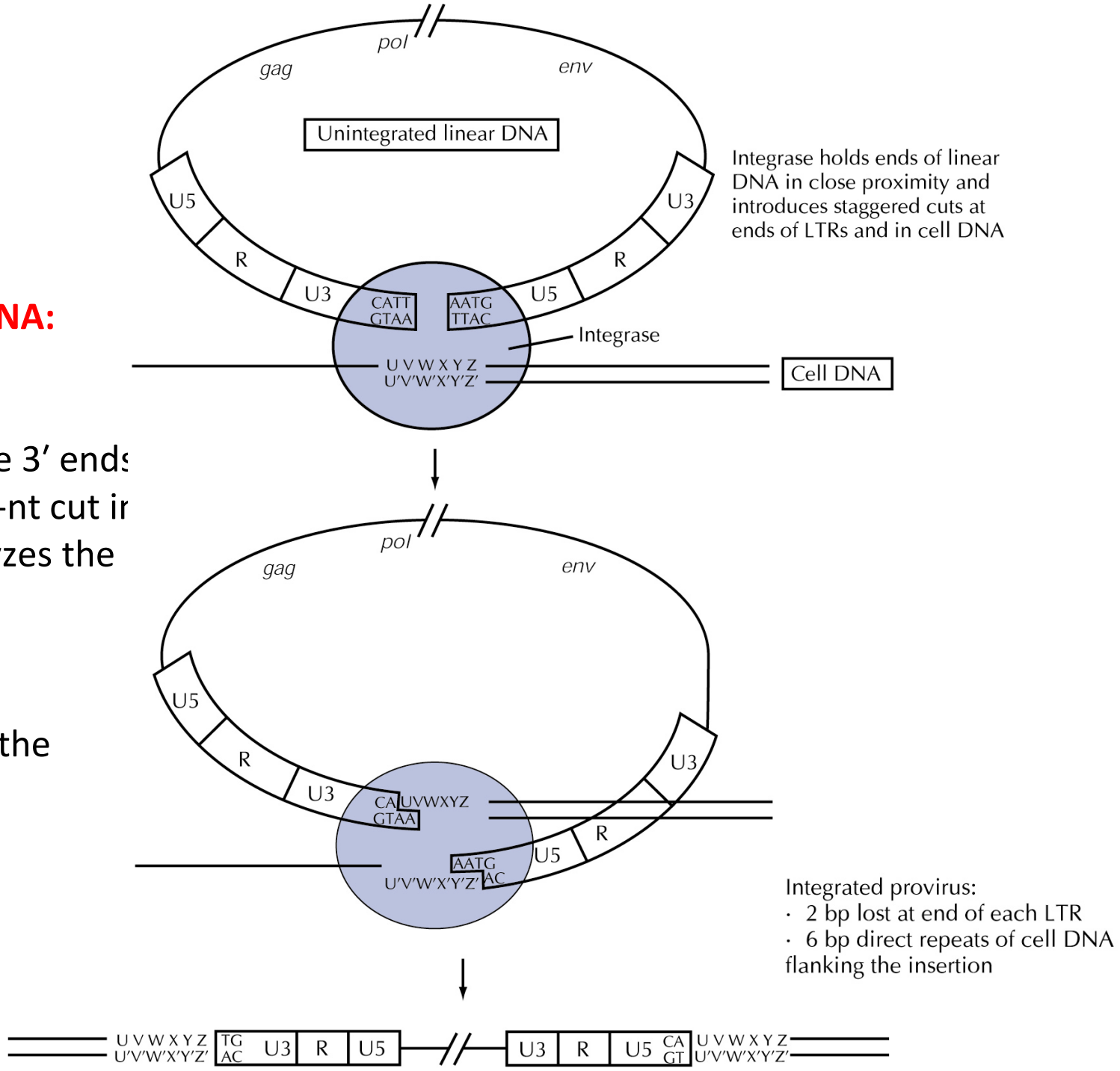


The nuclear envelope is intact during interphase. During cell division (mitotic phase), the nuclear envelope completely breaks down at metaphase and is re-formed at telophase. Whereas Moloney murine leukaemia virus (MoMLV) requires the disassembly of the nuclear envelope at mitosis to enter the nucleus, lentiviruses (human, feline and simian immunodeficiency viruses; HIV, SIV and FIV) and some other retroviruses (avian sarcoma virus, ASV) can cross the intact nuclear envelope during interphase.

Integration into the host DNA:

The integrase performs a 2-nucleotide cleavage at the 3′ ends of the proviral DNA and a 6-nt cut in the cellular DNA, and catalyzes the integration.

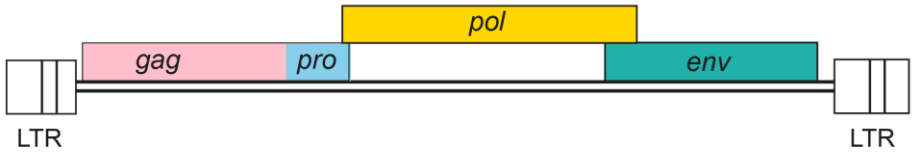
The repair is carried out by the cellular machinery



From Cann *Principles of molecular virology* (2001). Academic Press

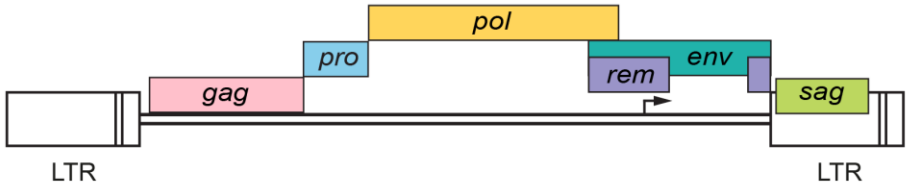
Genome architecture of retroviruses

Alpharetrovirus (ALV, 7.2 kbp)

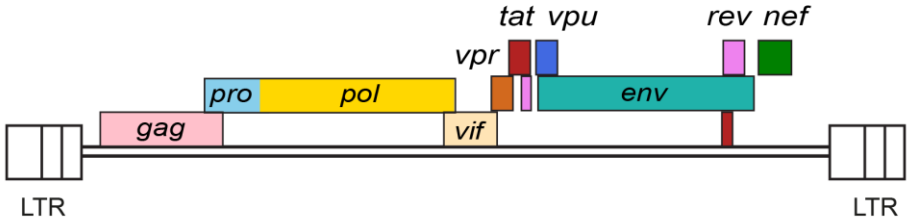


Epsilonretrovirus (WDSV, 12.3 kbp)

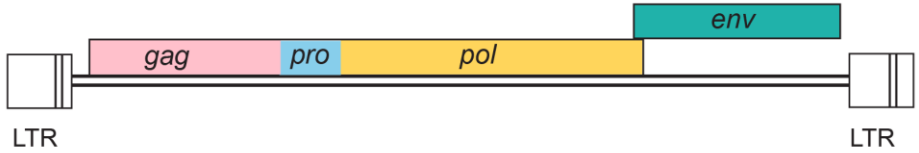
Betaretrovirus (MMTV, 10 kbp)



Lentivirus (HIV-1, 9.3 kbp)

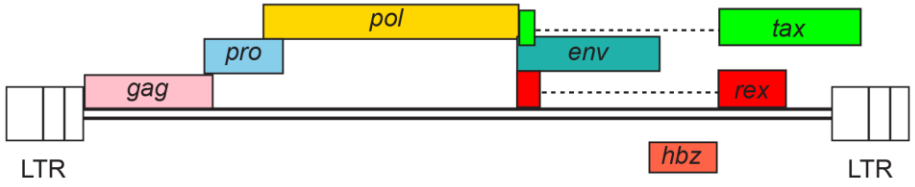


Gammaretrovirus (MMLV, 8.3 kbp)



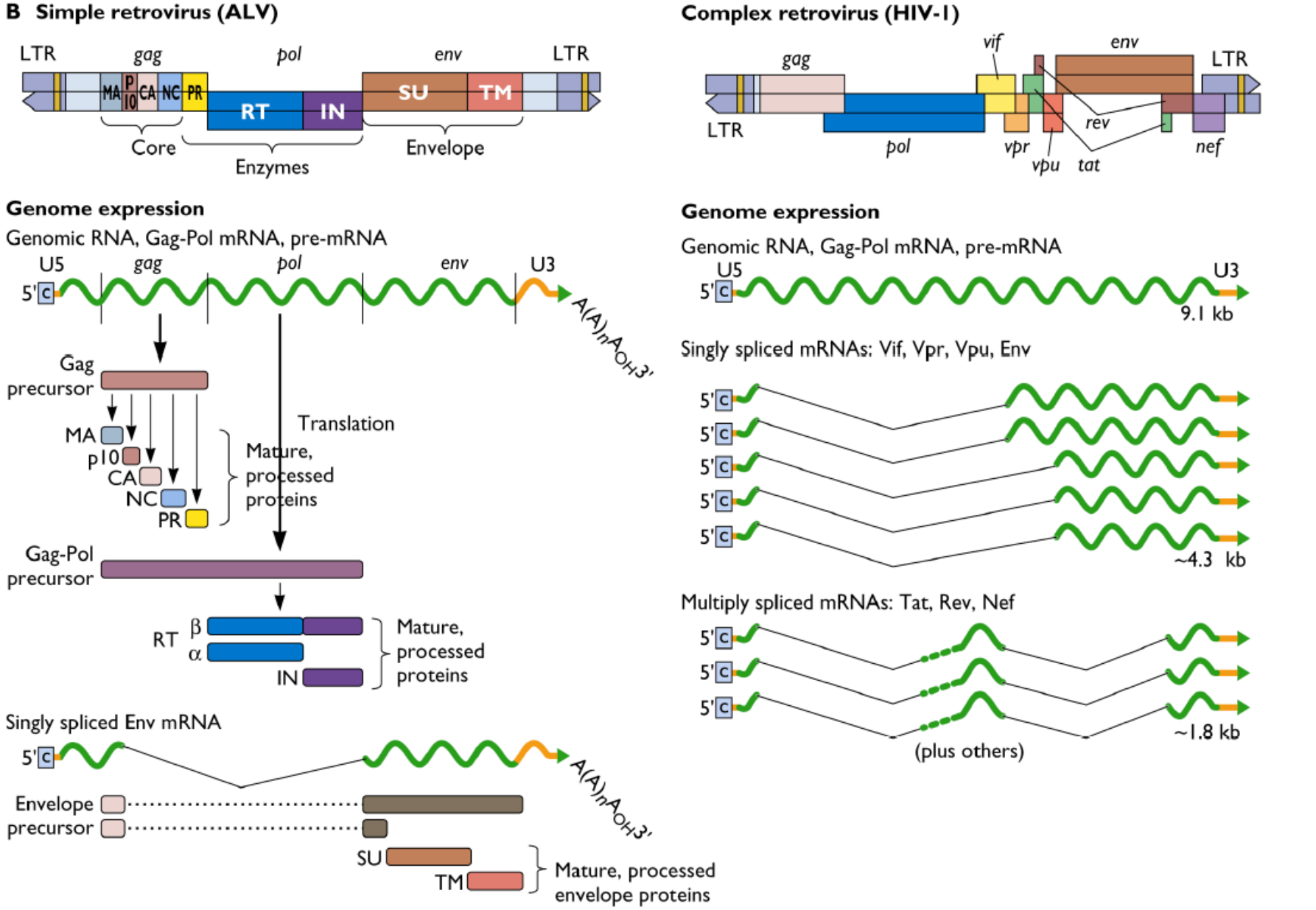
Simiispumavirus (SFV, 13.2 kbp)

Deltaretrovirus (HTLV-1, 8.7 kbp)

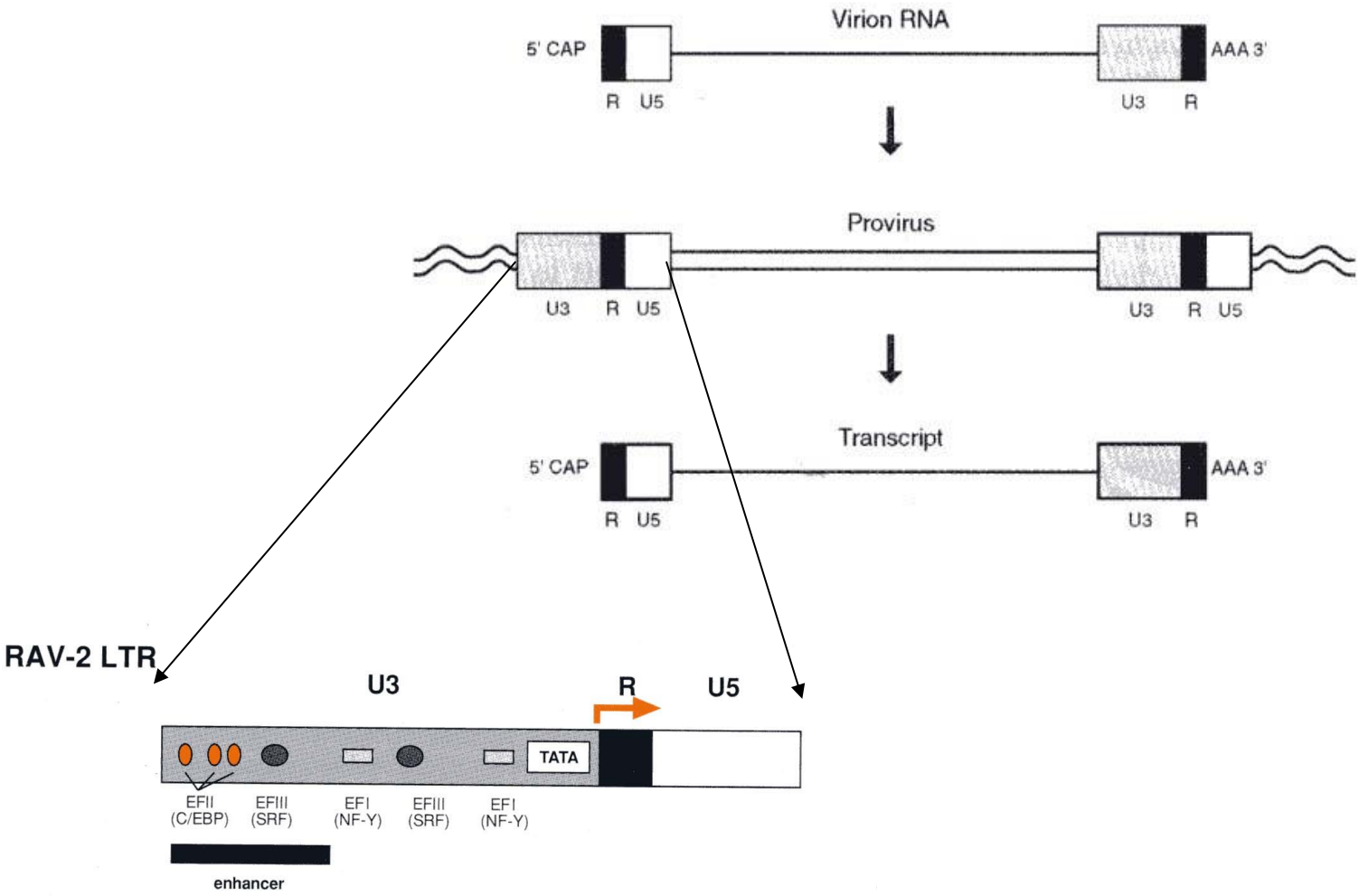


Provirus structures for representative viruses of the *Alpharetrovirus*, *Betaretrovirus*, *Gammaretrovirus* *Deltaretrovirus*, *Epsilonretrovirus*, *Lentivirus* and *Simi ispumavirus* genera

Genome of Simple vs. Complex Retroviruses



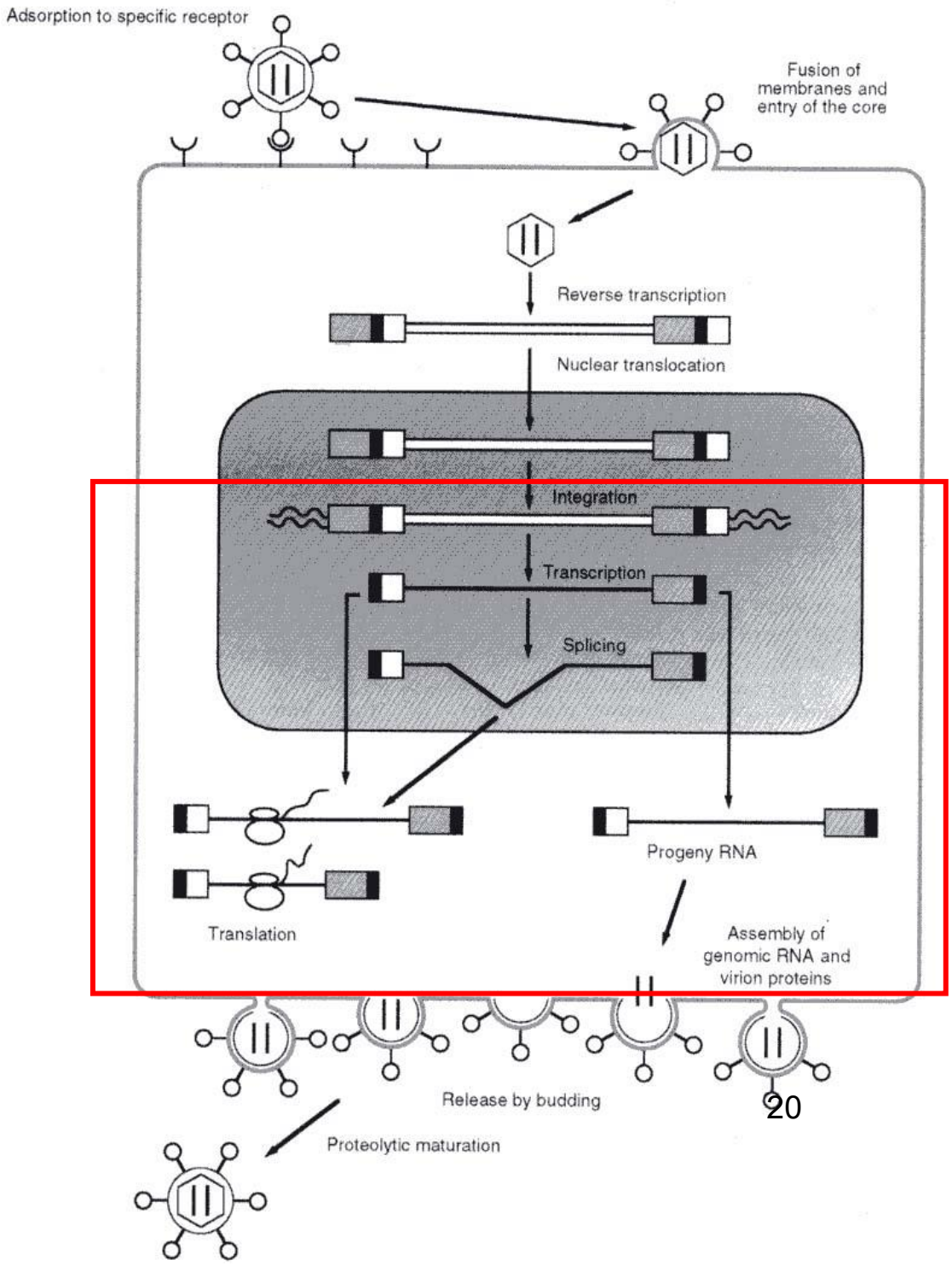
Genome expression



LTR di un retrovirus aviario. Nella regione U3 sono indicati importanti sequenze regolatrici e siti specifici per fattori di trascrizione cellulari. Il legame di tali fattori è determinante per l'attivazione della trascrizione del DNA provirale. Questi fattori sono sia ubiquitari che tessuto-specifici, così che il repertorio di fattori di trascrizione usati da un particolare retrovirus rappresenta un ulteriore grado di specificità per un determinato tipo cellulare.

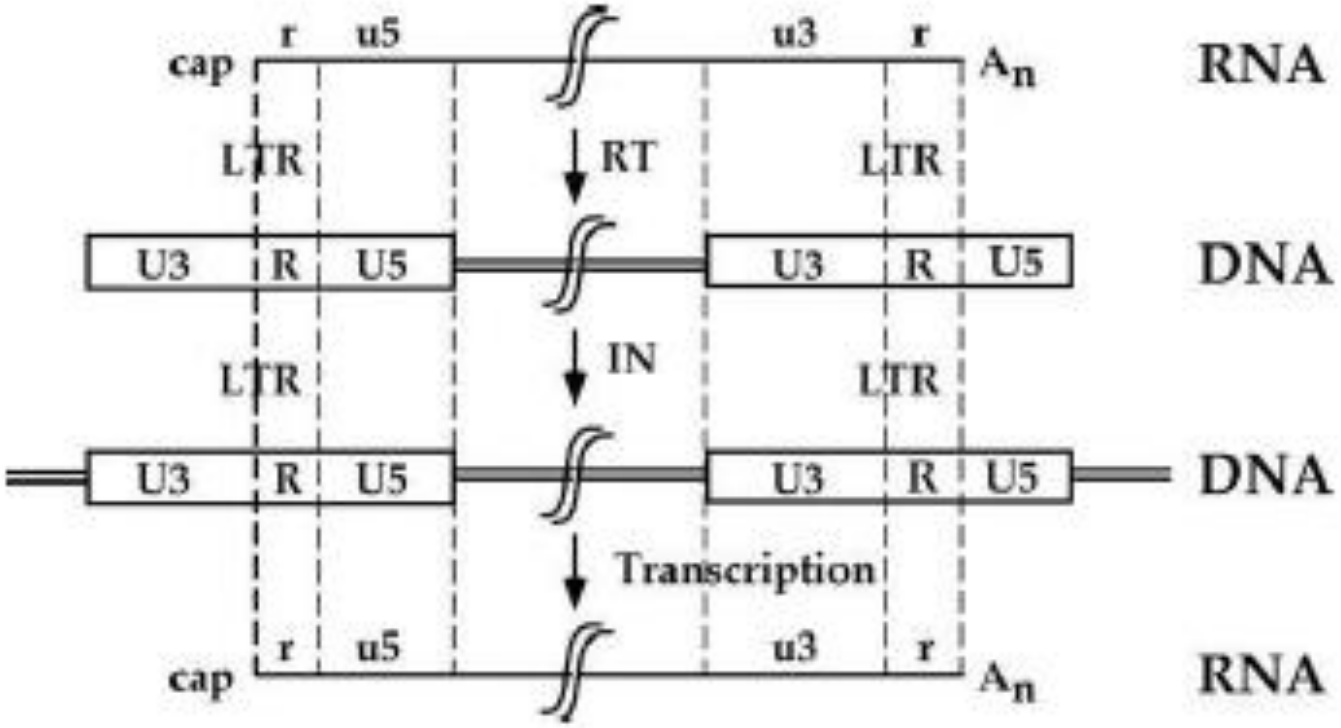
Proviral DNA transcriptiom

Transcription of the integrated DNA begins at the R regions, generating the genomic mRNA and, following splicing reactions, the subgenomic mRNA



U5 poliadenilazione

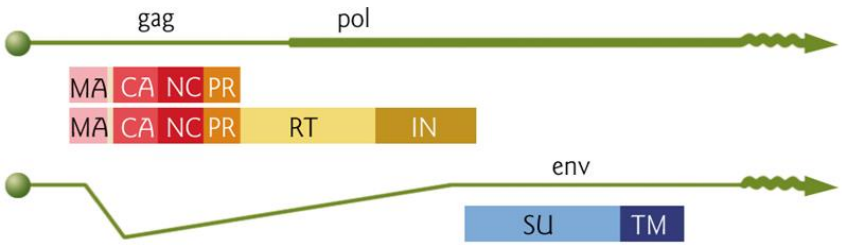
From the genome to the provirus and the transcripts



Fields virology, editors-in-chief, David M. Knipe, Peter M. Howley. – 6th ed. Lippincott Williams & Wilkins press 2013

Proviral DNA expression

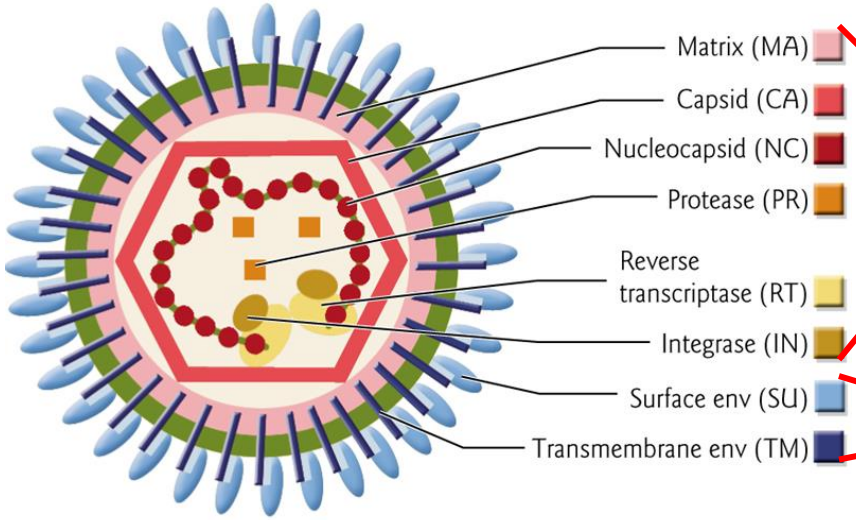
Proviral DNA



Genomic mRNA

Sub-genomic mRNA

(a)



Proteasi virale

Proteasi cellulare

(b)

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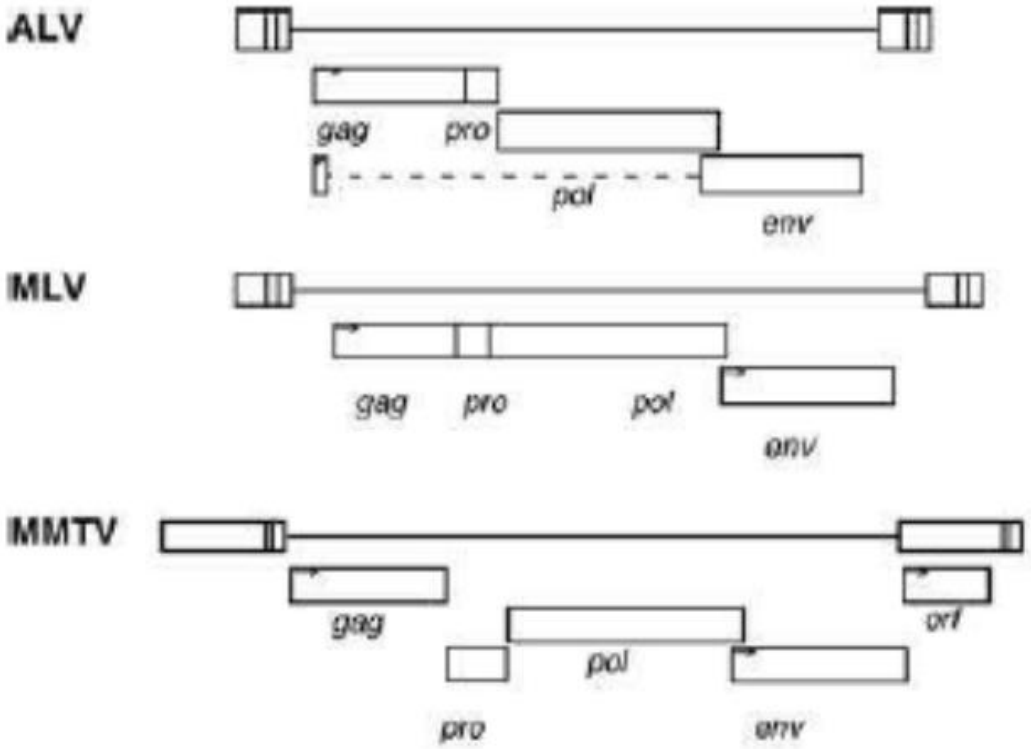
Retrovirus expression

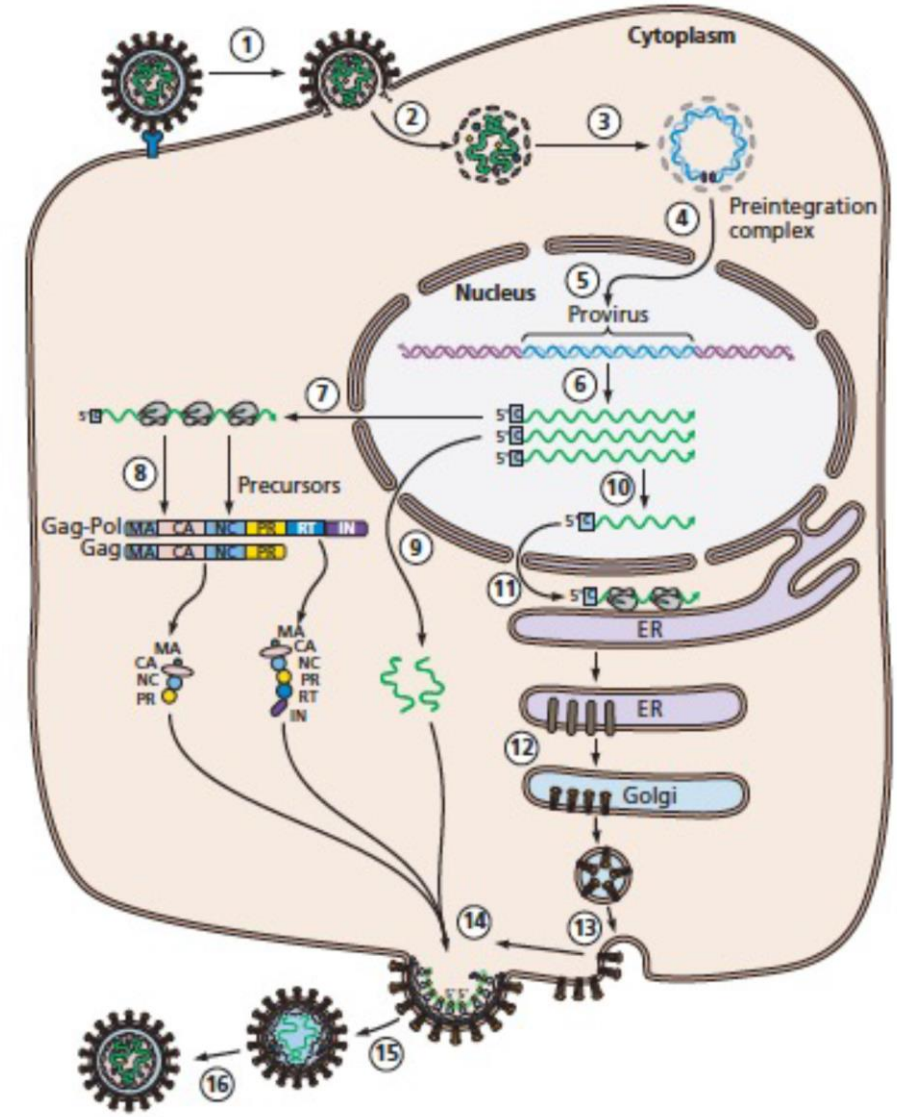


For the alpharetroviruses, *gag* and *pro* are fused and expressed as a single polyprotein; *pol* is in a different reading frame, and a frameshift is used to express the Gag-Pro-Pol polyprotein.

For the gammaretroviruses, *gag and a pro-pol* fusion are in the same reading frame and separated by a stop codon, and translational readthrough is used to make Gag-Pro-Pol.

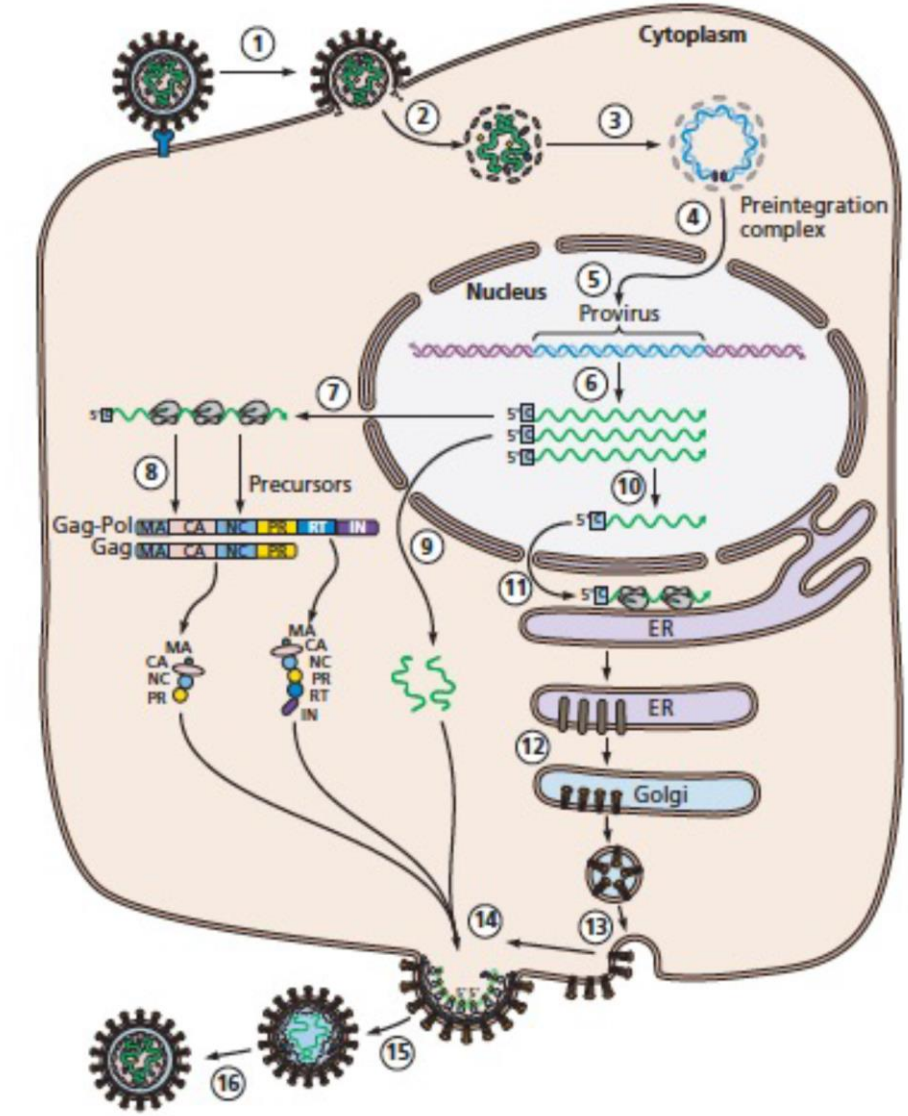
For the betaretroviruses *gag, pro, and pol* are all in different frames and successive frameshifts are used to express Gag-Pro and Gag-Pro-Pol polyproteins.

Life cycle



From Flint et al. *Principles of Virology* (2015), ASM Press

Life cycle



(6) Transcription of integrated viral DNA (the provirus) by the host cell RNA polymerase II system produces full-length RNA transcripts.

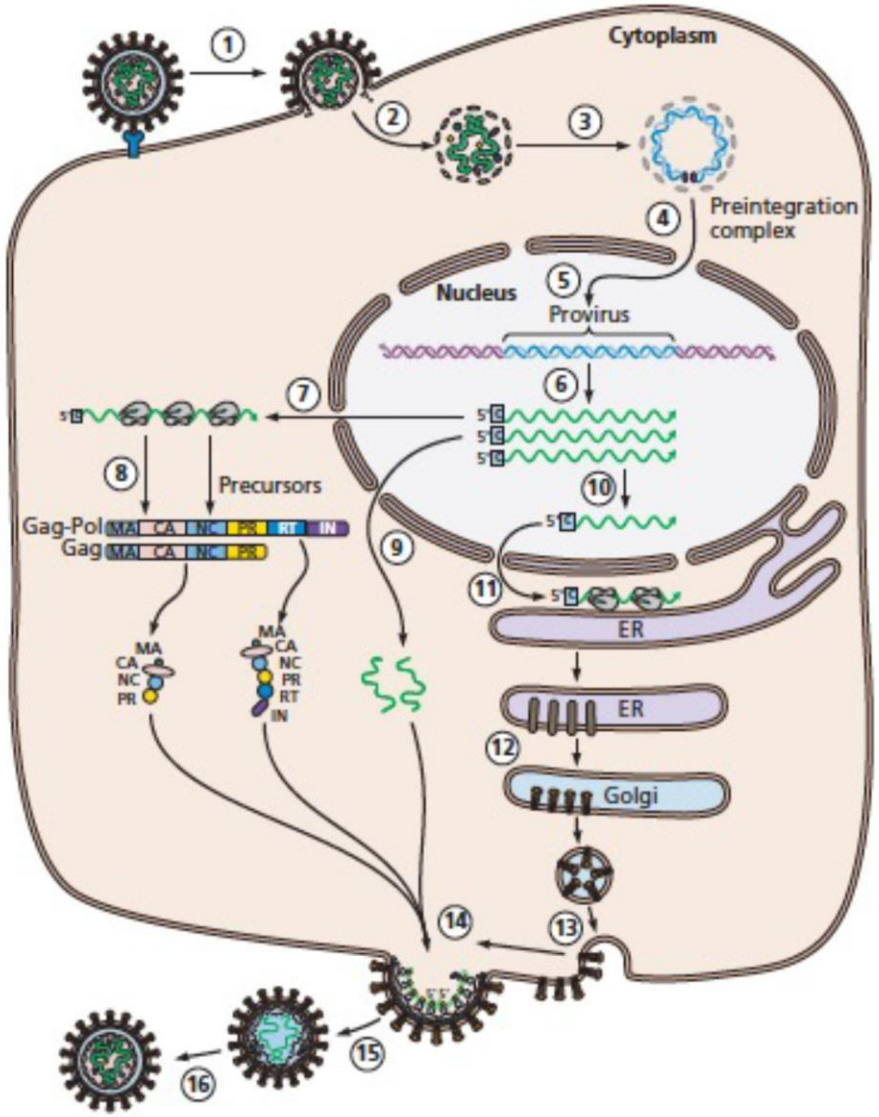
(7) Some full-length RNA molecules are exported from the nucleus to the cytoplasm and serve as mRNAs.

(8) These mRNAs are translated by cytoplasmic ribosomes to form the viral Gag and Gag-Pol polyprotein precursors at a ratio of approximately 10:1.

(9) Some full-length RNA molecules are destined to become encapsidated as progeny viral genomes

From Flint et al. *Principles of Virology* (2015), ASM Press

Life cycle



(10) Other full-length RNA molecules are spliced within the nucleus to form mRNA for the Env polyprotein.
(11) Env mRNA is translated by ribosomes bound to the endoplasmic reticulum (ER).
(12) The Env proteins are transported through the Golgi apparatus, where they are glycosylated and cleaved by cellular enzymes to form the mature SU-TM complex.
(13) Mature envelope proteins are delivered to the surface of the infected cell.
(14) Virion components (two copies of the viral RNA, Gag and Gag-Pol precursors, and SU-TM) assemble at budding sites with
the help of cis-acting signals encoded in each.
(15) The nascent particles bud from the surface of the cell.
(16) Maturation (and infectivity) requires the action of the virus-encoded protease (PR), which is itself a component of the core precursor polyprotein.

From Flint et al. *Principles of Virology* (2015), ASM Press

Retroviruses as oncogenic viruses

Table 6.3 The oncogenic retroviruses

Property or characteristic	Transducing viruses	Nontransducing viruses	Nontransducing, long-latency viruses
Example	Rous sarcoma virus	Avian leukosis virus	Human T-lymphotropic virus type 1
Efficiency of tumor formation	High (ca. 100% of infected animals)	High to intermediate	Very low (<5%)
Tumor latency	Short (days)	Intermediate (weeks, months)	Long (months, years)
Infecting viral genome	Viral-cellular recombinant; normally replication defective	Intact; replication competent	Intact; replication competent
Oncogenic element	Cell-derived oncogene carried in viral genome	Cellular oncogene activated in situ by a provirus	Virus-encoded regulatory protein controlling transcription?
Mechanism	Oncogene transduction	*cis*-acting provirus	*trans*-acting protein?
Ability to transform cells in culture	Yes	No	No

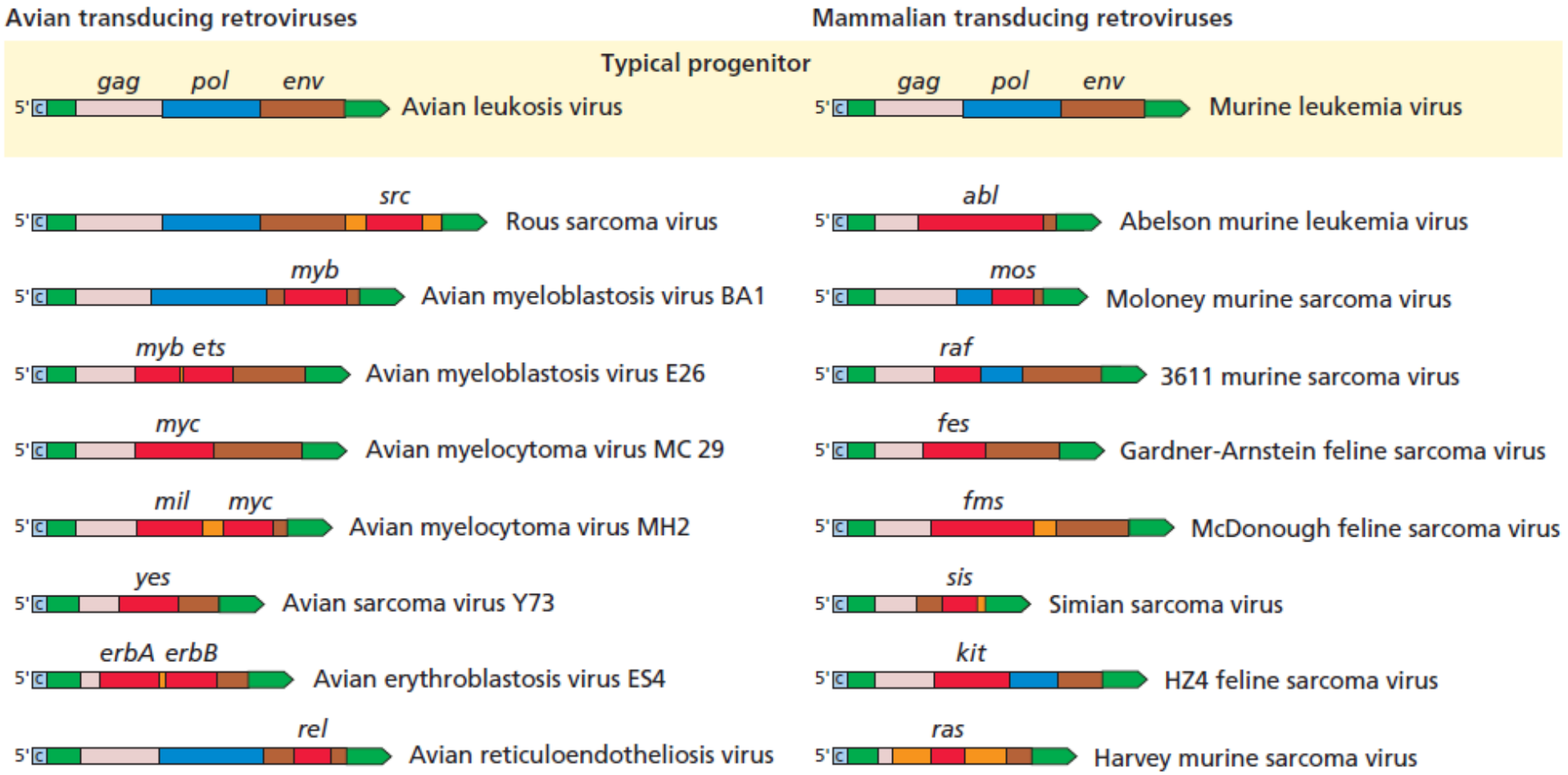
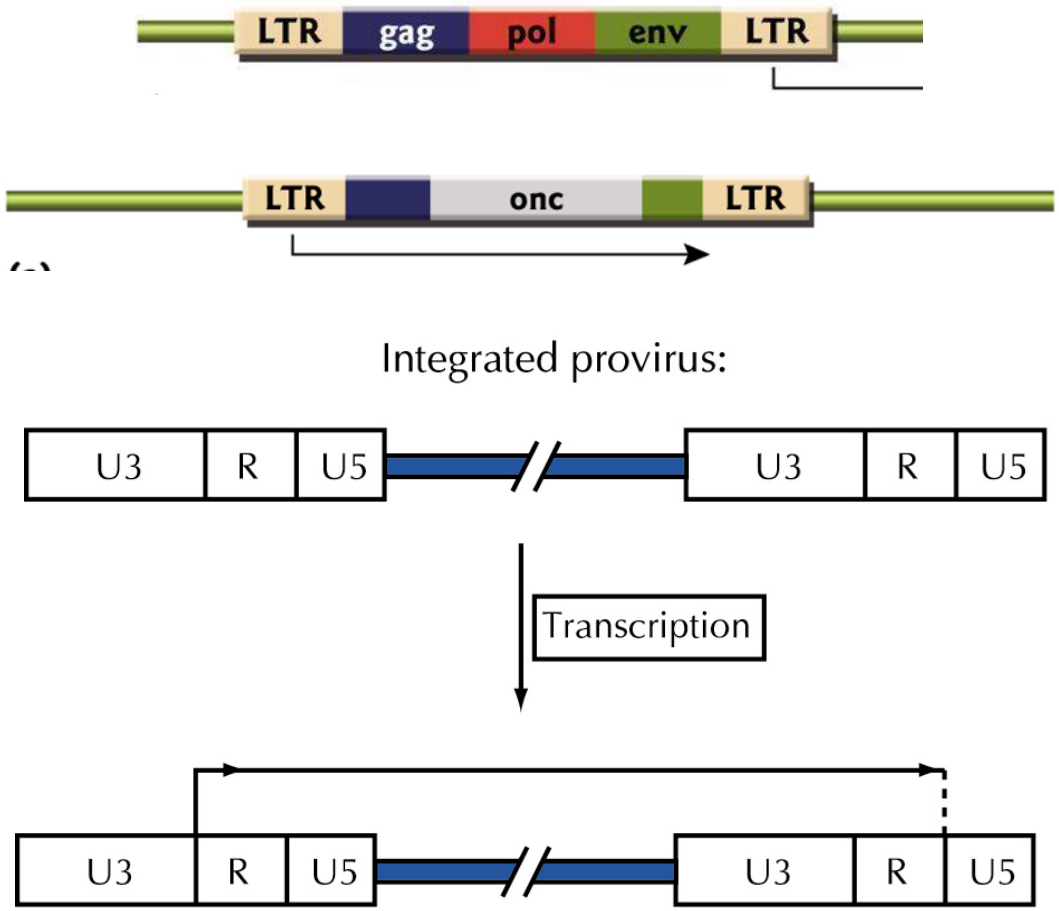
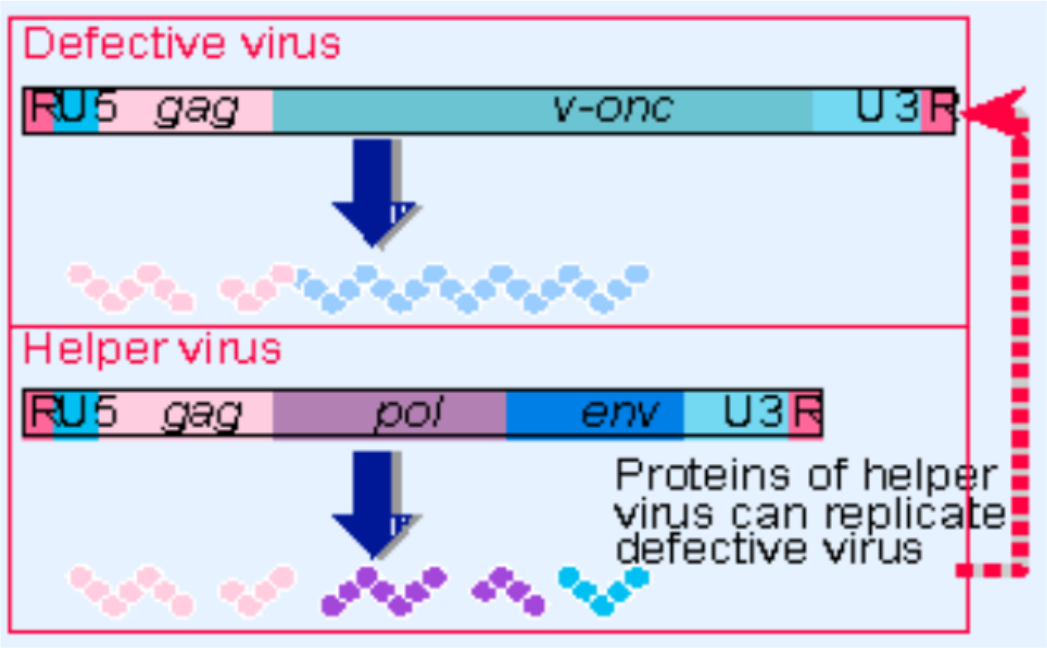


Figure 6.8 Genome maps of avian and mammalian transducing retroviruses. Avian leukosis virus (e.g., Rous-associated virus) and murine leukemia virus are prototypical retroviruses. Their genomes contain the three major coding regions: *gag* (pink), *pol* (blue), and *env* (brown). In Rous sarcoma virus, the oncogene *src* is added to the complete viral genome. In all other avian and mammalian transducing retroviruses, some of the viral coding information is replaced by cell-derived oncogene sequences (red). Consequently, such transducing viruses are defective in replication. The majority of the transducing retroviruses carry a single v-oncogene in their genomes, but some include more than one (e.g., *erbA* and *erbB* in avian erythroblastosis virus ES4). In such cases, one is sufficient for transformation, while the second accelerates this process. In some cases, additional cellular DNA sequences (orange) were also captured in the viral genome. Adapted from T. Benjamin and P. Vogt, p. 317–367, *in* B. N. Fields et al. (ed.), *Fields Virology*, 2nd ed. (Raven Press, New York, NY, 1990), with permission.

Transducing retroviruses: Acute transforming retroviruses



Defective retroviruses require helper virus to produce more virus particles



Replication-defective transforming viruses have a cellular sequence substituted for part of the viral sequence. The defective virus may replicate with the assistance of a helper virus that carries the wild-type functions.

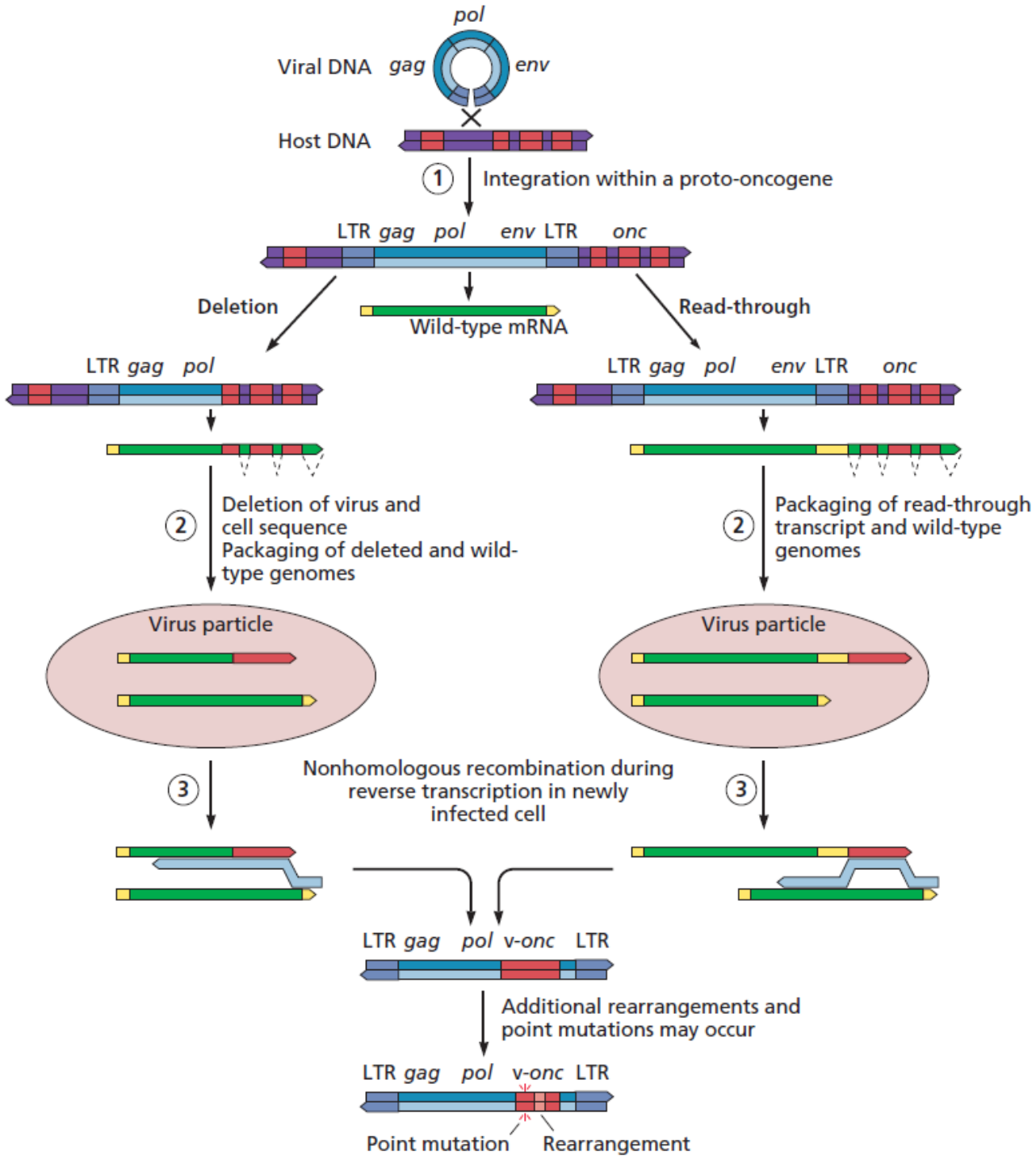
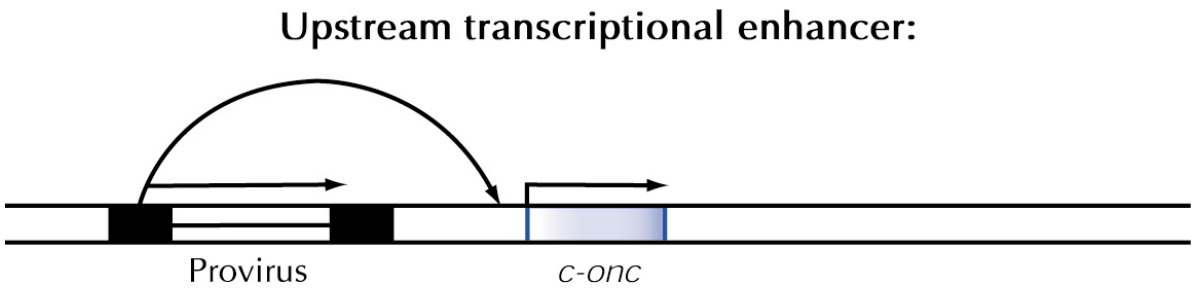
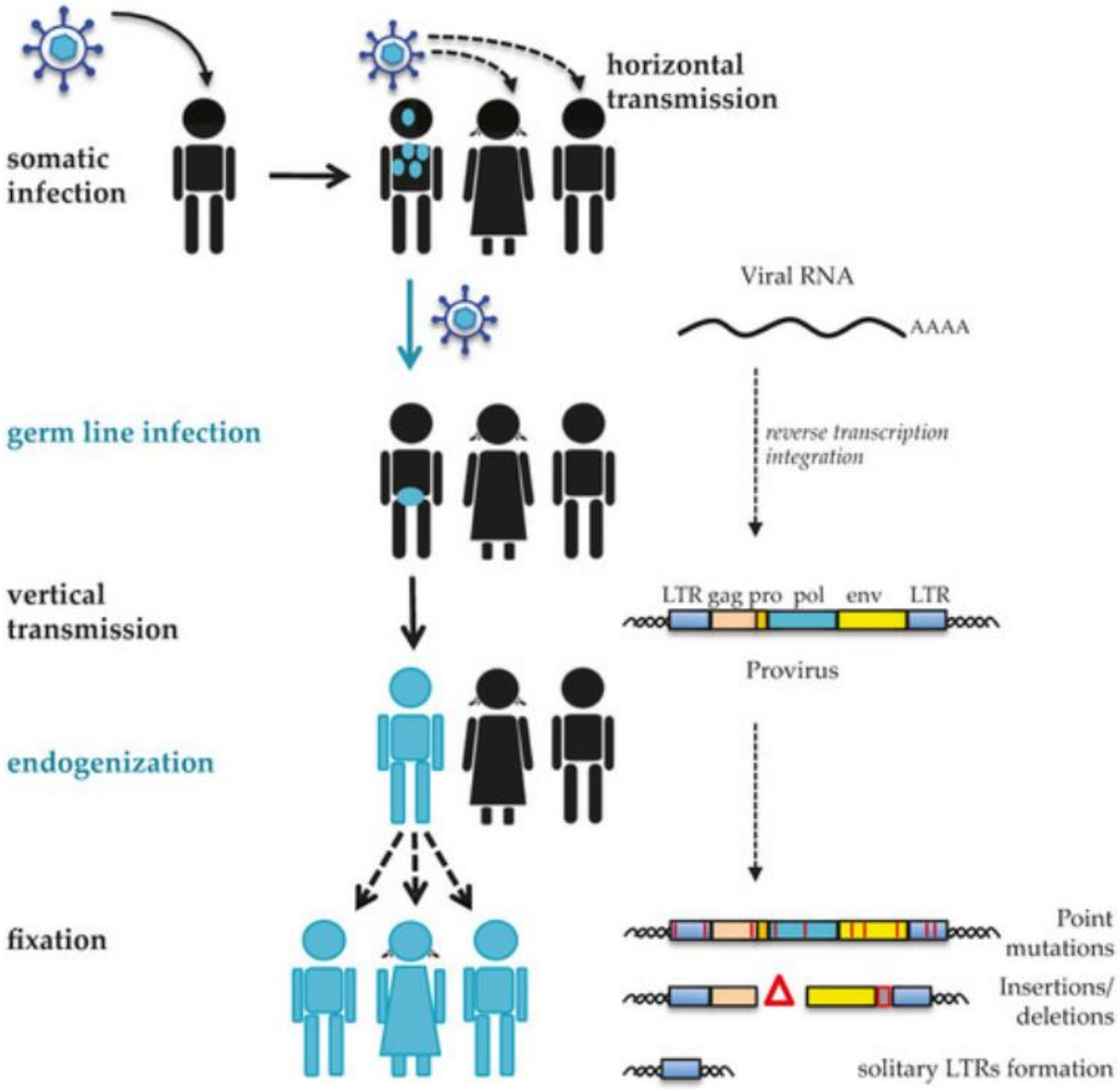


Figure 6.9 Possible mechanisms for oncogene capture by retroviruses. The first step in each of two mecha-

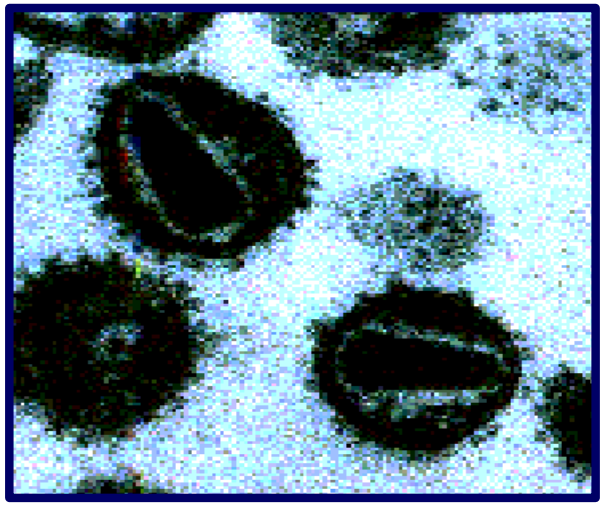
Slowly transforming retroviruses: insertional transformation

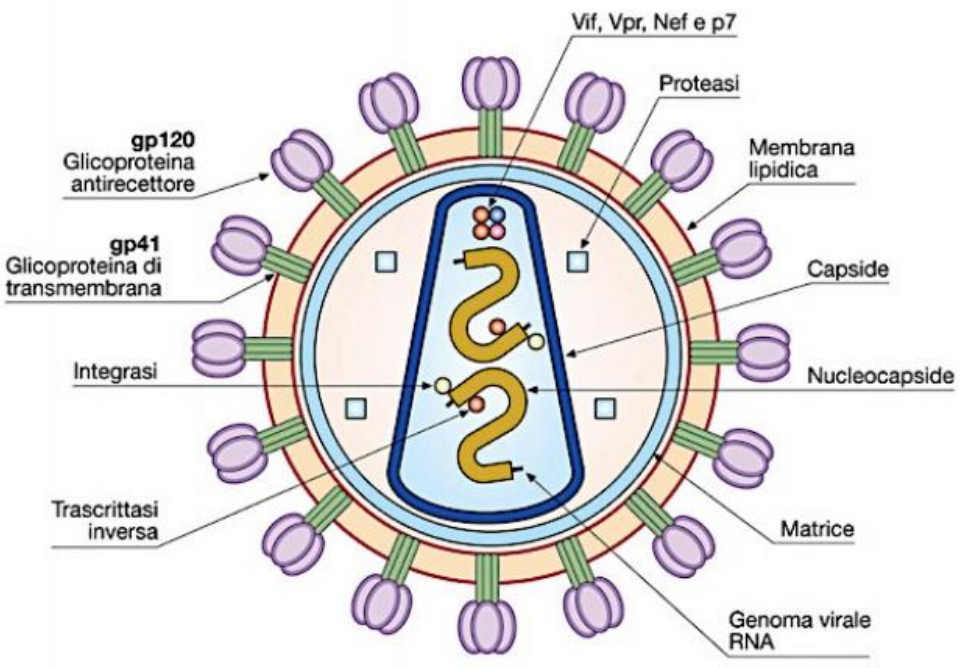


Endogenous retroviruses (ERV)

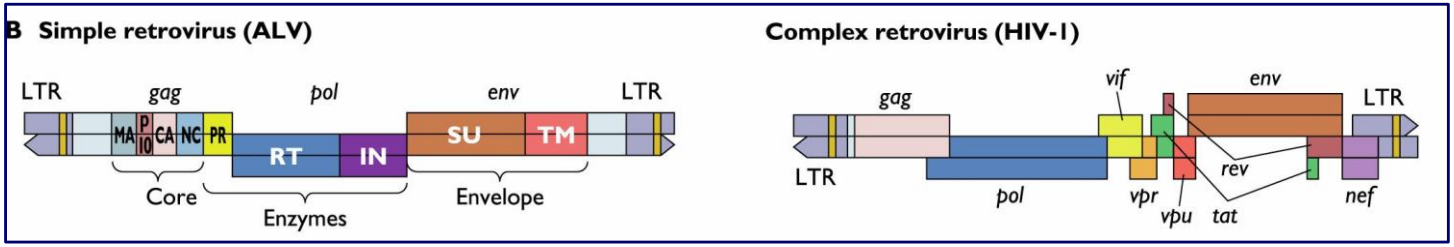


HIV





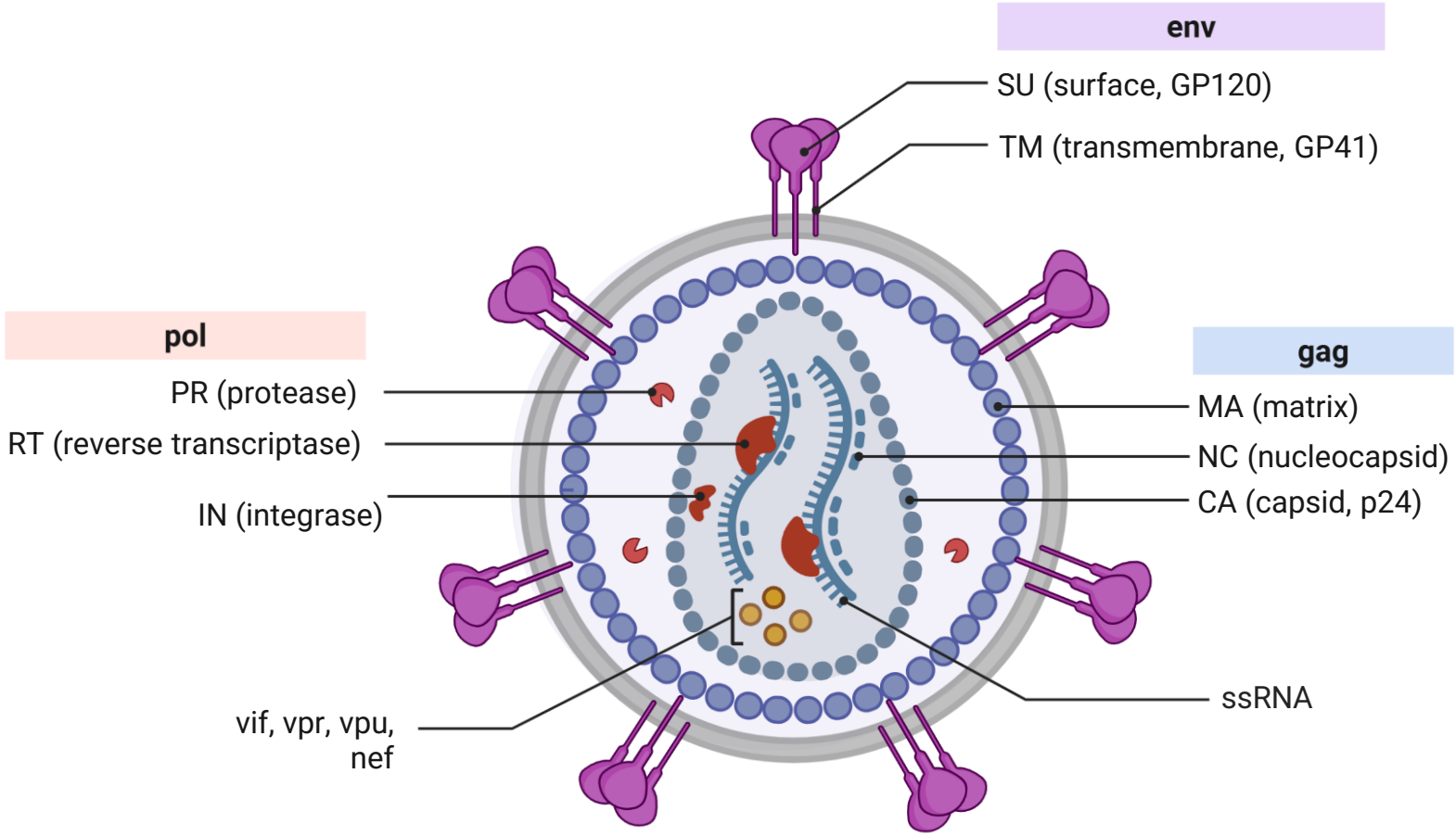
Multiple copies of the accessory proteins Vpr, Vif and Nef are also found within the virion



From Flint et al. *Principles of Virology* (2000), ASM Press

HIV-1 virion

Envelope: strato fosfolipidico nel quale sono mantenute certe proteine cellulari di superficie, come le molecole del complesso maggiore di istocompatibilità 1 e 2 (MHC), e nel quale sono inserite le proteine virali env, dette spikes. Tre molecole di gp41-TM (transmembrane), ancorate al bilayer fosfolipidico, sovrastate da tre molecole di gp120-SU (surface unit)



HIV-1 genome and provirus

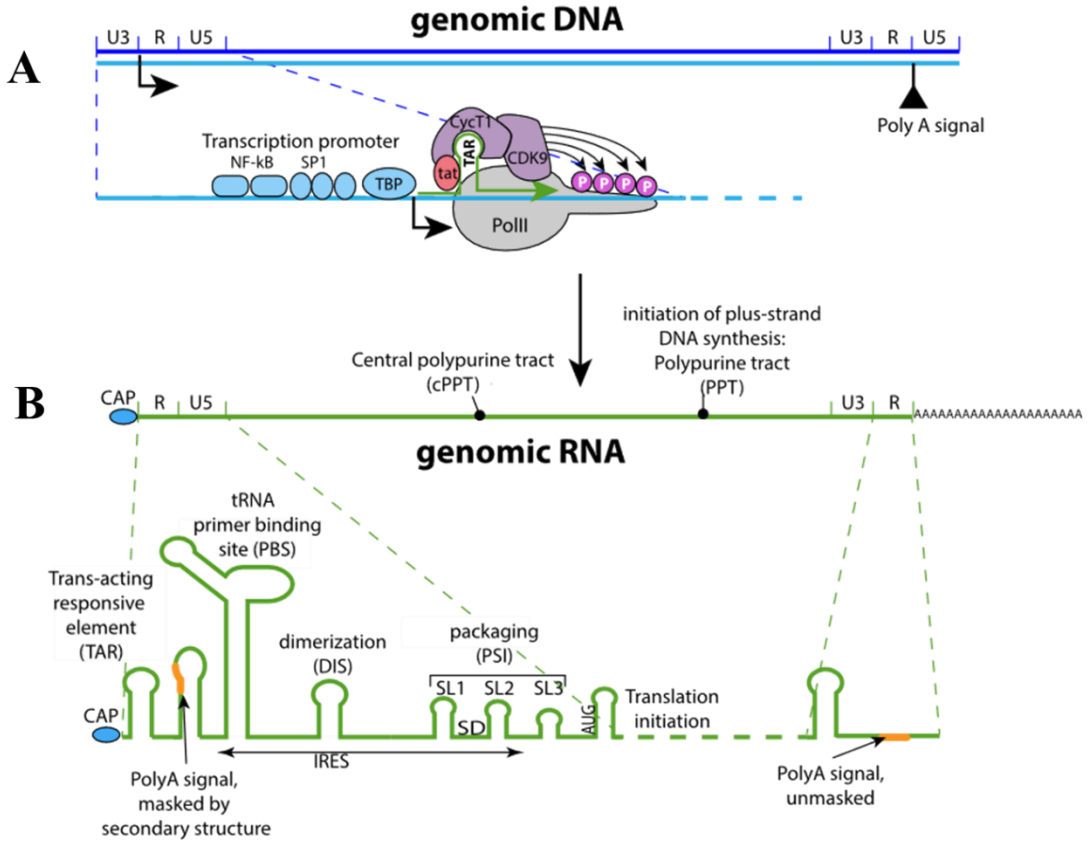
L'RNA genomico (gRNA) è lungo 9.75 kb e contiene nove ORF codificanti per 15 proteine

3 ORF, ovvero gag (Group of specific Antigen), pol (Polymerase) ed env (envelope), sono comuni a tutta la famiglia Retroviridae e codificano per le proteine strutturali e funzionali indispensabili per la formazione di nuovi virioni.

Le altre 6 ORF aggiuntive sono, invece, tipiche dei lentivirus e contengono le informazioni per le proteine accessorie nef, vif, vpr e vpu e per quelle regolatorie tat e rev.

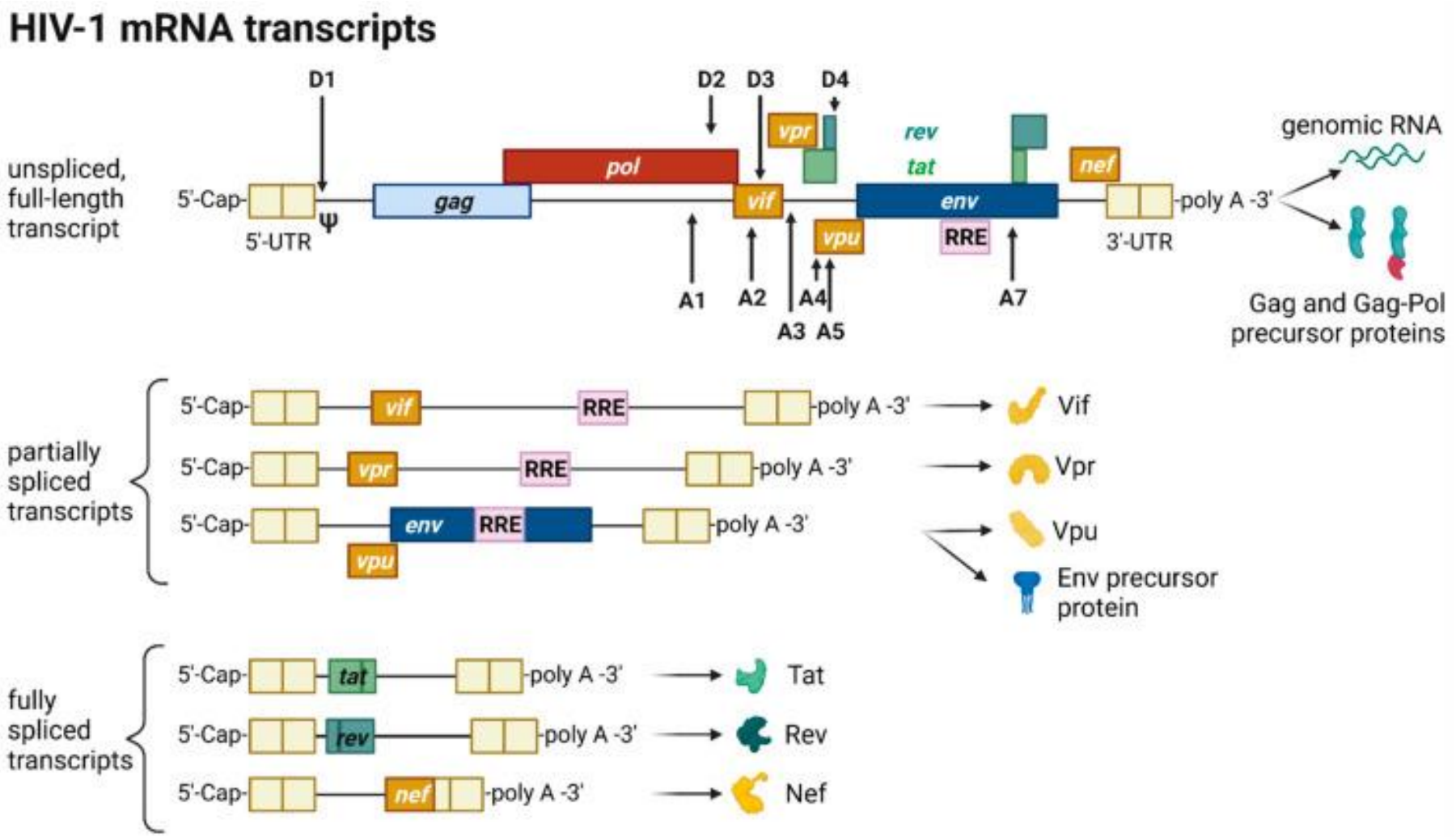
HIV-1 genome and provirus

LTR: Nella regione di giunzione tra U3 e R nell'LTR 5' si trova il promotore per l'inizio della trascrizione che lega l'RNA polimerasi II cellulare e che è costituito dalla TATA box (riconosciuta dal TF2D e TF2H), da *enhancers*, e da siti di legame per fattori di splicing e fattori di trascrizione cellulari (ad esempio, NFkB (*Nuclear Factor-kB*), NFAT (*Nuclear Factor of Activated T-cells*), sp1). Le regioni R e U5 nell'LTR 3', invece, possiedono i segnali di fine trascrizione.



Al 5'Major Splice Donor Site D1 (usato per generare tutti gli mRNA subgenomici)

HIV-1 transcripts and proteins



Full lenght: circa metà di questi trascritti farà o da copie del genoma o da stampo per l'espressione dei geni gag e pol; la restante metà, invece, farà da stampo per lo splicing alternativo.

HIV-1 proteins

La poliproteina *gag* (p55) : **p17-MA** importante per il trasporto nucleare del PIC e per l'indirizzamento dei componenti strutturali del virione alla membrana plasmatica; **p24-CA** importante per l'*uncoating*, il trasporto nucleare del PIC e l'integrazione del genoma; **p7-NC** responsabile dell'incapsidamneto del genoma; **p6** responsabile dell'incorporazione di *vpr* e della gemmazione del virione.

La poliproteina *pol,* dà origine ai tre enzimi virali:

l'integrasi (p32) è una proteina di 288 amminoacidi tetramerica nelle cui porzioni N- e C-terminali possiede domini a dita di Zn per il legame al DNA virale che andrà ad integrare nel genoma dell'ospite;

la proteasi (p10) è una proteina di 99 amminoacidi omodimerica

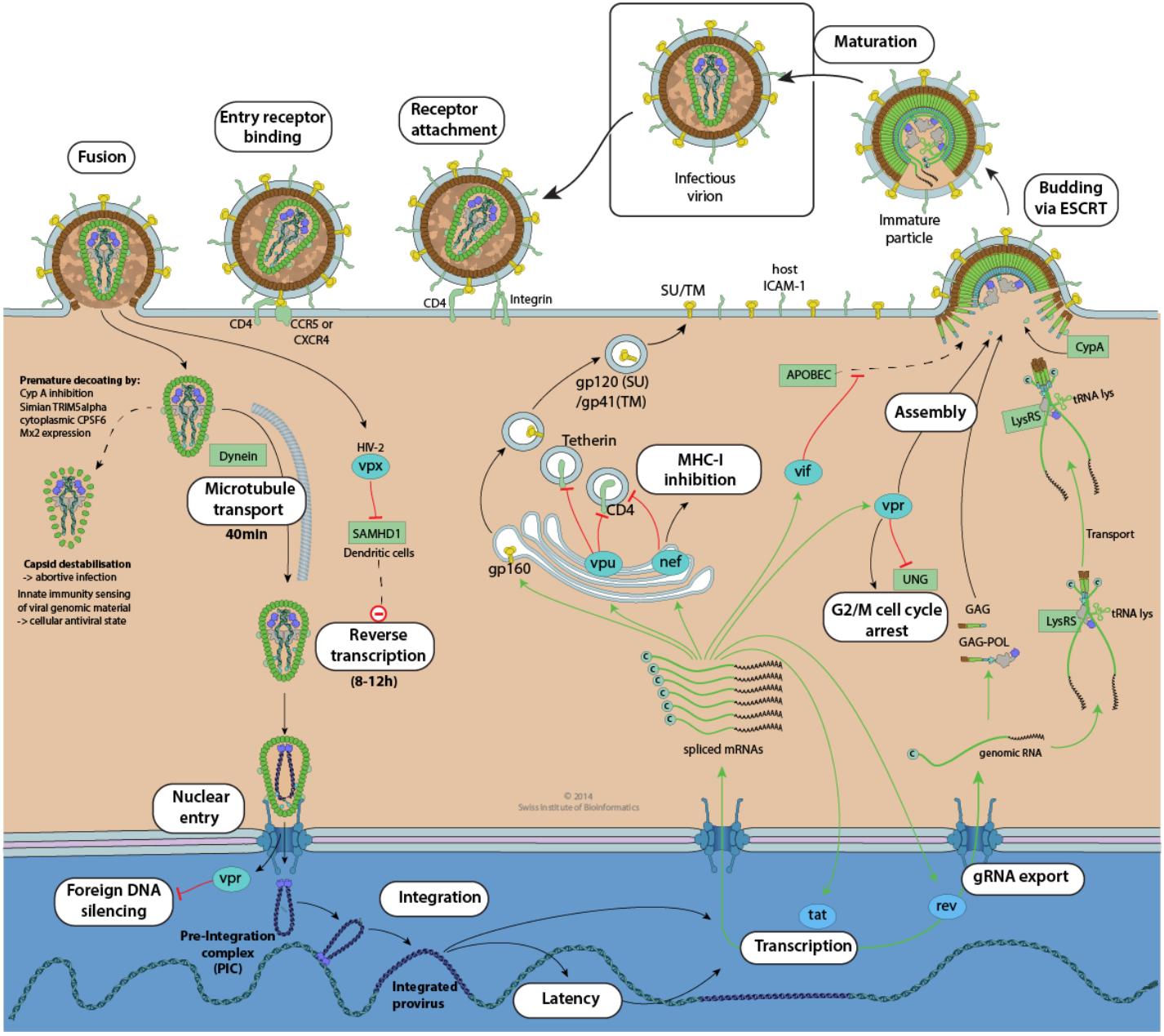
la trascrittasi inversa (RT) è una proteina di 355 amminoacidi eterodimerica formata da due subunità, ovvero la p51 che dà stabilità conformazionale e la p66 con triplice funzione (DNA polimerasi RNA-dipendente e DNA polimerasi DNA-dipendente per la sintesi del dsDNA, RNAsi-H

La poliproteina env (gp160) deriva da un mRNA biscistronico (env-vpu) che viene tradotto nel RER, dove si assembla in trimeri, glicosilato nell'apparato del Golgi e, infine, tagliato dalla furina cellulare nelle subunità **gp120 e gp41**

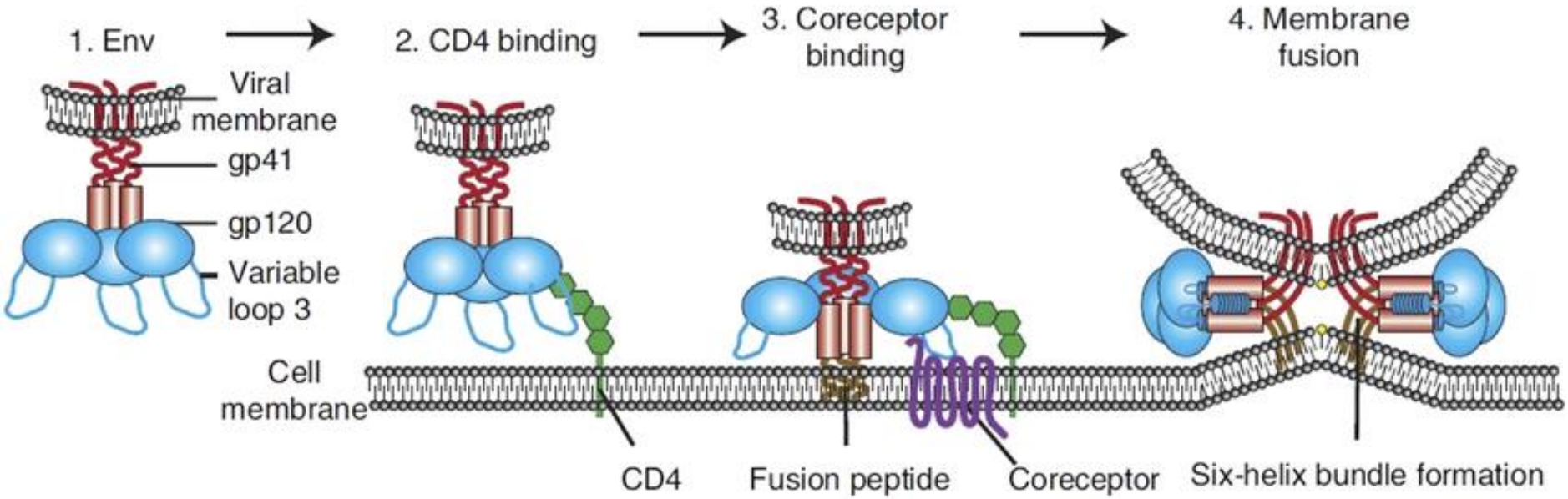
HIV-1 transactivation and accessory proteins

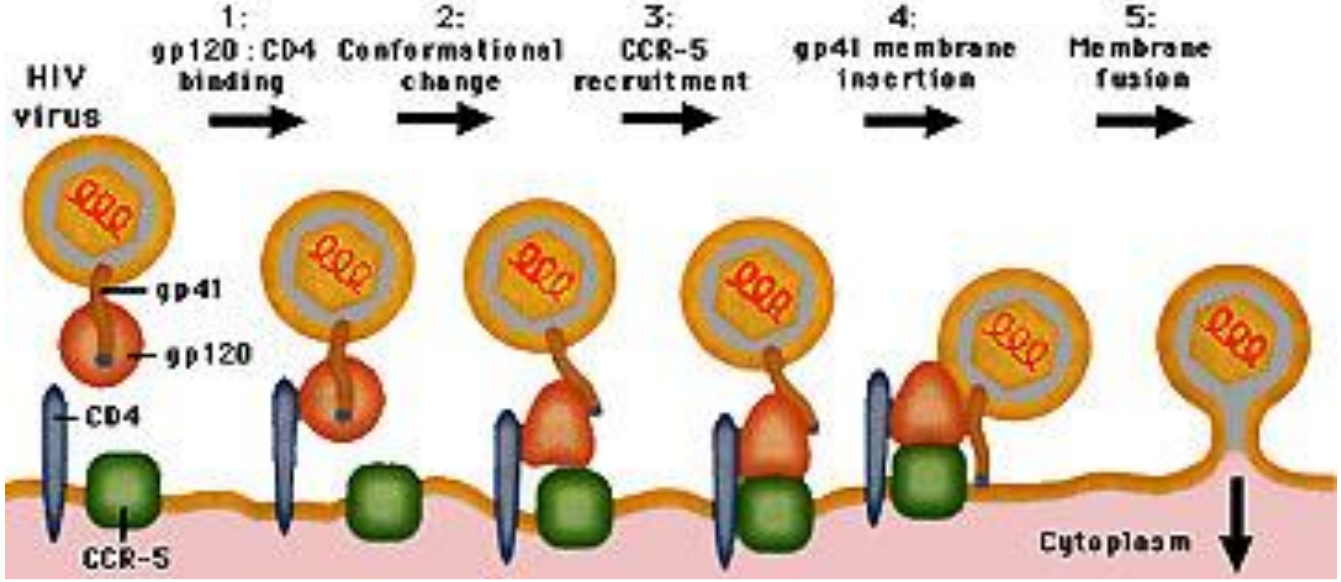
- TAT: ***T***rans-***A***ctivator of ***T***ranscription
- REV: ***Re***gulator of ***V***irion protein expression
- NEF: ***Ne***gative *R*egulatory ***F***actor
- VIF: ***V***irion ***I***nfectivity ***F***actor
- VPU: ***V***iral ***P***rotein ***U***
- VPR: ***V***iral ***P***rotein ***R***

HIV-1 life cycle

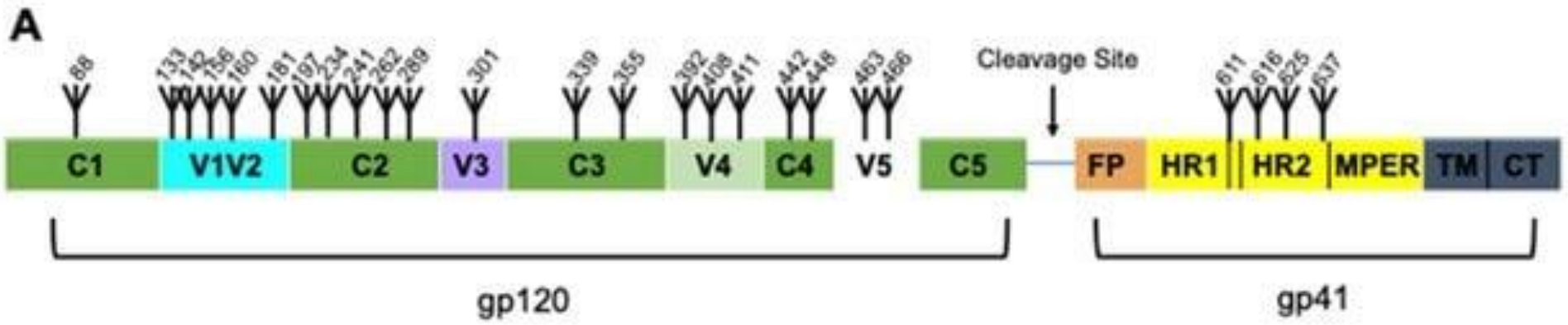


HIV entry into target cell





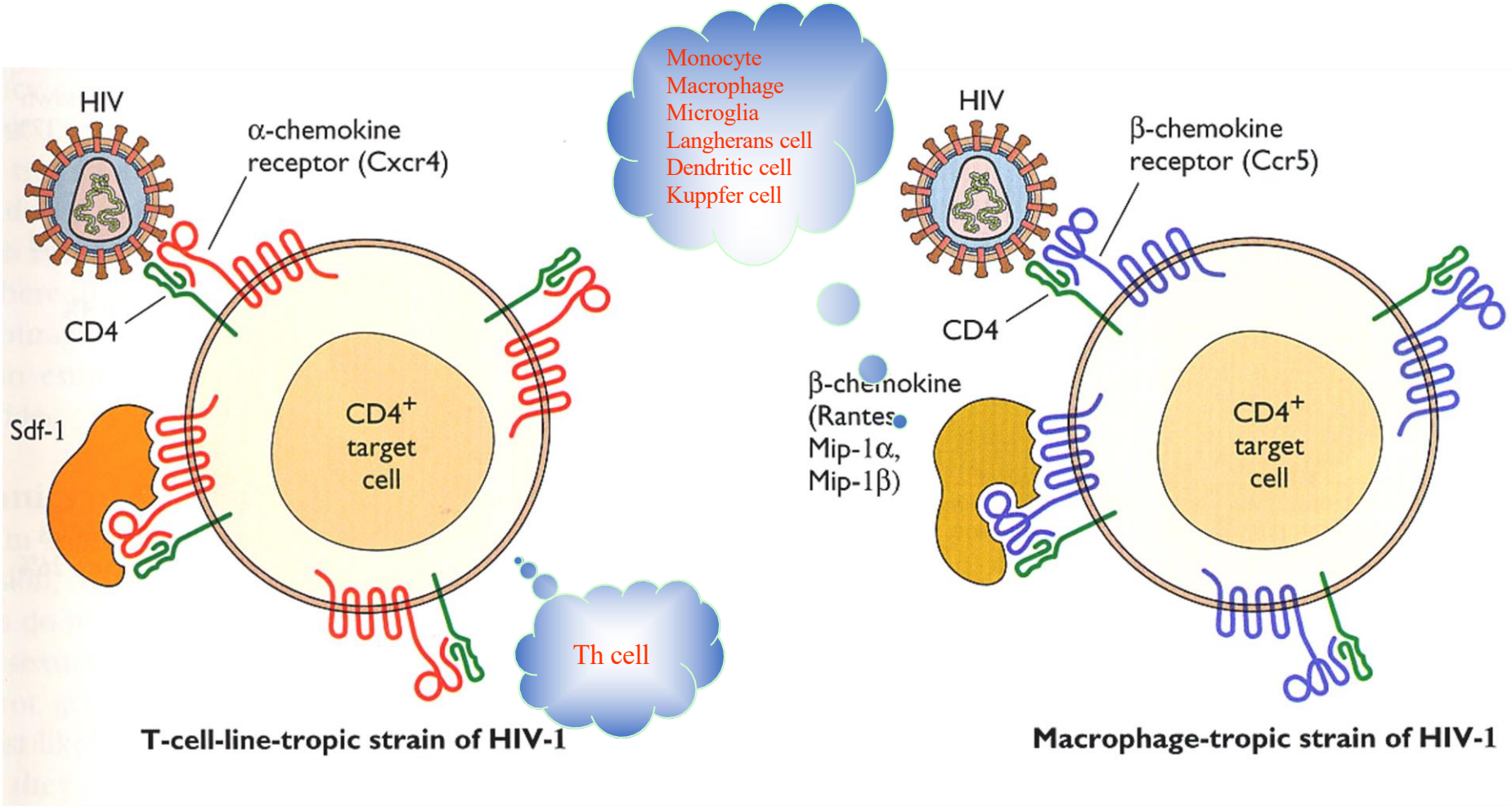
The env proteins



le regioni C3-C4 sono deputate al legame del recettore primario:
CD4 (espresso sui linfociti T CD4+, sulle cellule microgliali, sui macrofagi/monociti e sulle cellule dendritiche)

l'ansa V3 lega invece il recettore secondario o corecettore determinando il tropismo cellulare. Si distinguono i virus R5-tropici (o M-tropici), che riconoscono il recettore per beta chemochina CCR5 espressa su macrofagi, cellule dendritiche, cellule microgliali e linfociti CD4+ memory e CD4+ attivati, e quelli X4-tropici (o T-tropici), che riconoscono il recettore per l'alfa chemochina CXCR4 espressa sui linfociti CD4+naïve e memory. Esistono anche i virus dual tropici (o X4/R5-tropici) che riconoscono entrambi i corecettori.

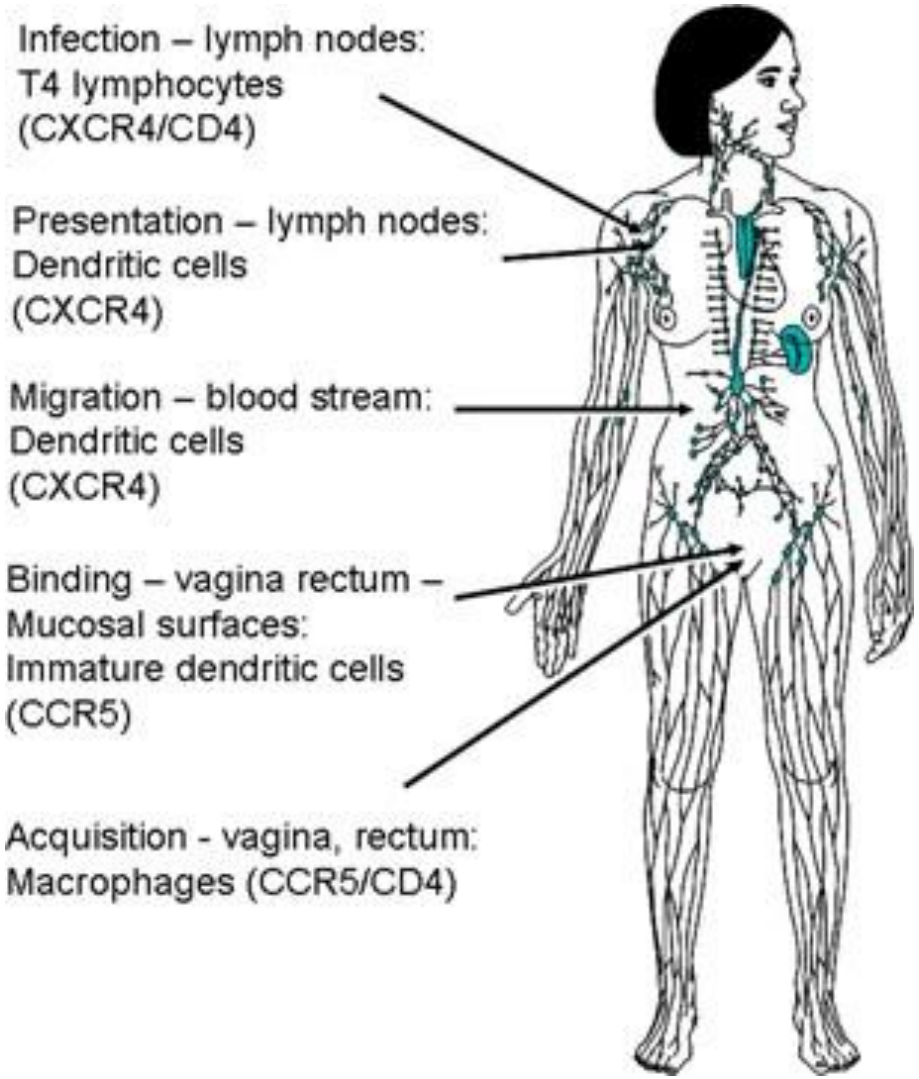
Chemokine receptors seem to be the key to the gateway of the cell



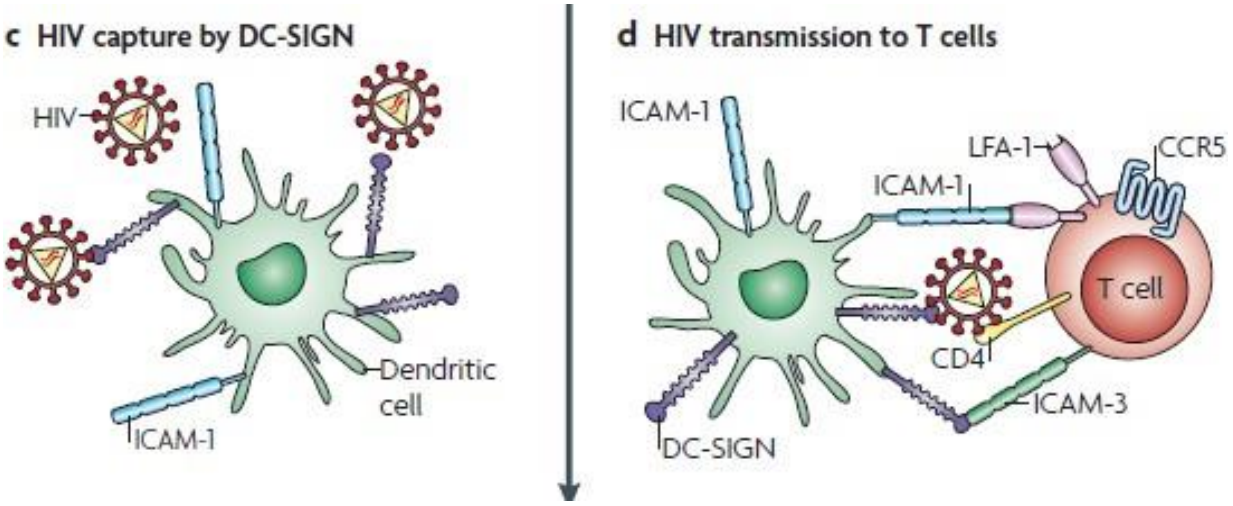
Chemokine receptors seem to be the key to the gateway of the cell

These co-receptors may explain the phenotypic switch during infection. Changes in the amino acid sequence of Gp120 occur in the progression of the disease. It is likely that HIV uses CCR5 in the early stages of disease and then switches to CXCR4, perhaps avoiding the suppressive activity of chemokines. This also explains the transition from non-syncytium-inducing to syncytium-inducing phenotype. CXCR4 and CCR5 are members of a large family of receptors and the spread of HIV through subtypes of T cells may reflect subtle changes on the variable loops of Gp120 allowing the infection of new CD4+ cells with different co-receptors.

Entry of HIV via the mucosal route and transit via dendritic cells to the lymph nodes



HIV-1 and Dendritic cells

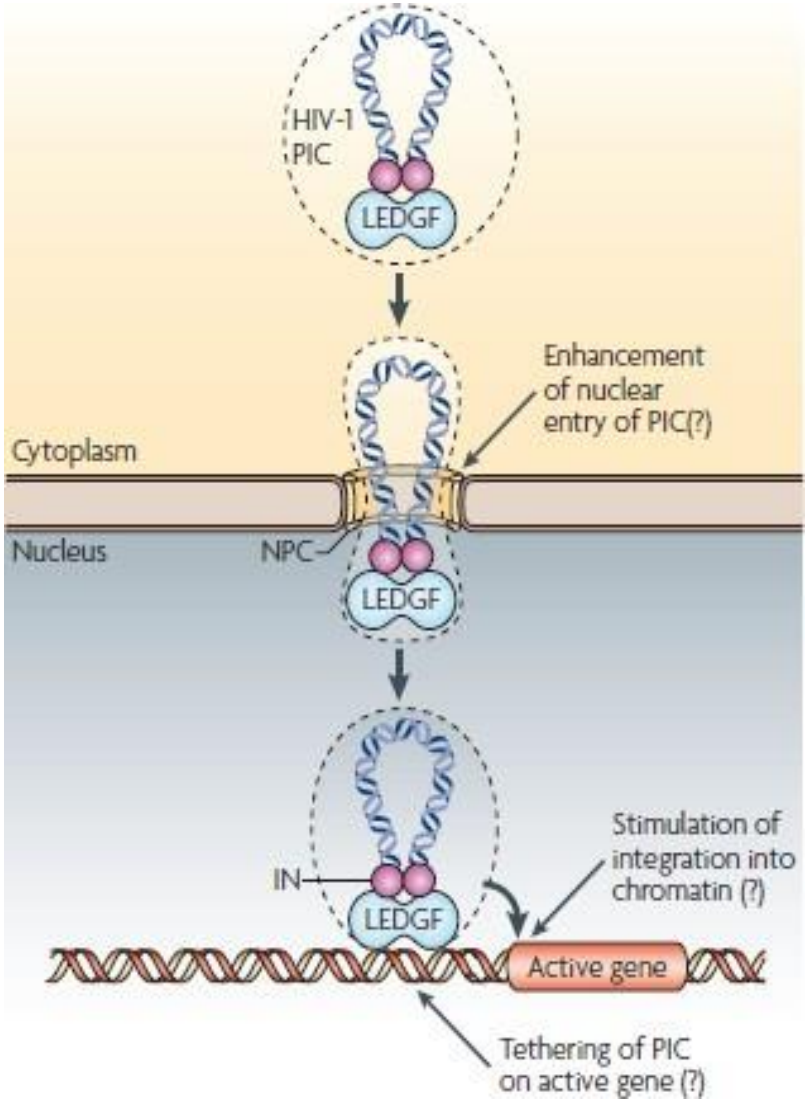


The capture of HIV particles by dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing non-integrin (DC-SIGN)-expressing cells such as DCs (c), and DC-SIGN-captured HIV transmission to T cells (d).

HIV-1 life cycle

Viaggiando lungo il network di microtubuli e in virtù di interazioni CA-nucleoporine/carioferine, il core virale raggiunge il poro nucleare a livello del quale si forma il complesso di retrotrascrizione (RTC, *Retrotranscription Complex*) costituito dal materiale genetico associato al primer tRNA, all'INT, all'RT, e alle proteine MA, NC e *vpr*. Ha così inizio la retrotrascrizione, durante la quale la sintesi dei filamenti antisenso e senso di DNA, rispettivamente dai primer tRNA e PPT, si ottiene con l'alternanza delle attività polimerasica e RNAsica dell'RT e con i trasferimenti dei filamenti alle estremità genomiche (*jump* o *strand transfer,* ovvero salto o trasferimento del filamento)

HIV-1 life cycle: nuclear import and integration



l'import nucleare è un meccanismo attivo mediato dai segnali NLS delle proteine CA, MA, INT e *vpr* che prendono contatto diretto col poro nucleare e con le importine

Una volta dentro il nucleo, il PIC si associa a cofattori cellulari quali proteine di riparo del DNA e gli chaperoni LEDGF/P75 e CPSF6, trasformandosi nel TCC (*Target Capture Complex*) Esso media l'inserzione del DNA virale nel cromosoma ospite

HIV-1 life cycle: vDNA integration

IN associates with the viral DNA in the cytoplasm to form the pre-integration complex (PIC).

1) IN cuts a dinucleotide from both ends of the viral DNA to produce hydroxylated 3' ends in the PIC (sticky ends)

2) IN binds the host DNA, catalyses a staggered cleavage in the cellular target DNA (5nt),

3) The 3' recessed ends of viral DNA are joined to the 5' "overhanging" termini of the cleaved cellular DNA.

4) gaps and any mismatched bases at the newly created junctions are repaired by host DNA repair machinery irreversible integration.

HIV-1 life cycle: synthesis of the viral proteins

If integration occurs in quiescent cells or in transcriptionally inactive sites, the provirus (the integrated form of HIV) enters a latent state, generating a reservoir that does not produce progeny.

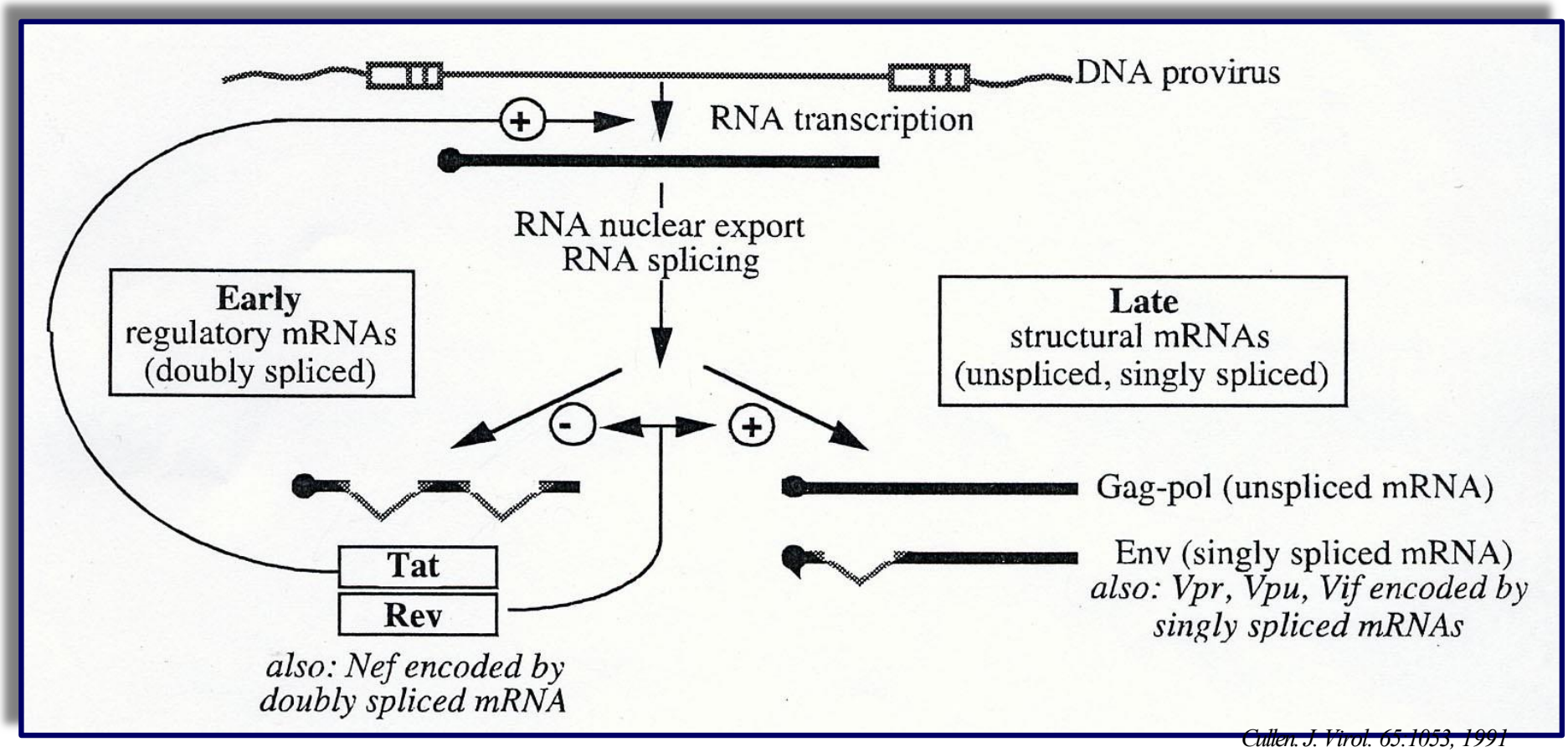
If, instead, integration takes place in euchromatic regions or in immunologically stimulated cells, viral protein synthesis begins.

This process is subject to temporally regulated post-transcriptional control:

an early phase, in which the poorly processive cellular RNA polymerase generates many fully spliced transcripts and few full-length transcripts,

and **a late phase, in which—thanks to the increased polymerase efficiency driven by Tat and Rev—the other classes of transcripts also appear.**

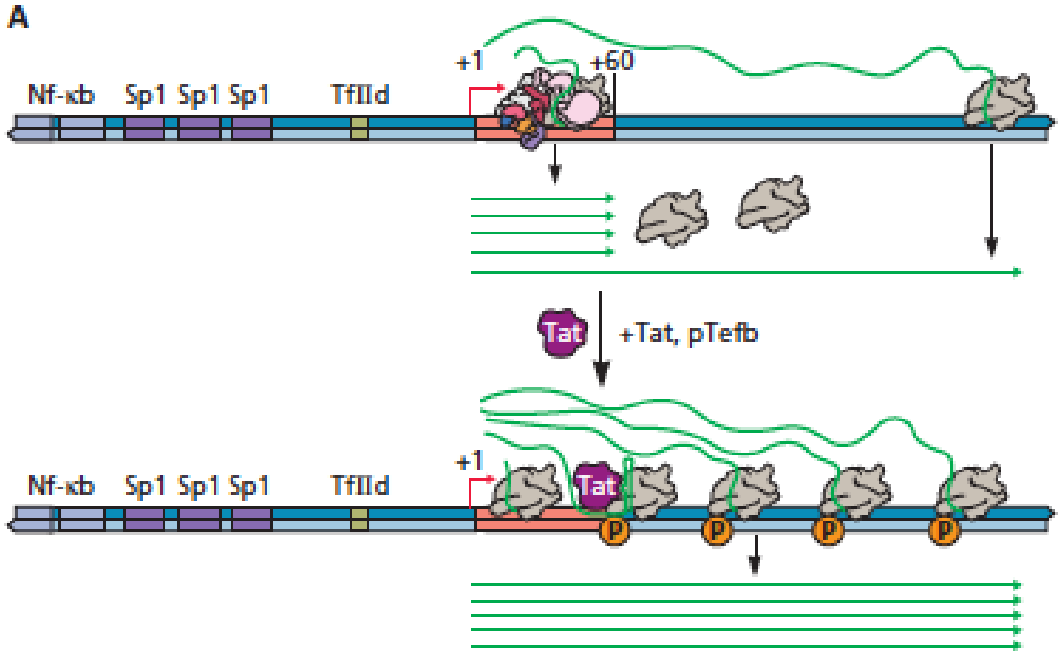
HIV-1 Gene Expression



In una fase precoce i piccoli RNA riescono a essere esportati dal nucleo

HIV provirus transcription

Tat plays a crucial role in synthesis of full-length HIV-1 mRNA transcripts



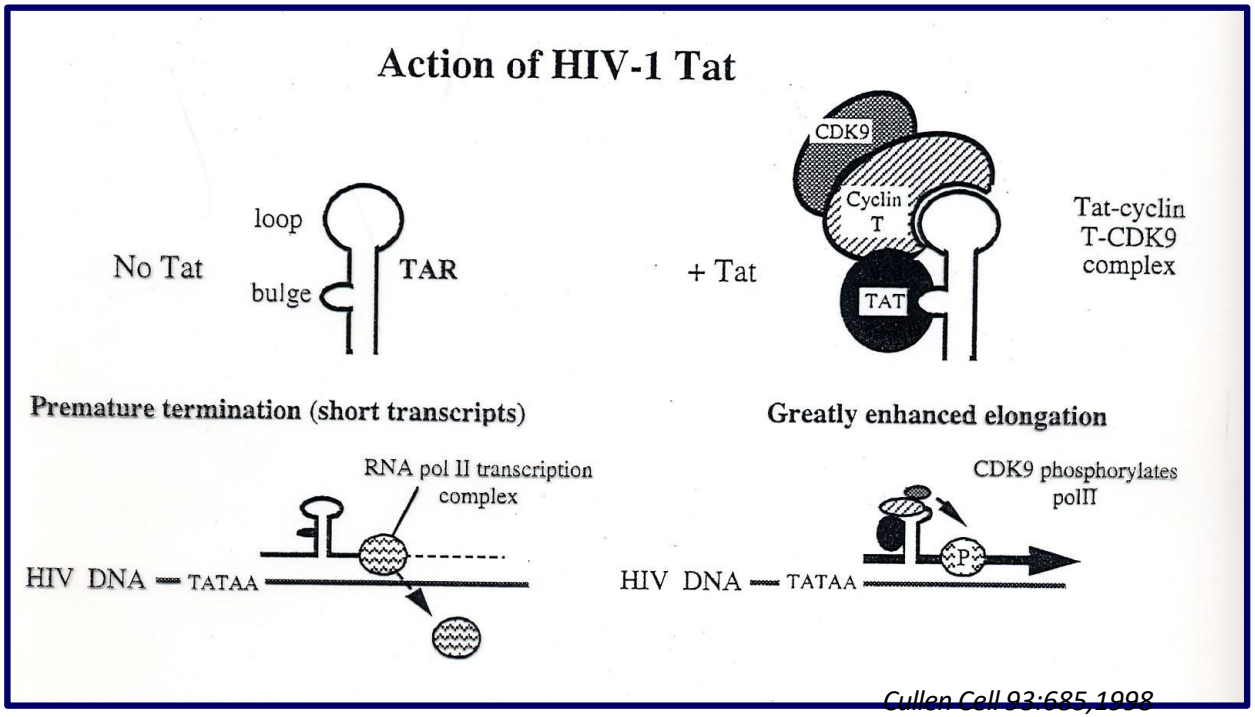
In the absence of Tat, transcription complexes are poorly processive, and the great majority (9 of 10) terminate within 60 bp of the initiation site, releasing transcription components and short transcripts.

Production of the Tat protein and its recruitment of p-Tefb and other regulators of elongation to nascent RNA allow transcriptional complexes to pass through the elongation block and synthesis of full- length viral RNA.

From Flint et al. *Principles of Virology* (2015), ASM Press

Action of TAT

- Nuclear activity
- Recognizes and binds the TAR sequence (Tat-responsive element) located in the R region of the nascent viral RNA
- Interacts with the RNA polymerase II complex by increasing the efficiency of the elongation



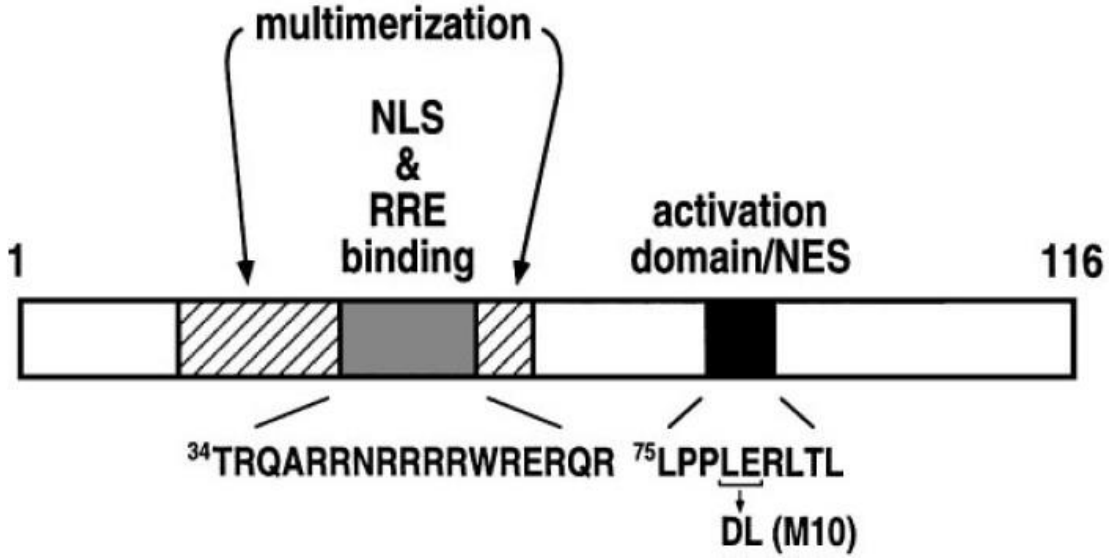
1. Establishes a cooperative interaction with the TAR sequence and the human cyclin T1 (the regulative component of the elongation factor b, P-TEFb)
2. Recruitment of the hCycT1/ CDK9 complex on the TAR sequence allows phosphorylation of Pol II C-terminus by CDK9, resulting in productive transcription

TAT is much more than just a HIV transactivator

- ✓ Released in soluble form is able to enter into uninfected cells by interacting with and crossing the membrane and affect the expression of target genes also in uninfected cells
- ✓ Interacts with fibronectin receptor and other integrins through the RGD motif
- ✓ Stimulates the expression of immunoregulatory cytokines: TNF, IL-2, IL- 6, TGF-a, TGF-b, IL-8 [immunomodulation]
- ✓ Induces the expression of adhesion molecules such as fibronectin and collagen type I and III [migration of infected cells]
- ✓ Regulates the expression of proteins involved in apoptosis: the CD95 ligand (FasL) and Bcl-2 [apoptosis of activated T cells]
- ✓ Inhibits the transcription of p53 and MnSOD (manganese superoxide dismutase) [cancer and oxidative stress]
- ✓ Represses the expression of MHC class I [escape from IS]

REV

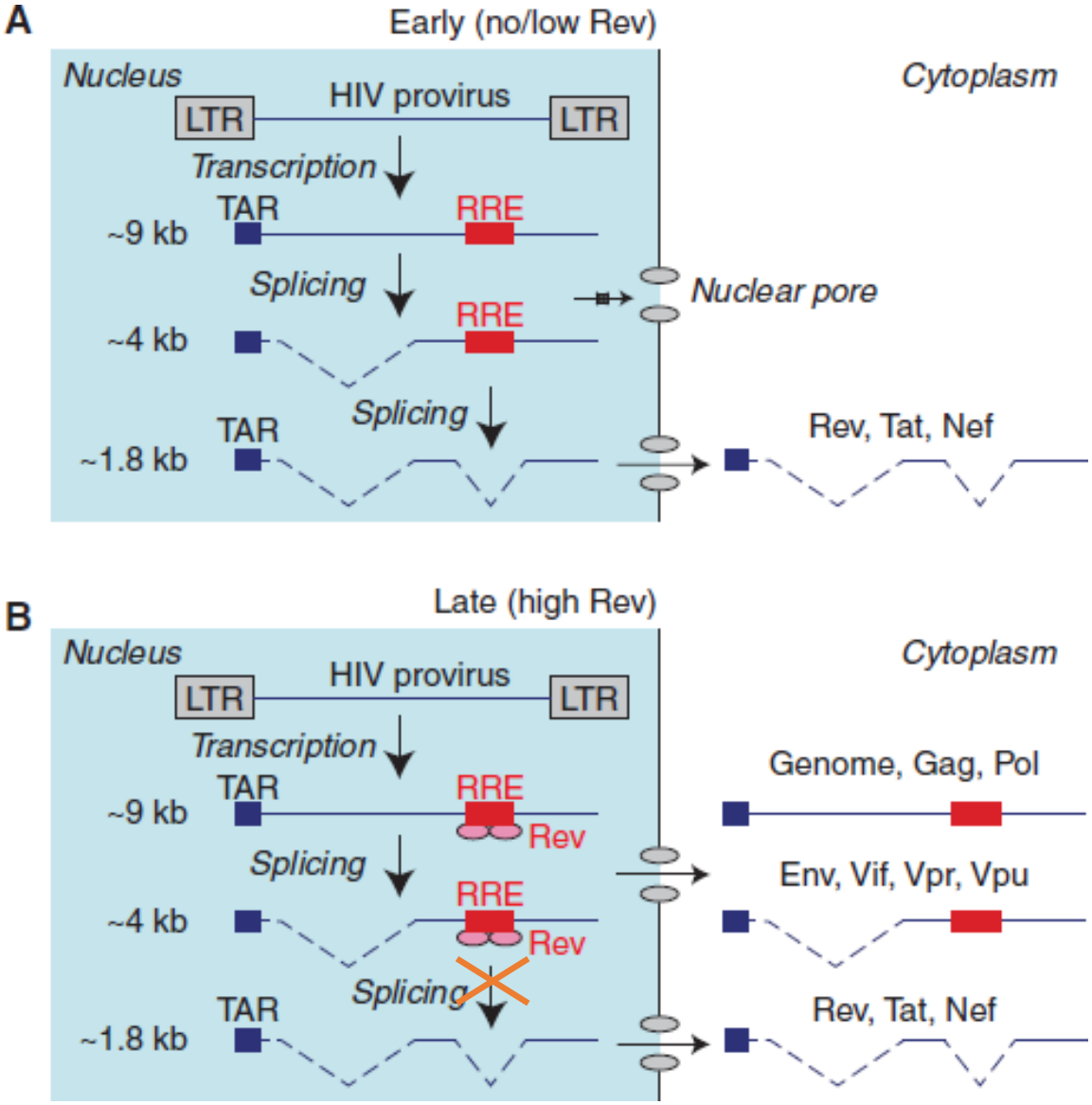
Regulator of virion protein expression: regulates the splicing and transport of viral RNA. Rev facilitates the transport of intron- containing viral RNA (unspliced or singly spliced) out of the nucleus, promoting viral structural protein synthesis.



- The arginine rich, N-terminal domain harbors regions that mediate RNA (RRE) binding through the RRE (Rev-responsive element) sequence on the env gene and nuclear localization (NLS, gray box), and regions required for protein multimerization (hatched boxes).

- The leucine rich central domain harbors the NES (nuclear export signal)/activation domain (solid box).

Early and late phases of HIV-1 mRNA expression

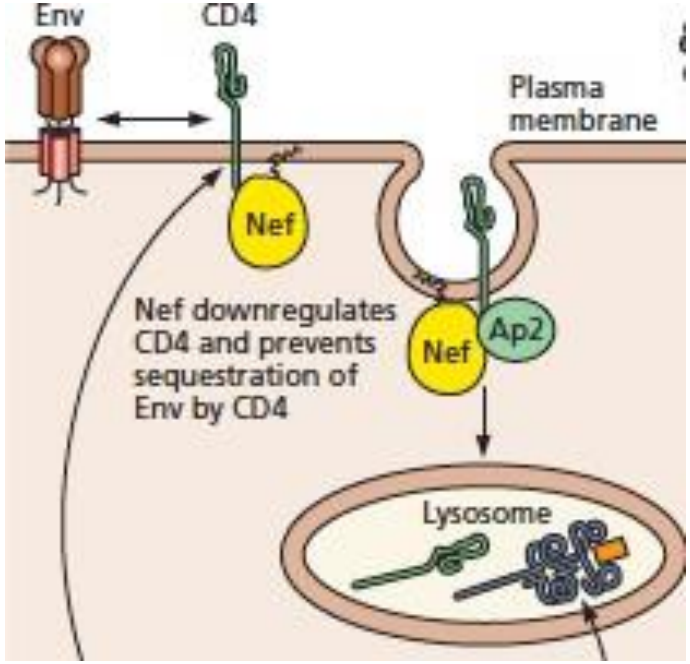


Full-length unspliced 9-kb, incompletely spliced 4-kb mRNA, and completely spliced 1.8-kb mRNAs are transcribed at both early and late times. (A) In the absence of Rev or when Rev is below the threshold necessary for it to function, the 9-kb and 4-kb mRNAs are confined to the nucleus and either spliced or degraded. Completely spliced 1.8-kbmRNAs are constitutively exported to the cytoplasm and translated to yield Rev, Tat, and Nef. (B) When the levels of Rev (shown as a pink oval) in the nucleus exceed the threshold necessary for function, the 9-kb and 4-kb mRNAs are exported to the cytoplasm and translated. The Rev-response element (RRE) is shown as a red rectangle.

Cold Spring Harb Perspect Med 2012

NEF: Negative Factor (27 kDa)

Nef protein is synthesized early in infection.
Nef is a myristoylated protein localized at the cell membrane of infected cells.
NEF is important for HIV replication in vivo



NEF causes the internalization of CD4 antigen from the cell surface and its destruction in lysosomes, enhancing Env incorporation into budding virus particles.

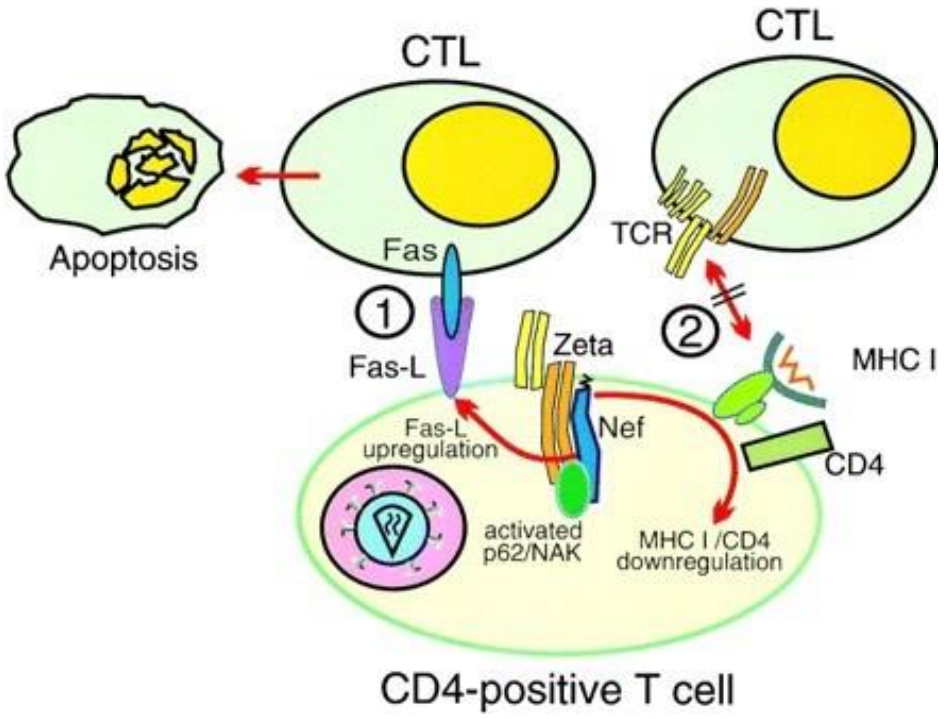
NEF reduces surface expression of MHC class I molecules. This alters antigen presentation by the infected cell and is proposed to protect the infected cell from attack by cytotoxic T cells.

NEF: Negative Factor (27 kDa)

SERINC3 and 5 reduce the efficiency of virus fusion with target cells. Nef induces SERINC to move from the cell surface to an intracellular compartment, preventing it from being incorporated into the budding virus.

Pro-apoptotic Role

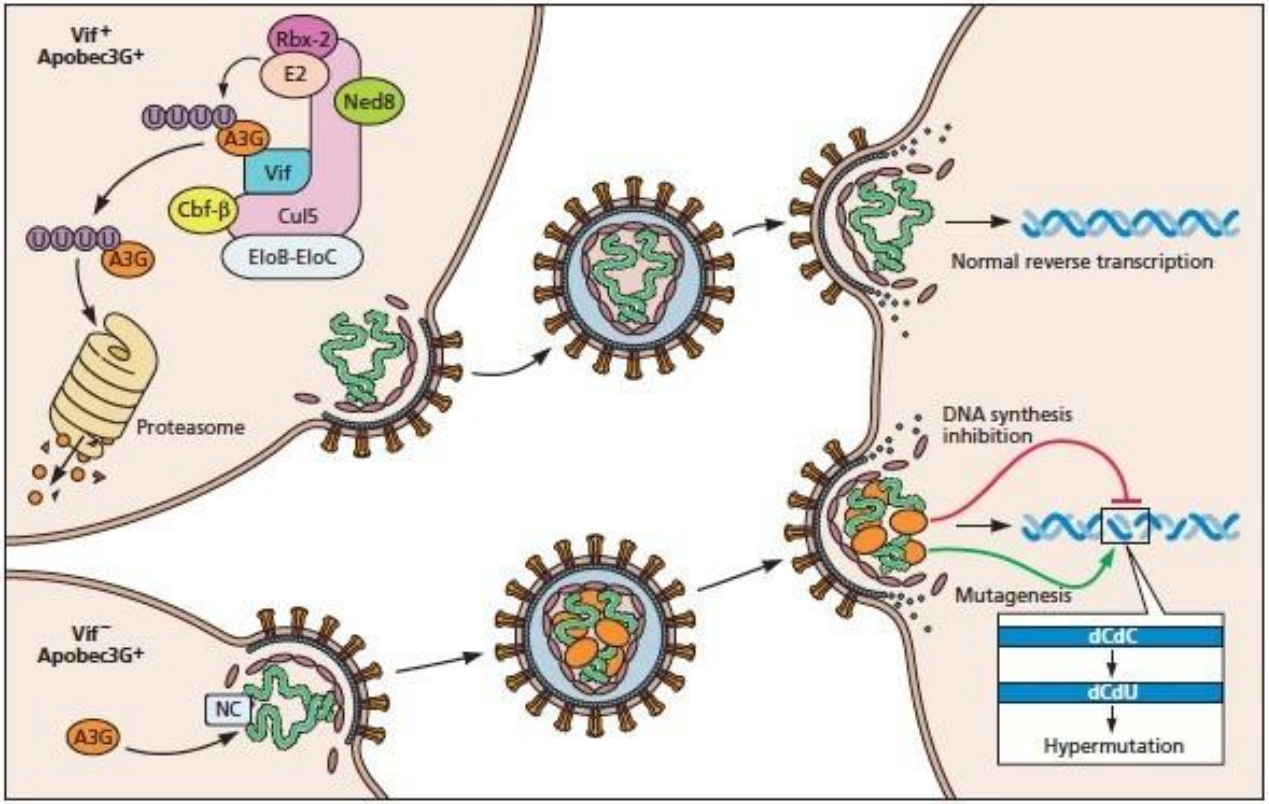
Nef stimulates expression of FasL on the surface of infected cells (the mechanism involves the signaling pathway of the TCR).
This results in the protection of infected cells from CTL attack by killing Fas+ viral-specific CTLs in the process (1)



Anti-apoptotic role

In infected cells, Nef blocks apoptotic pathways mediated by FAS and tumour-necrosis factor receptor (TNFR)
(through inhibition of apoptosis signal- regulating kinase 1, ASK1) and by p53 (through direct binding), and unleashes the anti- apoptotic effects of BCL-2 and BCL-XL (by inducing the PAK-mediated phosphorylation of BAD, releasing the anti-apoptotic effectors, and thereby mimicking cytokine-induced signals).

VIF: Virion Infectivity Factor (23 kDa)



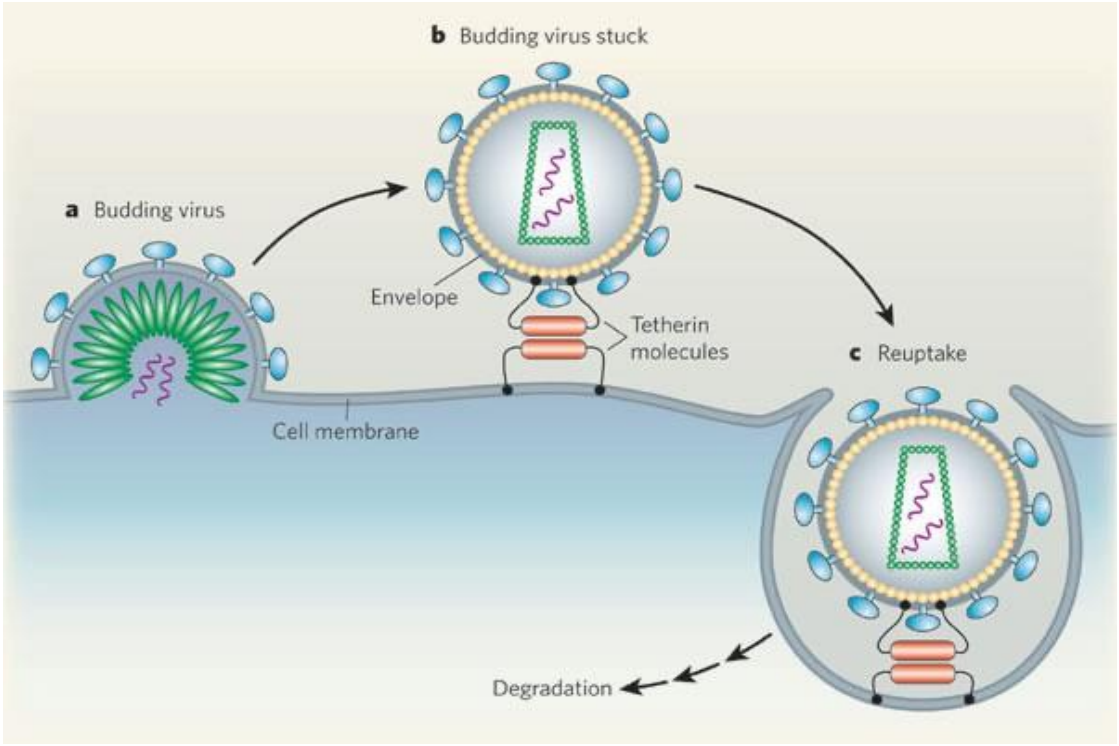
(Top) Vif counteracts the antiviral effects of Apobec3G (A3G) by mediating its polyubiquitination, which leads to proteosomal degradation. (Bottom) In the absence of Vif, A3G is incorporated into newly formed virus particles through interaction between viral RNA and NC protein. In the newly infected cell, reverse transcription is inhibited by A3G, and cytosines in the newly synthesized DNA are converted to uracil, causing hypermutation through eventual C to A transversions.

VPR (15kDa)

VPR : ***Viral Protein, Regolatory (15 kDa)***

- Is a late HIV product packaged into the virion nucleocapsid
- Contains a nuclear localization signal (NLS) and is involved in the pre-integration complex (PIC) formation and nuclear transport
- Induces cells to arrest in the G2 phase of the cell cycle (LTR promoter is more active in G2-arrested cells)
- Negatively modulates the expression of CD4 on the cell surface
- Acts as a weak transactivator of viral transcription. It is important for proviral DNA expression occurring before integration

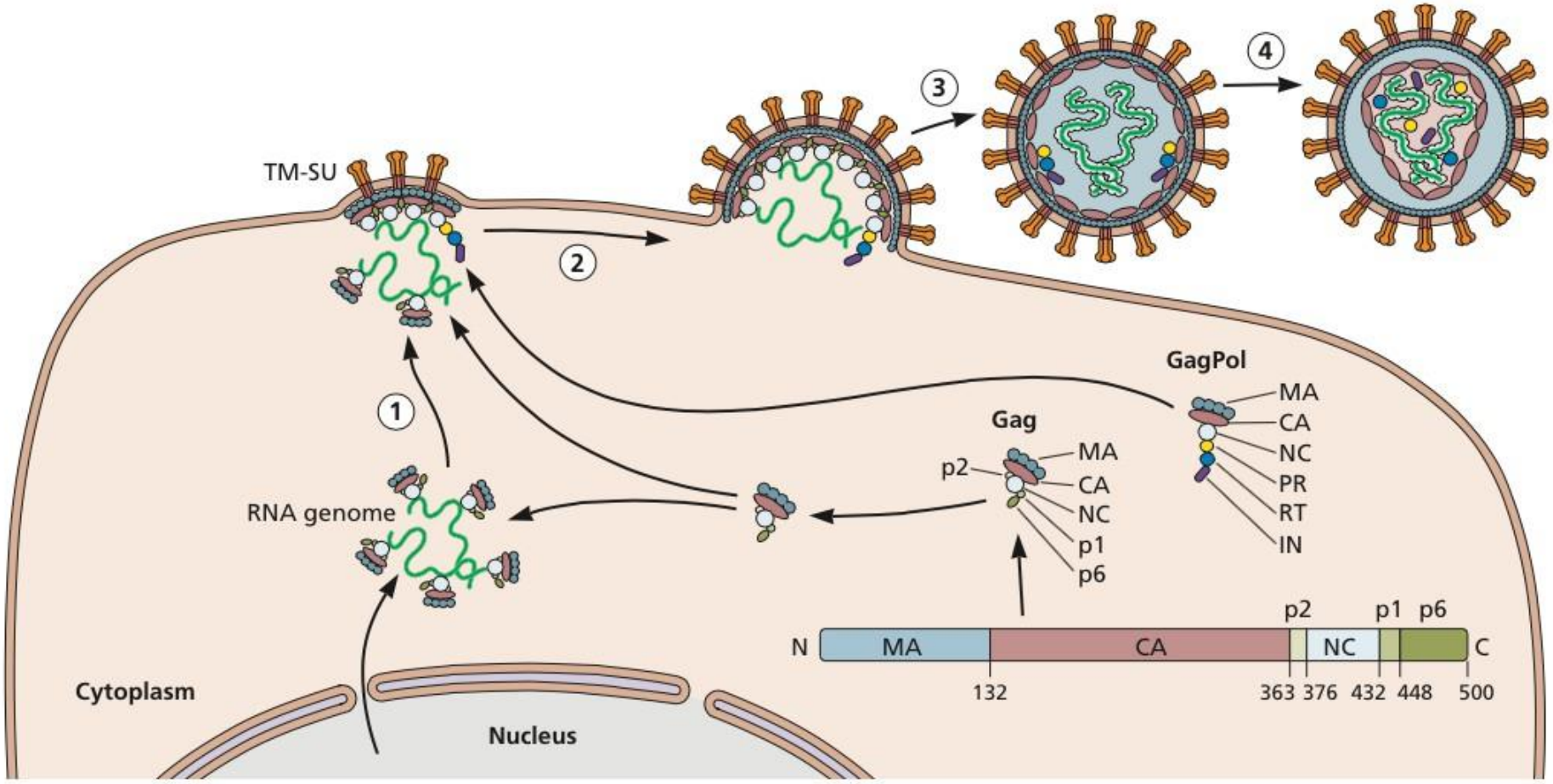
VPU: Viral Protein Unknown (9,2 kDa)



- Promotes the degradation of the CD4/gp160 complex in the endoplasmic reticulum, facilitating the gp160 transport to the plasma membrane for assembling of new viral particles.
- Reduces CD4 and MHC-I expression promoting the virion budding and the escape of the virus from IS
- Contrast the action of Tetherin

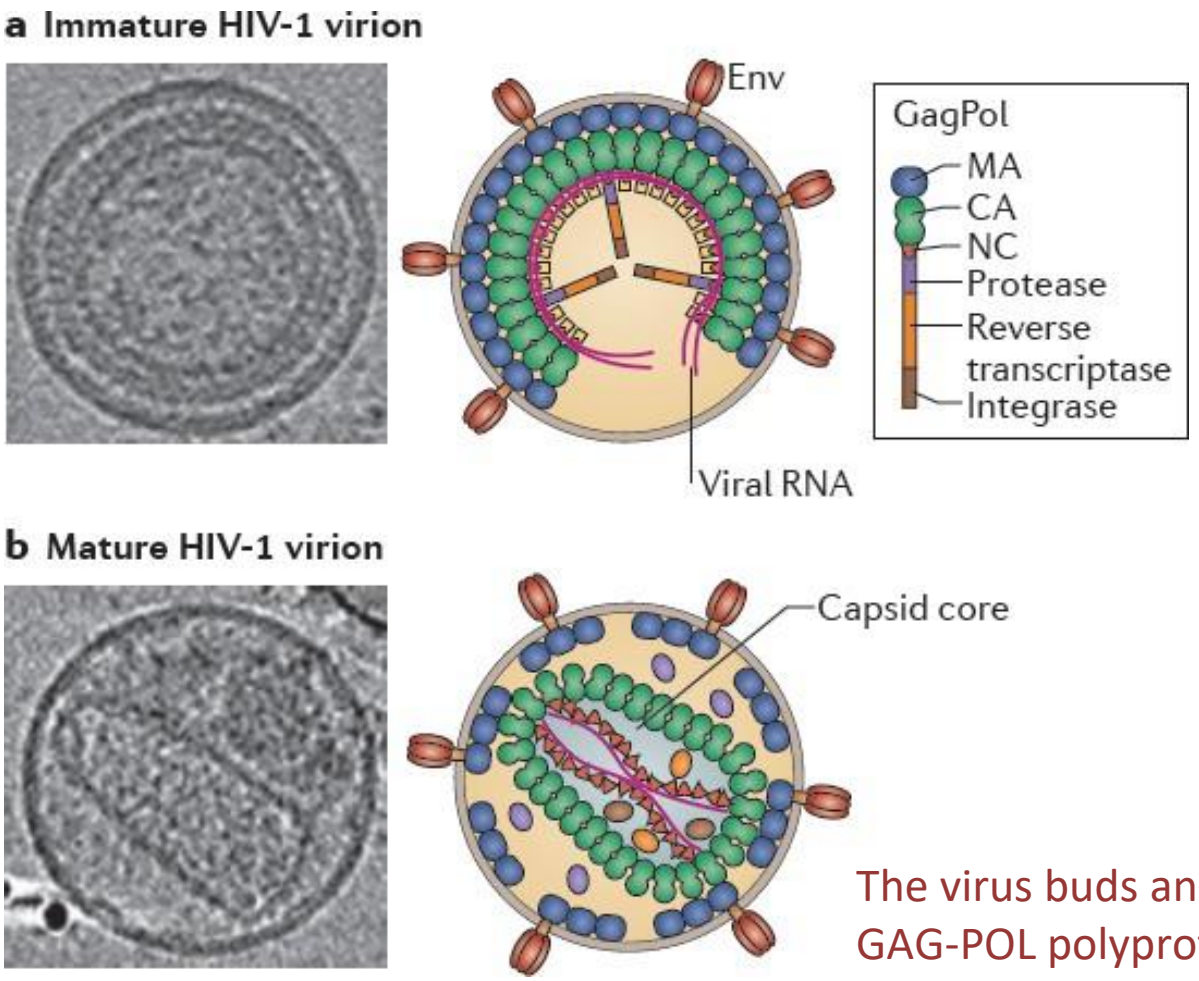
Nature **451**, 406-408(24 January 2008)

Assembly and release



The NC domain of Gag is the primary viral determinant that drives RNA packaging.
Gag NC domains direct the packaging of viral genomic RNA by binding to the packaging signal (ψ site).
An RNA dimer is the recognition unit for packaging into assembling virions.

Maturation



The virus buds and the protease cuts itself free of the GAG-POL polyprotein Further proteolytic cleavage occurs and the virion matures