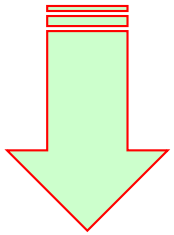
Human Immunodeficiency Virus

The discovery

1981 AIDS described for the first time as a result of a new infection (anomalous cases of Kaposi's sarcoma and pneumonia induced by Pneumocystis carinii in young men who have sex with men in the areas of New York, Los Angeles and San Francisco)

1983 Francoise Barre-Sinoussi, Claude Chermann and Luc Montagnier (Nobel Prize, 2008), at the Pasteur Institute in Paris, isolate a virus from the lymphonodes of a patient with generalized lymphadenopathy of unknown etiology and called the virus Lymphadenopathy-Associated Virus (LAV). The virus, identified as a retrovirus, exhibits characteristics similar to the known human retrovirus HTLV-1

1984 Robert Gallo and coworkers at NIH confirmed the discovery of a human lymphotropic retrovirus (HTLV-III) that unlike HTLV-1 kills CD4 + T cells rather than stabilize them and establish the relationship of the virus with the immune deficiency syndrome AIDS (Acquired immunodeficiency Syndrome)



LAV and HTLV-III represent two strains of the same virus, the Human Immunodeficiency Virus HIV

1986 A second type of HIV is isolated in West Africa (HIV-2).

HIV types and groups

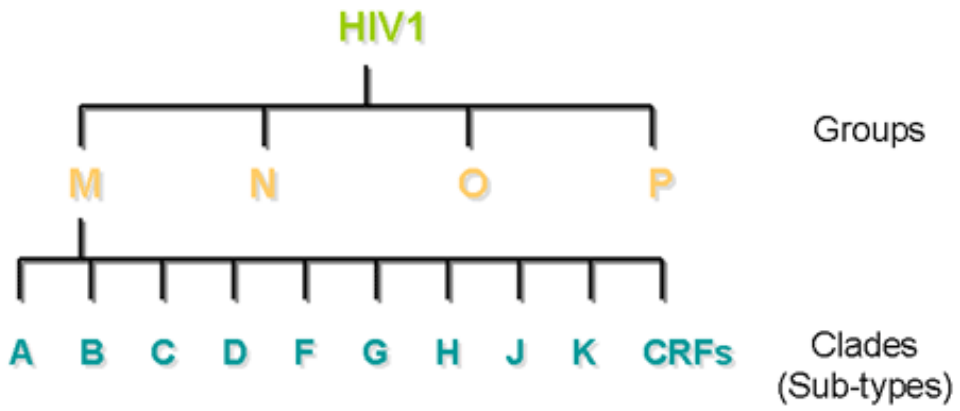
HIV-1 and HIV-2 cause clinically indistinguishable disease, although the time to disease onset is longer for HIV-2. The worldwide epidemic of HIV and AIDS is caused by HIV-1, while HIV-2 is mostly restricted to West Africa.

There are three groups of HIV-1, M (main or major), N (new) and O (outlier). Type O HIV-1 is mostly found in Cameroon and Gabon while the rare N sub-group is also found in Cameroon.

In 2009 a new strain closely related to gorilla simian immunodeficiency virus was discovered in a Cameroonian woman. It was designated HIV-1 group P.

HIV-1 groups and sub-types

Based on nucleotide sequence analyses of the *env* and *gag* genes, it has been found that there are also at least ten different HIV-1 subtypes within the M group - these are designated A to K.



CRF-Circulating recombinant form. In some countries, mosaics (recombinants) between different subtypes have been found. These arise when two different subtypes infect a person at the same time and recombination occurs.

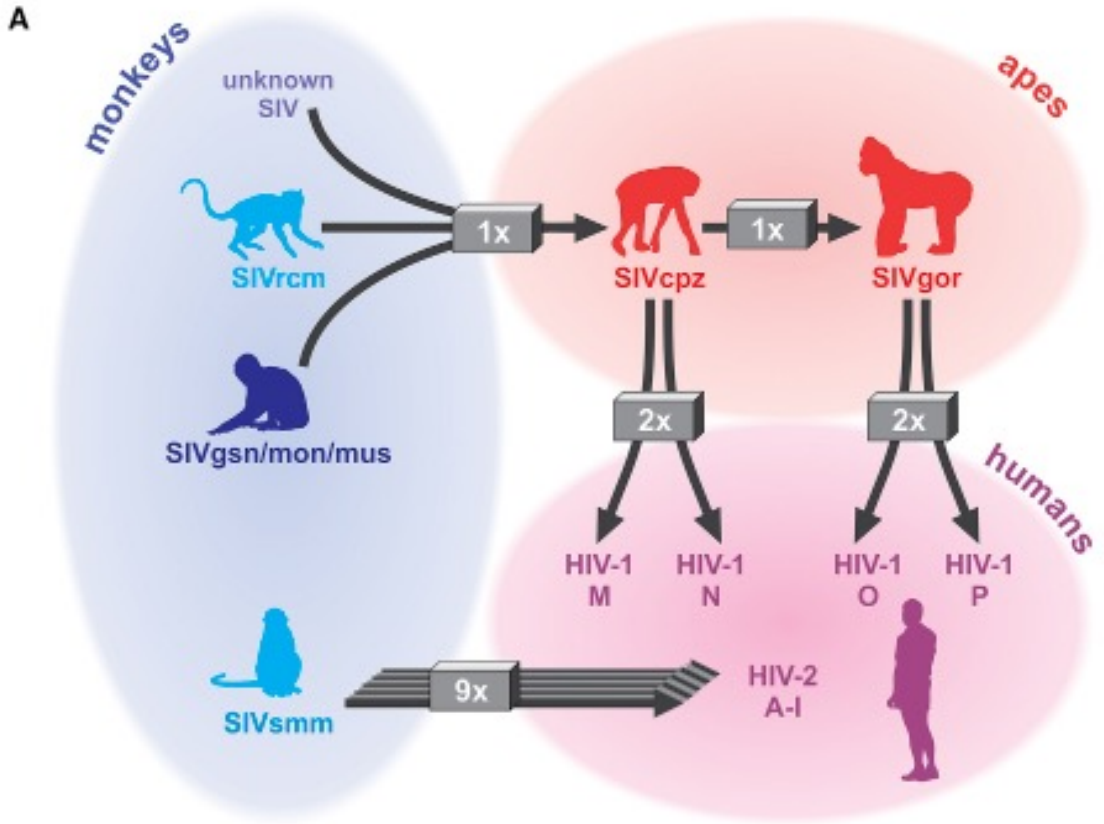
Origins of HIV

It is likely that HIV first appeared in humans in Africa near the beginning of the twentieth century as a result of infection by simian immunodeficiency virus (SIV) from chimpanzees. Since there are several groups of HIV-1, it is likely that humans became infected by SIV on more than one occasion.

The earliest proven case of infection by HIV-1 is currently a person in central Africa in 1959, from whom blood samples were stored at the time.

The cross-species transmission of lentiviruses from African primates to humans has selected viral adaptations which have subsequently facilitated human-to-human transmission. HIV adapts not only by positive selection through mutation but also by recombination of regions of its genome in individuals who become multiply infected. Naturally infected nonhuman primates are relatively resistant to AIDS-like disease despite high plasma viral loads and sustained viral evolution.

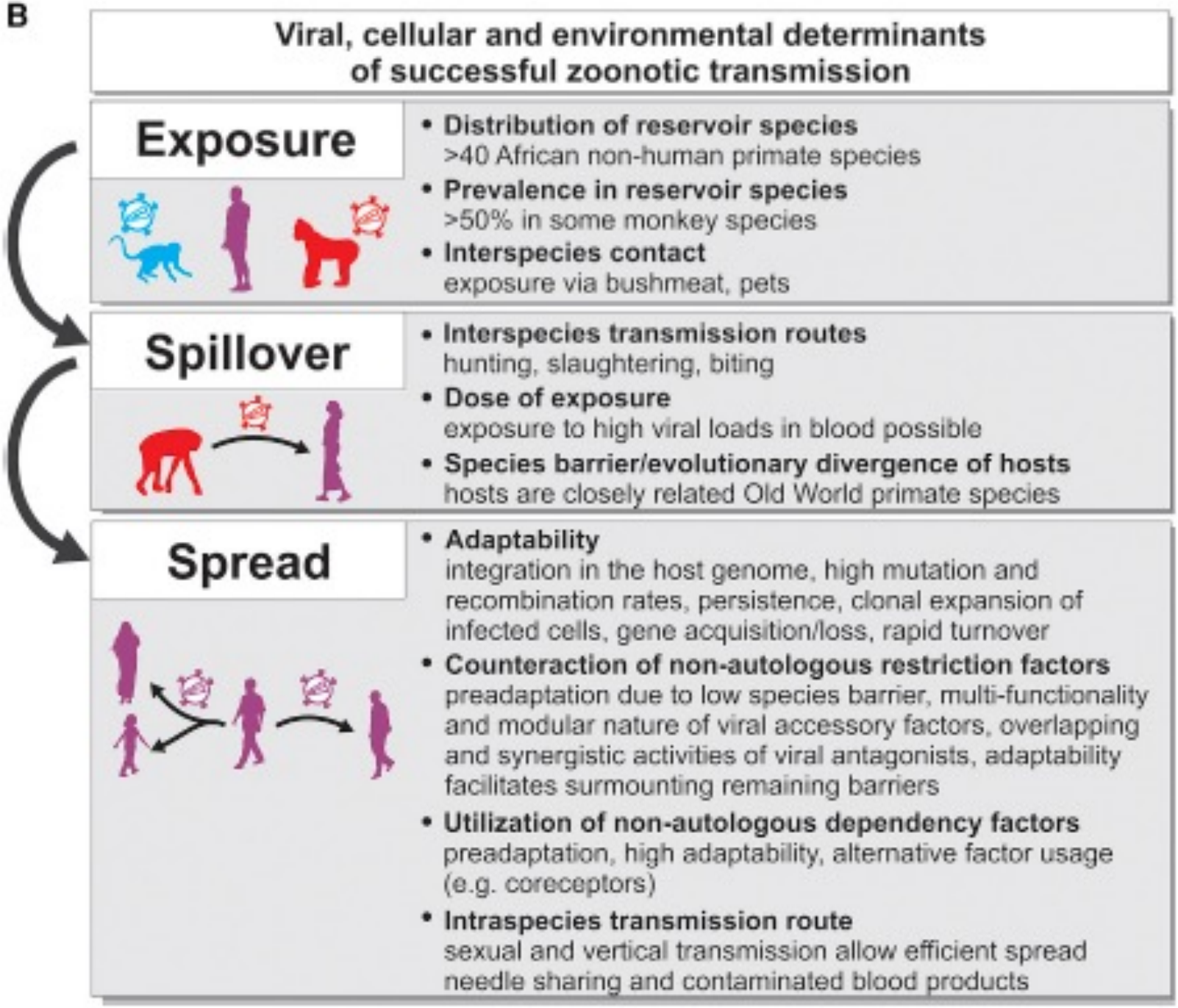
Origins of HIV



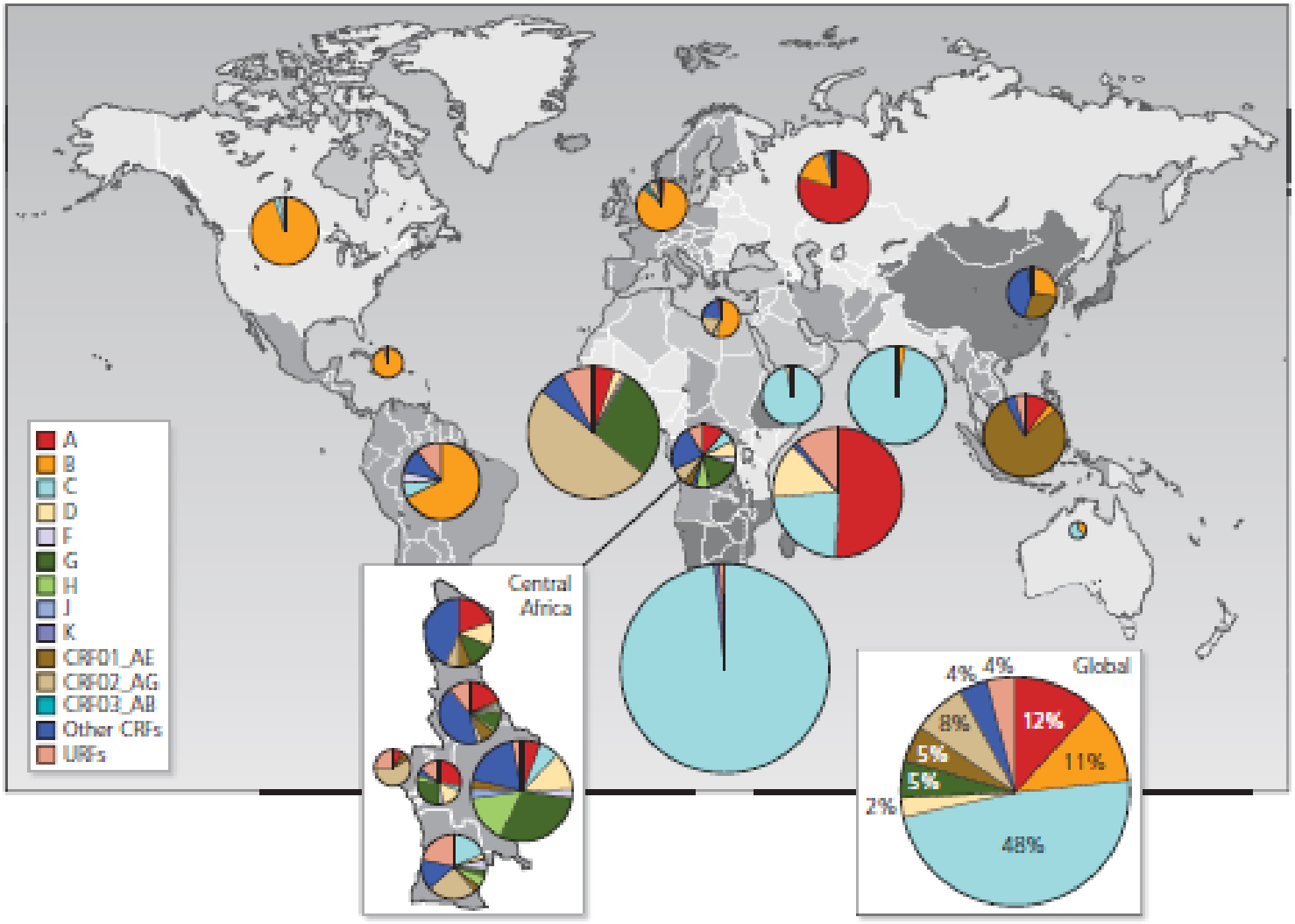
SIVcpz in chimpanzees is the result of recombination events between three different SIV strains. Besides an unknown SIV strain, recombination involved a precursor of today's SIVgsn/mon/mus clade infecting Cercopithecus monkeys and possibly a precursor of today's SIVrcm from redcapped mangabeys. The chimpanzee virus was subsequently transmitted to gorillas and humans, giving rise to SIVgor and HIV-1 groups M and N, respectively. HIV-1 groups O and P are the result of two zoonotic transmission events of SIVgor, while SIVsmm infecting sooty mangabeys was transmitted to humans on at least nine occasions, resulting in the emergence of HIV-2 groups A through I.

Cell Host & Microbe 25, January 9, 2019

B



Cell Host & Microbe 25, January 9, 2019



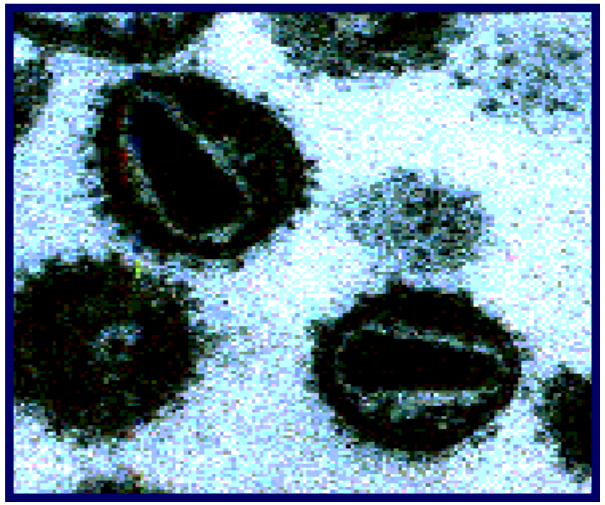
World map showing the relative contribution of different HIV-1 subtypes to the total burden of infection globally (inset) and in different regions (main map) from 2004–7. The overall size of each pie chart reflects the numbers of infected people in each region, with the coloured segments of the charts representing the particular subtypes (A–K) and recombinant forms (CRF) present. The situation in Central Africa is represented in greater detail in an inset which shows the greater heterogeneity in the virus present in this region as compared with other parts of the world.

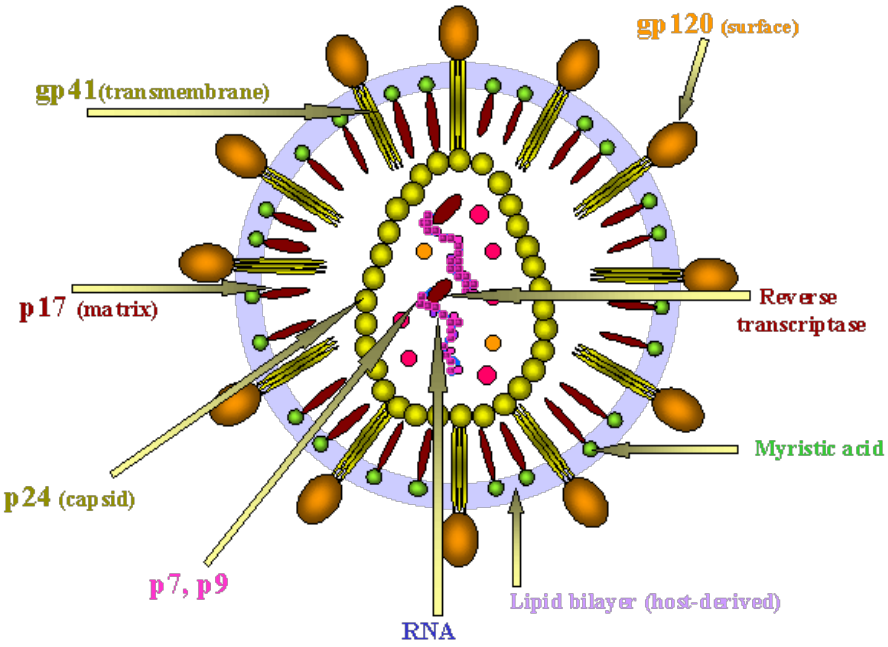
Trends in Molecular Medicine, 18: 2012

PROPERTIES OF LENTIVIRUSES

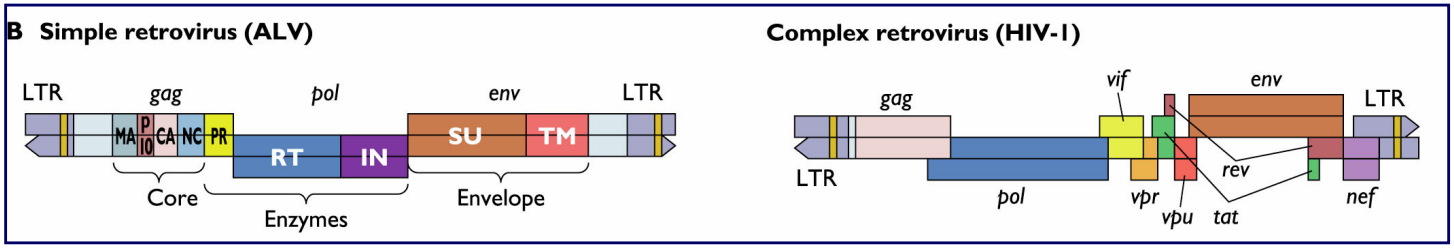
Family	*Retrovir*idae
Major human	HIV-1, HIV-2
Size	80-130 nm
Capsid symmetry	Icosahedral
Envelope	Yes
Genome	Diploid linear (+)sense ssRNA; 10 Kb
Genome replicated	Nucleus
Virus assembly	Cytoplasm
Common features	Slow Disease
Diseases	AIDS; neurologic; arthritis; pneumonia

The virion



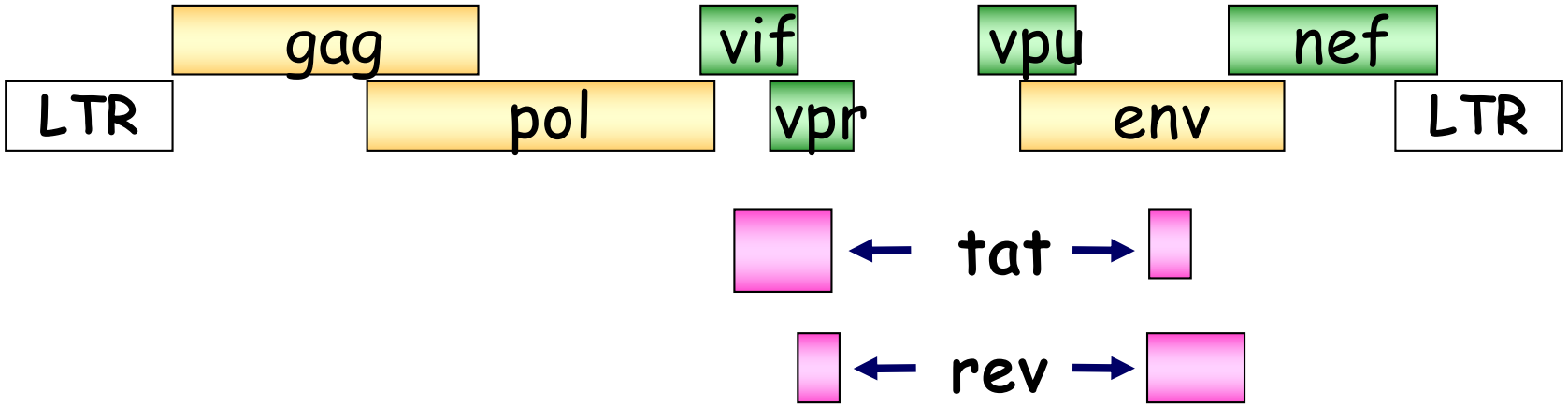


Multiple copies of the accessory proteins Vpr, Vif and Nef are also found within the virion



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

HIV-1 genome

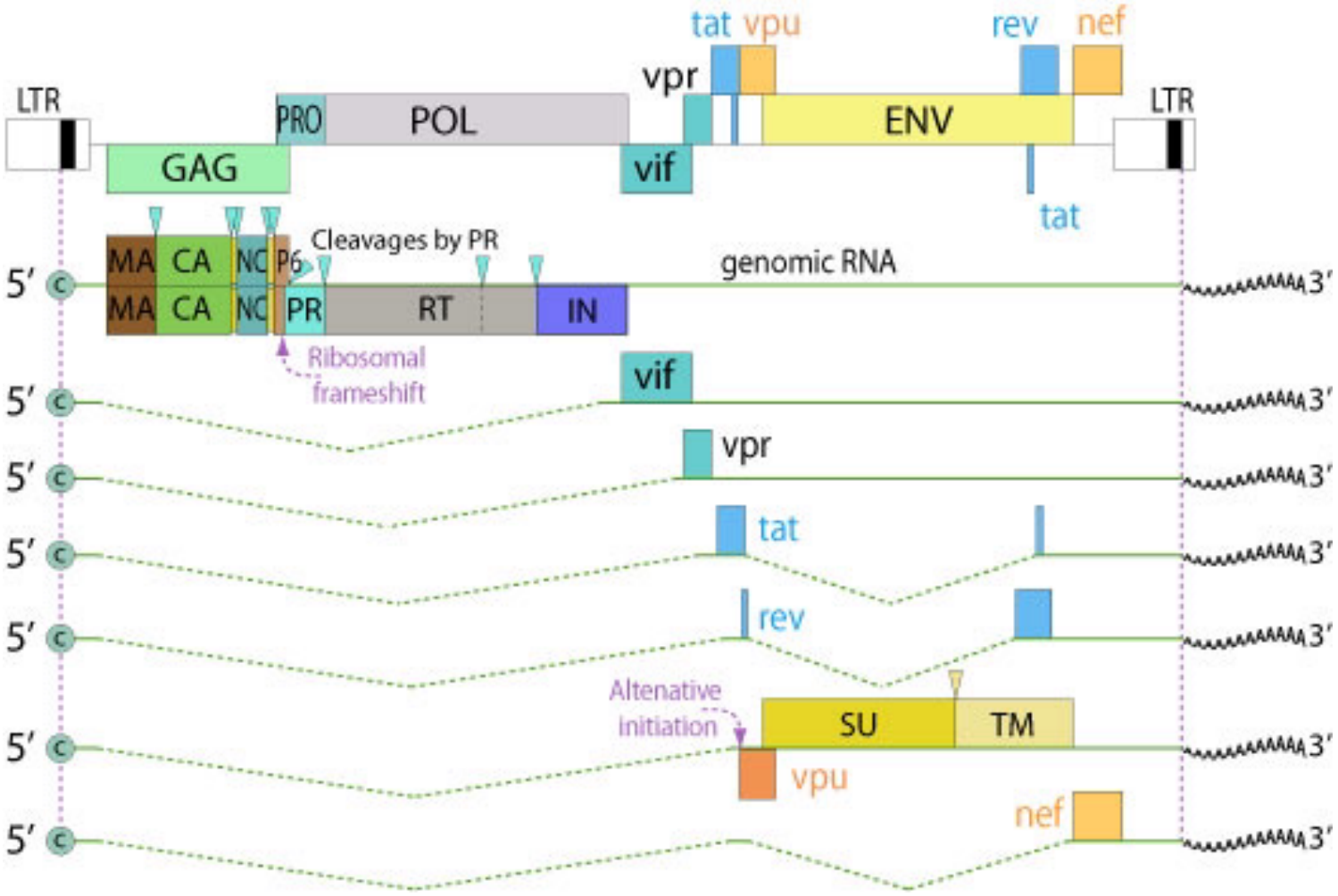


Structural genes

Transactivation genes

Accessory genes

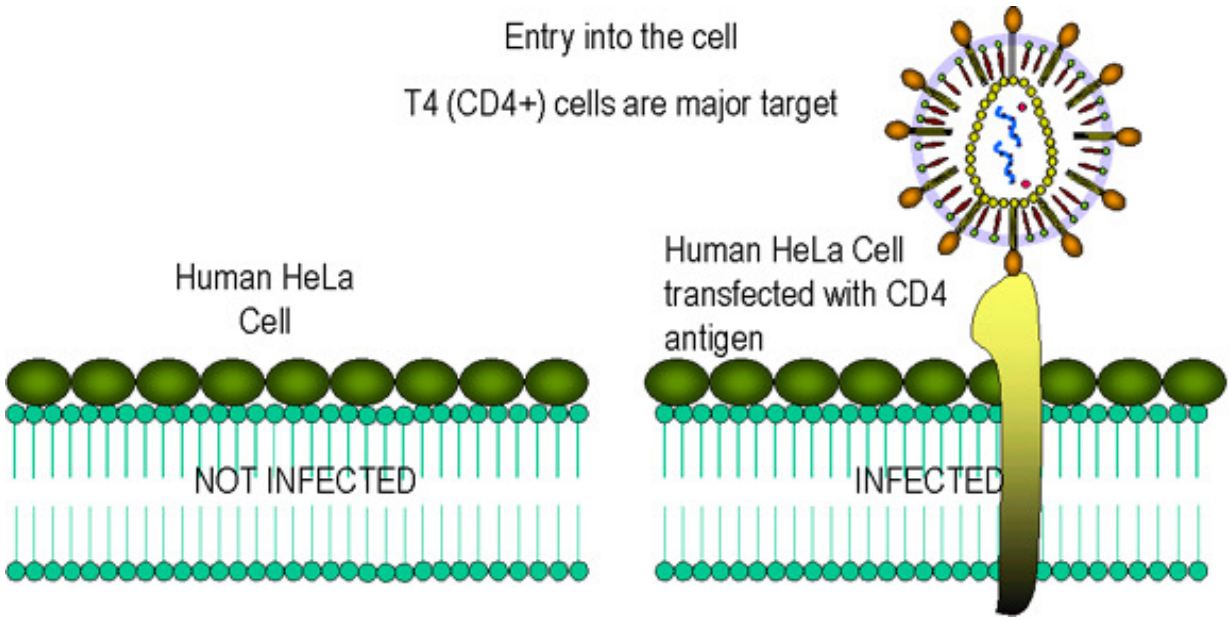
HIV-1 transcripts and proteins



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***

CD4 ANTIGEN IS THE HIV RECEPTOR

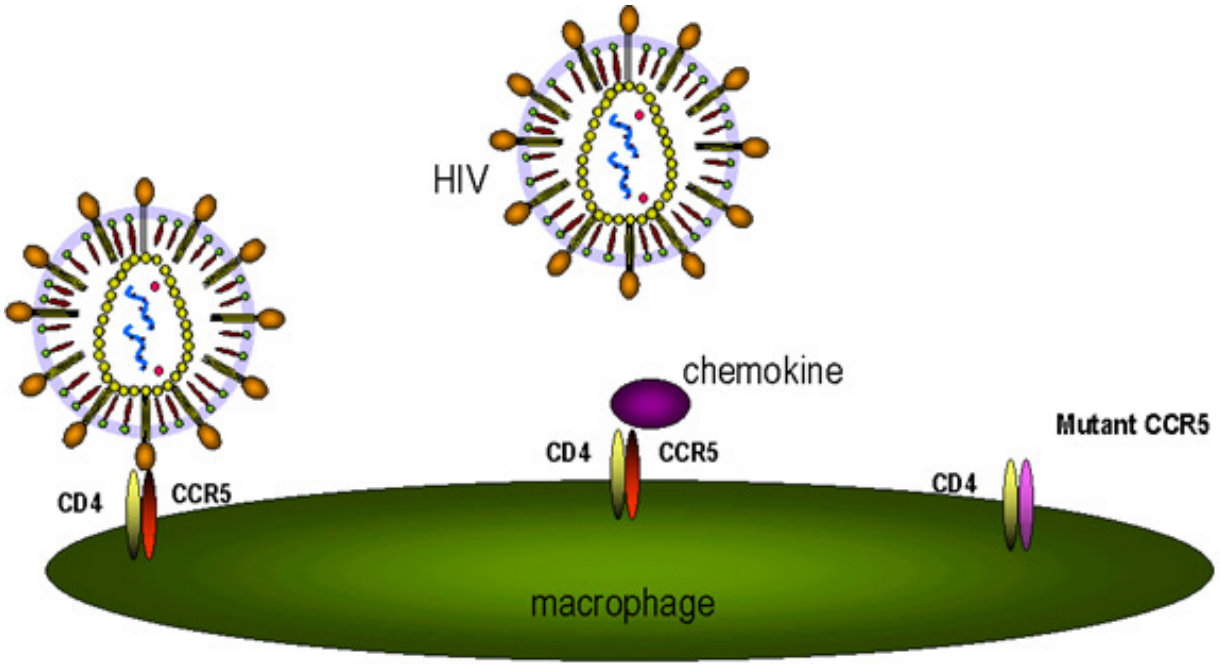


The necessity for CD4 antigen expression for entry of HIV into a human cell. HeLa cells do not have CD4 antigen and are not infected. HeLa cells transfected with CD4 gene are infected

A CO-RECEPTOR FOR INFECTION BY HIV

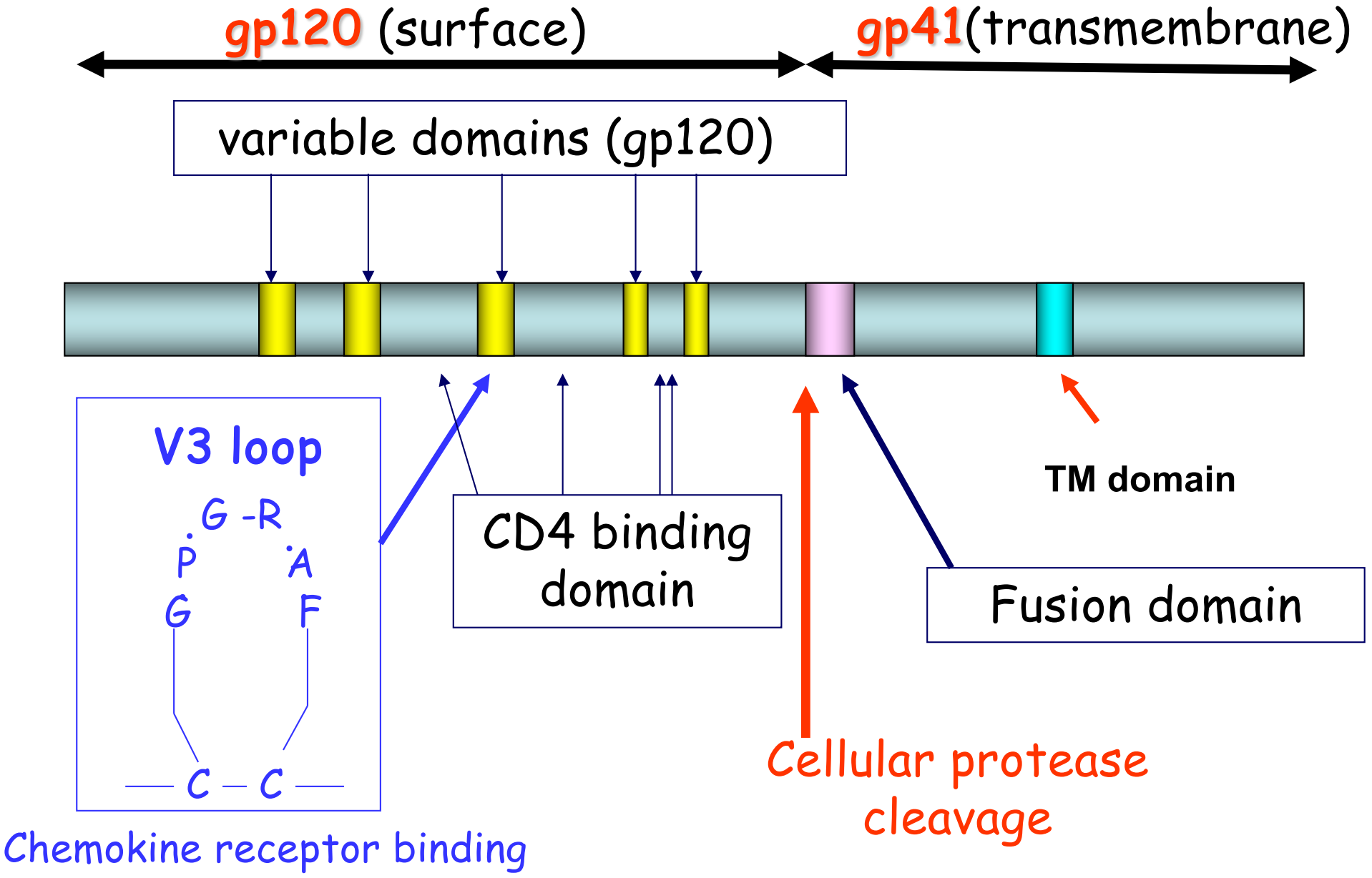
Some strains of HIV (those adapted for life in T cells) could infect and replicate in activated human T cells but not in monocytes or macrophages. Conversely, those adapted for life in macrophages are less efficient in replicating in activated T cells. Yet both macrophages and T4 cells possess CD4 antigen. The differences in tropism of the viral strains mapped to the V3 region of Gp120 suggesting that molecules other than CD4 antigen have an important role in infection and this role is CD4+ cell type-specific

A CO-RECEPTOR FOR INFECTION BY HIV

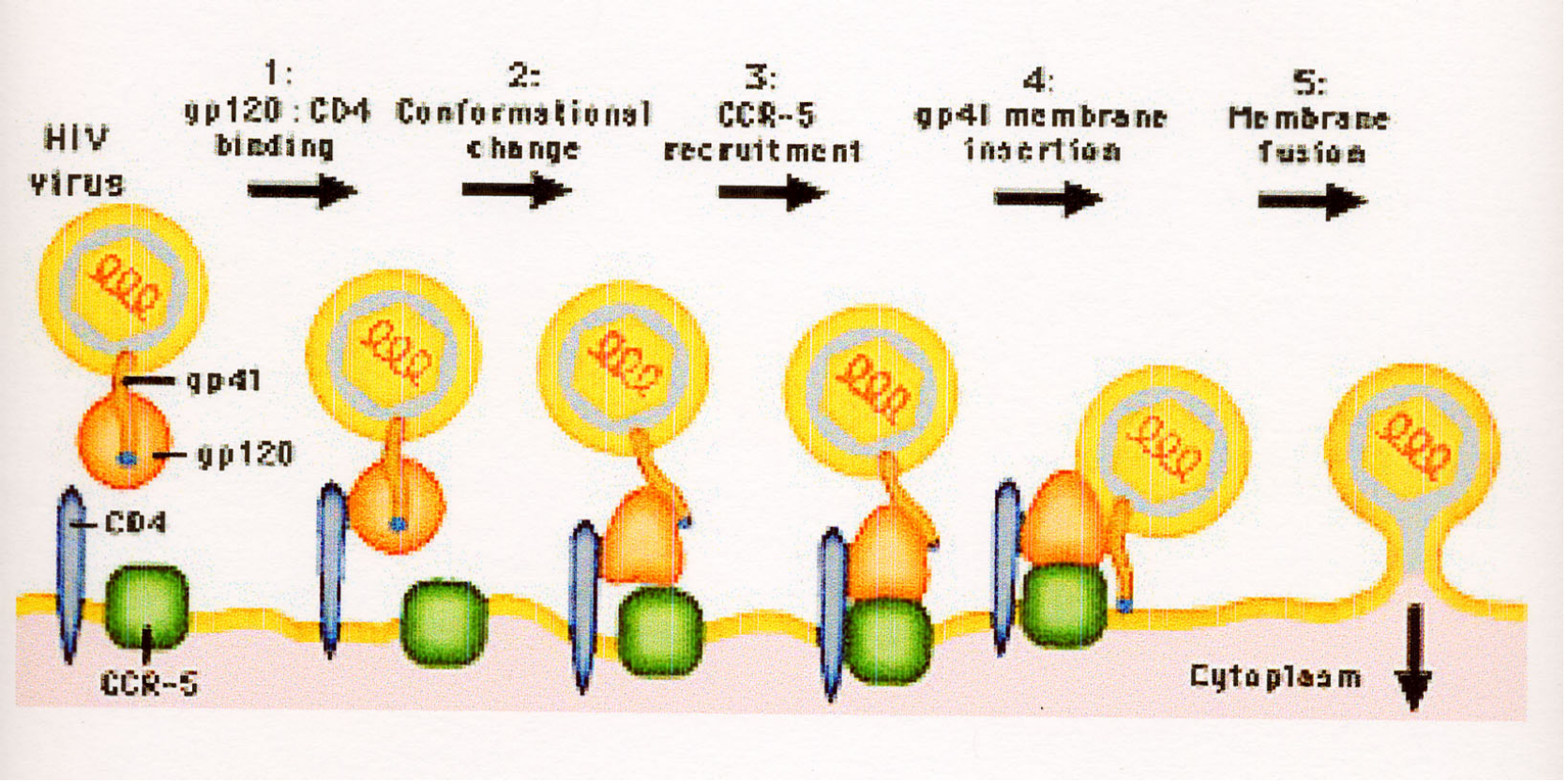


Chemokine receptors are involved, in association with CD4 antigen, in infection by HIV. The chemokine can block attachment of the virus to its receptors (i.e. RANTES, MIP-1a and MIP-1b secreted by CD8 T lymphocytes). Mutations in the chemokine receptor can lead to resistance to HIV infection

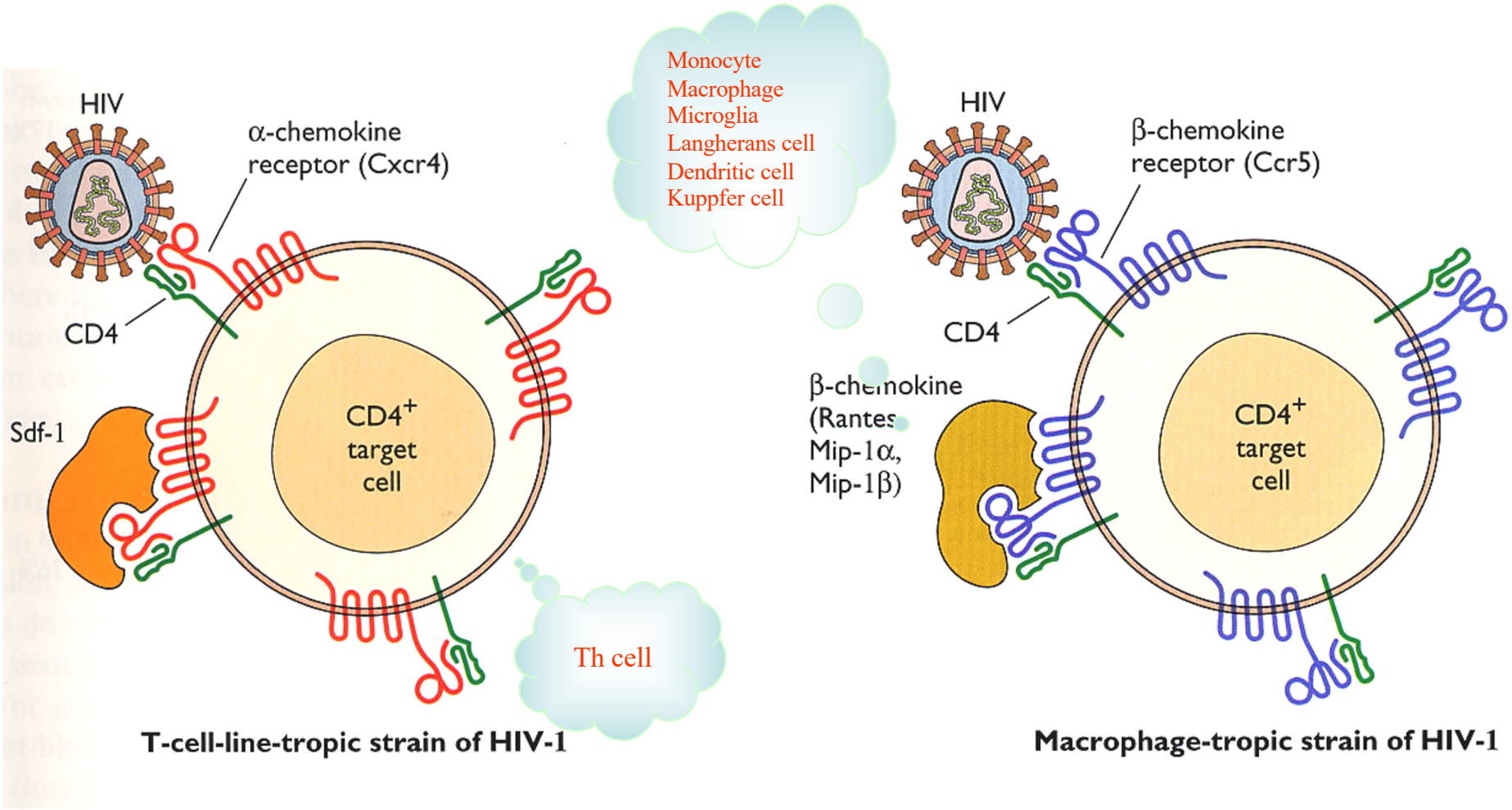
The env proteins



HIV-1 attachment and membrane fusion



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.

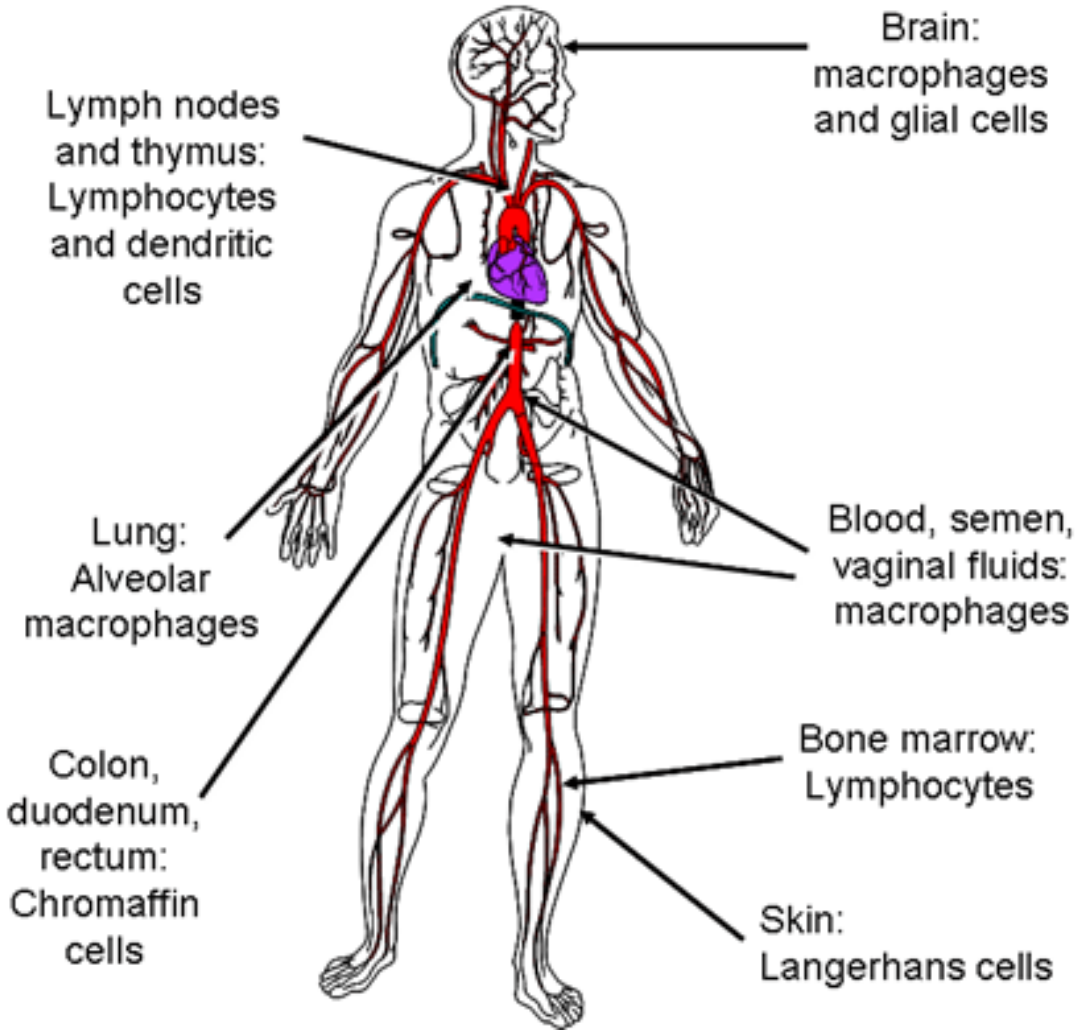
HIV-1 remission following CCr5Δ32/Δ32 haematopoietic stem-cell transplantation

Ravindra K Gupta[1,2,3,4]*, Sultan Abdul-jawad[1], Laura E McCoy[1], Hoi Ping Mok[4], Dimitra Peppa[3,5], Maria Salgado[6], Javier Martinez-Picado[6,7,8], Monique Nijhuis[9], Annemarie M. J. Wensing[9], Helen Lee[10], Paul Grant[11], Eleni Nastouli[1,11], Jonathan Lambert[12], Matthew Pace[5], Fanny Salasc[4], Christopher Monit[1], Andrew Innes[13,14], Luke Muir[1], Laura Waters[3], John Frater[5,15], Andrew ML Lever[4,16], SG Edwards[3], Ian H Gabriel[13,14,17,18] & Eduardo Olavarria[13,14,18]

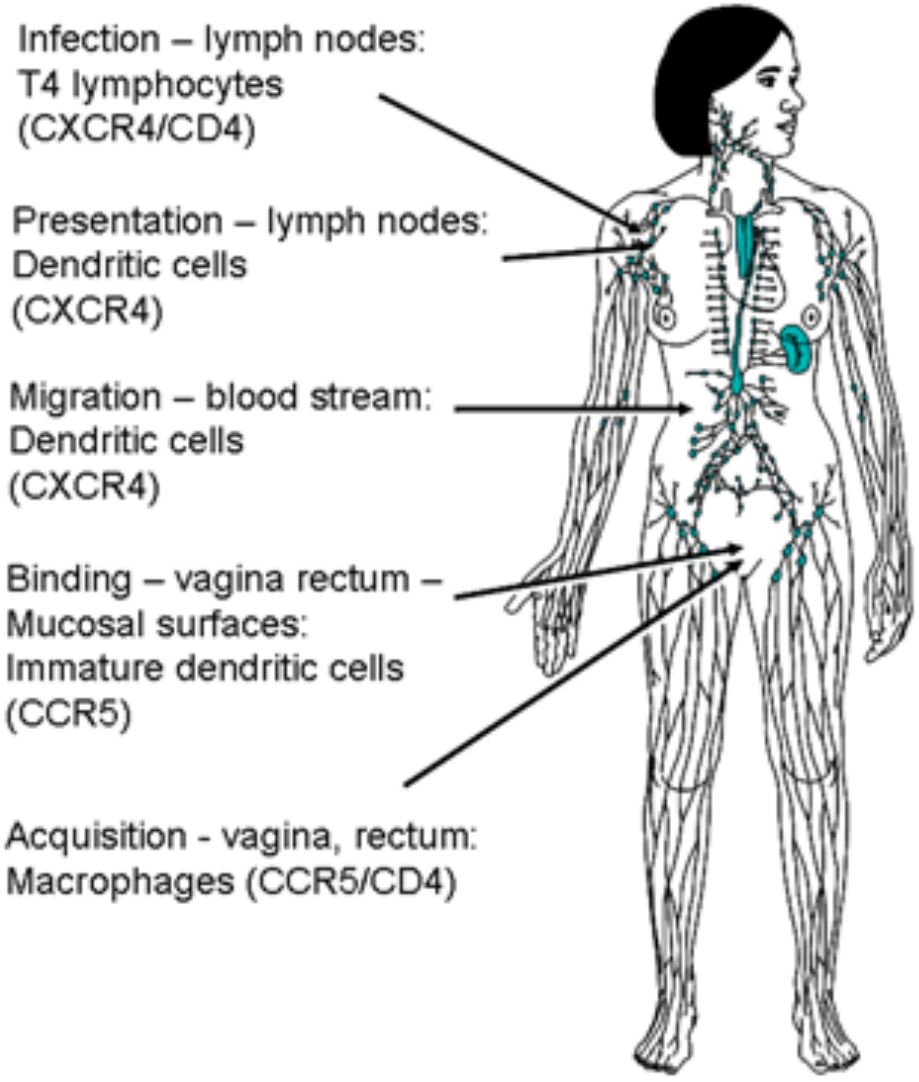
Tabella 1. Caratteristiche del “paziente di Berlino” e del “paziente di Londra”

Note	paziente di Berlino	paziente di Londra
Alleli CCR5 del paziente	CCR5/CCR5Δ32	CCR5/CCR5
Mantenimento della mancata espressione di CCR5 nel tempo (chimerismo totale)	presente	presente
Presenza di varianti virali "X4" (DNA)	nessuna	nessuna
neoplasia	Leucemia Mieloide Acuta	Linfoma di Hodgkin
n. di trapianti di cellule staminali da donatore CCRΔ32 omozigote	2	1
Regime di condizionamento	Irradiazione corporea totale (due volte)	A bassa intensità (Alemtuzumab, anti-CD52)
GVHD	Grado 1	Grado 1
ART post-trapianto (durata)	nessuna	16 mesi
Tempo di remissione senza evidenza di replicazione virale	>10 anni	18 mesi
Negativizzazione di Ab e risposte immunitarie anti-HIV	presente	presente

Cells that are infected by HIV

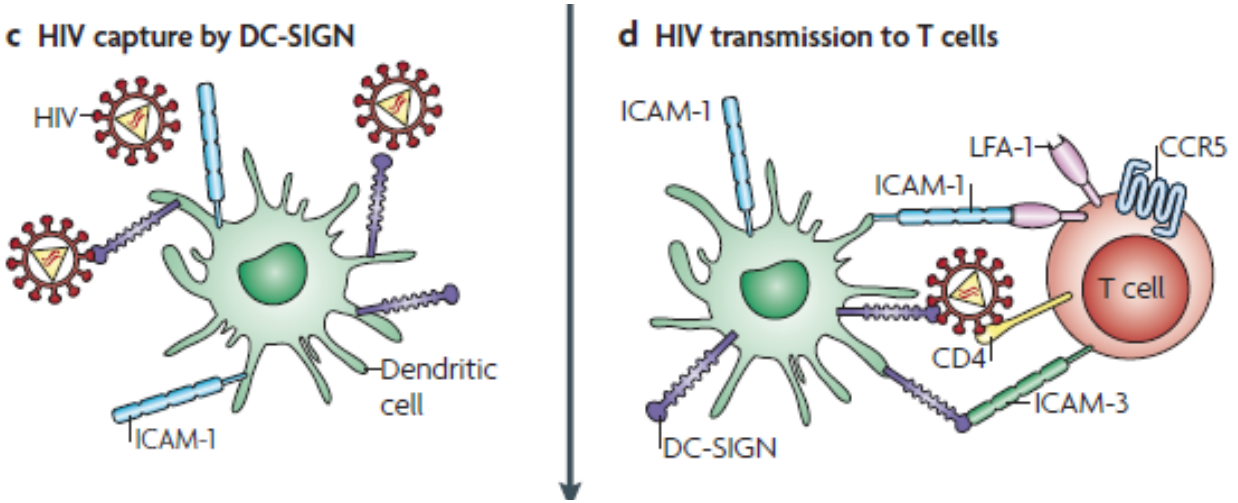


Entry of HIV via the mucosal route and transit via dendritic cells to the lymph nodes



HIV-1 and Dendritic cells

When HIV enters the body via the mucosal route (epithelia of the vagina, penis or rectum), it is bound by DCs that migrate to the lymphonodes; here the DCs present HIV to T4 cells, which become infected.

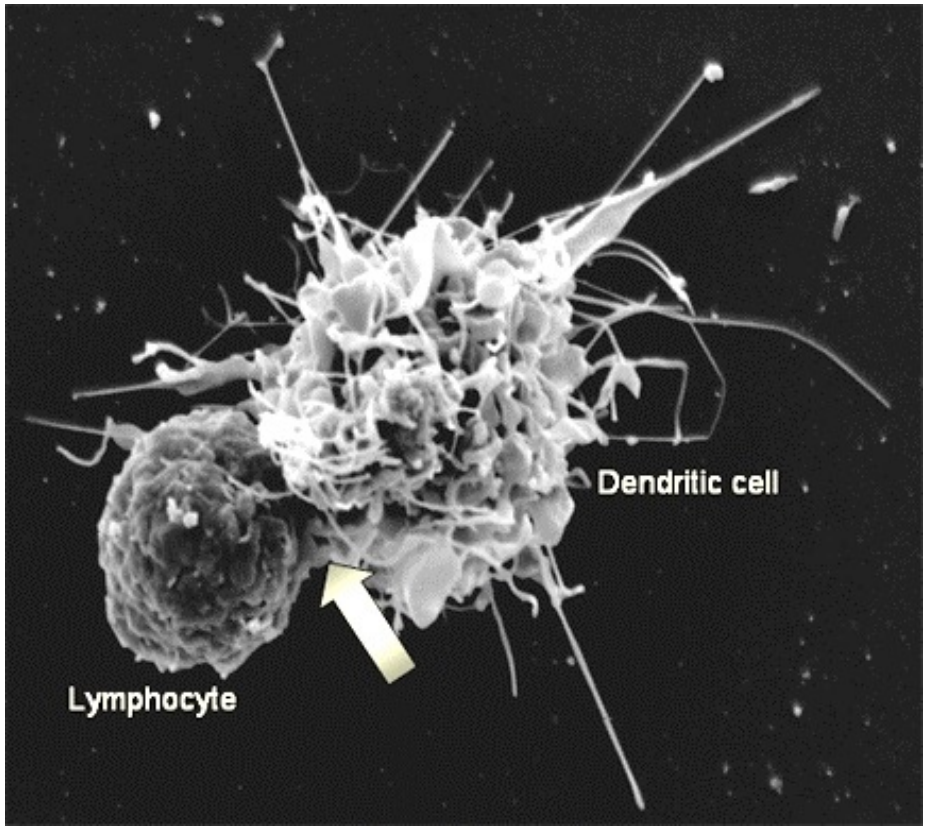


The capture of HIV particles by dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing non-integrin (DC-SIGN)-expressing cells such as DCs (c), and DC-SIGN-captured HIV transmission to T cells (d).

HIV-1 and Dendritic cells

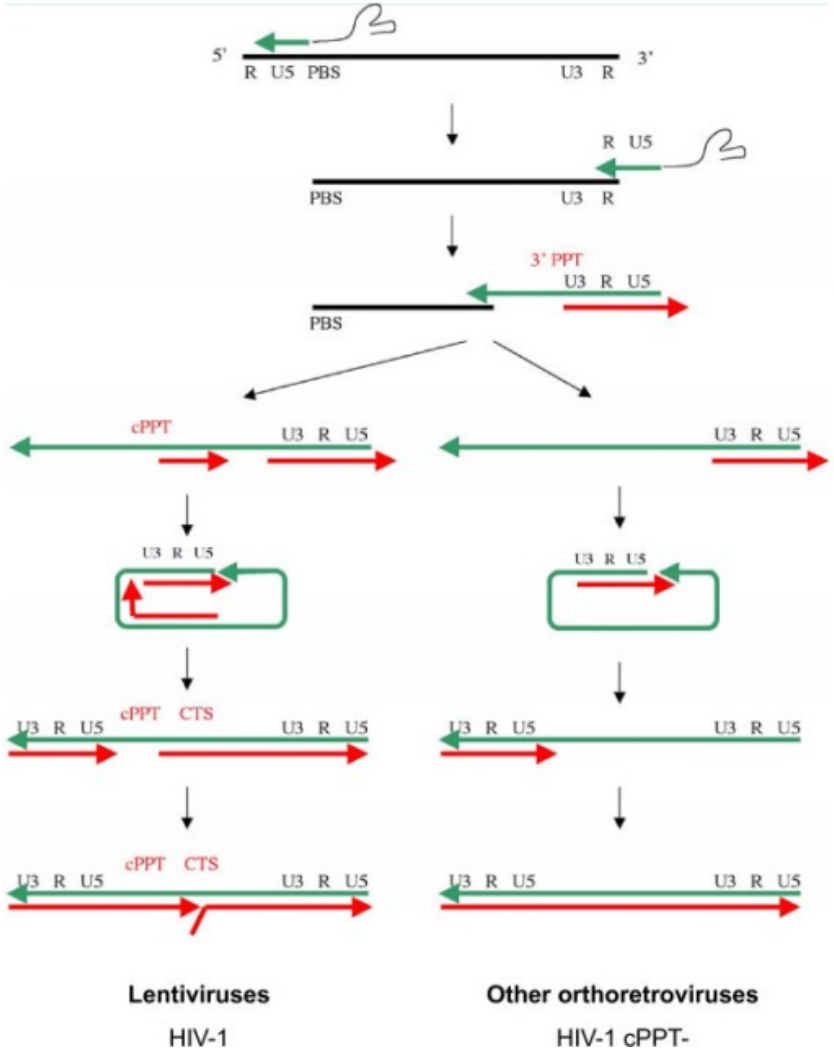
DCs are not readily infected by HIV, though they can be productively infected as a result of having low levels of HIV receptors (CD4 antigen and the co-receptors CCR5 and CXCR4). Importantly, these cells trap HIV on their surfaces since they possess a surface lectin (called dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin or DC-SIGN) that binds to the carbohydrate components of HIV gp120. Binding by DC-SIGN does not allow fusion of the membrane of the virus with the DC (which requires CD4 antigen) and so infection does not occur by this route; however, this protein also participates in the association of DCs with lymphocytes and clusters at the sites of DC-lymphocyte interactions. Thus, the bound virus is concentrated just at the site of interaction of the DC with the CD4+ cell.

HIV-1 and Dendritic cells



The interaction of a dendritic cell (right) with a lymphocyte (left). HIV bound to the surface of the dendritic cell is clustered at the site of interaction between the two cells (arrow), thereby facilitating the infection of the lymphocyte. On T4 cells, HIV receptors also concentrate here

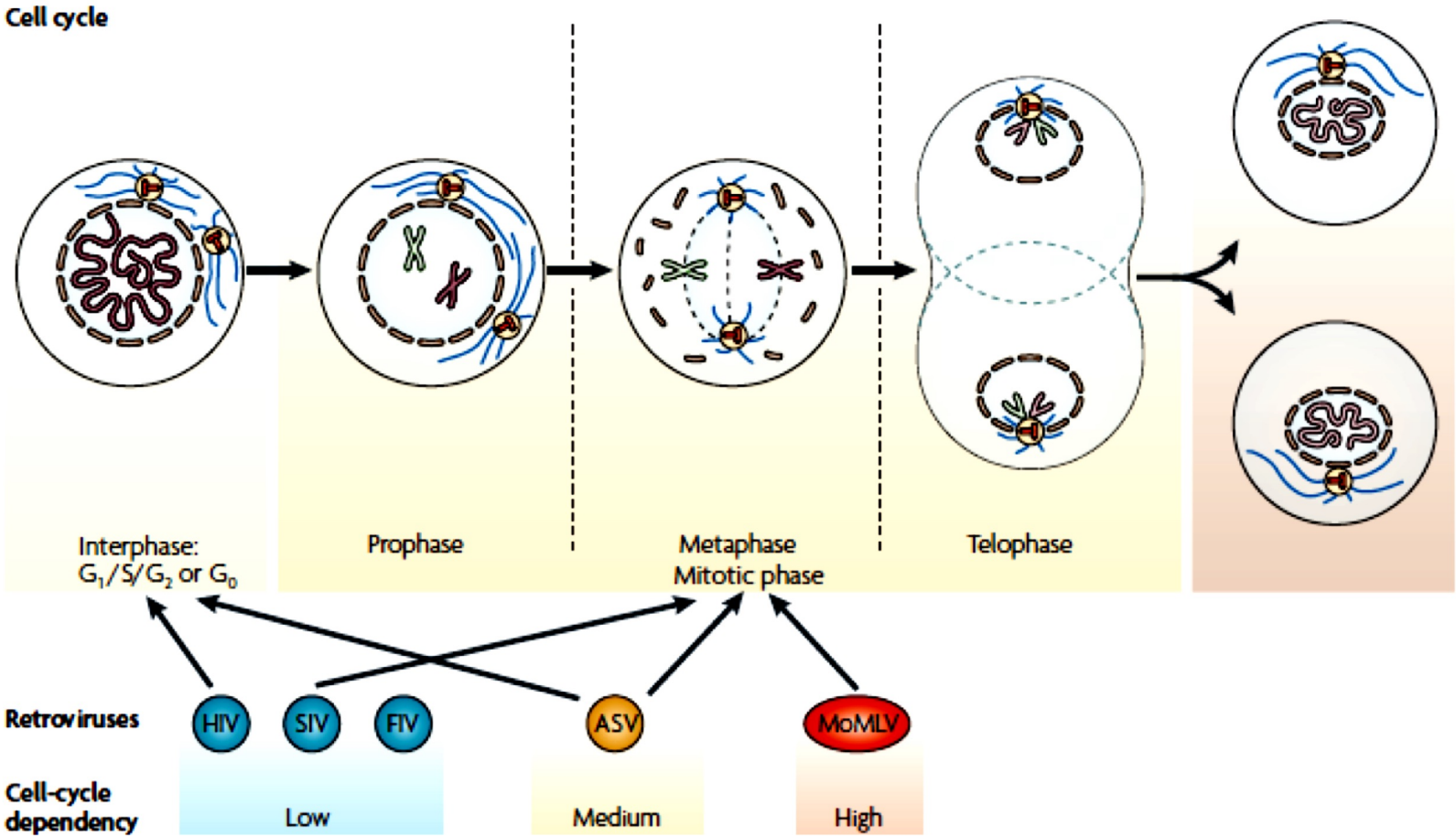
HIV-1 reverse transcription

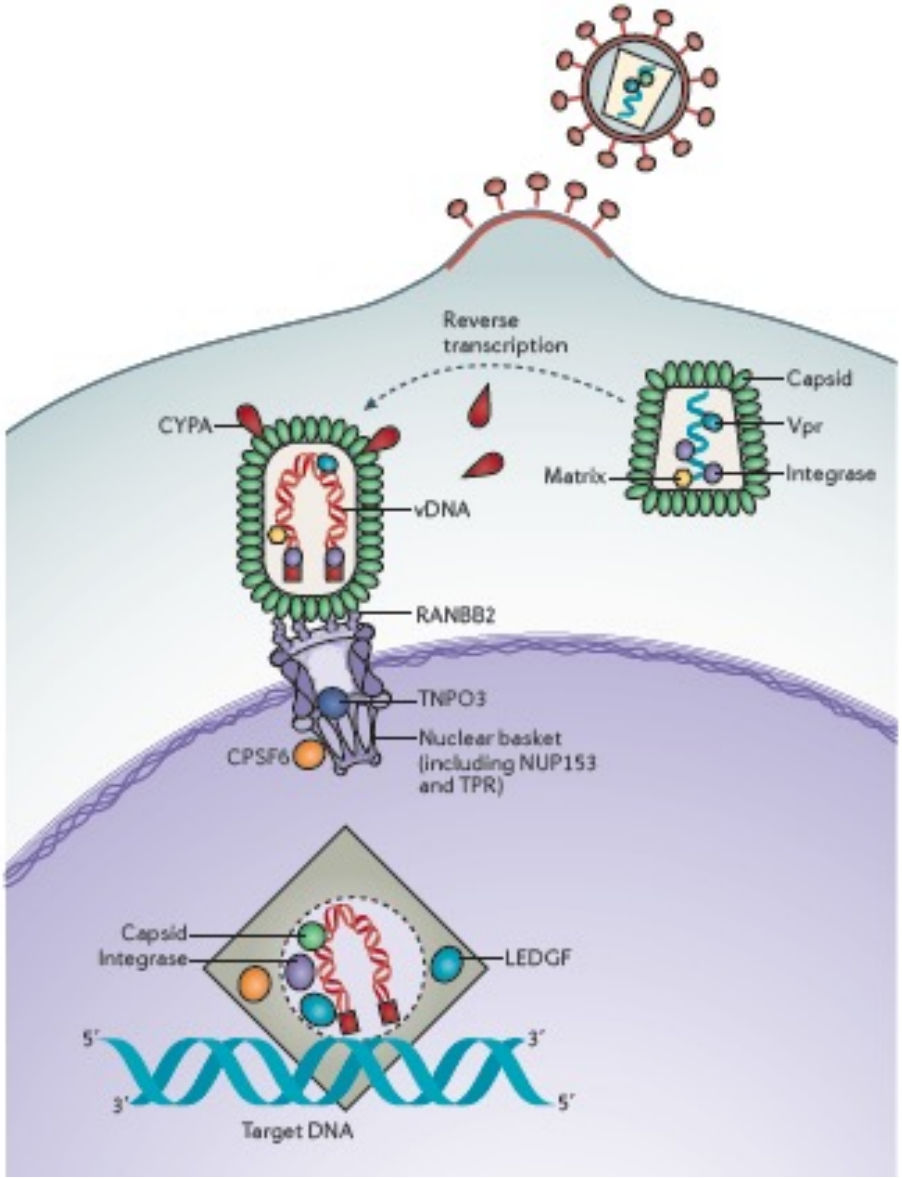


Comparison of retroviral and lentiviral reverse transcription. Retroviral DNA synthesis is initiated by a host-encoded tRNA primer that anneals the primer binding site. Copying of the genomic RNA template until the 5' cap is reached, results in the formation of a short minus-strand DNA, usually called minus-strand strong-stopDNA (-sssDNA). This -sssDNA anneals to the 3' end of the viral RNA. The jump from one end of the RNA copy to the other end is referred to as 'strand transfer'. After the first jump, the 3' end of the minus strand can be extended to produce the minus-strand DNA. Termination occurs near the PBS region of the genomic RNA. During synthesis of the minus-strand DNA, RNase H digests the RNA template. However, upstream of the U3 region, the RNA template is not digested and serves as a primer for plus-strand DNA synthesis (3' PPT). The initial plus-strand DNA product is termed plus-strand strong-stop DNA (+sssDNA). This process also produces a DNA copy of the PBS allowing second strand transfer to occur. The DNA copy of the viral genome is completed by RT that copies the entire plus and minus strands. The final product is a blunt-ended linear duplex DNA with a long terminal repeat (LTR) at each end. Formation of the plus strand of lentiviruses proceeds differently. **In these viruses, next to the 3' PPT, a second central polypurine tract (cPPT) is present in the pol open reading frame in the center of the genome. As a consequence, plus strand DNA synthesis is initiated both at the 3' PPT and the cPPT. When the DNA strand initiated by the 3' PPT reaches the DNA stretch initiated by the cPPT, strand displacement takes place until the central termination sequence (CTS) is reached. A single-stranded gap and a single-stranded overlapping DNA of 90 or 99 nucleotides, the DNA flap, are formed.**

Retrovirology 2010, 7:96

Nuclear import of pre-integration complexes and the cell cycle.



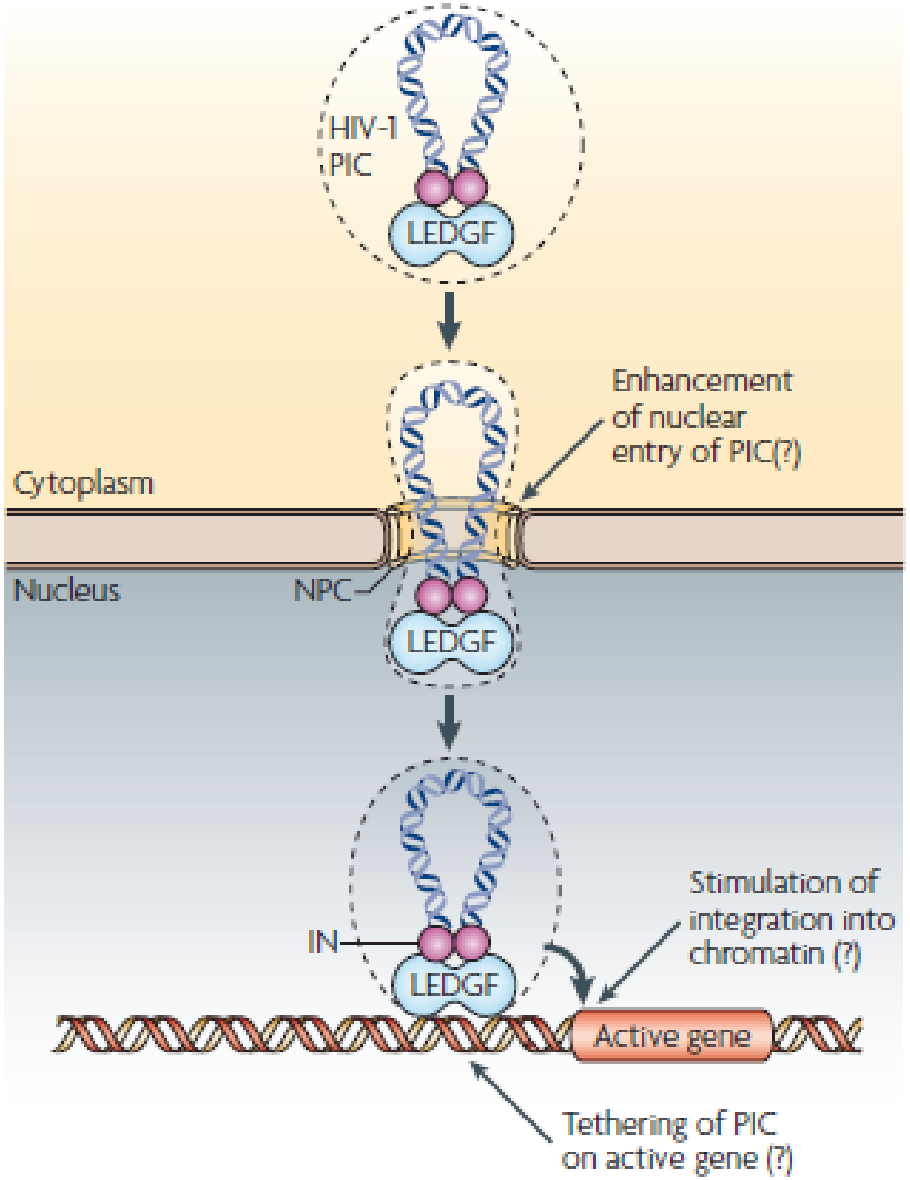


Nuclear entry and the selection of integration site

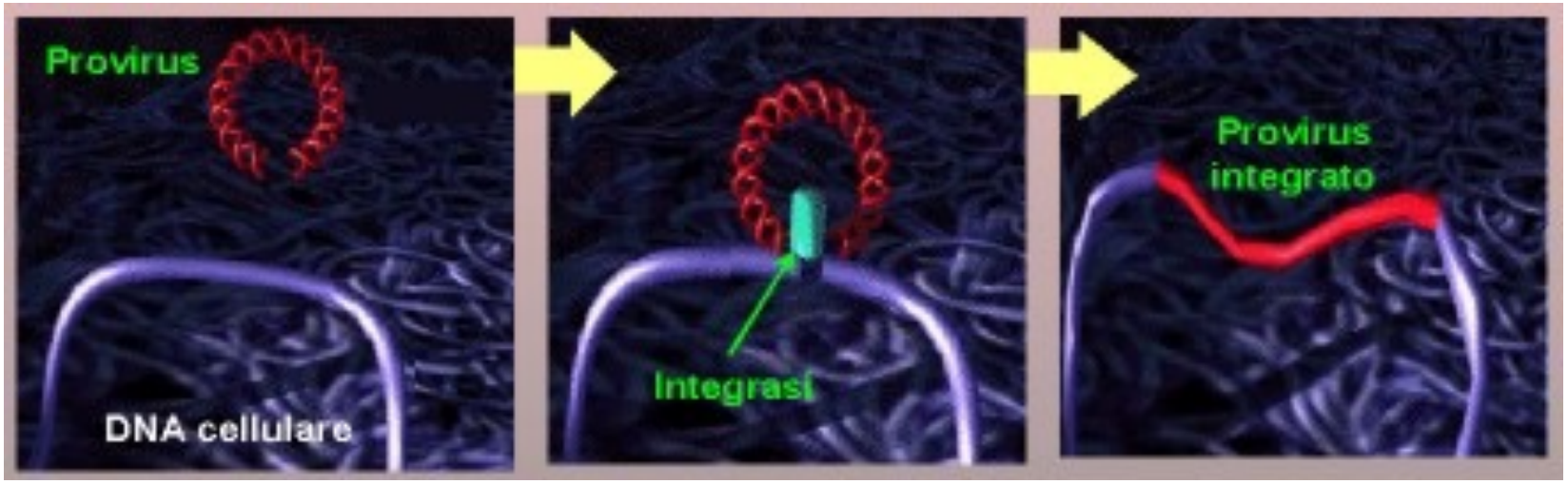
The capsid of HIV-1 seems to be a crucial viral factor for both uncoating and the entry of the pre-integration complex (PIC) into the nucleus. Other than capsid, the viral factors Vpr and matrix, as well as different cellular partners are involved in the process of nuclear entry on the cytosolic side of the nuclear pore complex (NPC). Cyclophilin A (CYPA; also known as PPIA) and RANBP2 have roles in the nuclear entry of the PIC on the cytosolic side of the NPC, transportin 3 (TNPO3) is involved in shuttling through the NPC, and nucleoporin 153 (NUP153) and cleavage and polyadenylation specificity factor 6 (CPSF6) are involved in the import of HIV-1 into the nucleus. Both NUP153 and CPSF6 have a role in target site selection, together with lens-epithelium-derived growth factor (LEDGF), which is the most prominent chromatin-tethering factor that is involved in HIV-1 integration.

LEDGF/p75 and nuclear entry of PICs.

Several roles for lens-epithelium-derived growth factor (LEDGF/p75) have been proposed for human immunodeficiency virus 1 (HIV-1) DNA integration.
LEDGF/p75 might regulate HIV-1 replication through the tethering of integrase protein (IN) and chromatin. NPC, nuclear pore complex; PIC, pre-integration complex.

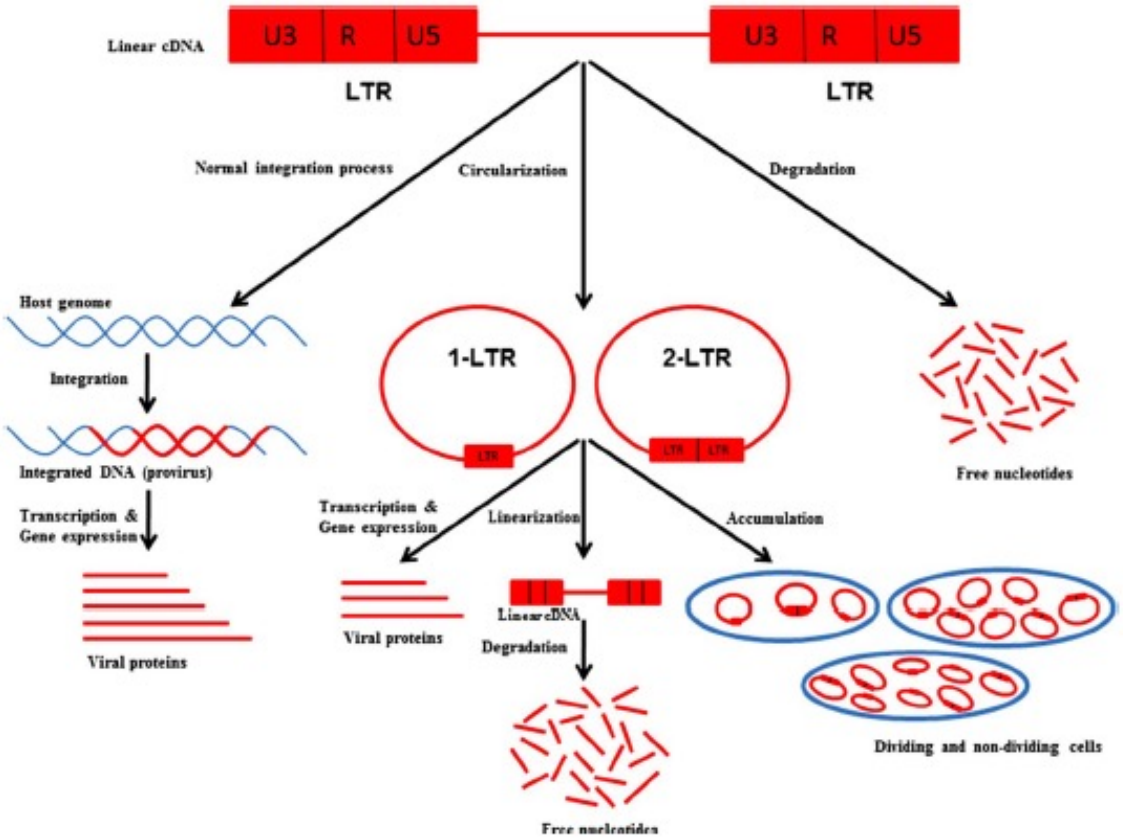


Viral DNA integration requires at least four steps



IN associates with the viral DNA in the cytoplasm to form the pre-integration complex (PIC).
In a first catalytic step IN cuts a dinucleotide from both ends of the viral DNA to produce hydroxylated 3' ends in the PIC
In the nucleus IN binds the host DNA, catalyses a staggered cleavage in the cellular target DNA, The 3' recessed ends of viral DNA are joined to the 5' "overhanging" termini of the cleaved cellular DNA.
Finally, gaps and any mismatched bases at the newly created junctions are repaired by host DNA repair machinery⟶irreversible integration.

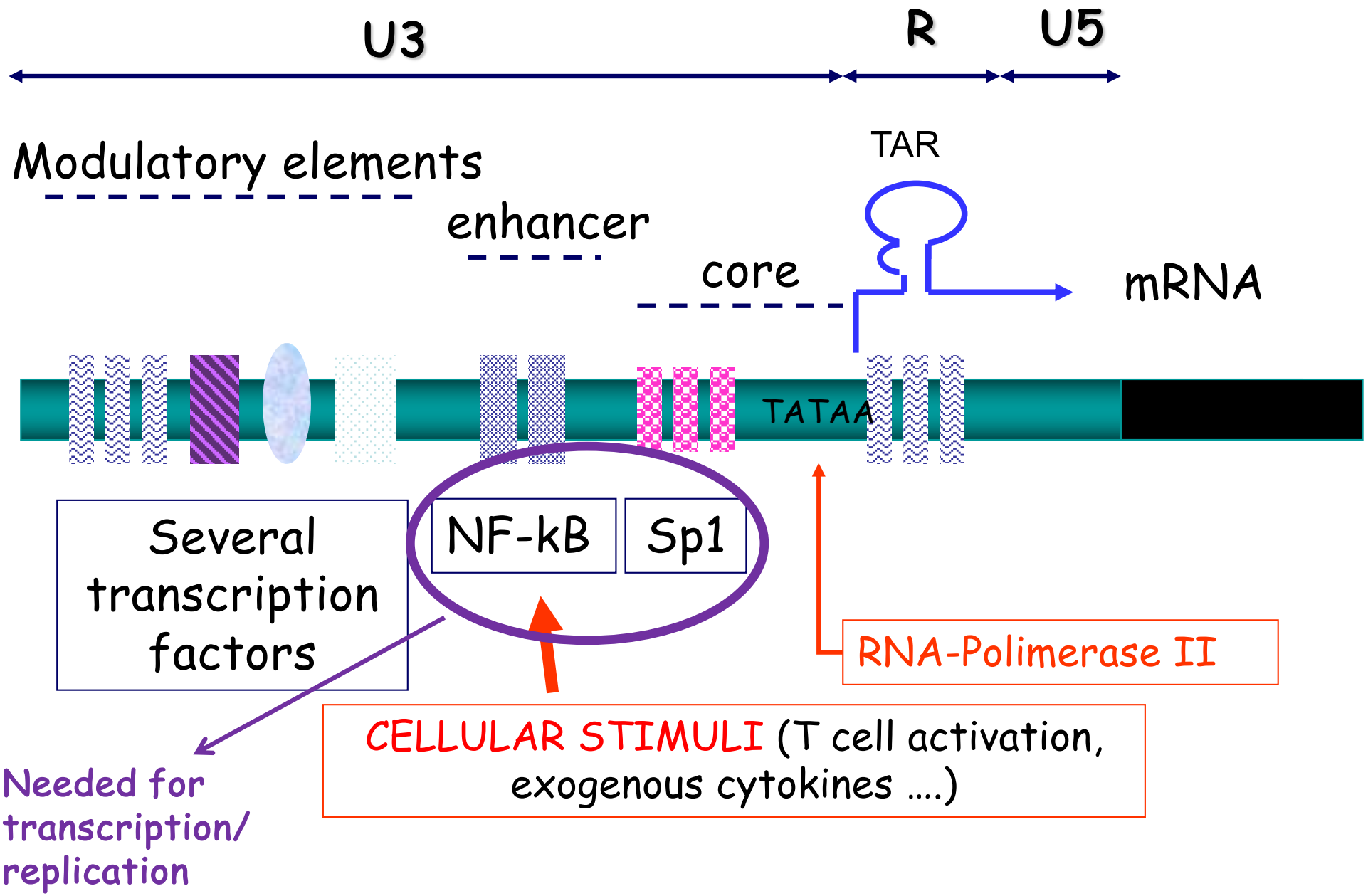
Fates of viral cDNAs after reverse transcription.



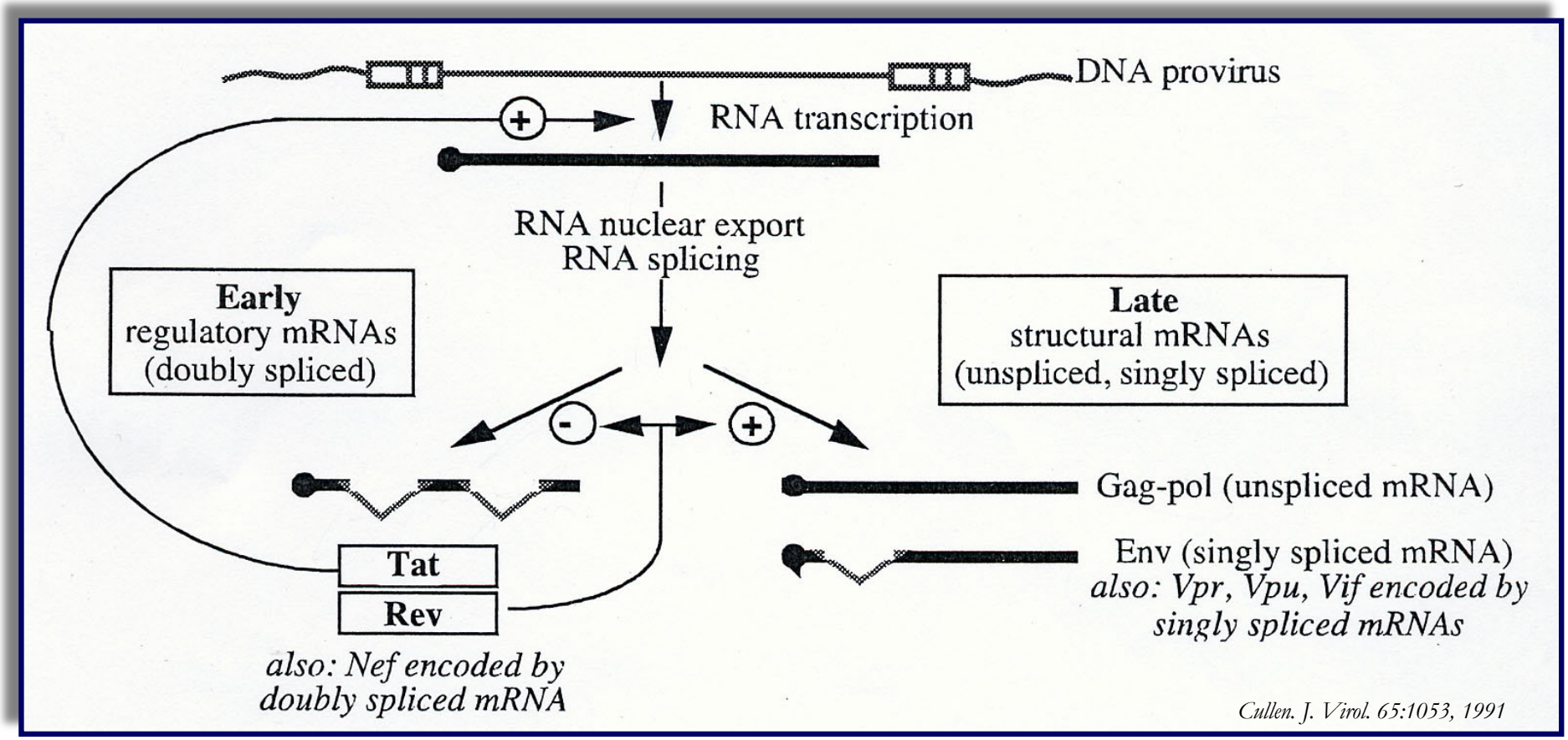
After reverse transcription, HIV-1 produces cDNAs that may either be integrated into host genome or circulate themselves. Otherwise, they are degraded into free nucleotides. Circular DNAs have been reported to actively participate in current gene expression similar to the integrated DNAs followed by linearization. They can also undergo to degradation, or accumulation as a reservoir. HIV-1 IN required for linearization of 2-LTR circles was found at the palindromic junction, recognized as integration site, and subsequently executed a de novo integration process.

AIDS Research and Therapy 2017 **14**:9

HIV-1 LTR ORGANIZATION

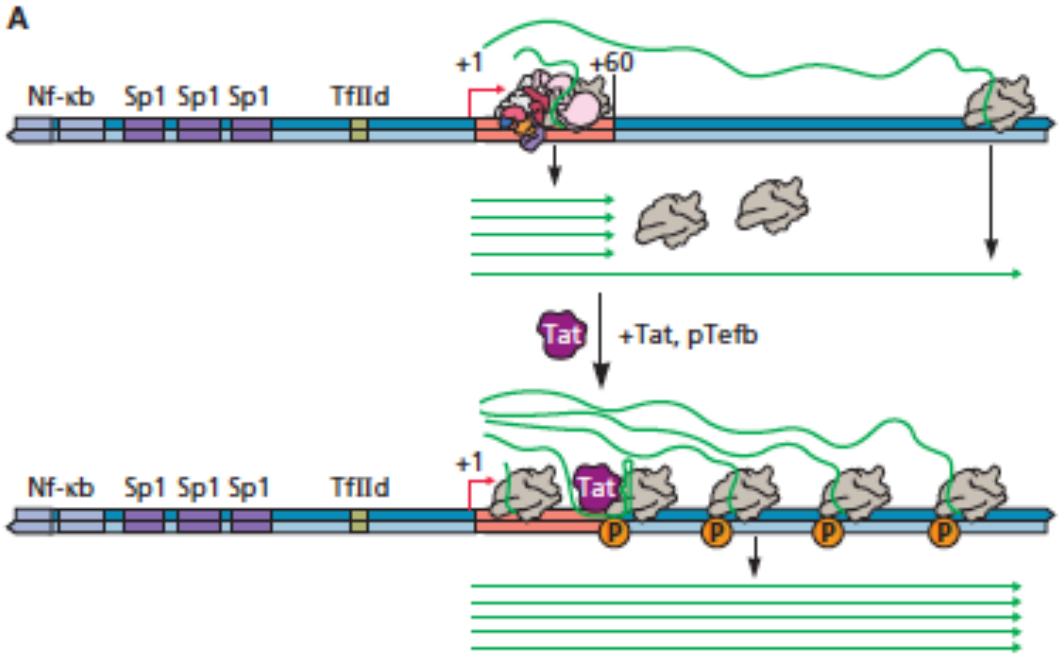


HIV-1 Gene Expression



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

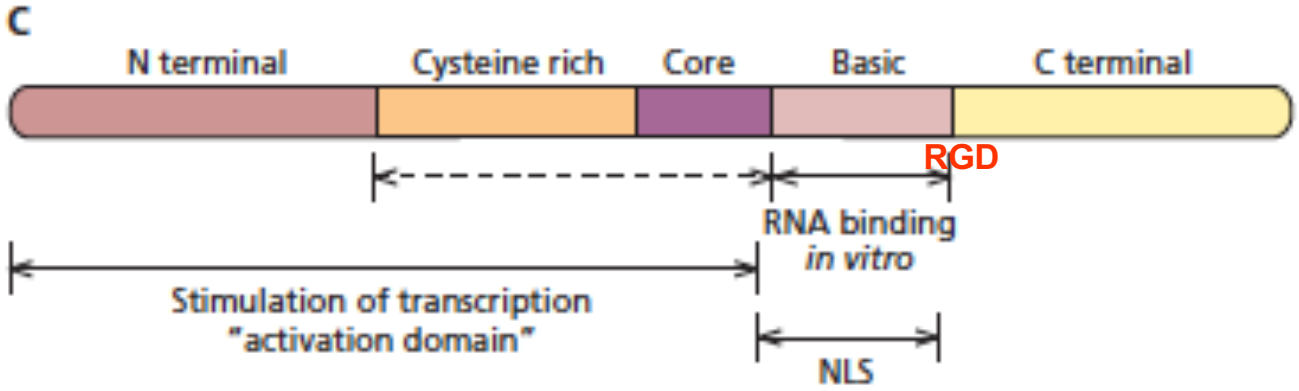
Model for the stimulation of elongation by Tat



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 upon translation of mRNAs spliced from rare, full-length transcripts 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

Tat - Trans-Activator of Transcription (14-16kDa)

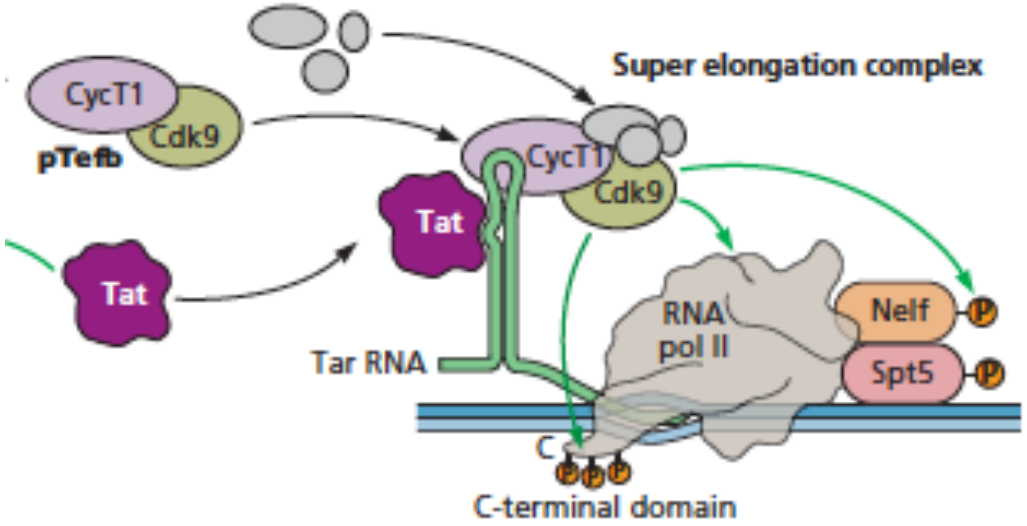


The Tat protein is made from several different, multiply spliced mRNAs and therefore varies in length at its C terminus. The regions of the protein are named for the nature of their sequences (basic, cysteine rich) or greatest conservation among lentiviral Tat proteins (core). Experiments with fusion proteins containing various segments of Tat and a heterologous RNA-binding domain identified the N-terminal segment as sufficient to stimulate transcription. **The basic region, which contains the nuclear localization signal (NLS), can bind specifically to RNA containing the bulge characteristic of TAR RNA. However, high-affinity binding, effective discrimination of wild-type TAR from mutated sequences in vitro, and RNA-dependent stimulation of transcription within cells require additional N-terminal regions of the protein, shown by the dashed arrow.**

TAT interacts with the ciclina T1/cdk9 complex through the cysteine rich domain.

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

Model for the stimulation of elongation by Tat

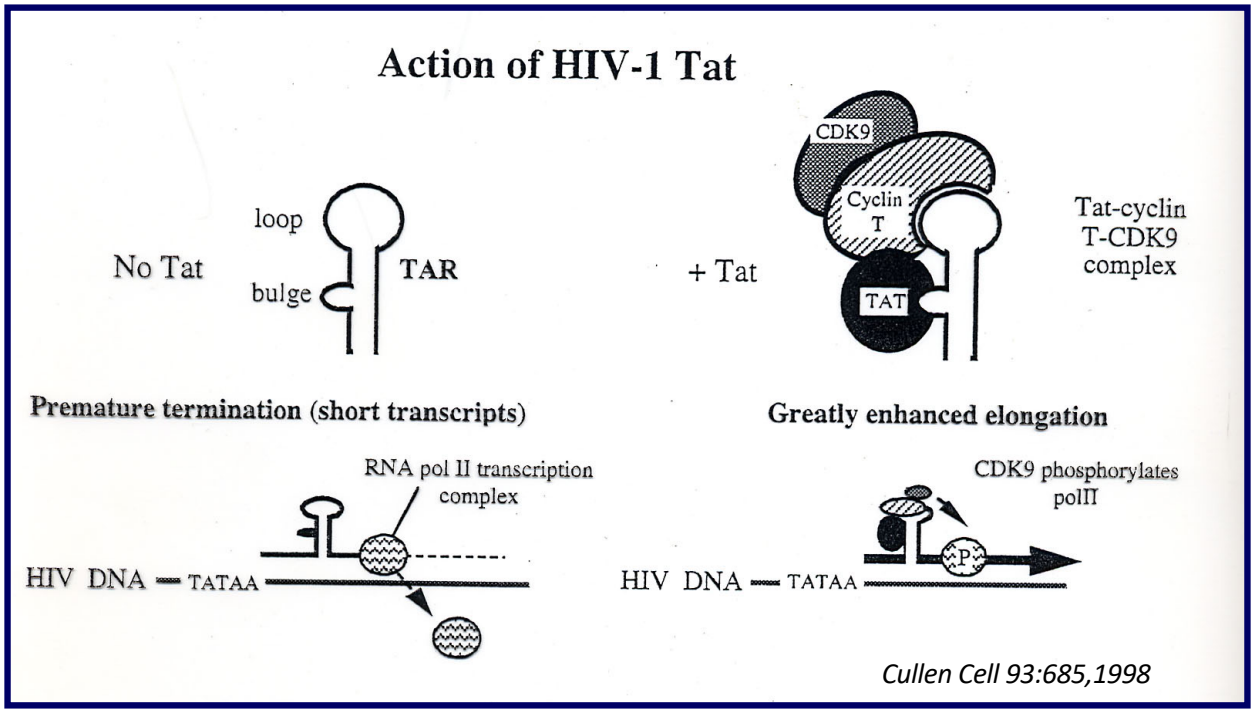


Cooperative binding to TAR of Tat and p-Tefb (via its cyclin T1 subunit) leads to phosphorylation (P) of the C-terminal domain of the largest subunit of RNA polymerase II by the Cdk9 kinase subunit of p-Tefb. This enzyme also phosphorylates and inactivates negative regulators of transcriptional elongation (e.g., transcription elongation factor [Spt5] and negative elongator factor complex [Nelf]). Positive regulators of elongation, such as RNA polymerase II elongation factor 2 (Ell2), are also recruited to form a super elongation complex. The net result is that transcriptional complexes become competent to carry out highly processive transcription. Adapted from M. Ott et al., Cell Host Microbe 10:426–435, 2011, with permission.

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



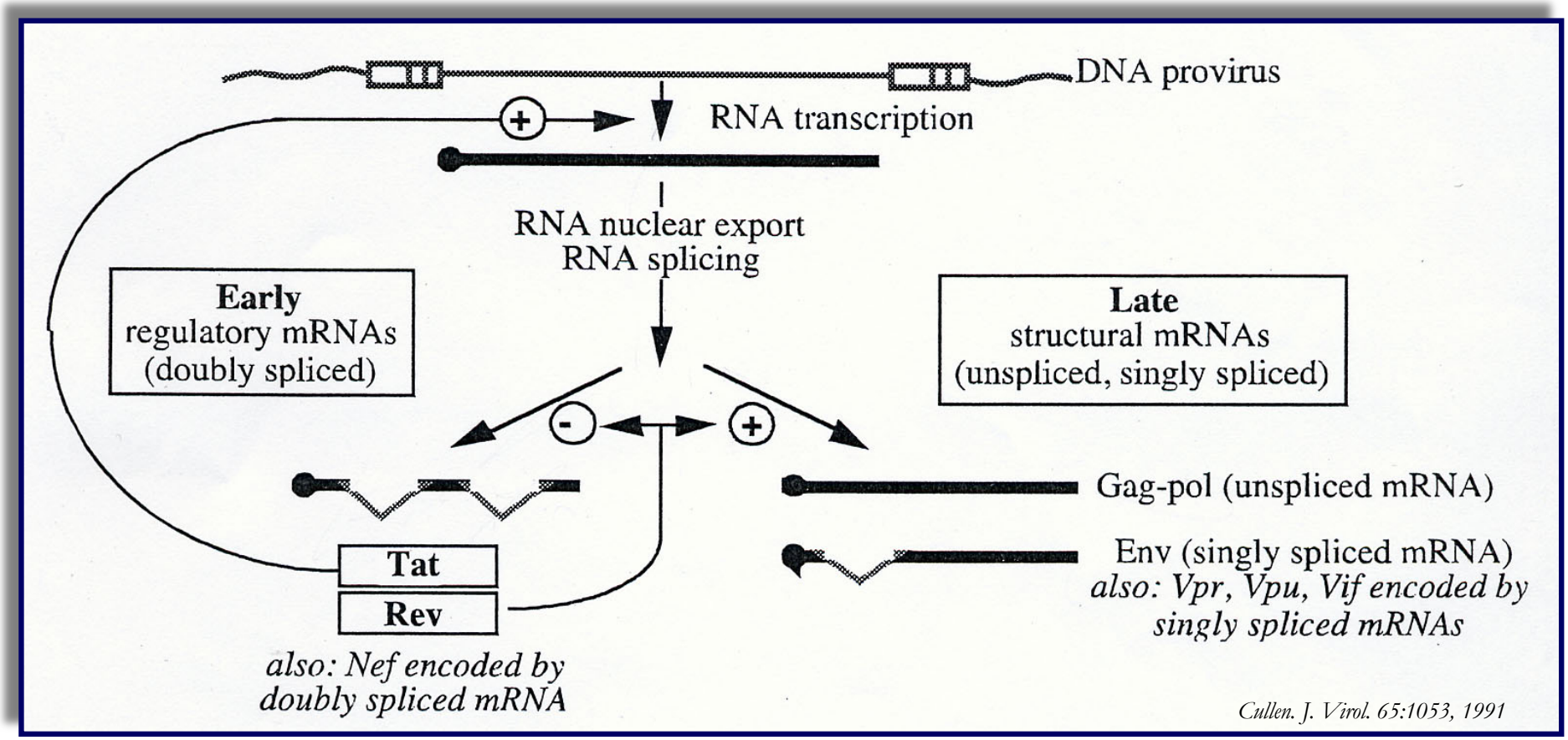
1. Establishes a cooperative interaction with the TAR sequence and the human cyclin T1 (the regulative component of the elongation factor b, P-TEFb)
2. Recruitment of the hCycT1/ CDK9 complex on the TAR sequence allows phosphorylation of Pol II C-terminus by CDK9, resulting in productive transcription

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]

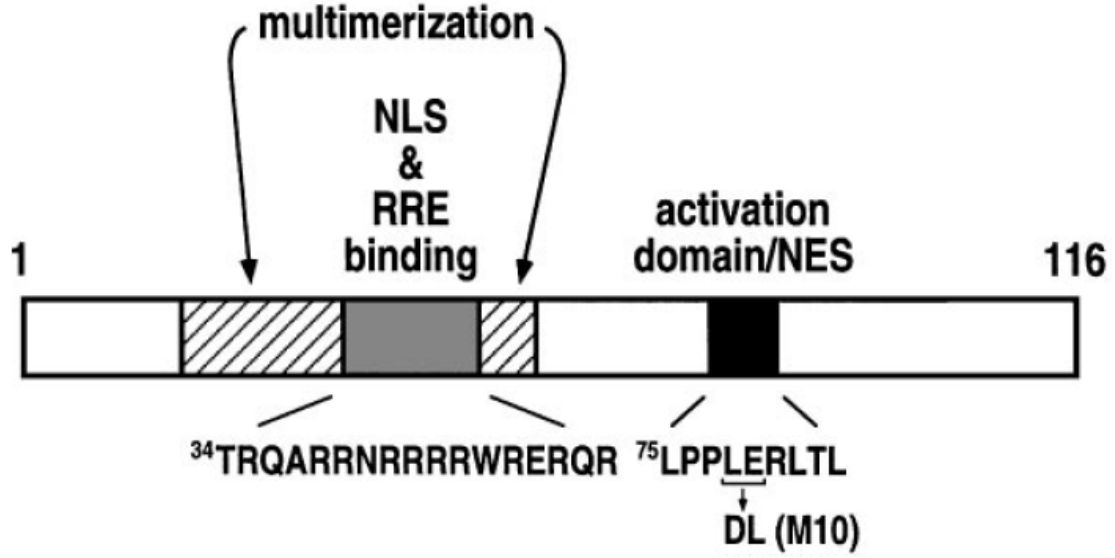
HIV-1 Gene Expression



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

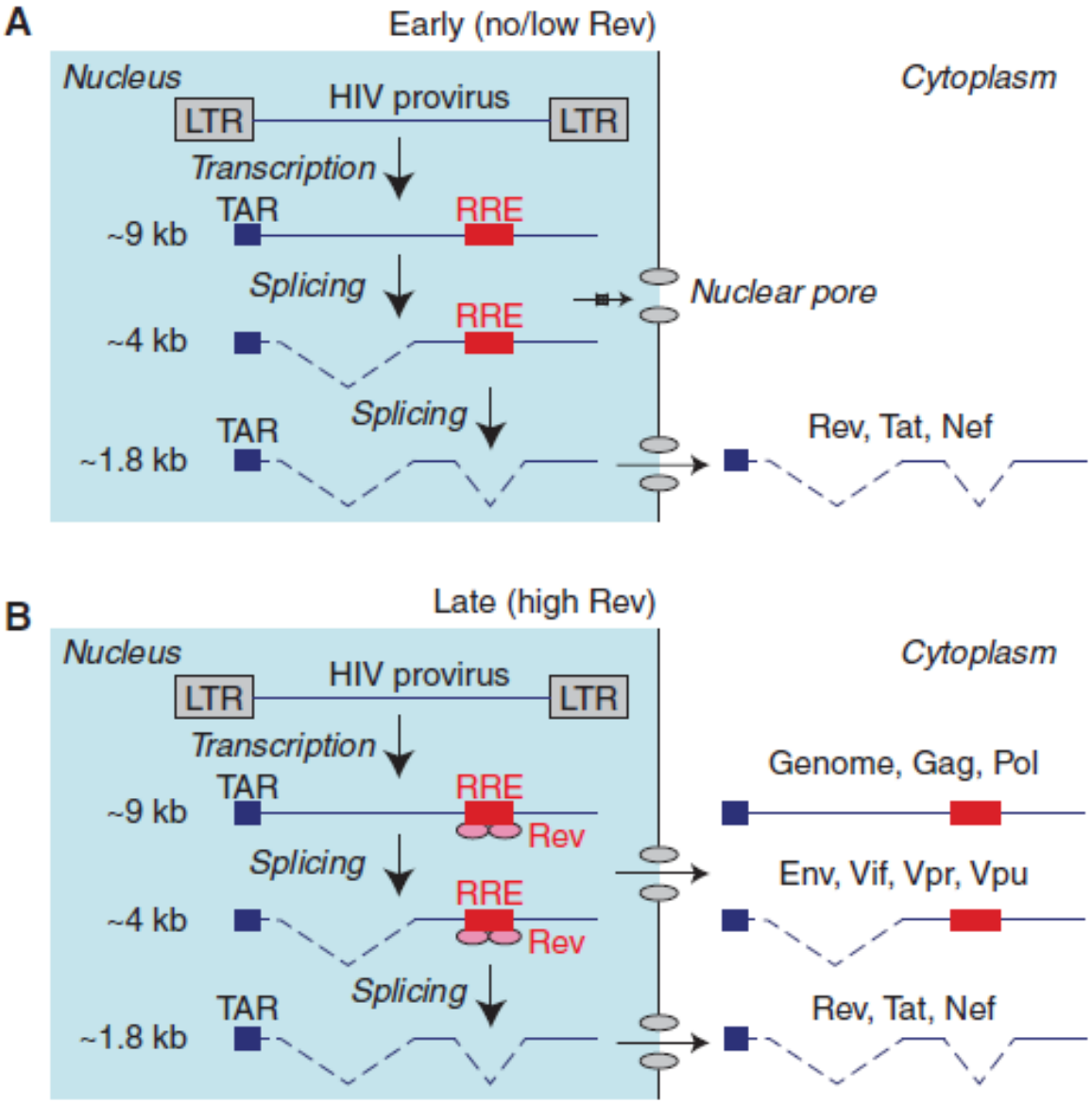
REV

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.

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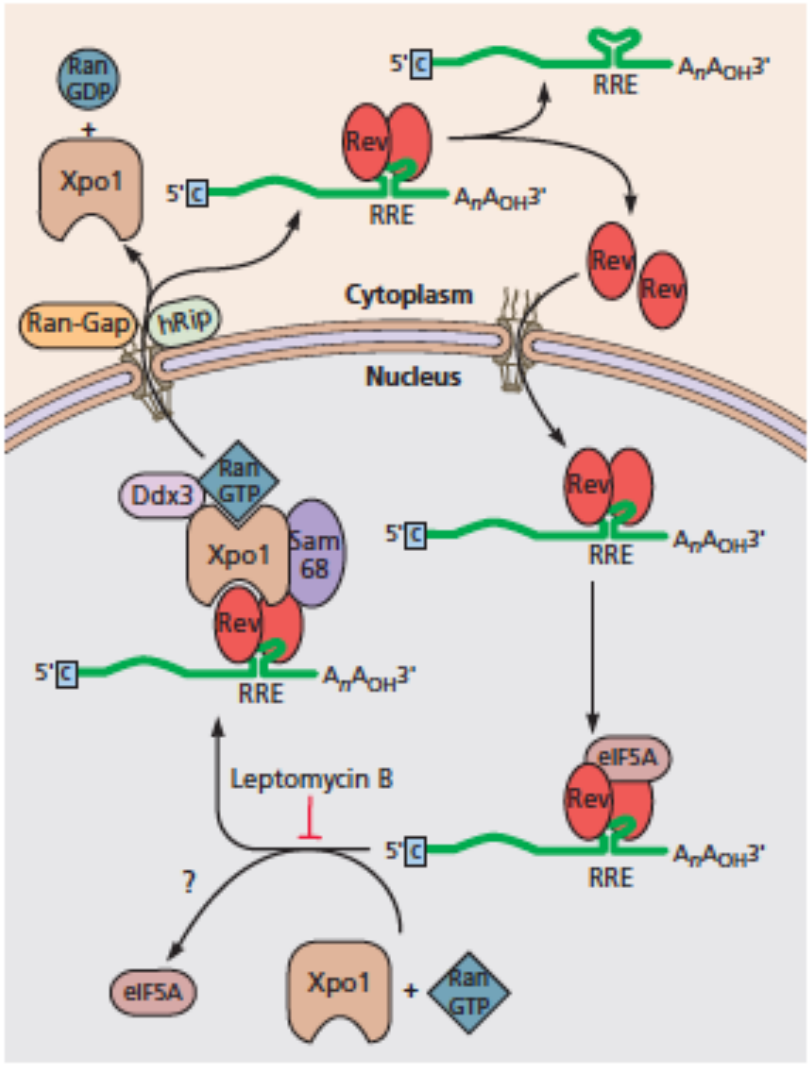
Action of Rev



The cellular nuclear proteins exportin-1 (Xpo1), the GTP-bound form of Ran (Ran-GTP), and the 68-kDa Src-associated protein in mitosis (Sam68) have been implicated in Rev-dependent mRNA export, for example, by analysis of the effects of dominant negative forms of the proteins. In the presence of Ran-GTP, Rev binds to Xpo1. This protein interacts with nucleoporins. The complex containing Rev, Xpo1, and Ran-GTP bound to the Rev-responsive element and RNA is translocated through the nuclear pore complex to the cytoplasm via interactions of Xpo1 with nucleoporins, such as Can/Nup14 and Nup98. Translocation may be facilitated by the action of Ddx3, an ATP-dependent RNA helicase. The Sam68 protein can bind to the Rev nuclear export signal, but does not appear to shuttle between nucleus and cytoplasm. It may therefore act prior to docking of the viral RRE-containing RNA complex at the nuclear pore. The human Rev-interacting protein (hRip) appears to act following translocation, as it is essential for efficient release of Rev-associated RNA into the cytoplasm. Hydrolysis of GTP bound to Ran to GDP induced by the cytoplasmic Ran GTPase-activating protein (Ran-Gap) is presumed to dissociate the export complex, releasing viral RNA for translation or assembly of virus particles, and Ran, Xpo1, and other proteins for reentry into the nucleus.

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