Taxonomy:

Group VI: RNA Reverse Transcribing Viruses			
Family	**Genus**	**Type Species**	**Hosts**
Retroviridae	*Alpharetrovirus*	*Avian leukosis virus*	Vertebrates
	Betaretrovirus	*Mouse mammary tumor virus*	Vertebrates
	Gammaretrovirus	*Murine leukernia virus*	Vertebrates
	Deltaretrovirus	*Bovine leukemia virus*	Vertebrates
	Epsilonretrovirus	*Walley dermal sarcoma virus*	Vertebrates
	Lentivirus	*Human immunodeficiency virus 1*	Vertebrates
	Spumavirus	*Chimpanzee foamy virus*	Vertebrates
Metaviridae	*Metavirus*	*Saccharomyces cerevisiae Ty3 virus*	Fungi
	Errantivirus	*Drosophila melanogaster gypsy virus*	Invertebrates
Pseudoviridae	*Pseudovirus*	*Saccharomyces cerevisiae Ty1 virus*	Invertebrates
	Hemivirus	*Drosophila melanogaster copia virus*	Invertebrates

retroviruses target mitotically active cells, this limits the use of these vector

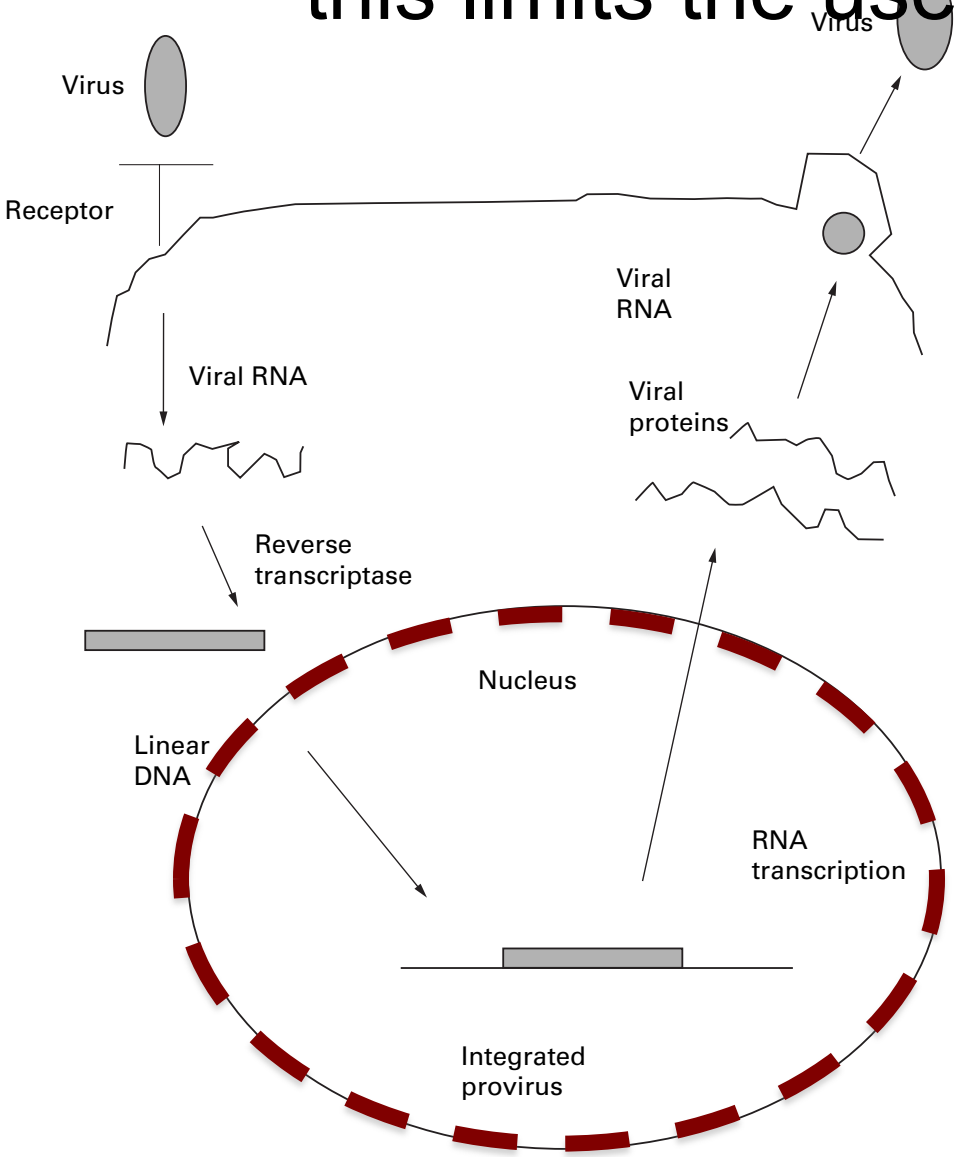


Figure 1 The murine leukaemia retrovirus life cycle.

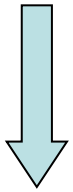
The retrovirus consists of two copies of a single stranded RNA genome with sequences known as gag, pol, and env, which encode viral structural and catalytic proteins. These are surrounded by a glycoprotein envelope.

At the onset of infection, the surface glycoprotein envelope interacts with receptors on the surface of the target cell to gain entry. When inside the cell, the single stranded viral genome is converted into linear double stranded DNA by a virus encoded reverse transcriptase.

As the target cell undergoes mitosis, (the cell must divide to allow DNA entry into the nucleus) the viral DNA integrates with the target cell DNA at which point it is known as a provirus.

One of the major limit of retroviral vectors is their dependence on cellular replication. Thereby they can be used exclusively in mitotically active cells.

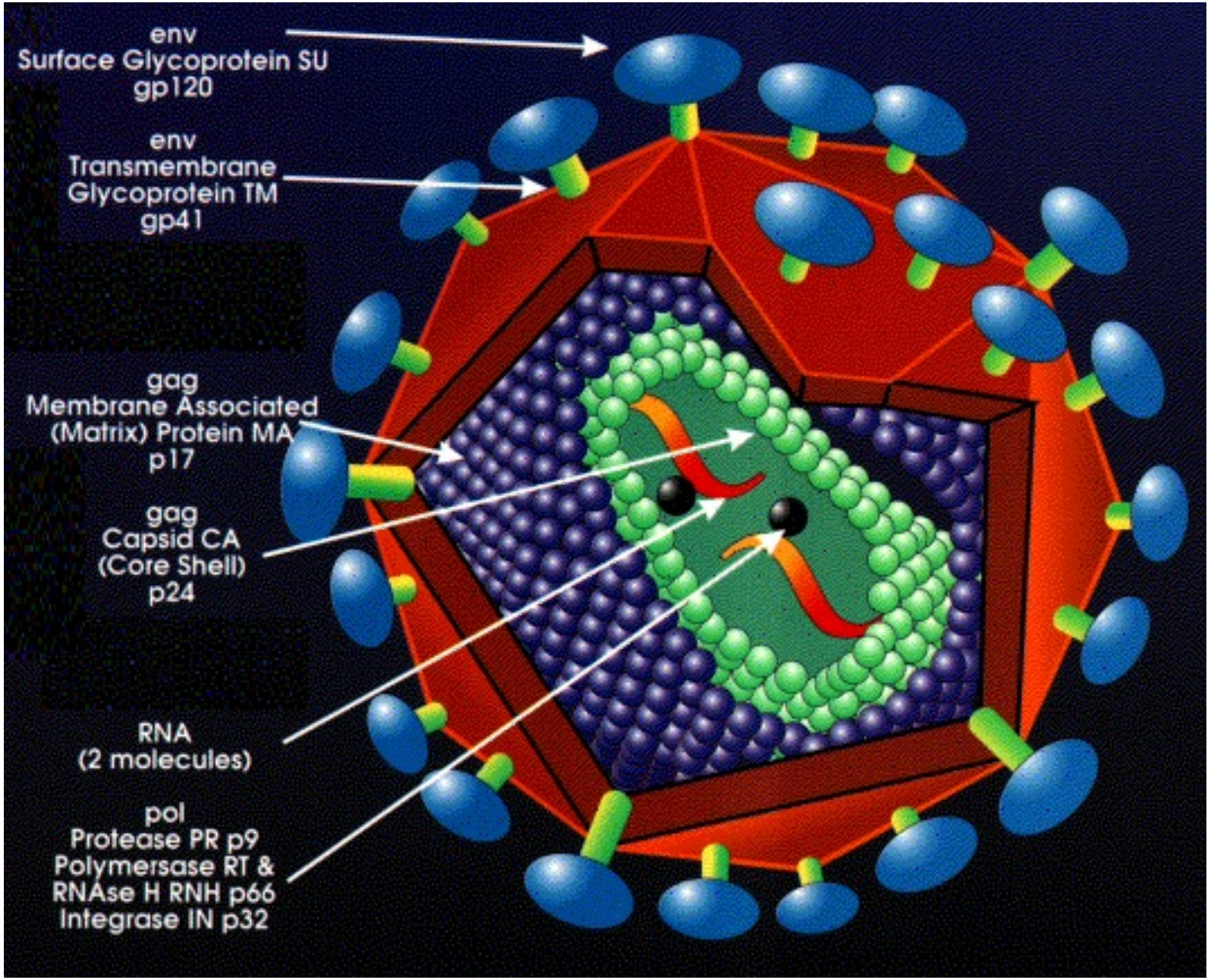
this can be overcome by:
inducing cell proliferation (in vitro)



can, onother virus be used?

HIV-based
gene transfer systems are popular due to their ability to tranduce terminally differentiated and non-dividing cells

HIV particles



The HIV genome is a typical retroviral genome

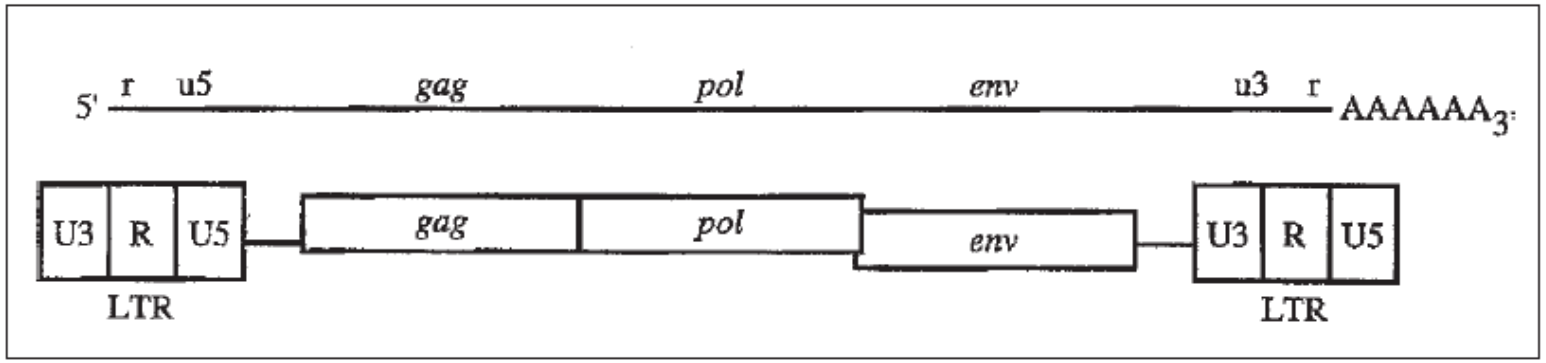
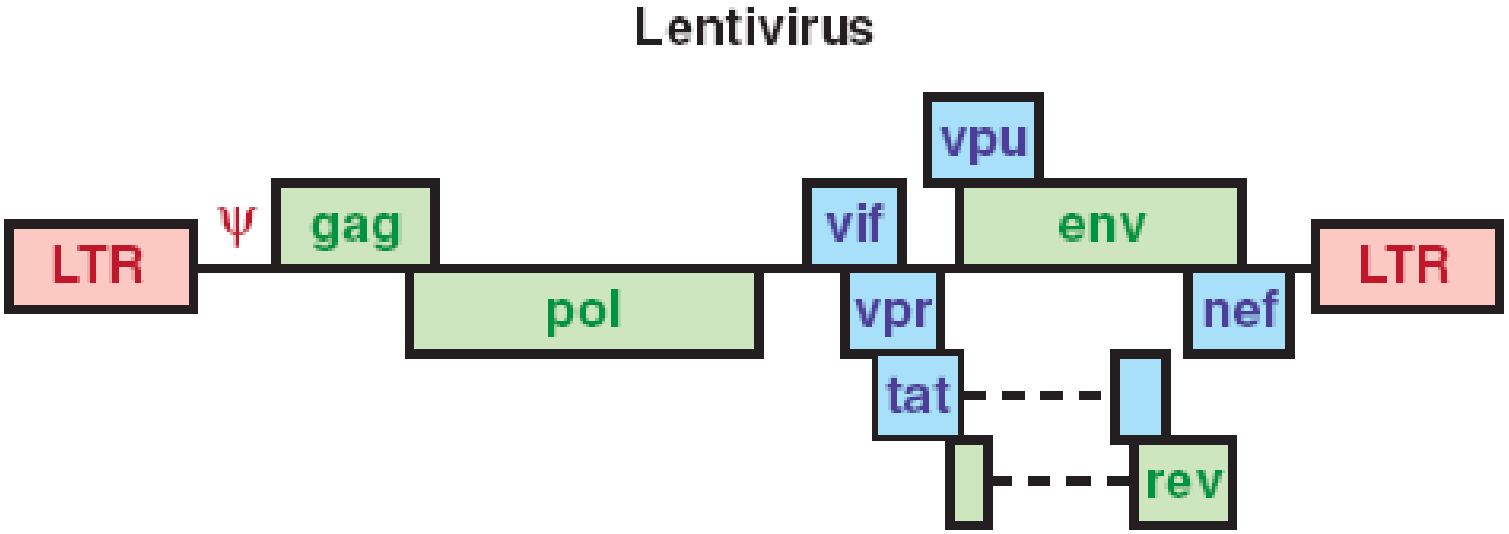


Fig. 1. Genome structure of a prototypical retrovirus. The genomic viral RNA, represented by a single black line, is shown at the top of the figure, with the structure of the resulting provirus after reverse transcription below. The locations of the open reading frames *gag*, *pol*, and *env* are shown. Reverse transcription of the RNA results in rearrangement of the termini of the genome, resulting in the structures of the LTRs (long terminal repeats) as indicated. *Cis*-acting sequences of the viral genome are shown in more detail in Figure 3.

the lentiviral genome in more details

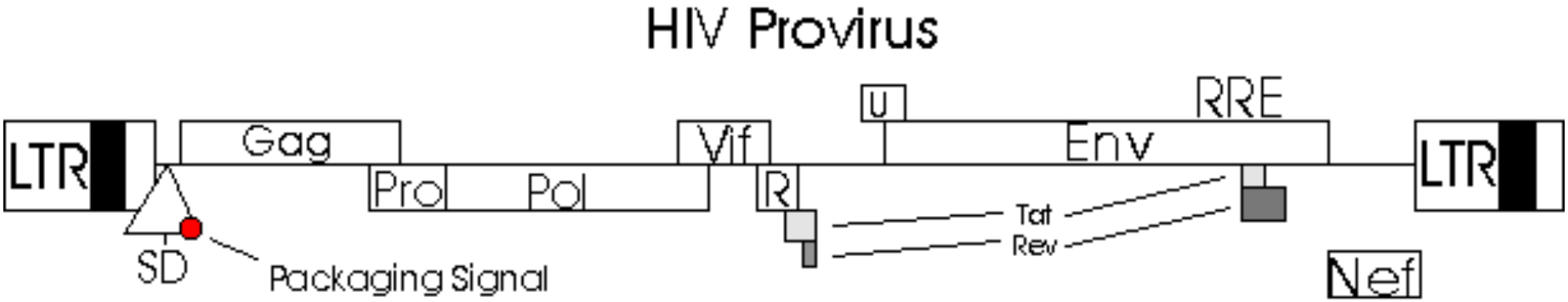


Cis acting

Geni essenziali, supportati dal costrutto di packaging

gag (group-specific antigen), *pol* (polymerase), *env* (envelope)

Geni non essenziali, eliminabili



Trans

Gag	capside, p24
	proteina MA, p17
Pol	proteasi PR. P9
	trascrittasi inversa
	RNAseH, p66
	integrase, p32
Env	proteina di superficie, gp120
	proteina transmembrana, gp41
TAT	seganle di localizzazione nucleare
Rev	export signal

Cis acting

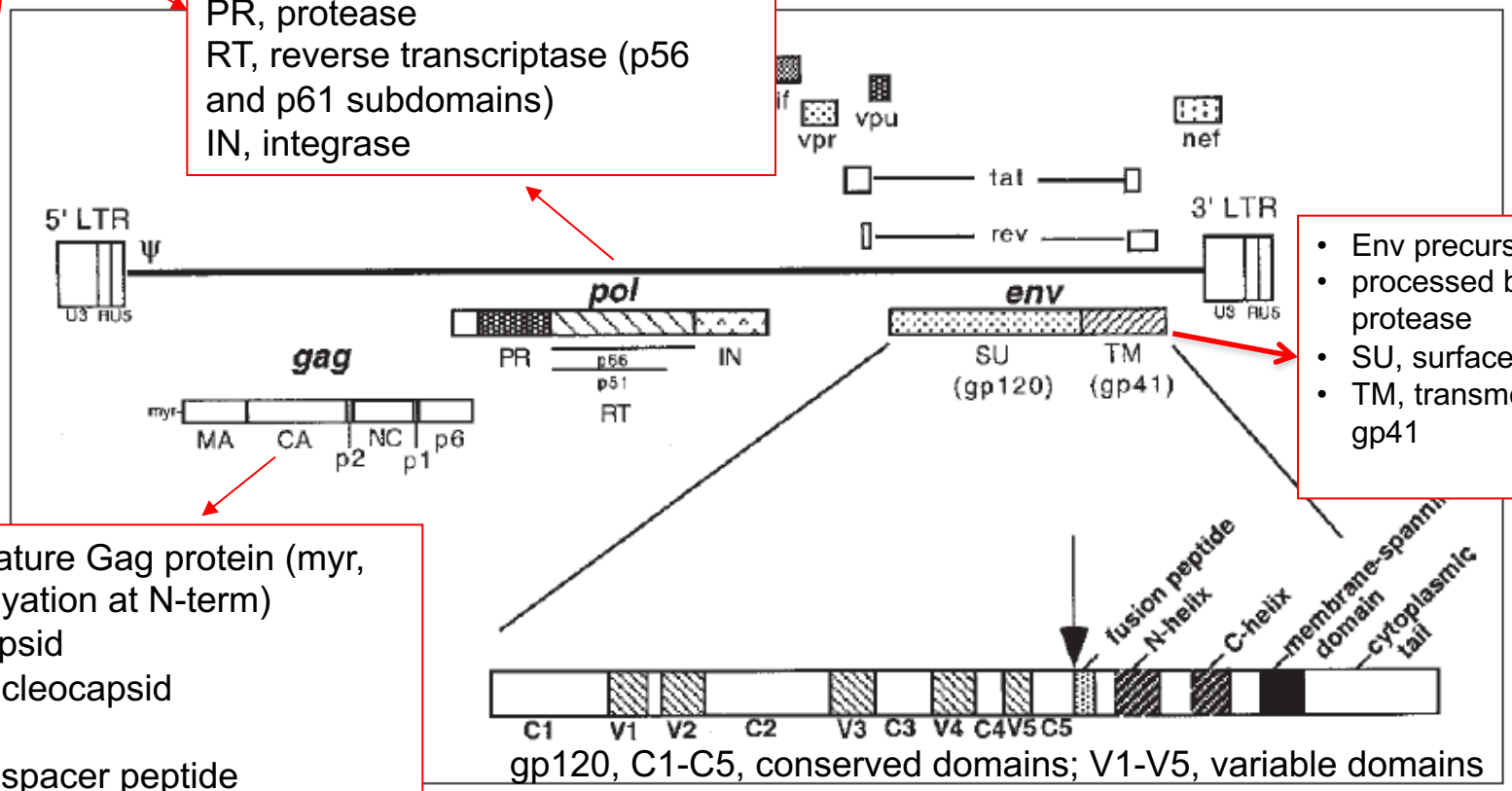
- LTR, integrazione
- ψ, segnale di packaging
- RRE, rev responsive element
- cPPT/CTS

Rev/RRE are required to get high titer preparations

The HIV-1 genes and proteins

Cleaved by PR

PR, protease
RT, reverse transcriptase (p56 and p61 subdomains)
IN, integrase

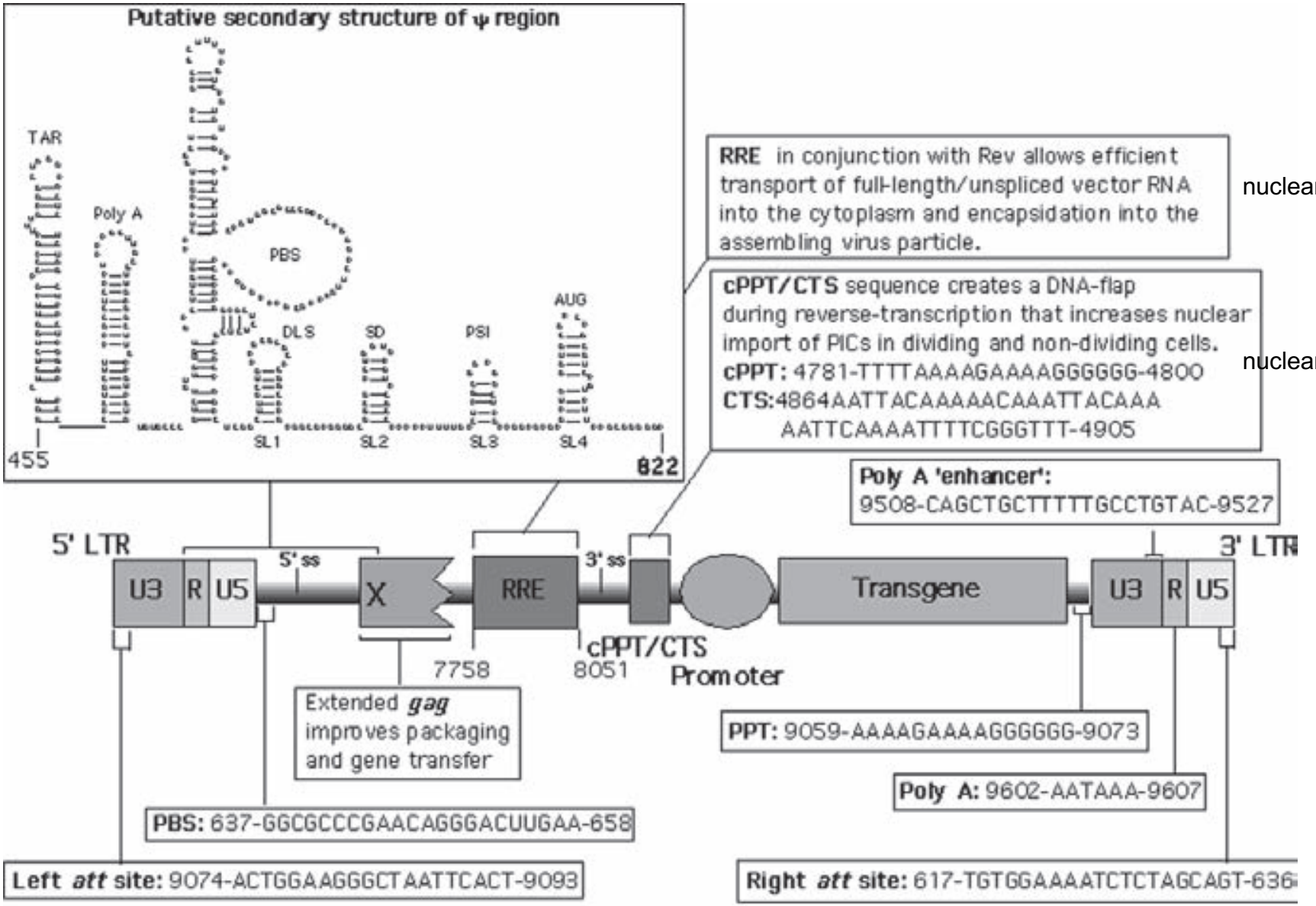


- Env precursor (gp160)
- processed by celular protease
- SU, surface gp120
- TM, transmembrane gp41

- MA, mature Gag protein (myr, myristilyation at N-term)
- CA, capsid
- NC, nucleocapsid
- P6
- p1-p2, spacer peptide

From Freed 2001

The HIV-1 *cis* acting elements to be considered for vector design



HIV regulatory and accessory proteins

Tat, transcription activation (activation domain,RNA-binding domain, overlapping nucelar localization signal)

Rev, Regulator of expression of viral proteins (act on RRE, Rev Responsive Element); RNA binding domain, and hydrophobic domain that mediates nuclear export.

Rev binds to RRE and the resulting complex binds to the nuclear export machinery

Nuclear Import
Assicura il trasporto dal citoplasma al nucleo del complesso di pre-integrazione, attraverso i pori nucleari.

TAT	Import
RRE-Rev	Export

trafficking to and from the Nucleus

Nuclear Export
Assicura il trasporto degli RNA virali dal nucleo al citoplasma attraverso i pori

HIV-1 particle

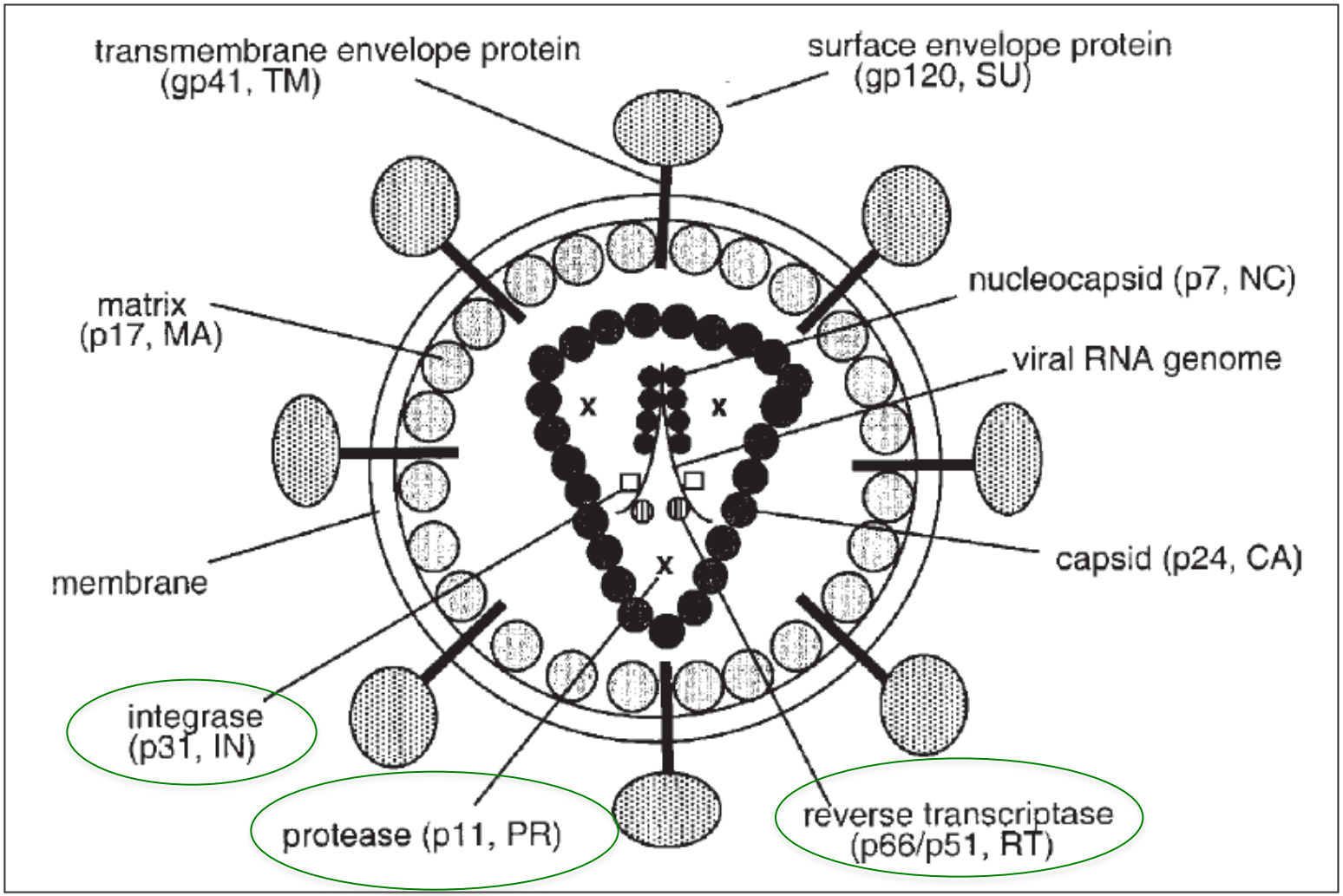
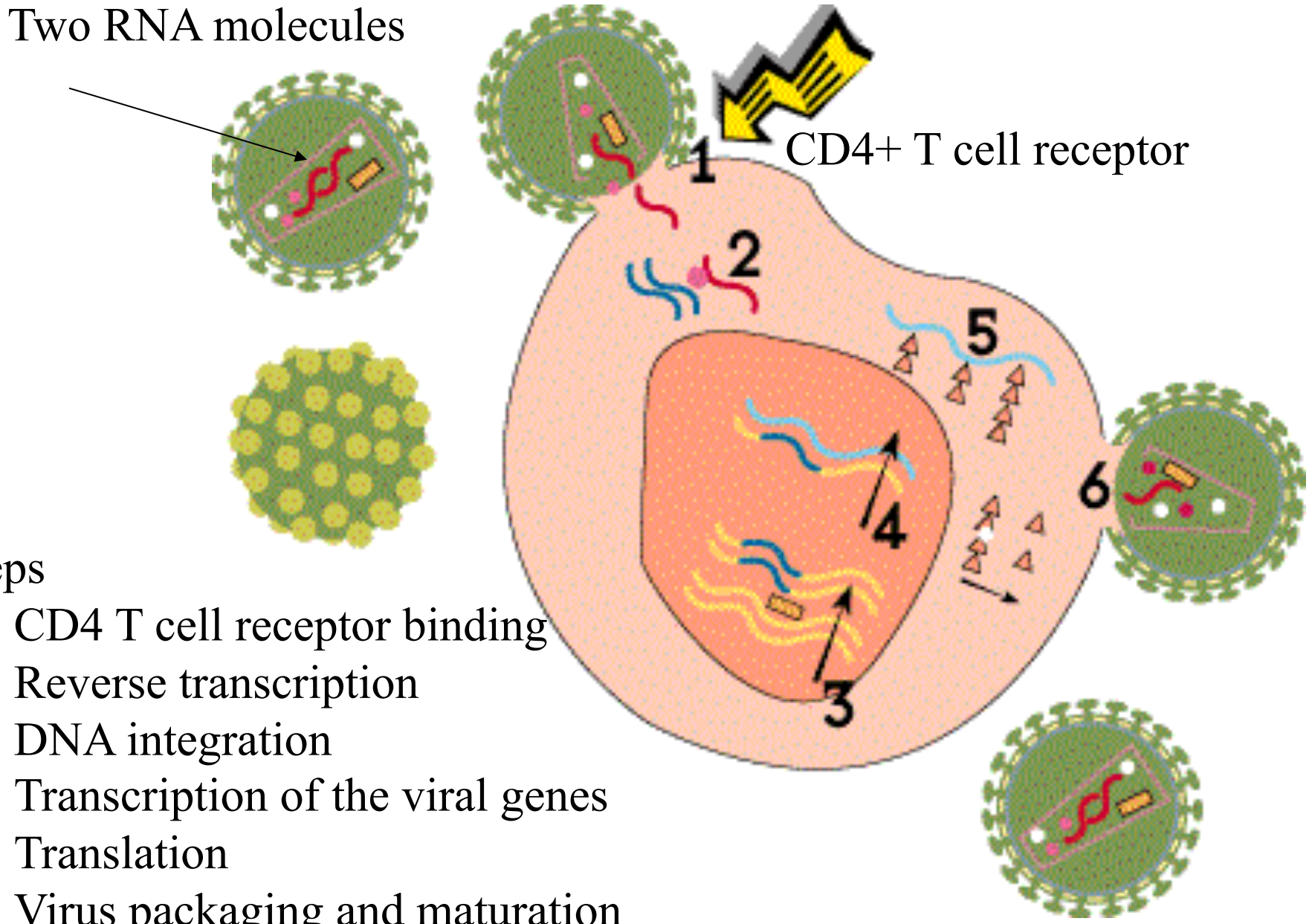


Fig. 2. Schematic representation of a mature HIV-1 particle. Positions of the major viral proteins, the lipid bilayer, and the genomic RNA are indicated. Modified from Freed, 1998 (ref. 22)

From Freed2001

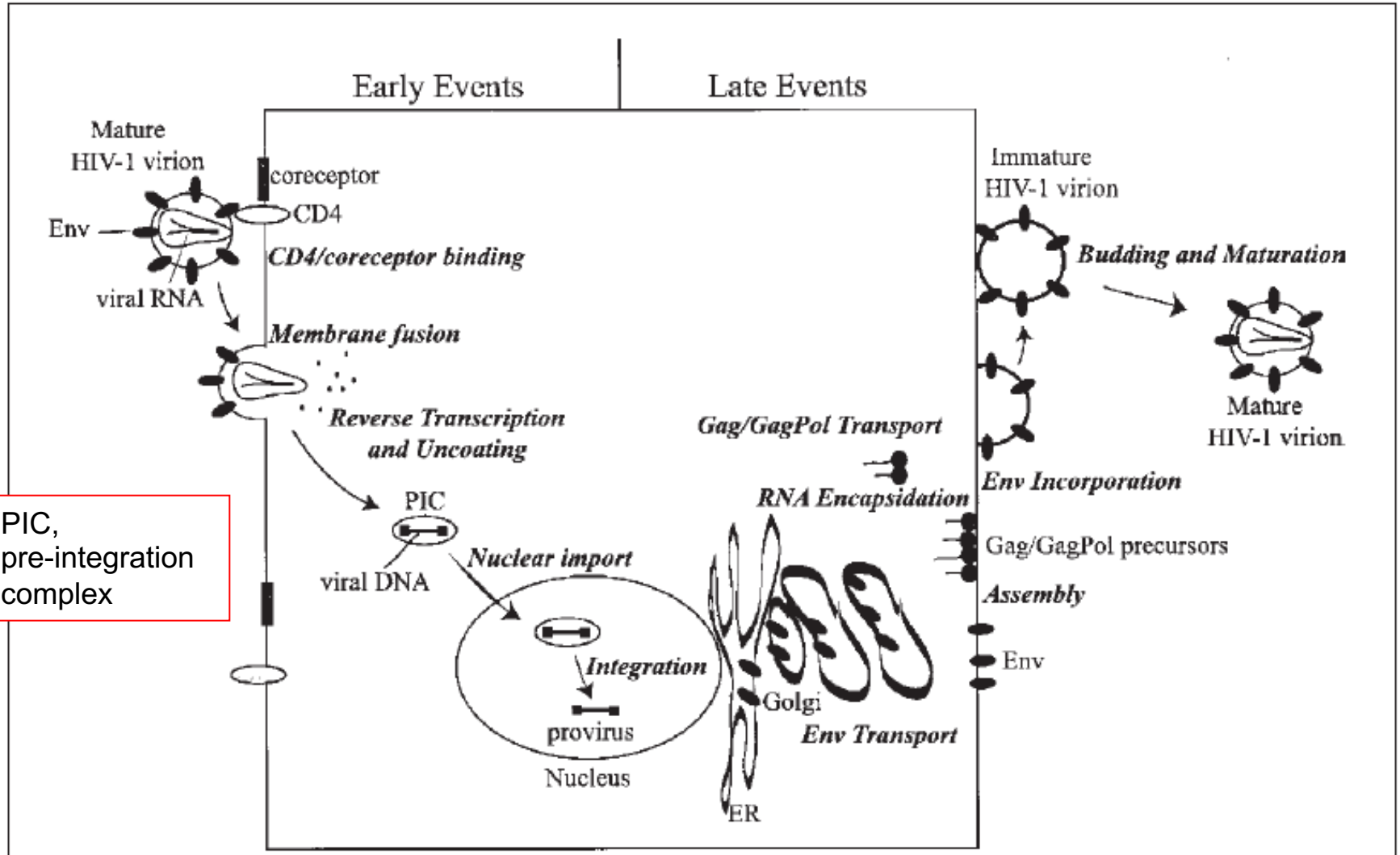
HIV life cycle



Steps

1. CD4 T cell receptor binding
2. Reverse transcription
3. DNA integration
4. Transcription of the viral genes
5. Translation
6. Virus packaging and maturation

HIV-1 life cycle

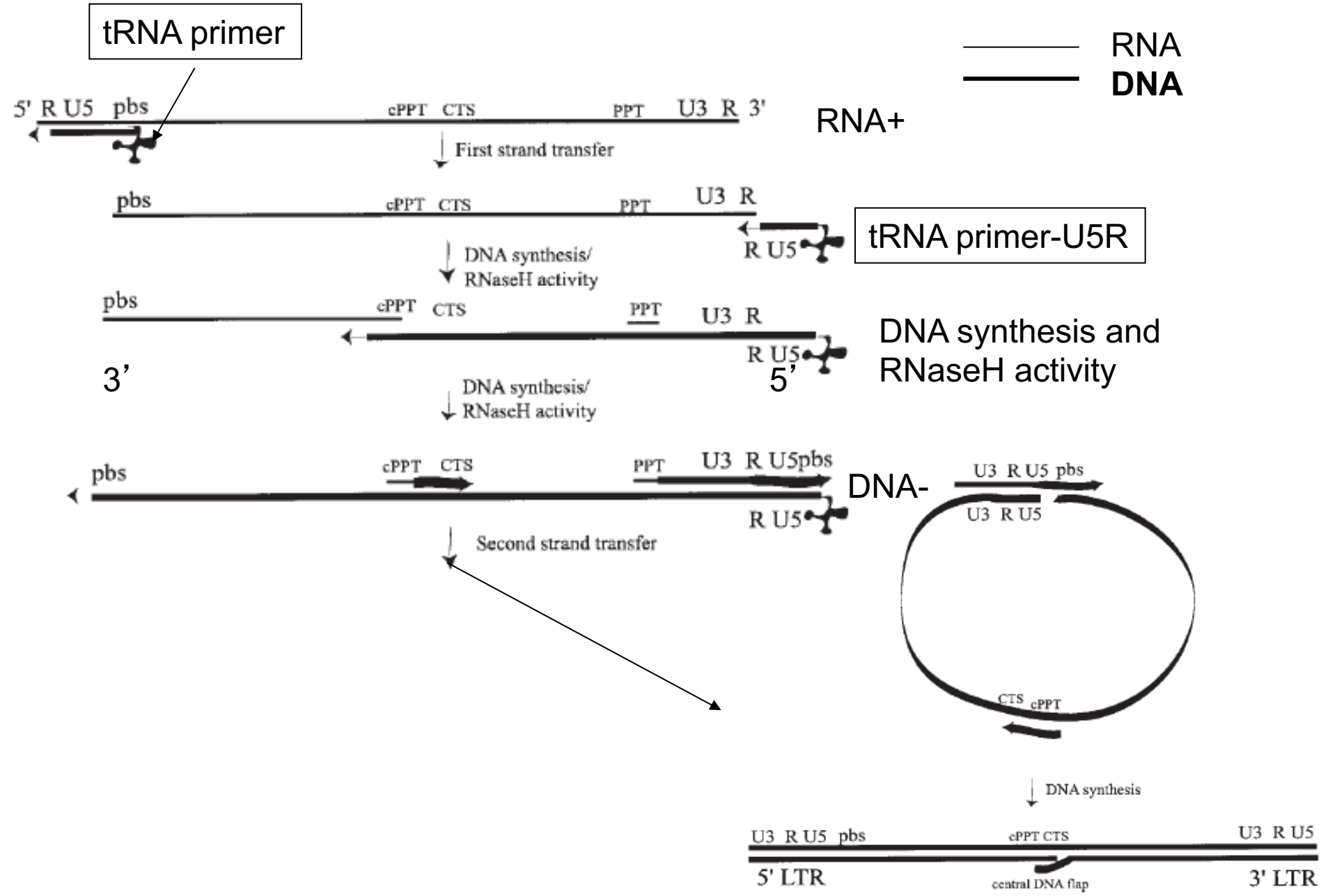


PIC, pre-integration complex

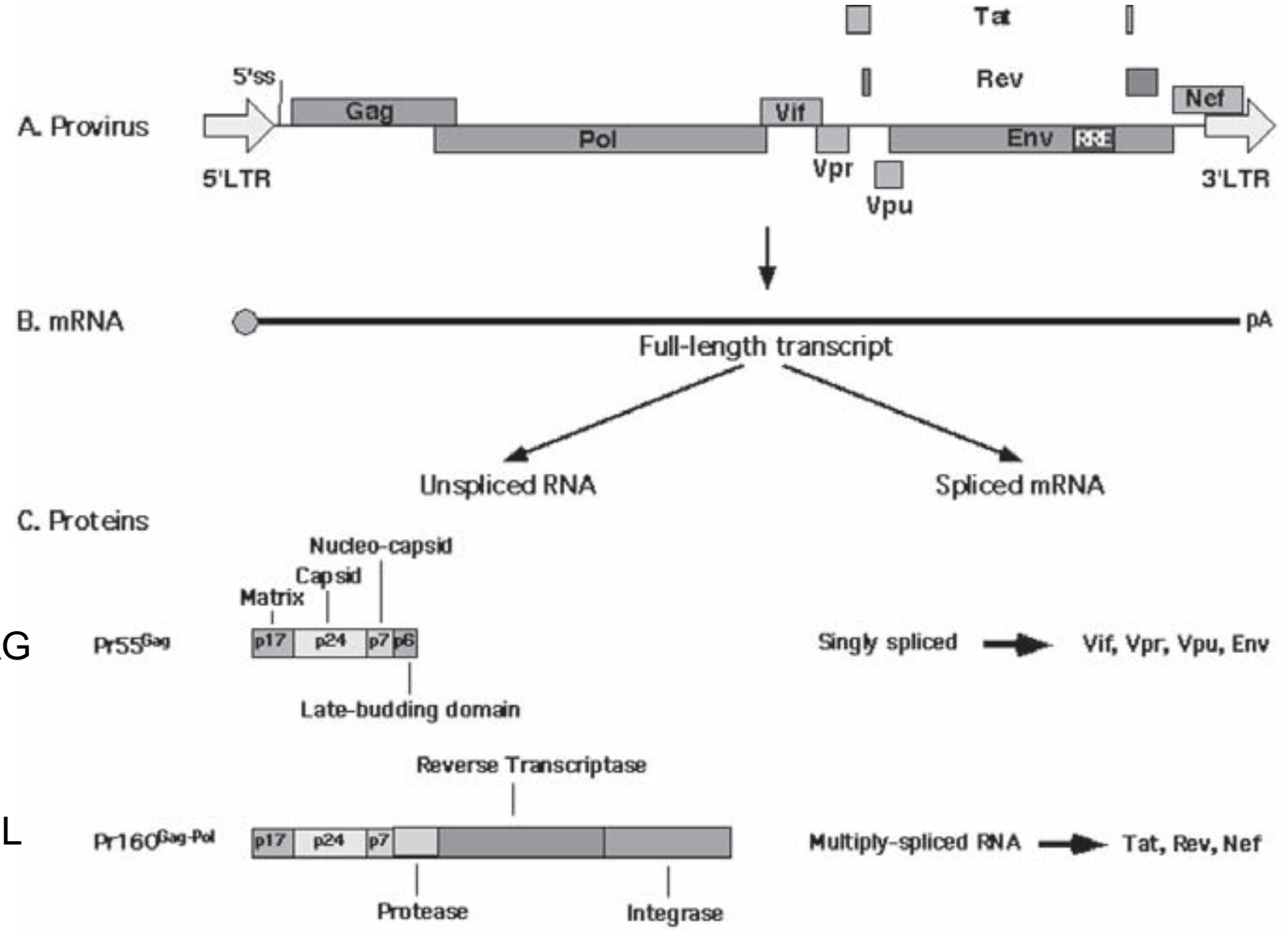
Fig. 3. Schematic representation of the HIV-1 life cycle. The major steps in the early and late stages of the replication cycle (described in detail in the text) are indicated.

From Freed2001

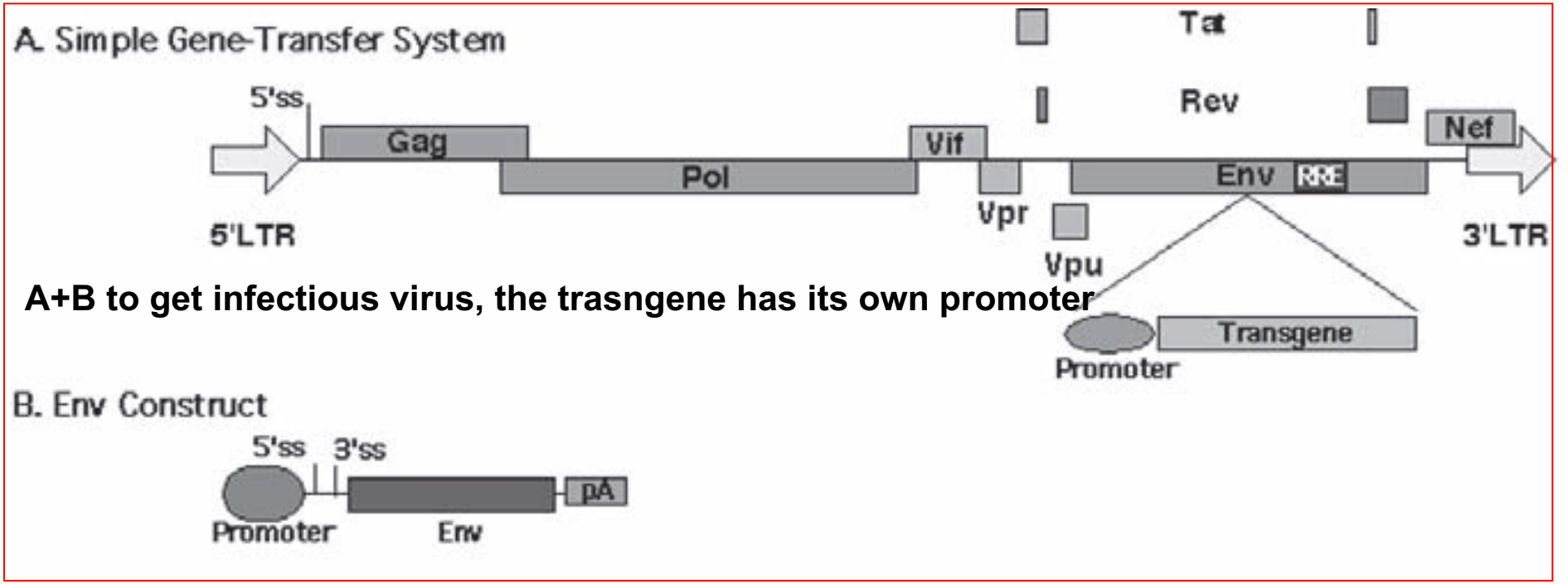
from the RNA viral genome to the DNA provirus



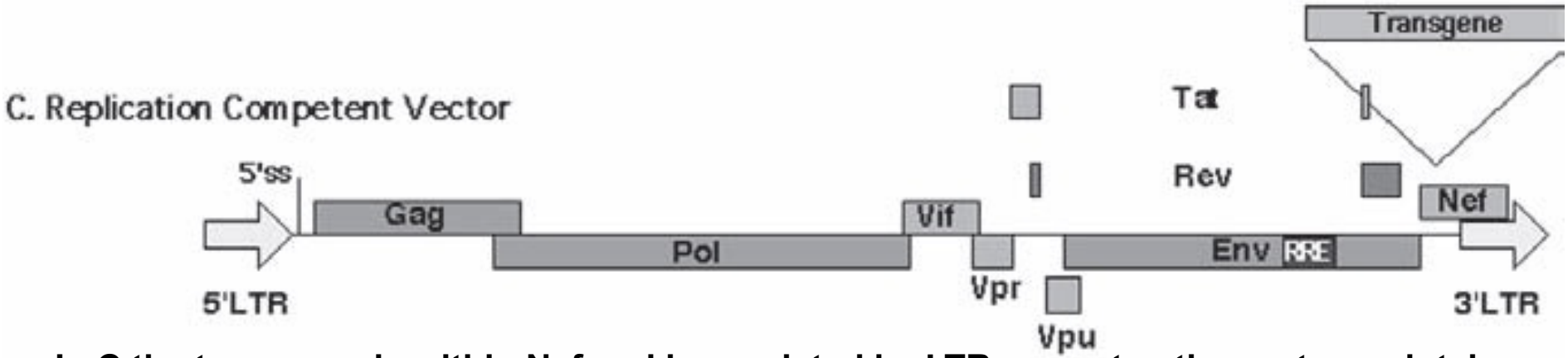
understanding the HIV-1 genome function: from the provirus to the viral transcripts



Prior to first generation HIV-based vectors

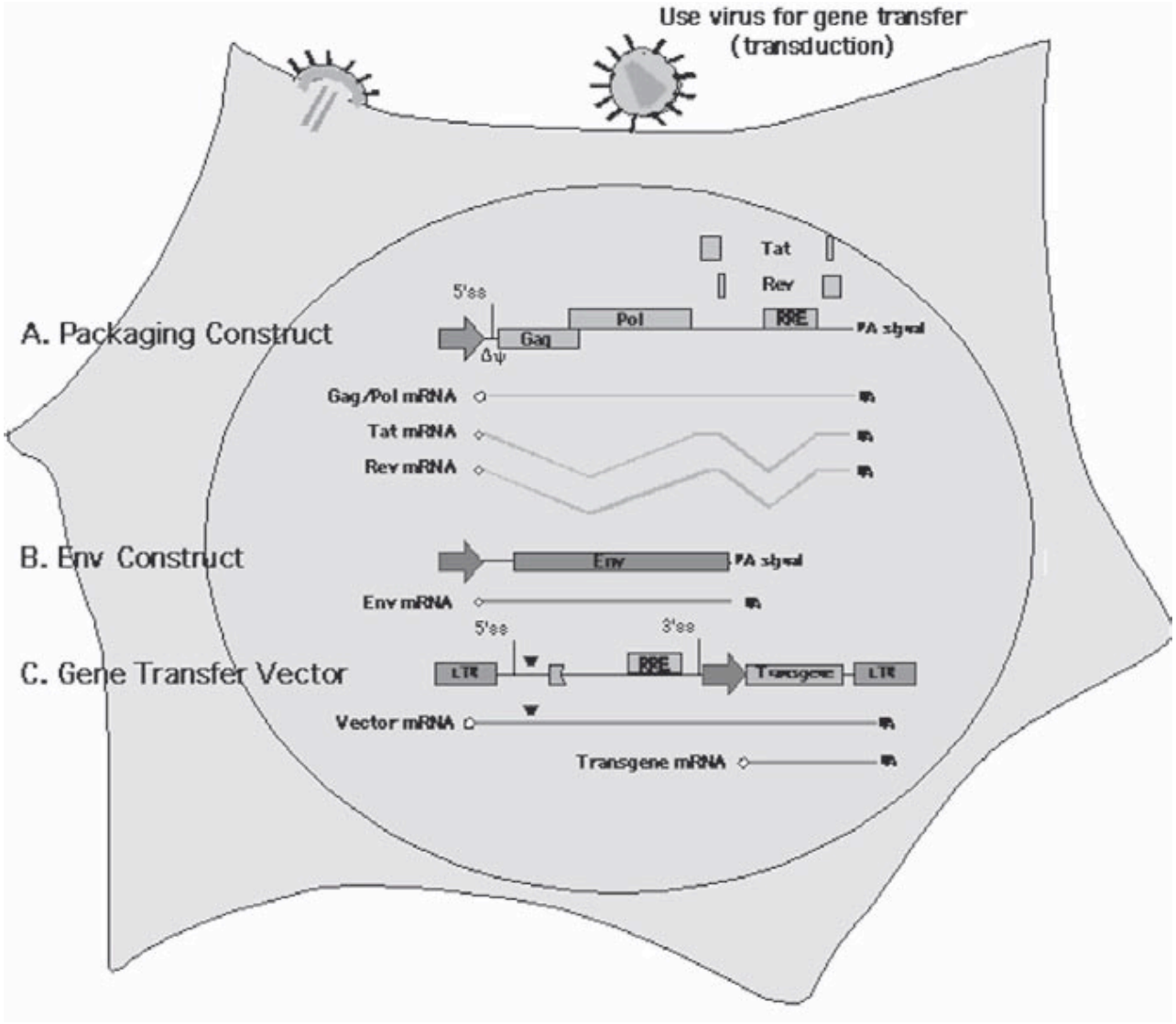


A+B to get infectious virus, the trasngene has its own promoter



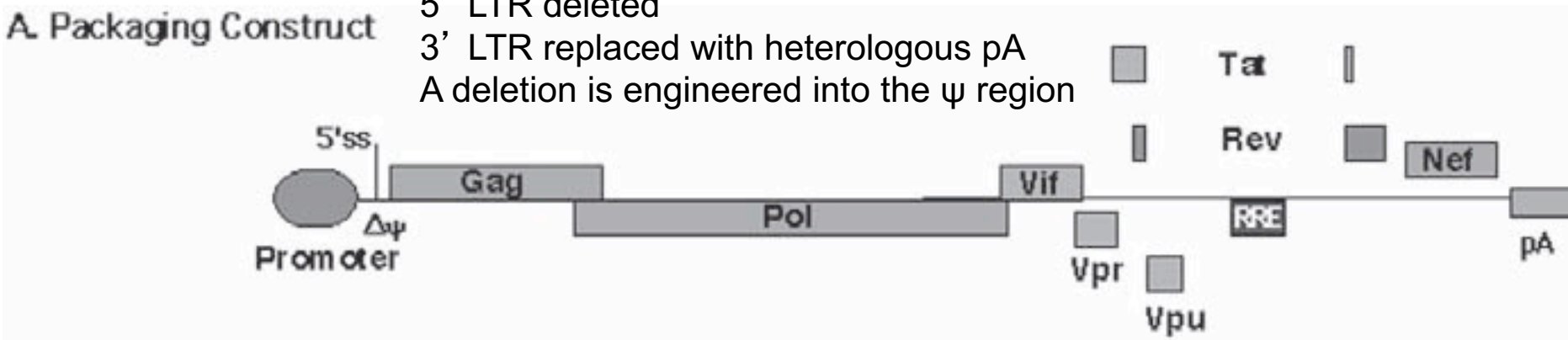
In C the trasngene is within Nef and is regulated by LTR promoter, the vector maintains all the viral functions

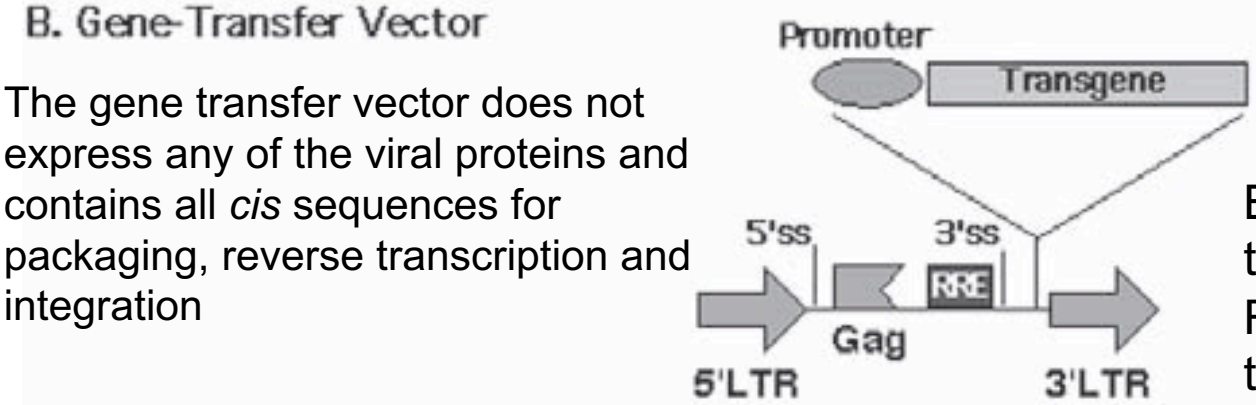
Hiv-1 vector system



First generation vectors

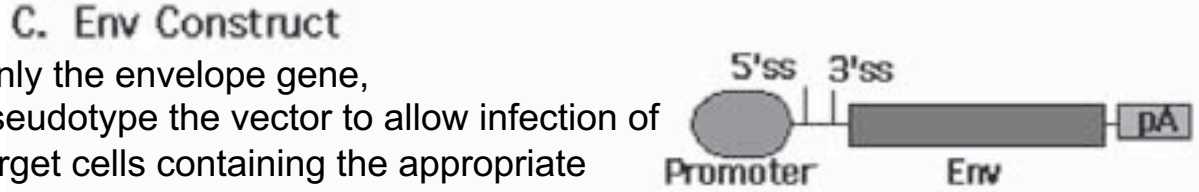
All proteins but Env,
5’ LTR deleted
3’ LTR replaced with heterologous pA
A deletion is engineered into the ψ region





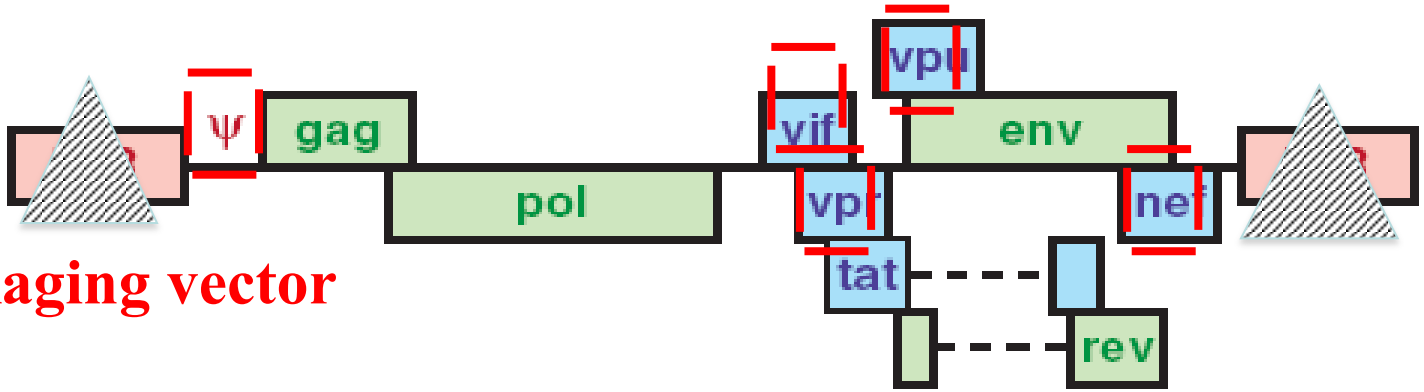
The gene transfer vector does not express any of the viral proteins and contains all *cis* sequences for packaging, reverse transcription and integration

Both the packging and the transfer vectors required the RRE element allowing transfer of the RNA from the nucleus to the cytoplasm

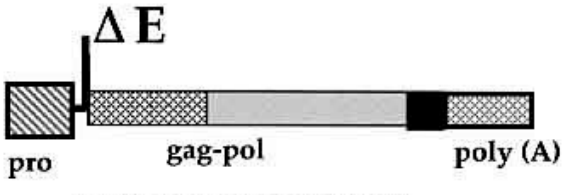


Only the envelope gene, pseudotype the vector to allow infection of target cells containing the appropriate receptor to which the Env protein can bind

First generation vector in detail



1. Packaging vector



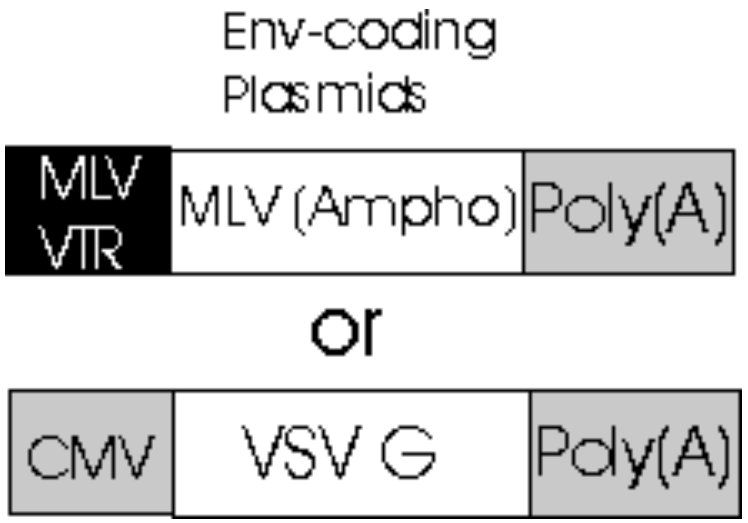
Δ LTR- ΔPsi, con promotore eterologo

le funzioni in trans (*gag, pol tat e rev*) sono trascritti da un promotore eterologo (CMV promoter, polyA dell'insulina di ratto)

Questo vettore non viene incorporato nelle particelle virali in quanto mancano i segnali di incapsidazione, si producono quindi paricelle non infettive

First generation vector in detail

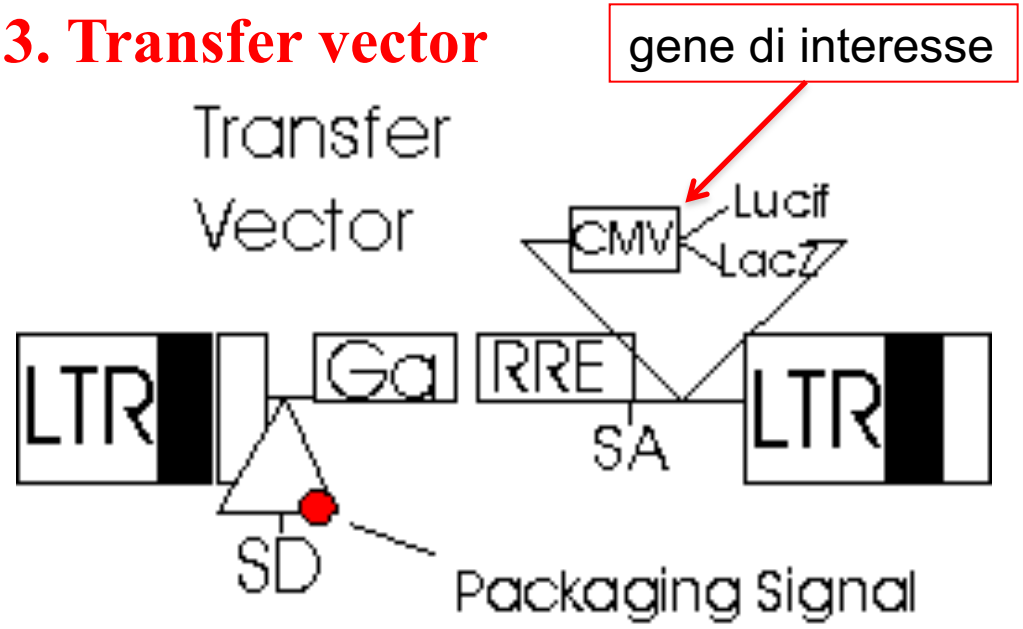
2. Envelop vector



MLV, proteina anfotropica del Murine Leukemia Virus - spettro d'ospite linfociti T CD4+

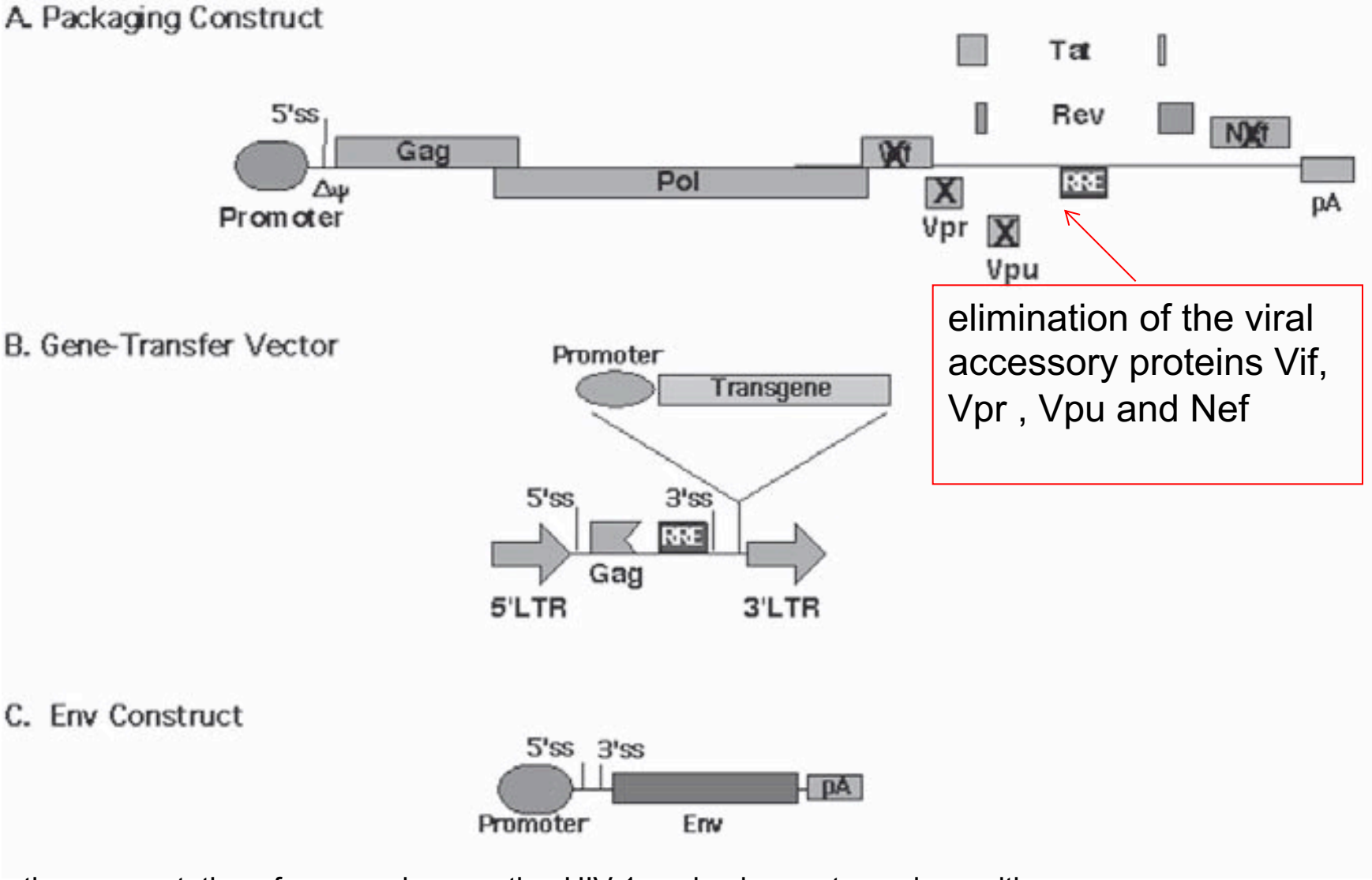
VSV G, proteina G del virus della stomatite vescicolare. Questo vettore permette di produrre particelle che infettano tutte le cellule di mammifero

3. Transfer vector



Contine solo le seq. cis ed i geni: Reporter e terapeutico.
Contiene tutti i segnali in cis (LTR e ψ) per produrre un RNA che viene impacchettato retrotrascritto ed inserito nel genoma dell'ospite.
RRE (Rev Responsive Element) permette una efficiente trascrizione e trasporto dal nucleo al citoplasma

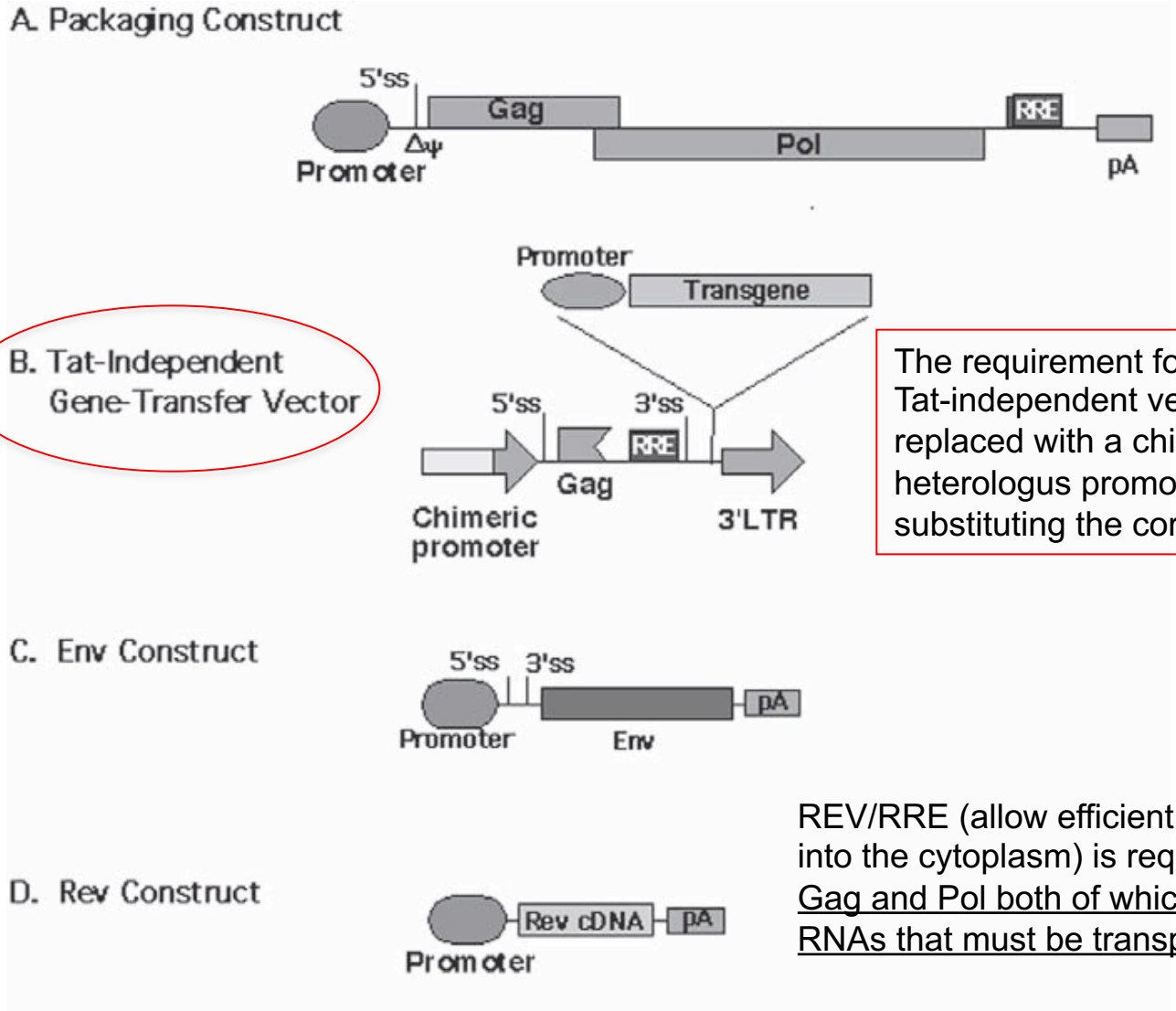
Second generation vectors: elimination of the viral accessory proteins Vif, Vpr , Vpu and Nef



Schematic representation of a second generation HIV-1 packaging system, along with a vector and Env-expressing construct. This is similar to the first generation packaging system but for the elimination of the viral accessory proteins Vif, Vpr , Vpu and Nef

Third generation vectors

This is a minimal HIV-1 packaging system that comprises three helper plasmids, in addition to the transfer vector: A, the packaging construct, Gag-Pol expression construct; B, the transfer vector (Tat-independent); C, the Env construct; D) the Rev expression construct.



The requirement for Tat is obviated by using a Tat-independent vector (B) in which the 5'LTR is replaced with a chimeric promoter consisting of heterologus promoter/enhancer elements substituting the corresponding viral elements.

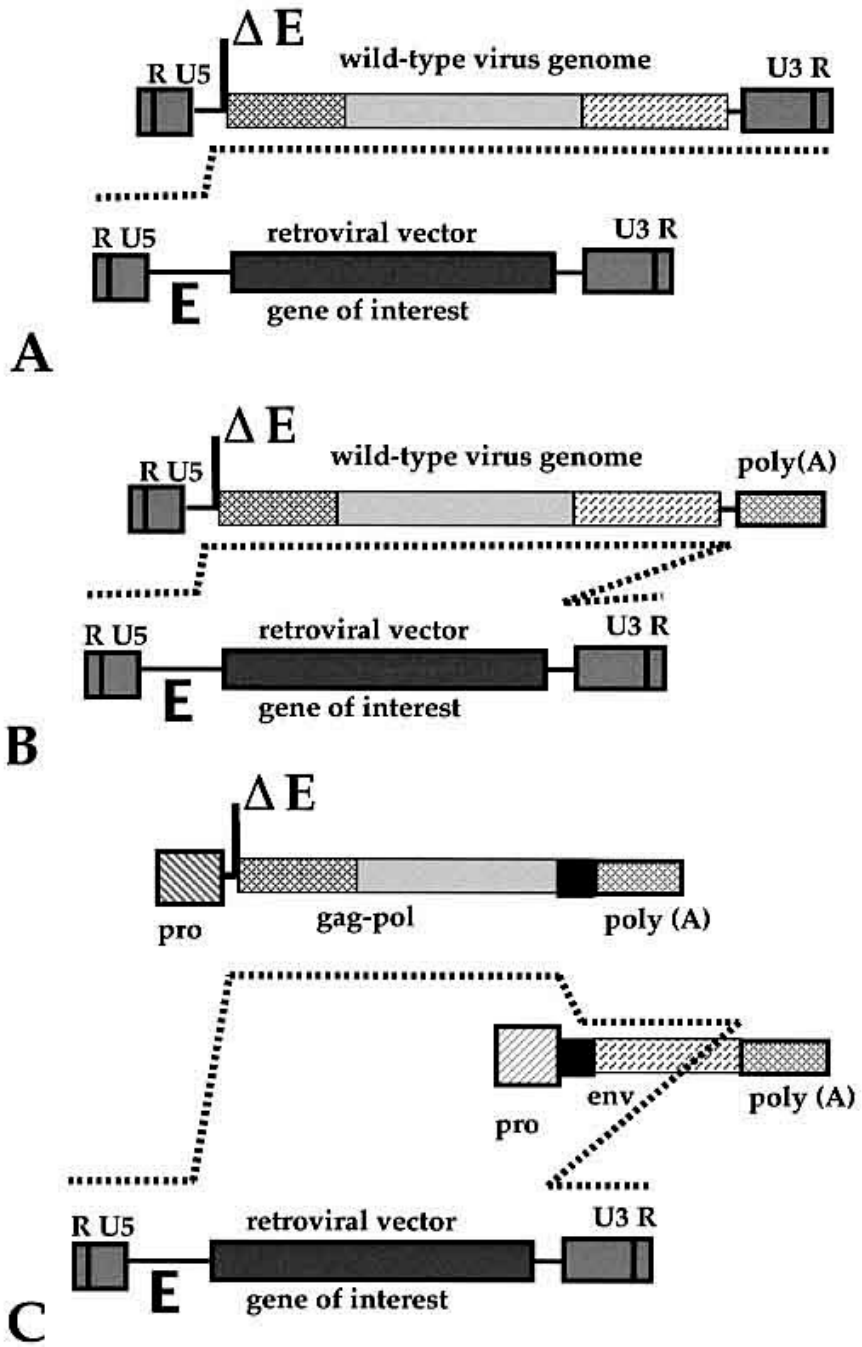
REV/RRE (allow efficient transfer of unspliced viral RNA into the cytoplasm) is required for efficient expression Gag and Pol both of which are produced from unspliced RNAs that must be transported to the cytoplasm.

vector components of the 1° , 2° and 3° lentiviral systems

- first generation – three vectors (packaging, transfer, envelope)
- second generation – three vectors (packaging deleted of Vif, Vpr, Vpu, Nef, transfer, envelope)
- third generation – four vectors (packaging*, Rev, transfer, envelope)

recombination events among the viral vector components may lead to *wt* viral genome formation

Generation of replication competent viruses



In the retroviral vector system (A), one recombination event was sufficient to generate a RCR.

In the second retroviral vector system (B), two recombination events were required to generate a RCR.

In the third retroviral vector system (C), three recombination events are required to generate a RCR.

Deleting viral controlling sequences and splitting the components of the helper virus into different plasmids reduces the regions of sequence overlap and increases the number of recombination events to generate a RCR. Consequently, in modern helper cells, the risk of RCR formation is greatly reduced (B and C).

strategies to reduce recombination events

splitting the packaging construct in two vectors

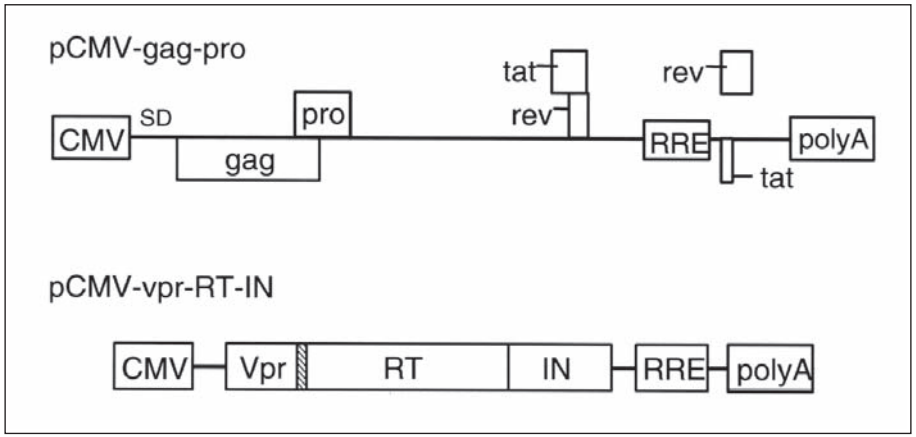
trans lentiviral packaging system

a new class of HIV-based vectors was designed that splits the gag-pol component of the packaging construct into two separate parts: one that expresses Gag/Gag-Pro and another that expresses Pol (RT and IN) fused with Vpr (Vpr-RT-IN)

packaging vectors:

Gag-pro, capside and protease

RT-IN: reverse transcriptase and integrase



Genetic components of the trans-lentiviral packaging system. The trans-lenti packaging construct is illustrated as pCMV-gag-pro. The pCMV-vpr-RT-IN construct encodes the Vpr-RT-IN fusion protein, which is packaged into the Gag/Gag-Pro particles, providing the reverse transcriptase and integrase function. Proteolytic processing by the viral protease liberates mature and enzymatically active RT (p51/ p66) and IN proteins.

Vector systems that use combination of two lentiviruses

HIV-2 and HIV-1
Based on the observation that Tat and Rev proteins of HIV-1 can also function in the context of HIV-2 LTR and RRE-2.

SIV (Simian Immunodeficiency Virus)
Use of helper construct derived from SIV (Simian Immunodeficiency Virus) to package HIV-1 gene transfer vector. This leads to a titer 10-time less than canonical system.

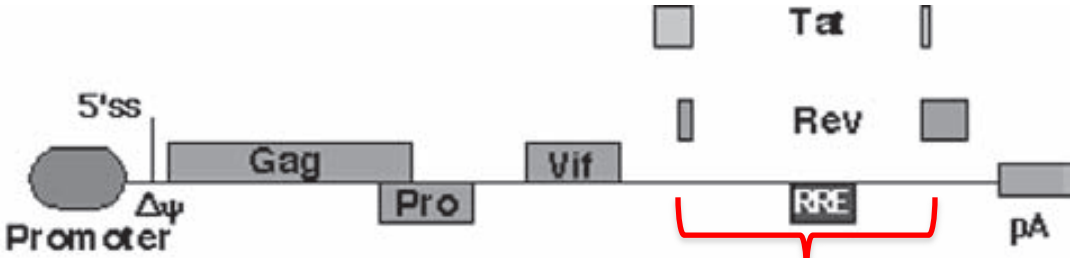
Trans-lentiviral heterogeneous packaging system

In this packaging system, the proteins encoded in the gag and pol coding regions are segregated in two different expression plasmids, one derived from HIV-1 and the other from HIV-2

GAG (HIV-1)

A. Gag-Pro Construct

One vector encodes for the Gag and Gag-Protease



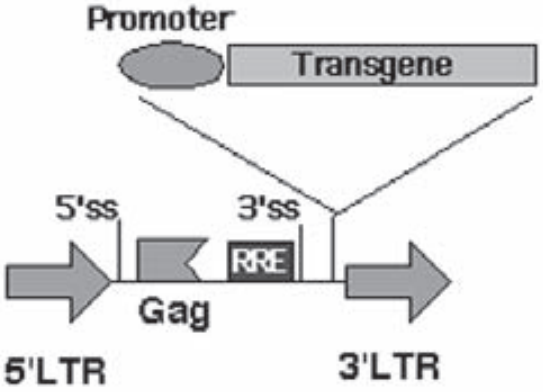
HIV-1 Tat and Rev can also function in the context of HIV-2 LTR and RRE-2.

POL (HIV-2)

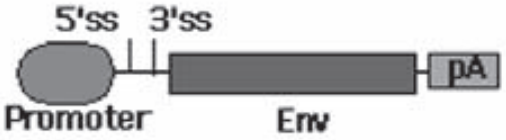
B. Vpr-RT-IN Construct

A second vector encodes for a Vpr-RT-IN fusion protein (HIV-2)

C. Gene-Transfer Vector



D. Env Construct



Heterologous vector system (HIV-MPMV)

alternative to the Rev/RRE system for the viral RNA transport nucleus ➔ cytoplasm

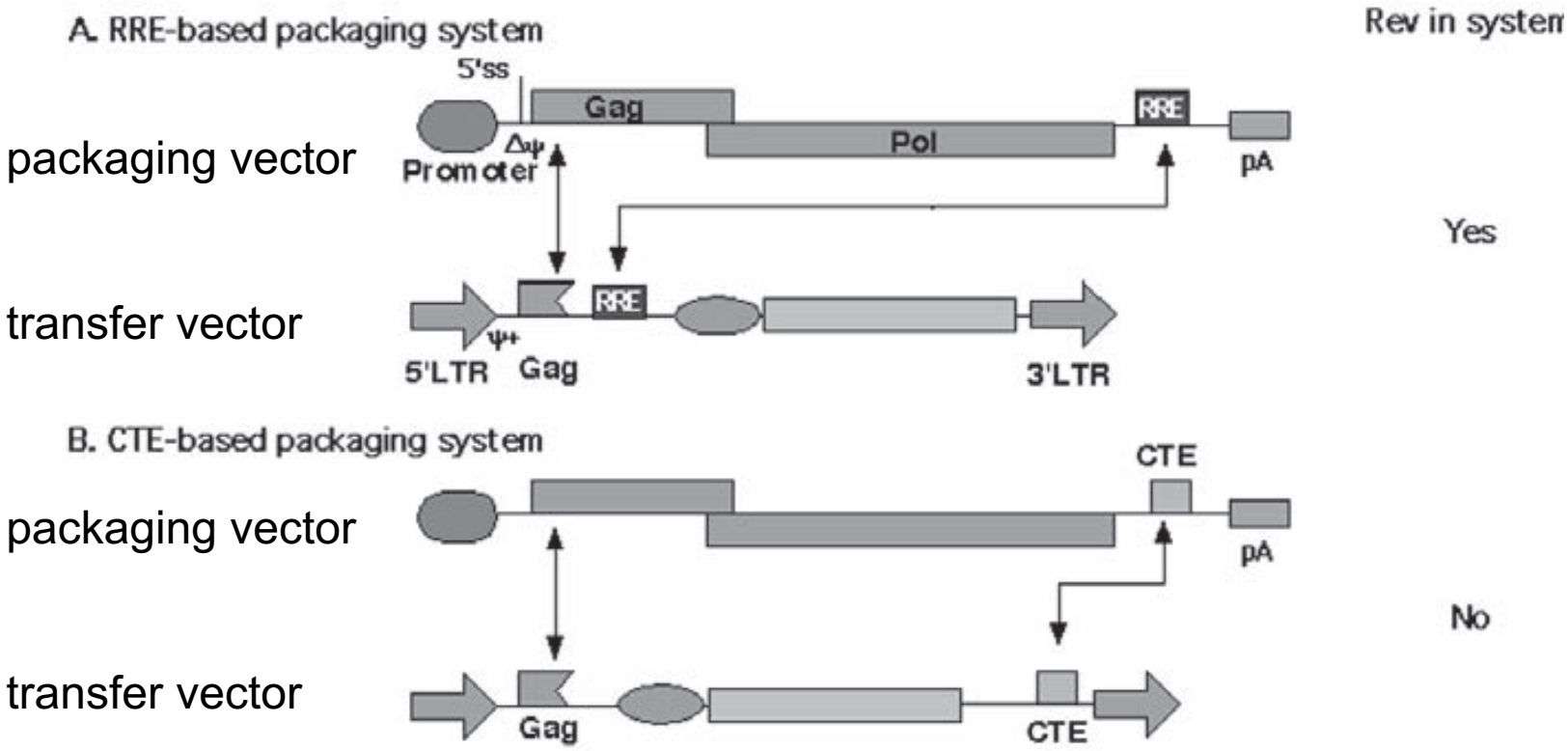
The **Constitutive Transport Element (CTE**) in Mason Pfizer Monkey Virus (MPMV) is a small structural RNA element that performs a similar function as Rev and RRE in HIV-1 (transport of unspliced RNA to the cytoplasm).
It has been shown that CTE can substitute Rev/RRE in HIV provirus and in the packaging and gene transfer vectors.

Changing the nucleic acid transport system allow to reduce the number of possible recombination events between the vectors in the packaging cells. In the following slides bidirectional arrows indicate region of homology.

Rev/RRE-dpendent and –independent vectors

Bidirectional arrows indicate region of homology between the packaging and the transfer vectors. Two such region are present in RRE-based and CTE-based system.

Packaging Systems Using Alternative RNA Transport Elements



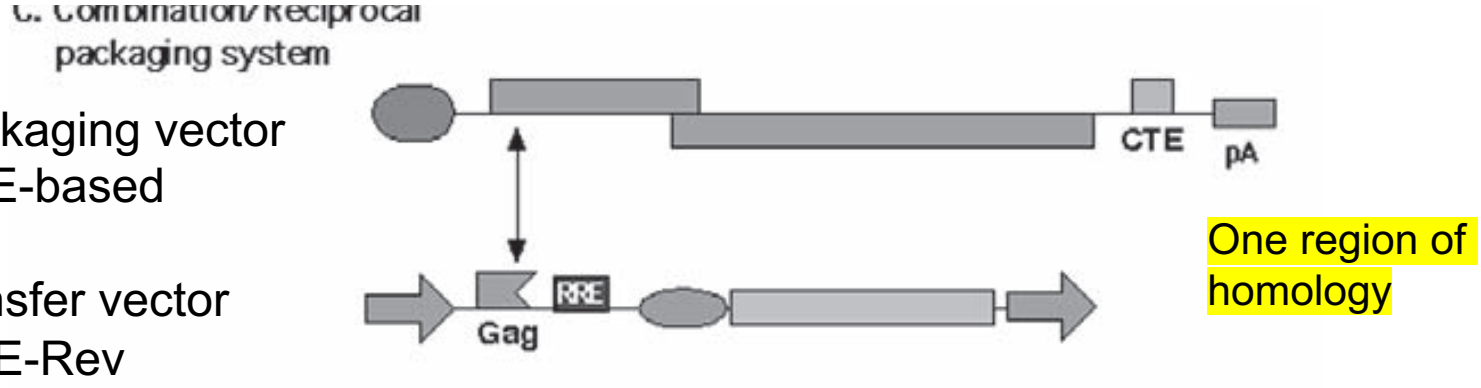
CTE, constitutive transport element of Mason-Pfizer monkey virus (MPMV) working similarly to Rev and RRE in HIV-1 (i.e. transport of unspliced RNA from the nucleus to the cytoplasm). CTE can replace Rev-RRE both in packaging and tramsfer vectors

For clarity, constructs expressing Env, accessory and regulatory proteins are not depicted

combining the RNA-transport system

The packaging vector is regulated by CTE

the gene transfer vector is controlled by RRE and Rev.



the packaging plasmid is regulated by CTE
the gene transfer vector is controlled by RRE and Rev.

bidirectional arrow:
possible site of recombination.
In C there is only one region of homology

For clarity, constructs expressing Env, accessory and regulatory proteins are not depicted

Lentiviral vectors

Vantaggi
Alta efficienza di integrazione nel genoma dell'ospite
Non si ha trasferimento di geni virali
Il genoma ed il ciclo virale del virus è ben conosciuto
La capacità di clonaggio e trasferimento è di circa 7 kb
Capacità di infettare cellule che non si stanno dividendo

Svantaggi
I vettori di seconda generazione non trasducono i macrofagi perché manca *vpr*
Mutagenesi da inserzione, attivazione di oncogeni mediata dalle LTR

Altri vettori lentivirali:
FIV, feline immunodeficiency virus, questo virus infetta 2-20% dei gatti domestici ma non ha mai infettato l'uomo

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