twe major classes of gene transfer vectors

Viral vectors

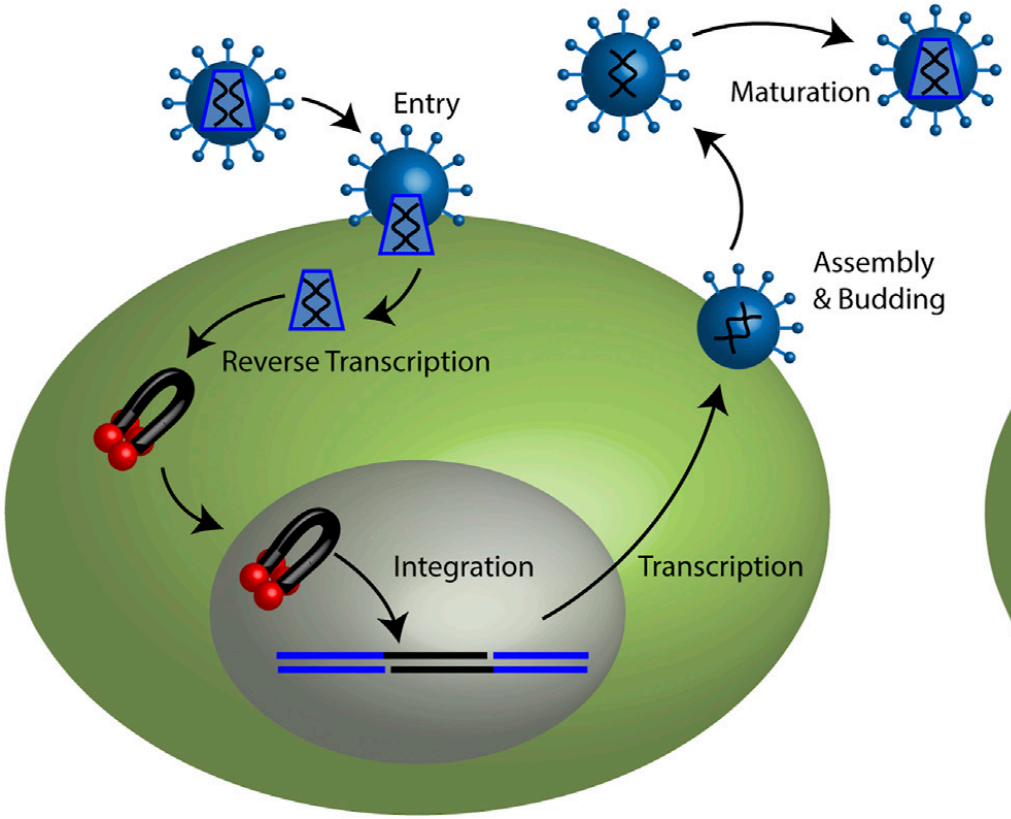
Viral-mediated gene transfer, use the method of entry and interaction (integration or episomal replication) with the host genome of animal viruses.

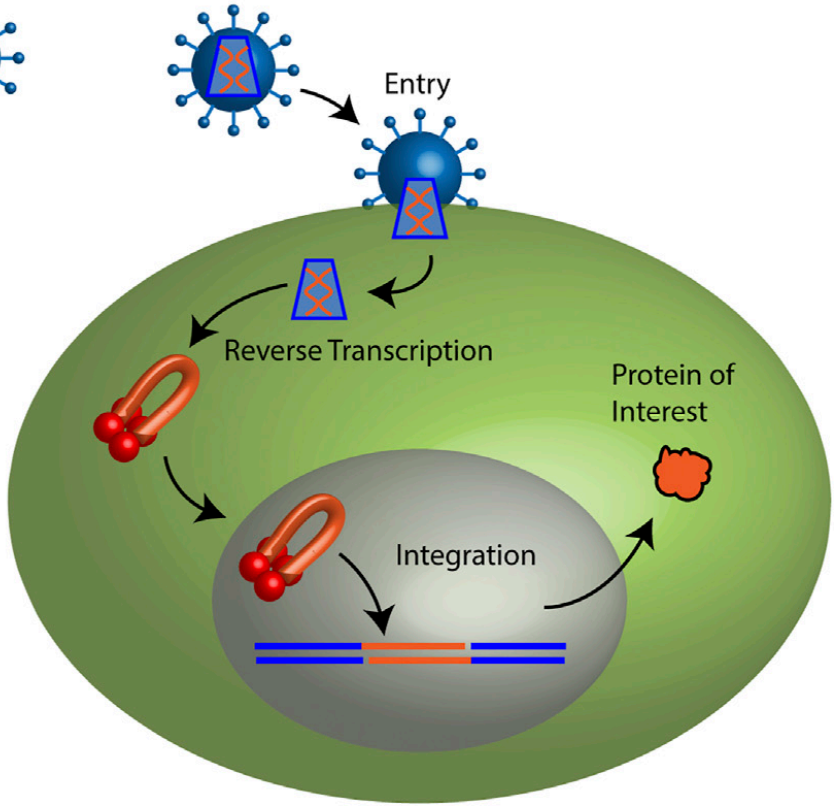
Non viral vectors

Nucleic acids are delivered by artificial vectors such as liposomes or cationic polimers

Infecting virus

Viral vectors





the most frequently used vectors

- Virali
  - **Integranti**
    - Retrovirus
    - Lentivirus
  - Adeno-associati
  - **Non integranti**
    - Adenovirus
    - Herpesvirus
- Non virali
  - Liposomi
  - Lipoplessi
    - Lipidi cationici-DNA
    - Polimeri cationici-DNA
  - DNA plasmidico "nudo"

Viral Vectors

Apparently they represent the ideal vectors for gene transfer in mammalian cells in fact they have developed very efficient systems to infect host cells with their own genome:

- Ligand-receptor interaction with the host cell
- Internalization by receptor mediated endocytosis
- Endosome escape
- Nuclear localization and integration of the genome into the chromosome

Host response

Activation of an immune response to neutralize the virus

Vector technology

A viral vector consists of proviral sequences that can accomodate the gene of interest (goi) and to allow entry of both DNAs (the viral and goi or the goi-only) to target cells.

Startegies to disarm animal viruses

- maintenance/substitution of the receptor mediated uptake of the virus
- insertion of the goi in the viral genome
- **development of a dual system, replication proficient in packaging cells and replication-deficient vector in target cells**

the general principle of viral vector assembly and production

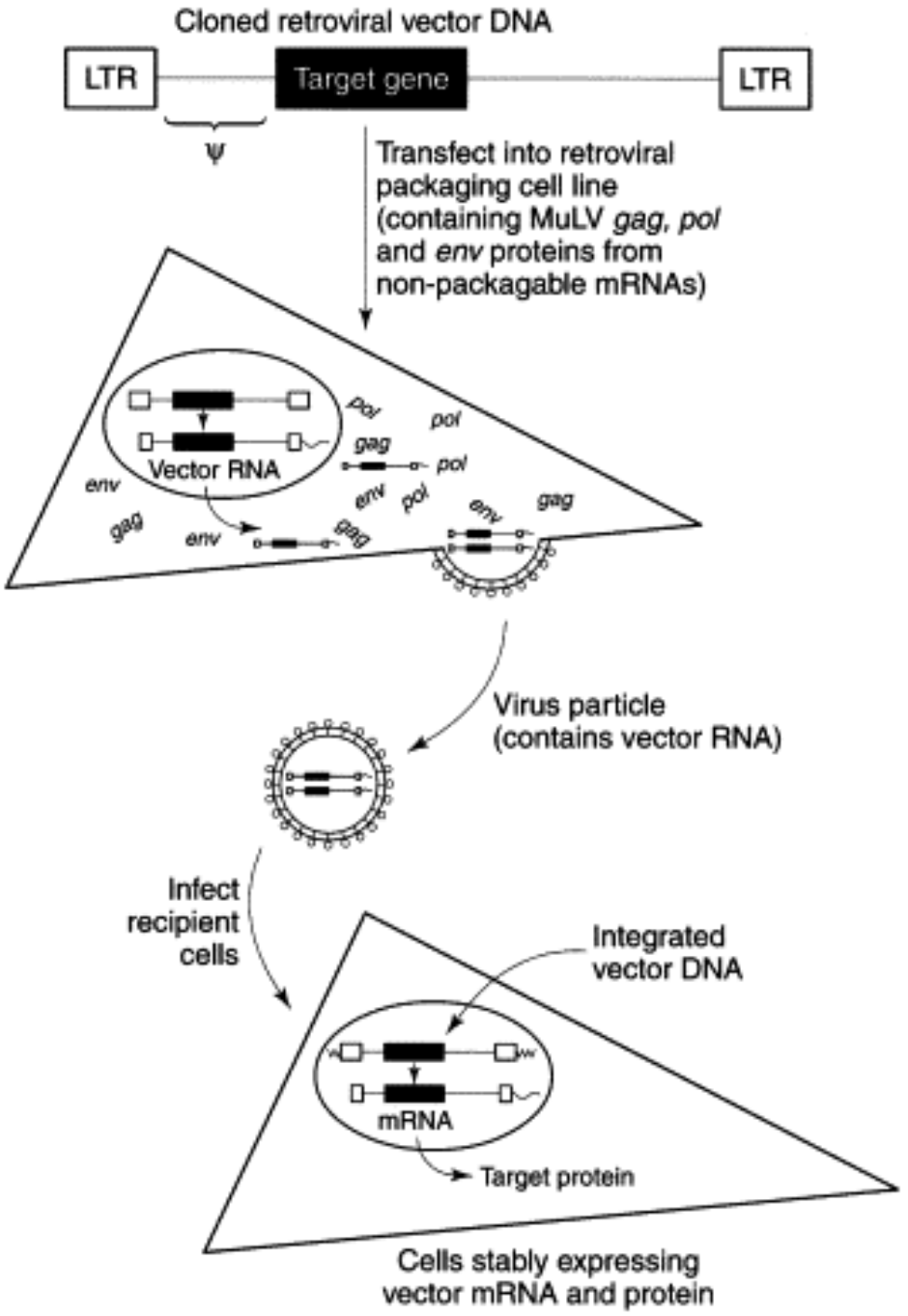
1) The viral vector is assembled by conventional recombinant DNA technology (step 1) and require knowledge of the viral genome and the proviral sequences (those inserted into the host genome, if applied);

2) the vector is transferred (as naked DNA by gene transfer reagents) into packaging cells (step2);

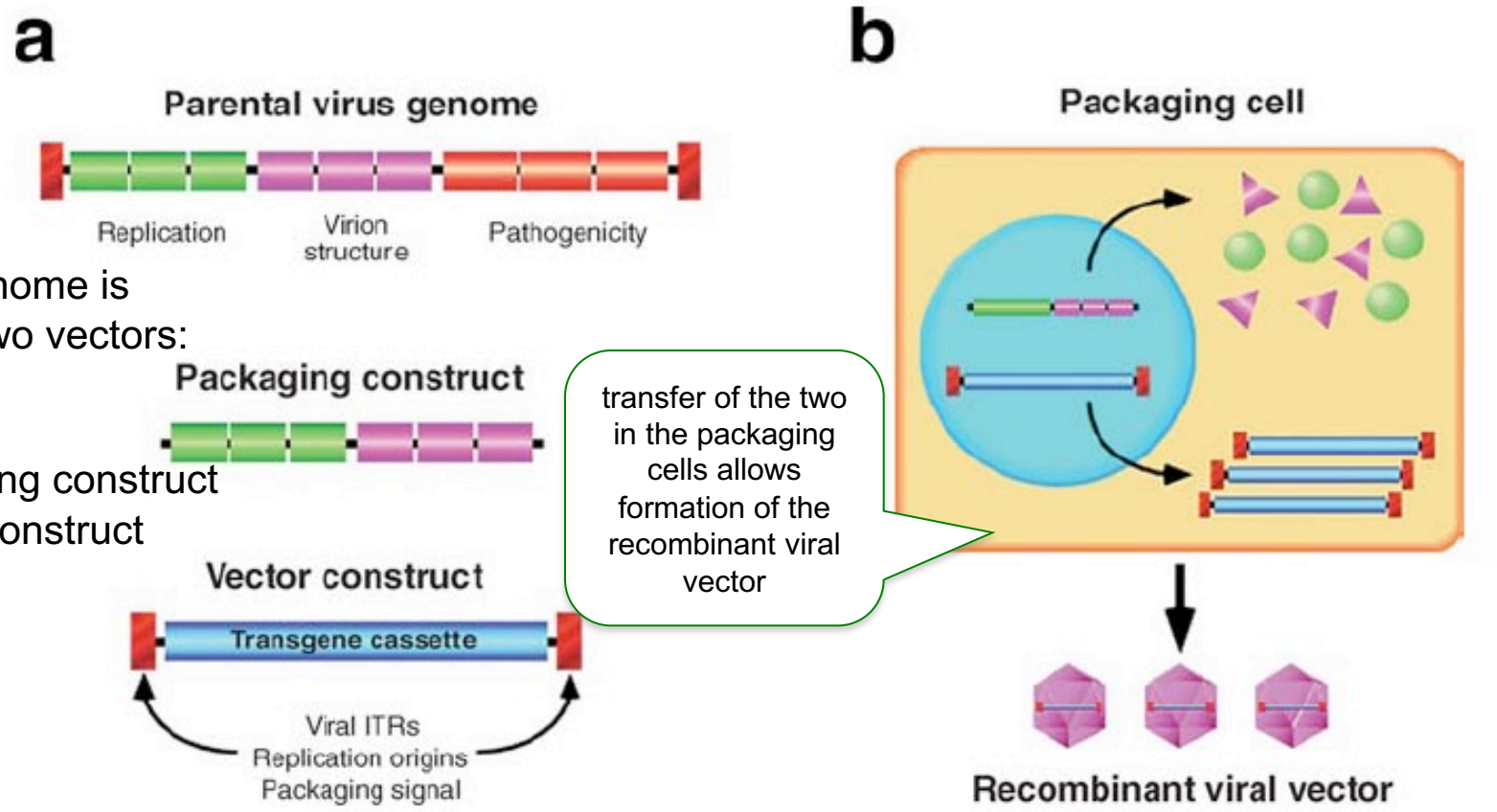
3) finally it is collected in culture supernatants, controlled and ready to use in target cells (steps 3-5)

recovery
purification
titration (pfu/ml)

the general principle of viral vector assembly and production



the general principle of viral vector assembly and production



the viral genome is divided in two vectors:

the packaging construct

the vector construct

1. Identification of the elements required for the viral genome replication and incapsidation
2. Remuval of the elements that are not strictly necessary for viral production
3. Cloning of the GOI into the “vector construct”
4. The elements required for recombinant viral vector production are assembled into one or more constructs, “ the packaging construct/s”
5. The transfer vector and the packaging constrcuts are introduced into the packaging cells for the production of the recombinant viral particles

Distinct retroviral genera (Retroviridae) have been classified

All the retroviruses shown belong to the subfamily of Orthoretrovirinae, with the exception of spumaviruses, which are separated into their own subfamily (Spumaretrovirinae) (http:// www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm).

TABLE 2: Taxonomy of the retroviral family (*Retroviridae*) and commonly used vector systems discussed in this article

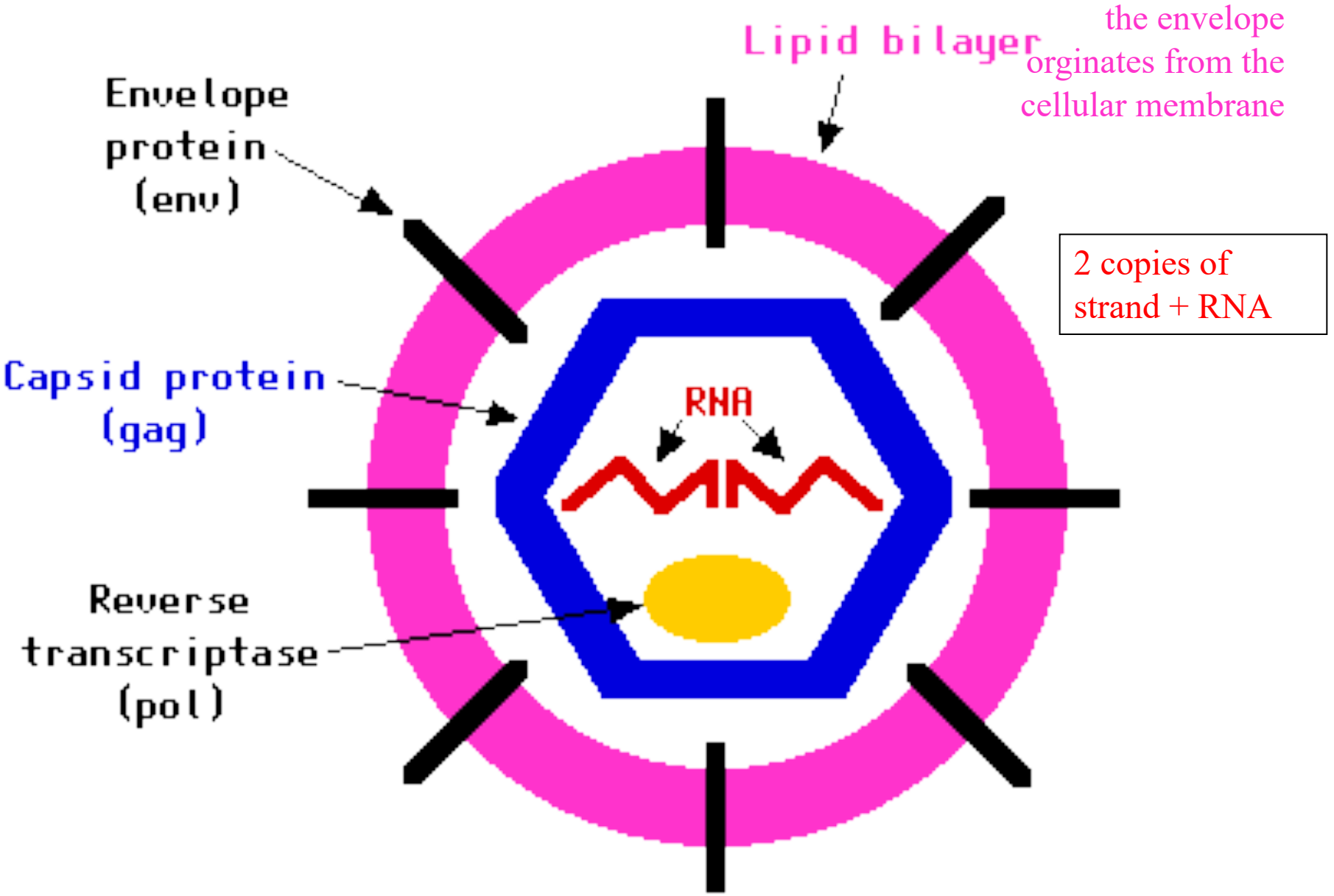
Genus	Species (examples)	Paradigmatic vector system discussed here	Key properties relevant for vector design
Alpharetrovirus	Avian leukosis sarcoma virus	—	Relatively unbiased integration pattern.
Betaretrovirus	Mouse mammary tumor virus (MMTV), Mason–Pfizer monkey virus (MPMV), human endogenous retroviruses K (HERV-K)	—	Tissue-specific promoter (MMTV); presence of constitutive (MPMV) or inducible (HERV-K) RNA export elements.
Deltaretrovirus	Human T cell leukemia virus, bovine leukemia virus	—	Poorly explored for vector design (replicates primarily as a provirus along with cellular DNA replication).
Epsilonretrovirus	Walleye dermal sarcoma virus	—	Poorly explored for vector design (infects fish).
Gammaretrovirus	Gibbon ape leukemia virus, feline leukemia virus	Mouse leukemia virus	Simple genome architecture, ''clean'' packaging system; powerful constitutive promoters; nontoxic Env proteins.
Lentivirus	Equine infectious anemia virus, feline immunodeficiency virus, simian immunodeficiency virus, bovine immunodeficiency virus	Human immunodeficiency virus type 1	Stabilization of genomic vector RNA in packaging cells; transduction of nondividing cells.
Spumavirus	Chimpanzee foamy virus	''Human'' foamy virus	Completion of reverse transcription prior to entry; relatively low preference for integration in active genes.

retroviral vectors are mainly based on

1.Simple gammaretroviruses with the paradigmatic vectors derived from **murine leukemia virus (MLV)**. The Moloney Murine Leukemia Virus (MoMuLV or MMLV) was the first used as vector. These vectors possess the most advanced packaging system.

1.Complex **lentiviruses** with the paradigmatic vectors derived from the human immunodeficiency virus type 1 (**HIV**). These vectors are able to transduce many types of nondividing cells.
2.The more distantly related **spumaviruses** with the paradigmatic vectors derived from **“human” foamy virus** (HFV). This virus is considered to be apathogenic in humans and has the unique property of completing reverse transcription prior to cell entry.

The retroviral particle: 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.



The murine leukaemia virus life cycle

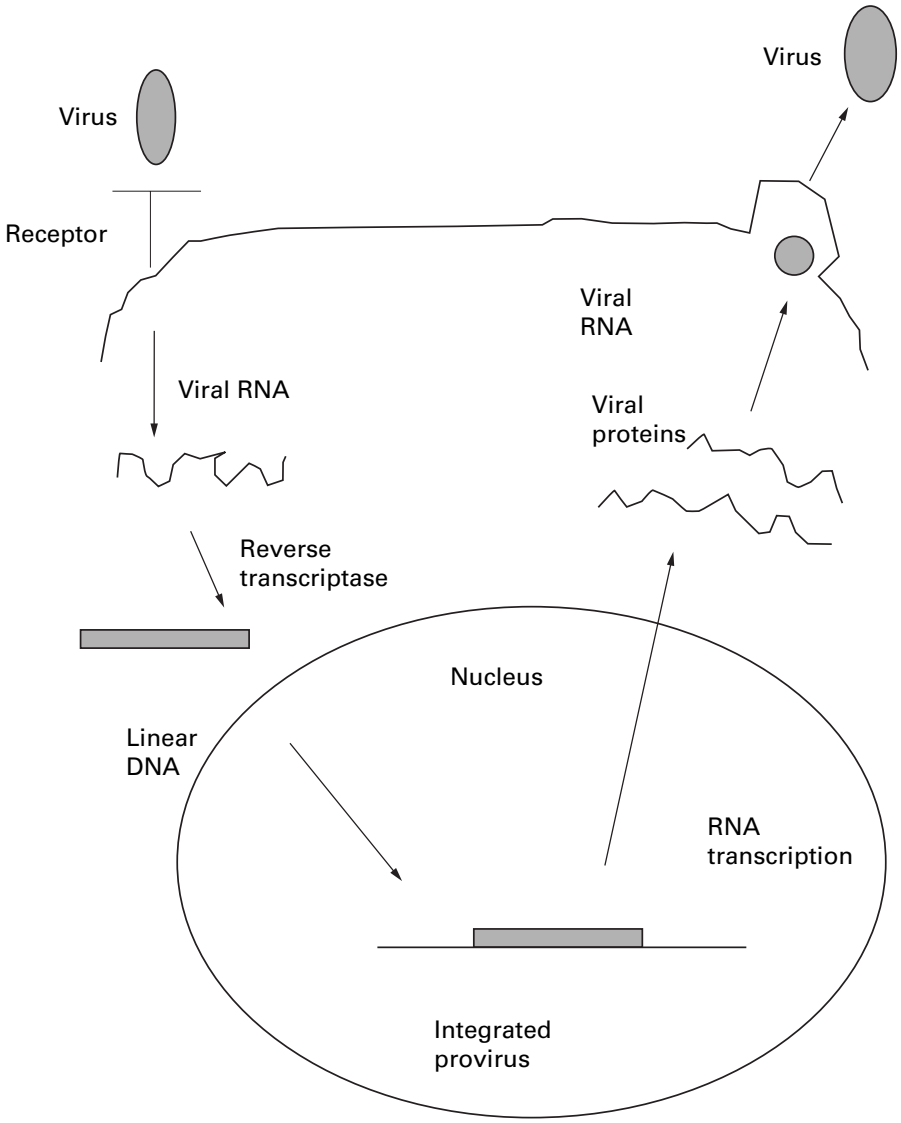


Figure 1 The murine leukaemia retrovirus life cycle.

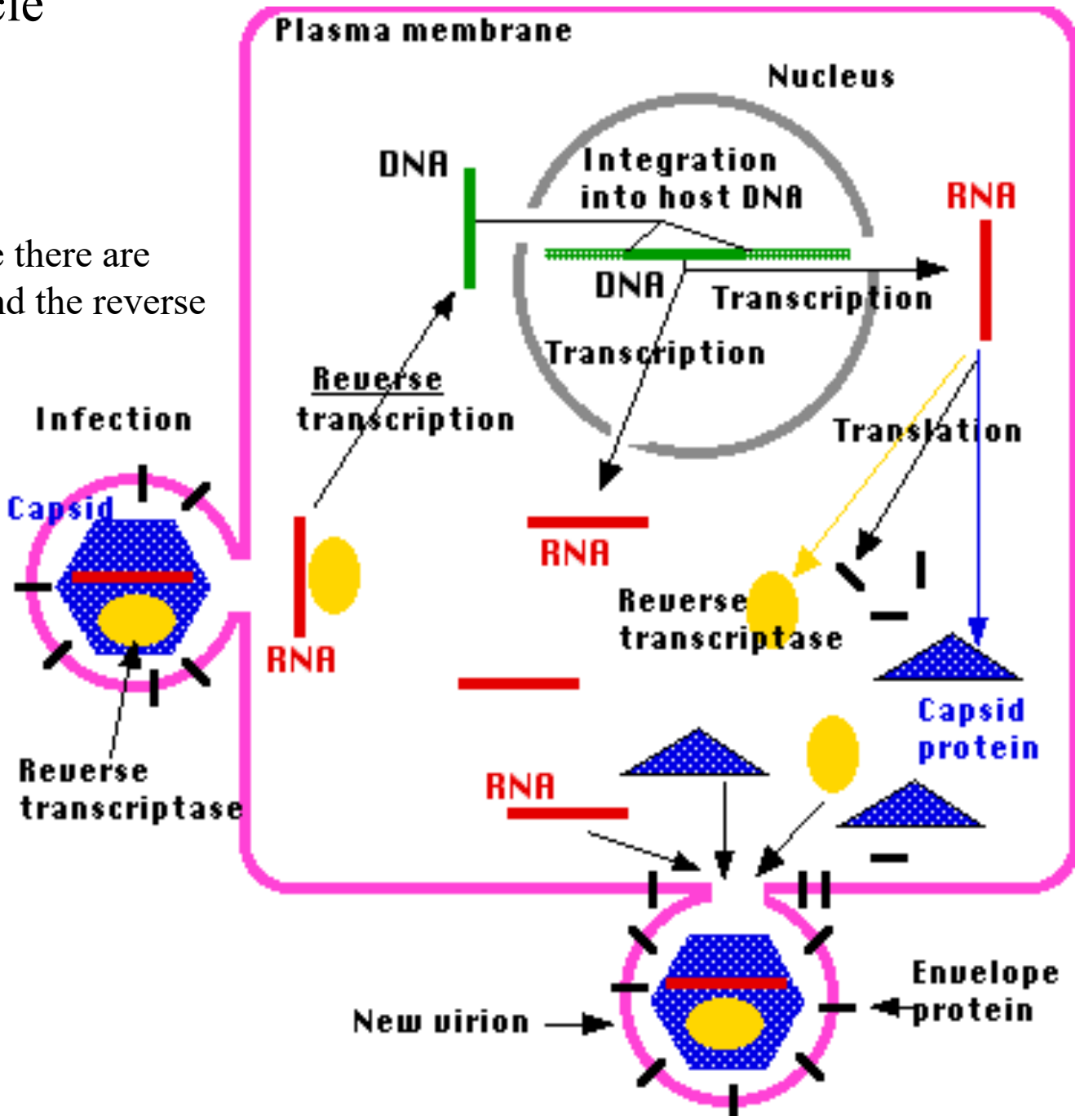
Infection and entry - At the onset of infection, the surface glycoprotein envelope interacts with receptors on the surface of the target cell to gain entry.

Integration into the host genome - 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 viral DNA integrates with the target cell DNA—at which point it is known as a provirus.
It is this proviral DNA that is manipulated to produce retroviral vectors for gene transfer.

virus production - The provirus then undergoes transcription and translation with the rest of the genome, resulting in the assembly of new viral particles that bud of the surface of the target cell to infect others cells.

retrovirus life cycle

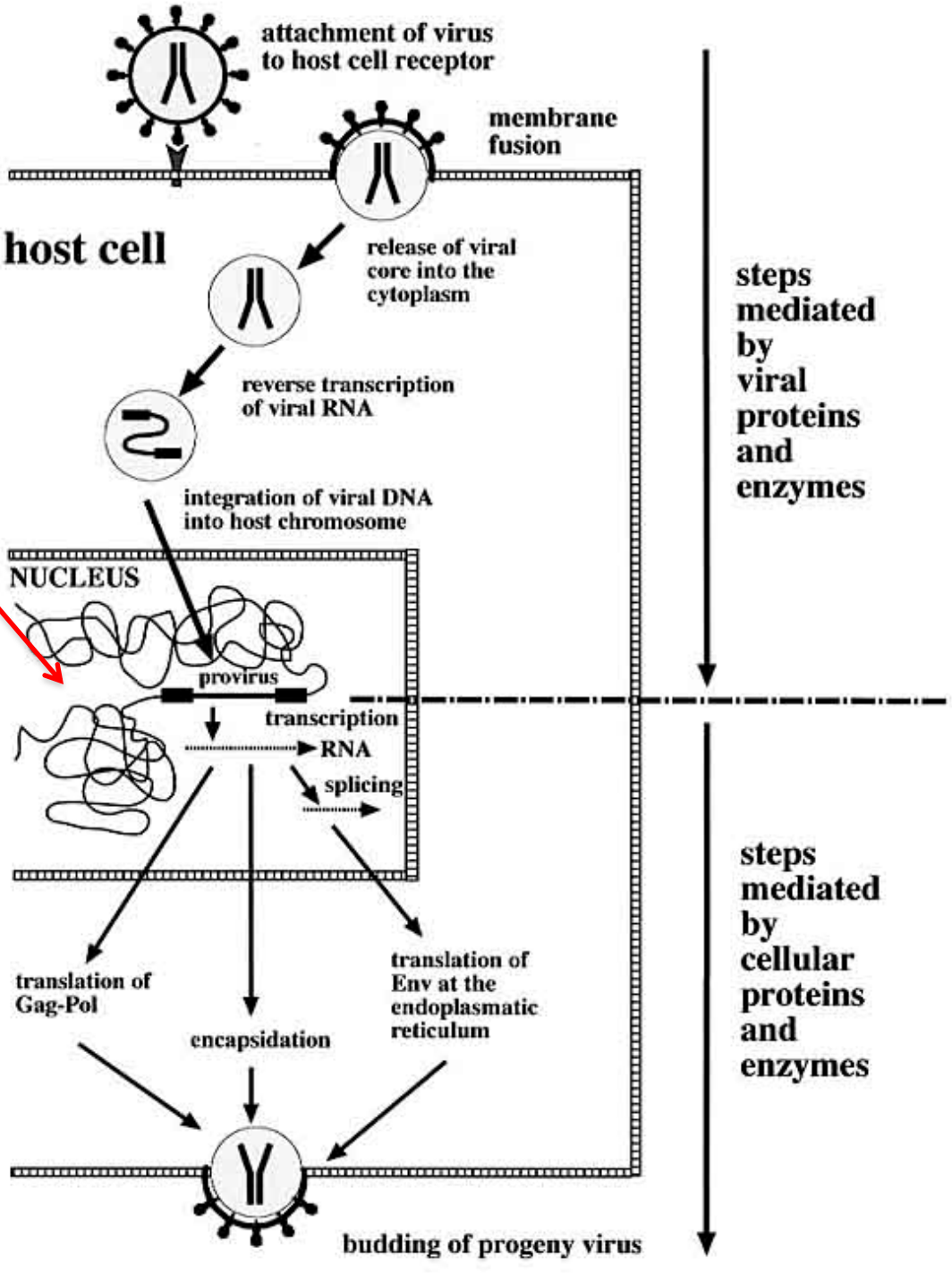
Inside the viral particle there are strand + viral RNAs and the reverse transcriptase



viral and host-dependent steps leading to virion production

It is this proviral DNA that is manipulated to form retroviral vectors for gene transfer

The **host cell is not lysed** by the virus and progeny viruses are released by budding.
However host cells infection gives rise to plaque formation which result from cell growth arrest of infected cells

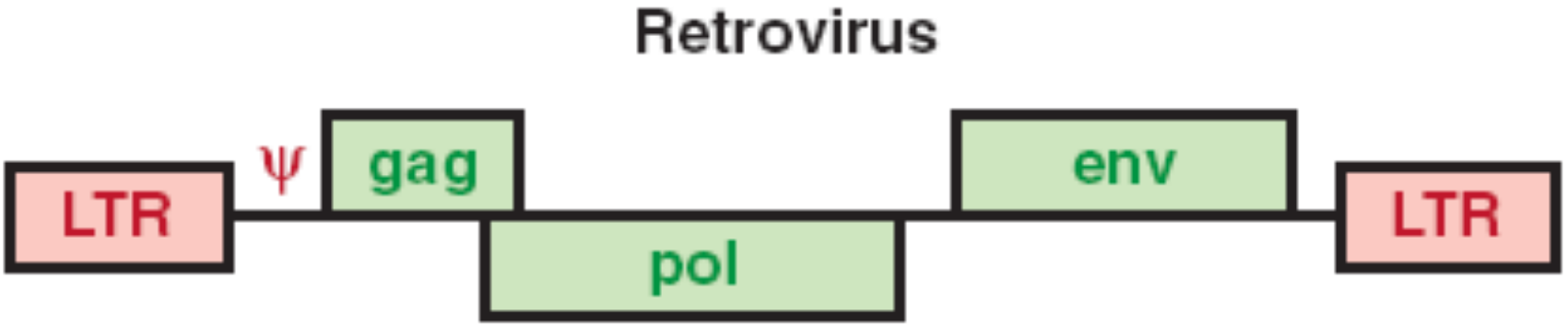


Schematic representation of a gammaretrovirus with its simple genomic architecture

Cis-regulatory sequences

LTR, long terminal repeat

Ψ, incapsidation signal



Three essential genes

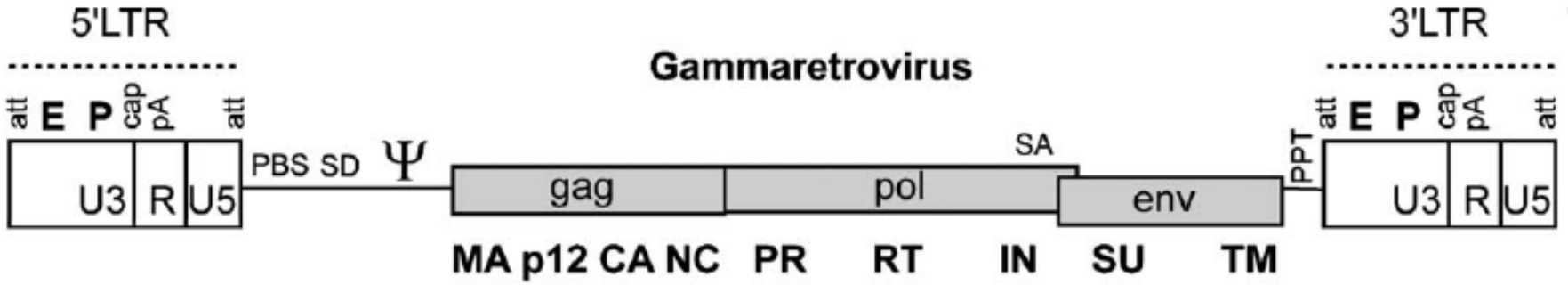
gag, viral matrix, capsid, and nucleocapsid proteins

pol, protease, reverse transcriptase, and integrase

env, membrane-anchored surface protein mediating target cell recognition and particle uptake

cis-acting regulatory sequences

EP, enhancer promoter
att, attachment site (integration)
pA polyadenylation site
PBS, primer binding site, 18 nt complementary to a tRNA used as a primer to begin reverse transcription
SD, splicing donor
SA, splicing acceptor
PPT, polypurine tract
Ψ, incapsidation site



gag, capside (**CA**), nucleocapside (**NC**) e matrix (**MA**)
pol, reverse transcriptase (**RT**), protease (**PR**), integrase (**IN**)
env, envelope protein consisting of the following domains: surface domain, **SU and** transmembrane domain, **TM**). It mediates interaction with cell receptors thus directing virus internalization

the genomic viral RNA
and
the proviral DNA genome

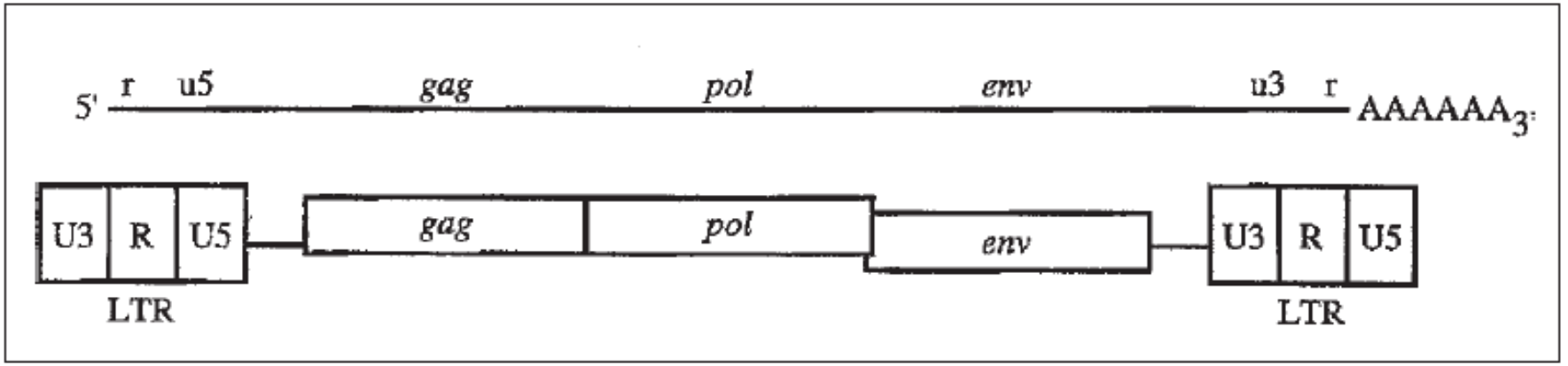
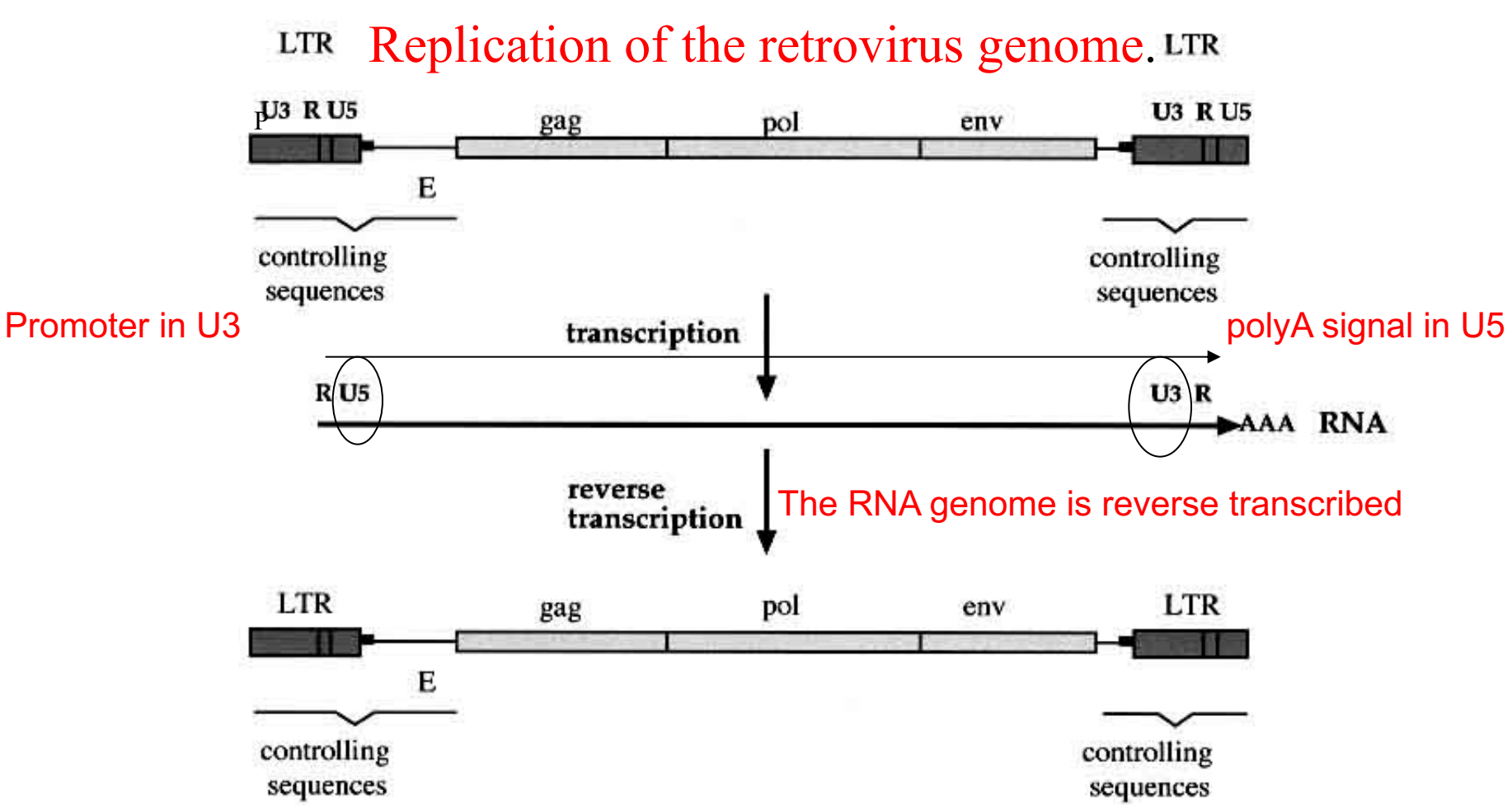


Fig. 1. Genome structure of a prototypical retrovirus. The genomic viral RNA, represented by a single black line, is shown at the top of the figure, with the structure of the resulting provirus after reverse transcription below. The locations of the open reading frames *gag*, *pol*, and *env* are shown. 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.

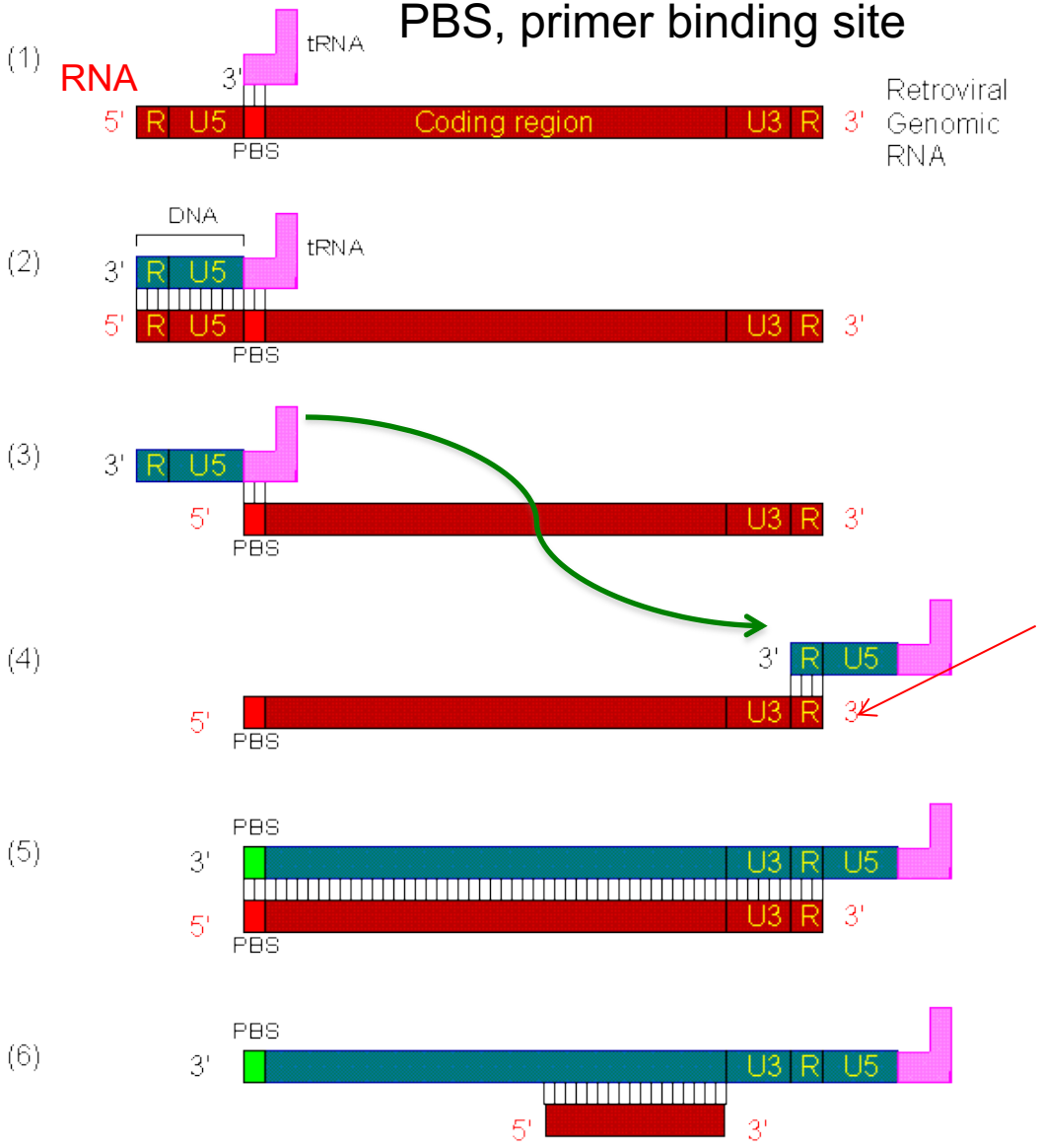
From Buchschacher 2001

Replication of the retrovirus genome.



Transcription from the viral long terminal repeat (LTR) promoter (located in the U3 region of the LTR) generates viral RNA which is polyadenylated like cellular mRNAs. Polyadenylation signal in the downstream (3') LTR (U5 located) mediates poly(A) tail downstream of R.

The RNA genome is reverse transcribed by the retroviral reverse transcriptase



Reverse transcriptase has two activities: DNA polymerase and RNaseH

1- A retrovirus-specific cellular tRNA hybridizes with a complementary region called the primer-binding site (PBS).

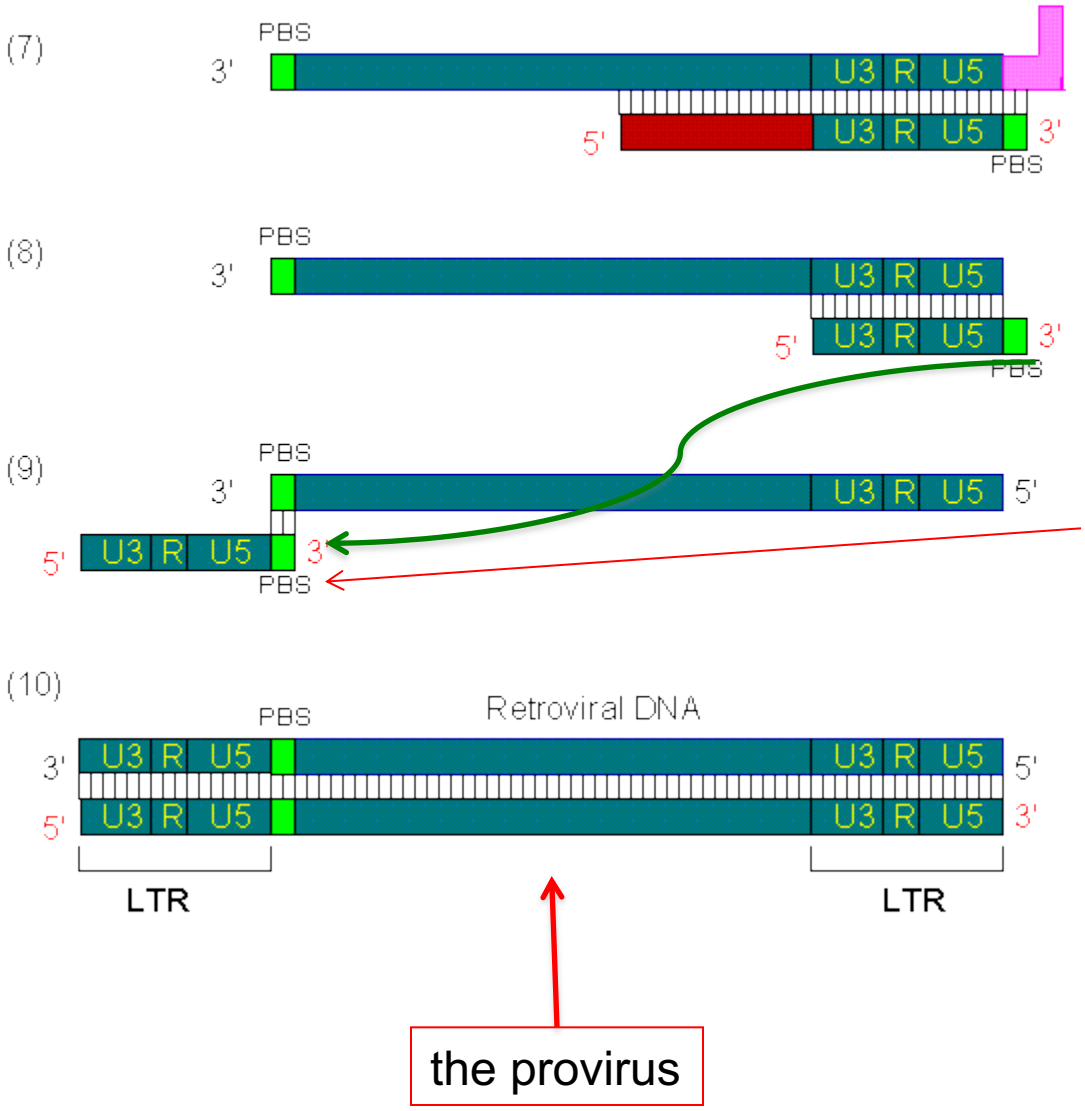
2- A DNA segment is extended from tRNA based on the sequence of the retroviral genomic RNA.

3- The viral R and U5 sequences are removed by RNase H.

4-First jump: DNA hybridizes with the remaining R sequence at the 3' end.

5-A DNA strand is extended from the 3' end.

6- Most viral RNA is removed by RNase H.



7- A second DNA strand is extended from the viral RNA.

8- Both tRNA and the remaining viral RNA are removed by RNase H.

9- Second jump: The PBS region of the second strand hybridizes with the PBS region of the first strand.

10- Extension on both DNA strands.

Perche' usare i VETTORI RETROVIRALI?

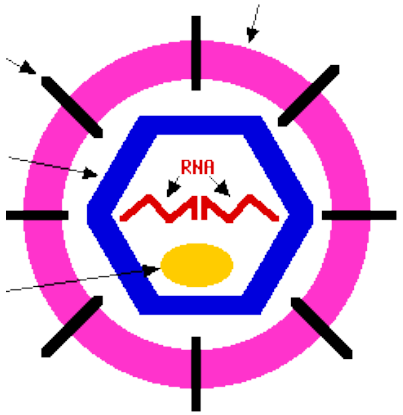
- Genoma semplice da modificare
- Efficiente integrazione (utile nella correzione delle malattie genetiche ereditarie)
- Non c'e' trasferimento di geni virali
- Non c'e' reazione immunitaria preesistente

retroviral vectors stably integrate into the dividing target cell genome so that the introduced gene is passed on and expressed in all daughter cells.

The main potential hazard of this technology is the production of replication competent virus, which can infect humans

which retrovirus?

Moloney Murine Leukimia Virus
Mo-MLV



- Il recettore per env di Mo-MLV è ubiquitario in cellule di mammiferi
- L'infezione non è patogena per l'uomo
- L'integrazione è altamente efficiente e non determina riarrangiamenti a carico del genoma virale
- Possono essere inseriti fino ad 8 kb di DNA
- LTR contengono un promotore ed un enhancer attivi in molti tipi cellula

general concepts

- the vectors are engineered to be **replication defective**, being able to complete only one round of replication (the transfer vector, infect the cells, enter the cytoplasm, as the cells divide, enter the nucleus and integrate).
- the integrated **vector genome, can express the foreign gene**/s but is **unable to replicate** and spread to other cells
- building a replication defective vector from the parental retrovirus necessitates separating cis- and trans-acting sequences. In a practical sense, this entails removal of the trans-acting genes and substituting with the gene of interest.
- The **trans-acting factors need the be provided for the production of the recombinant virions**, this can be done by transcomplementing vectors or using cells stably expressing the trans factors (packaging cells)

archetypal retrovirus vectors

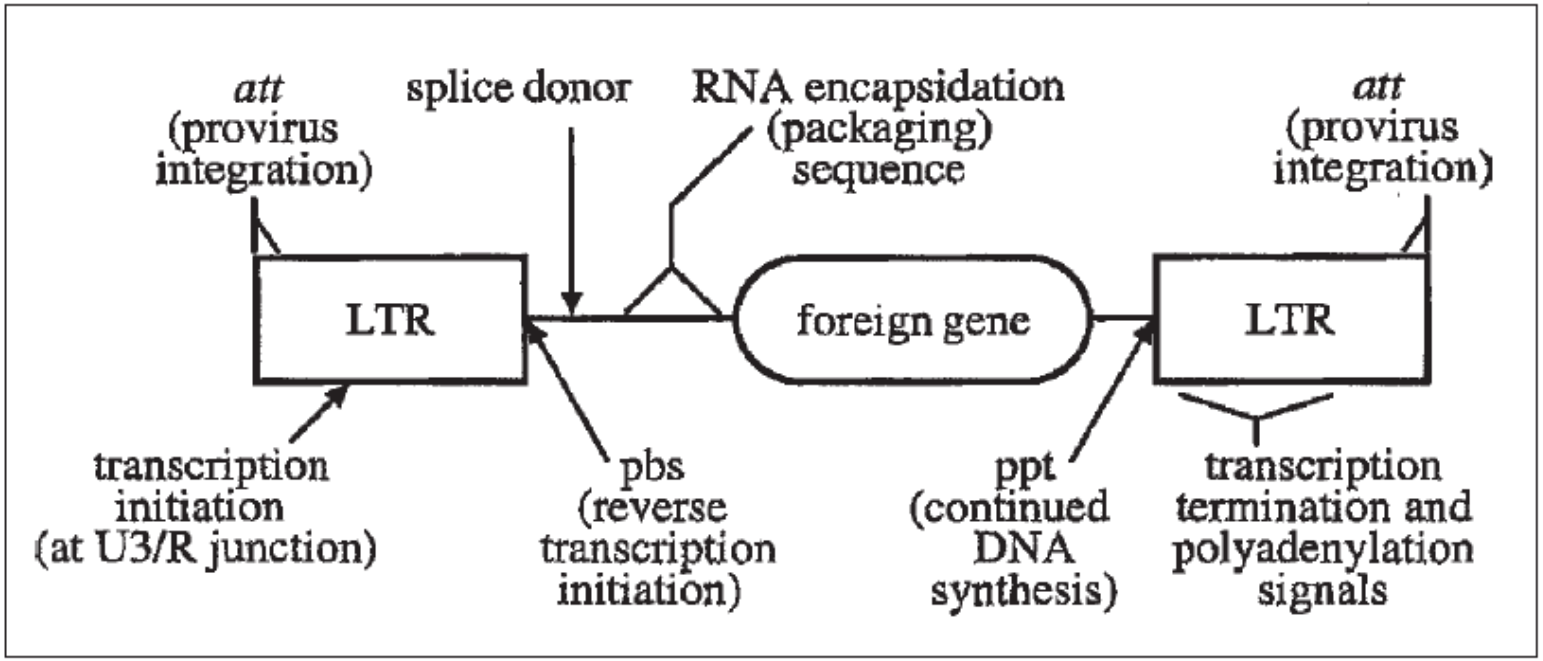
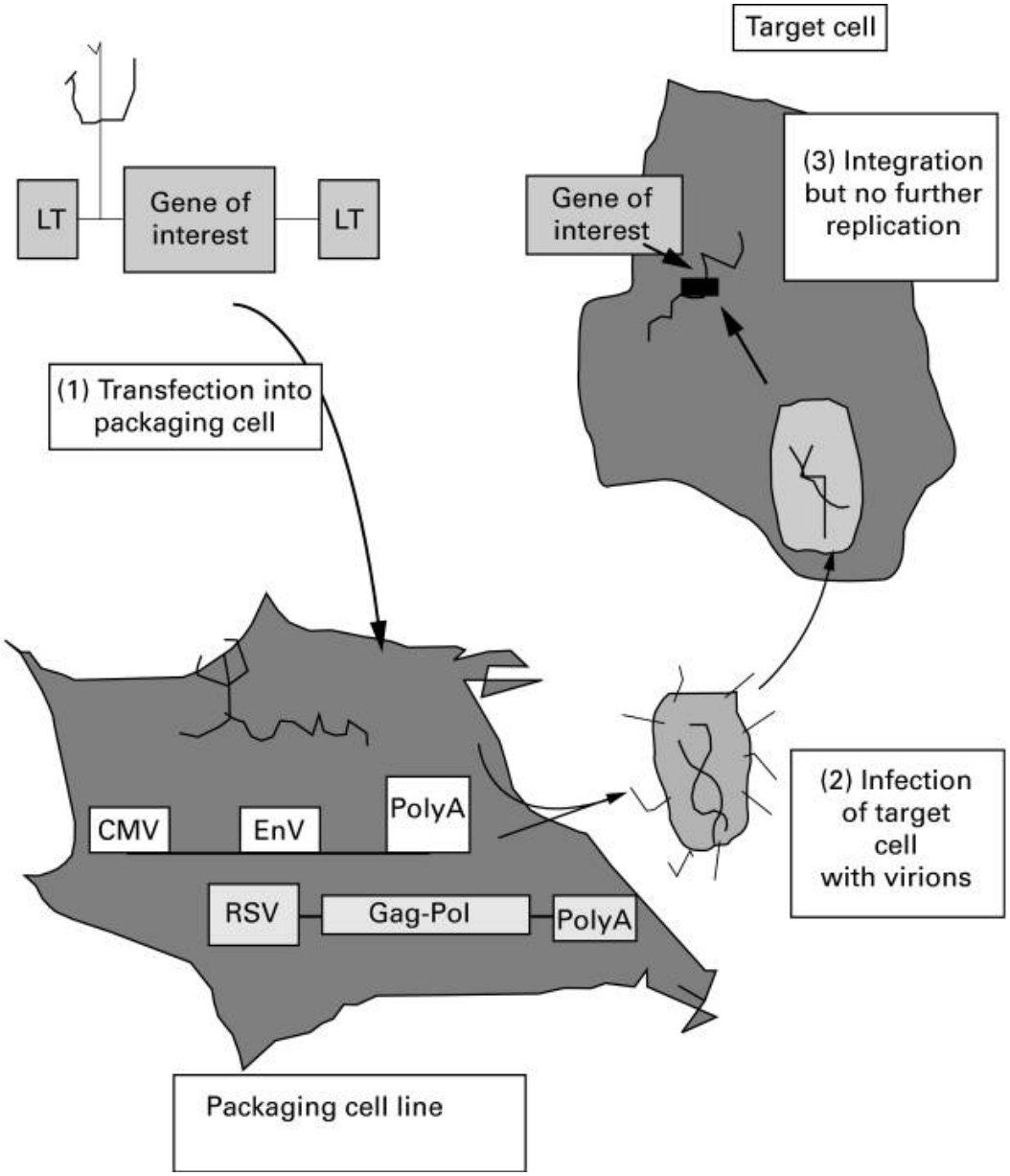


Fig. 3. Retroviral vector *cis*-acting elements. In this example of a "typical" retroviral vector, the viral genes have been removed and replaced with a foreign gene of interest. The viral sequences that remain as part of the vector construct are necessary in *cis* during various steps of the retroviral replication cycle. These sequences are necessary for vector production and for successful reverse transcription and integration of the vector genome, followed by expression of the foreign gene. Although in the example shown the foreign gene is expressed directly from the LTR, other strategies for expressing foreign genes exist and are illustrated in Figure 4. *att*, attachment site; pbs, primer binding site; ppt, polypurine tract. Modified from ref. 1.

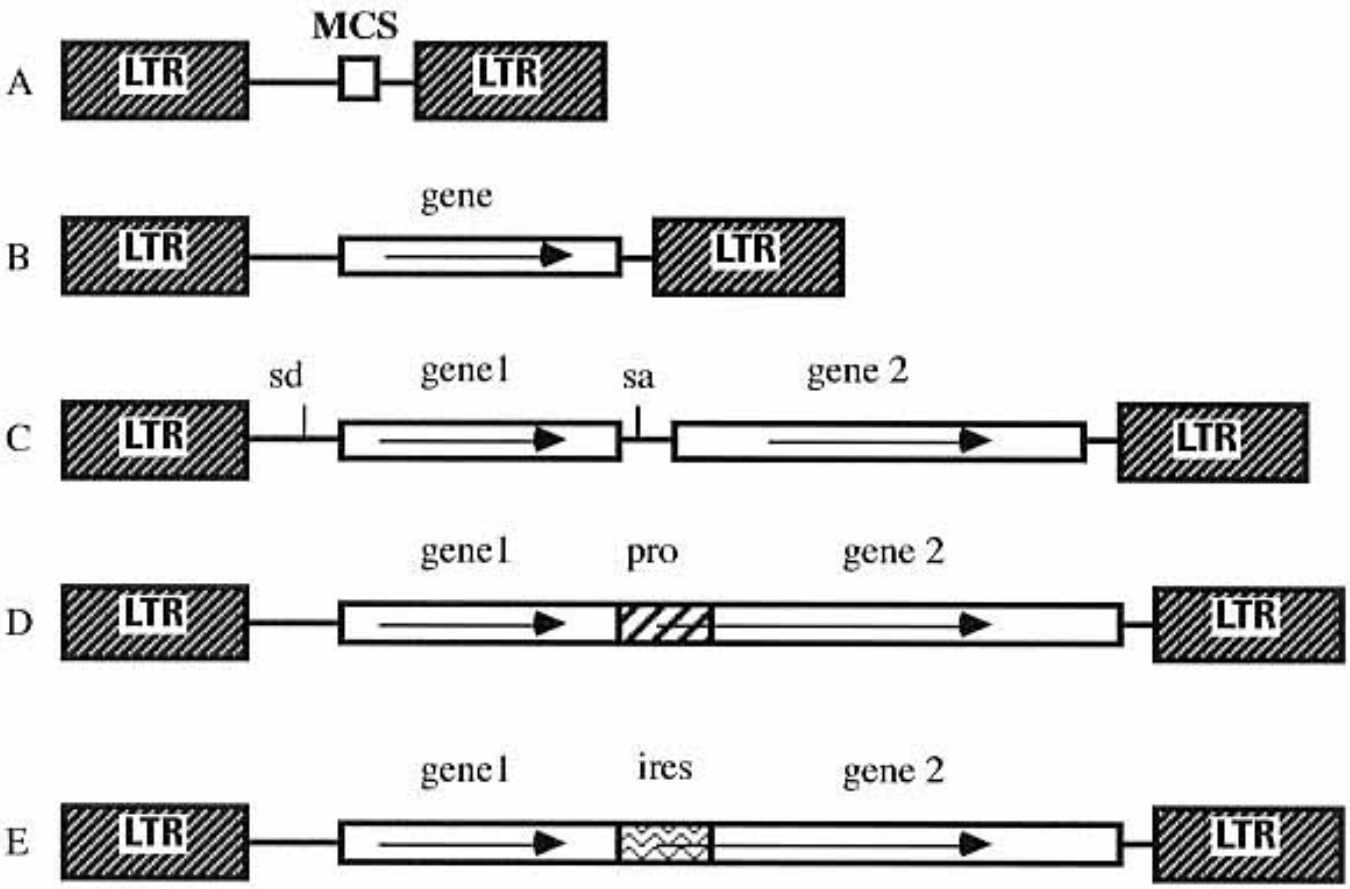
From Buchschacher 2001

Retroviral production and infection



Kurian et al 2000

Possible LTR-based Vectors



one vector – one gene

a second gene can be expressed:

from a spliced RNA (C),

an internal promoter (D),

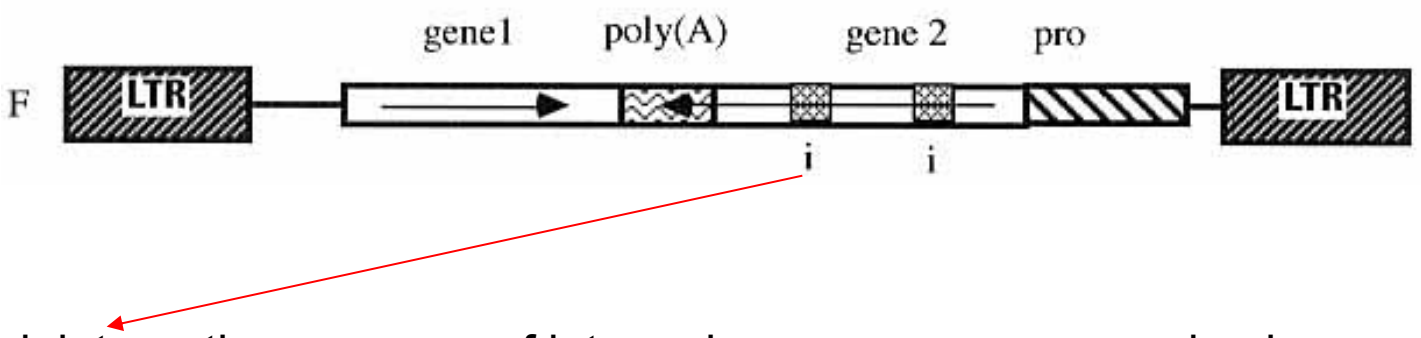
an internal ribosomal entry site (E)

improving transfer vector design

“promoter interference” although the phenomenon is not completely understood it has been observed that gene expression from a gene cloned under an heterologous promoter decreases

promoter interference can be reduced

to achieve sustained expression of the transgene **the gene cassette** (with heterologous promoter) **was cloned antisense to LTR.**

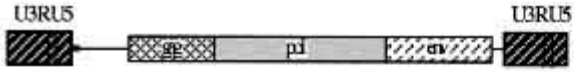


i, intron; the presence of introns increase gene expression in mammalian cells

safety concerns and genotoxicity

Promoter insertion, promoter activation and gene transcript truncation

Genotoxicity Mechanisms

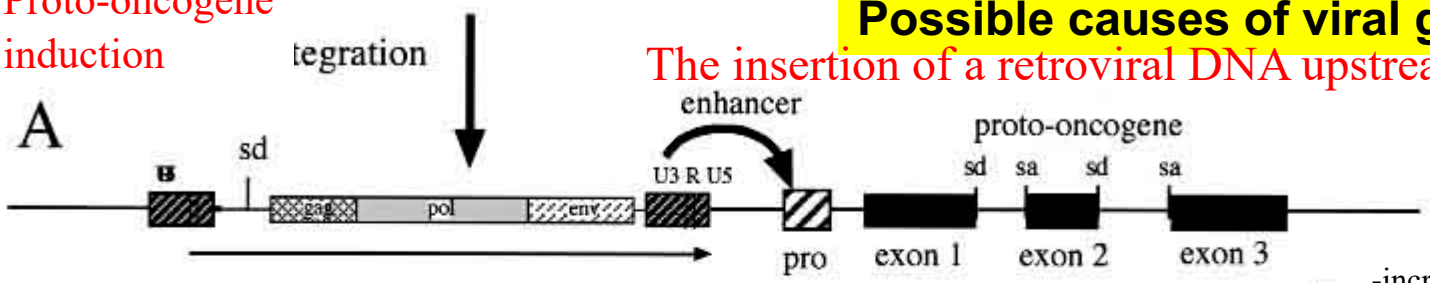


Possible causes of viral genome integration

Proto-oncogene induction

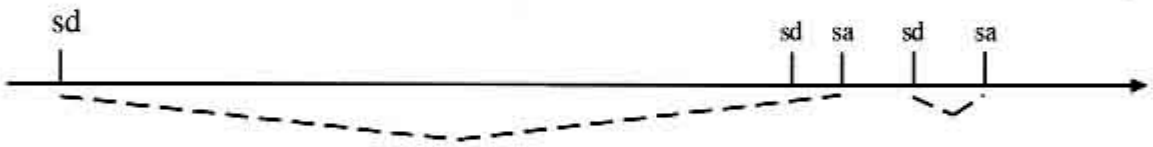
tegration

The insertion of a retroviral DNA upstream of a proto-oncogene



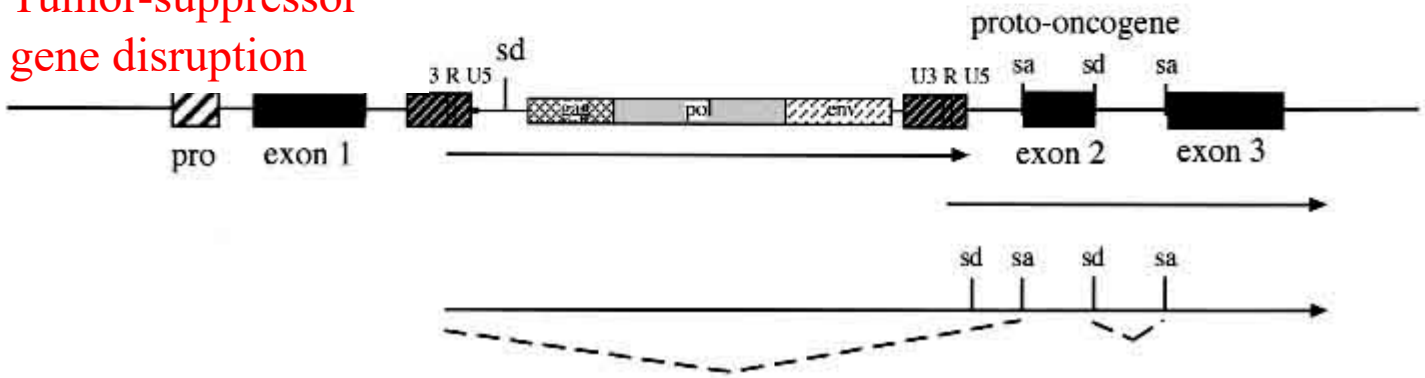
-increased transcription of the gene by the LTR induced promoter

Abnormal transcripts



- RNA read-through transcription and RNA splicing can generate abnormal products of the downstream gene

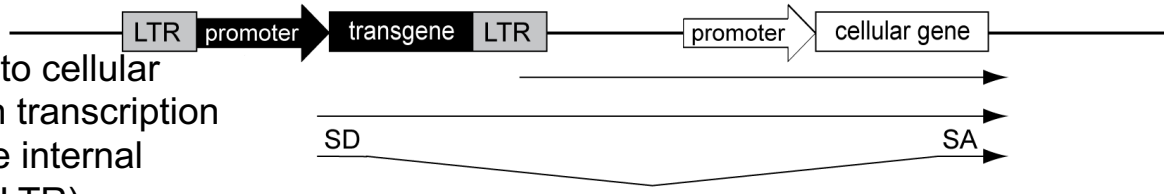
Tumor-suppressor gene disruption



The integrated provirus disrupts a proto-oncogene resulting in the transcription of an aberrant (truncated) proto-oncogene protein. This abnormal proto-oncogene may also lead to the disruption of a controlled cell-cycle mechanism of the host cell and, consequently, to continuous cell divisions.

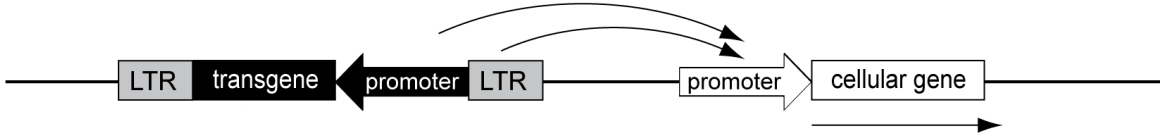
Genotoxicity Mechanisms

(i)



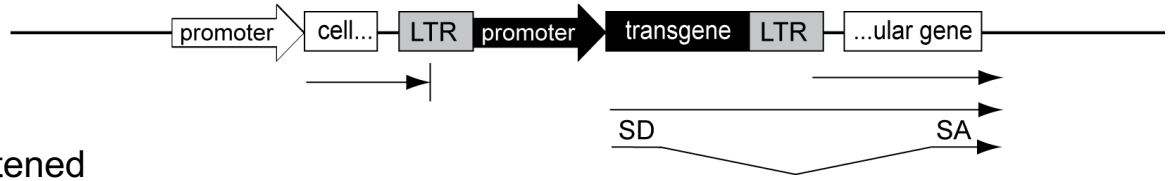
Promoter insertion upstream and in sense to cellular transcription units can lead to read-through transcription into adjacent cellular genes, either from the internal promoter or from the long terminal repeat (LTR) as indicated by the arrows

(ii)



promoter activation is mediated by enhancer-interactions by respective elements in the internal promoter or in the LTR with cellular promoters

(iii)



gene transcript truncation can lead to shortened cellular transcripts, either lacking 3' (left example) or 5' sequences (right example).

Promoter insertion, promoter activation and gene transcript truncation are the three most prevalent mechanisms of retroviral vector genotoxicity described to date.

Prevention strategies

Since insertional deregulation of cellular transcription is dependent on the presence of **strong promoter/enhancer sequences and splice sites**, optimized vector design should omit these elements.

Self-inactivating retroviral vectors designed for transfer of whole genes into mammalian cells

(neomycin-resistance selection/c-*fos*/gene therapy)

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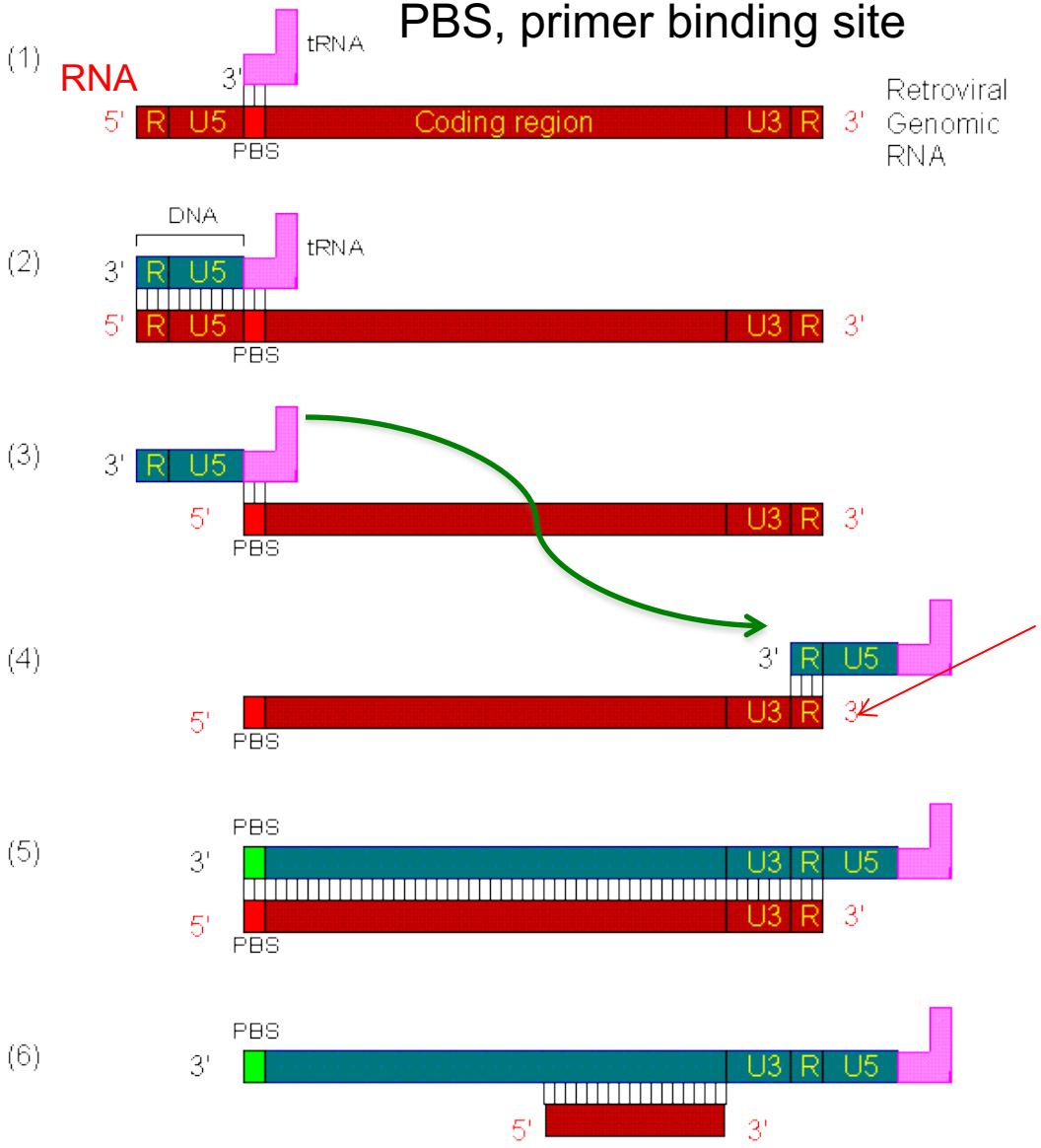
Vol. 83, pp. 3194–3198, May 1986
Biochemistry

ABSTRACT **A retrovirus-derived vector called *self-in*activating (SIN) vector was designed for the transduction of whole genes into mammalian cells. SIN vectors contain a deletion of 299 base pairs in the 3′ long terminal repeat (LTR), which includes sequences encoding the enhancer and promoter functions. When viruses derived from such vectors were used to infect NIH 3T3 cells, the deletion was transferred to the 5′ LTR, resulting in the transcriptional inactivation of the provirus in the infected cell. Introduction of a hybrid gene (human metallothionein-promoted c-*fos*) into cells via a SIN vector was not associated with rearrangements and led to the formation of an authentic mRNA transcript, which in some cases was induced by cadmium. SIN vectors should be particularly useful in gene transfer experiments designed to study the regulated expression of genes in mammalian cells. Absence of enhancer and promoter sequences in both LTRs of the integrated provirus should also minimize the possibility of activating cellular oncogenes and may provide a safer alternative to be used in human gene therapy.**



Reverse transcriptase has two activities: DNA polymerase and RNaseH

1- A retrovirus-specific cellular tRNA hybridizes with a complementary region called the primer-binding site (PBS).

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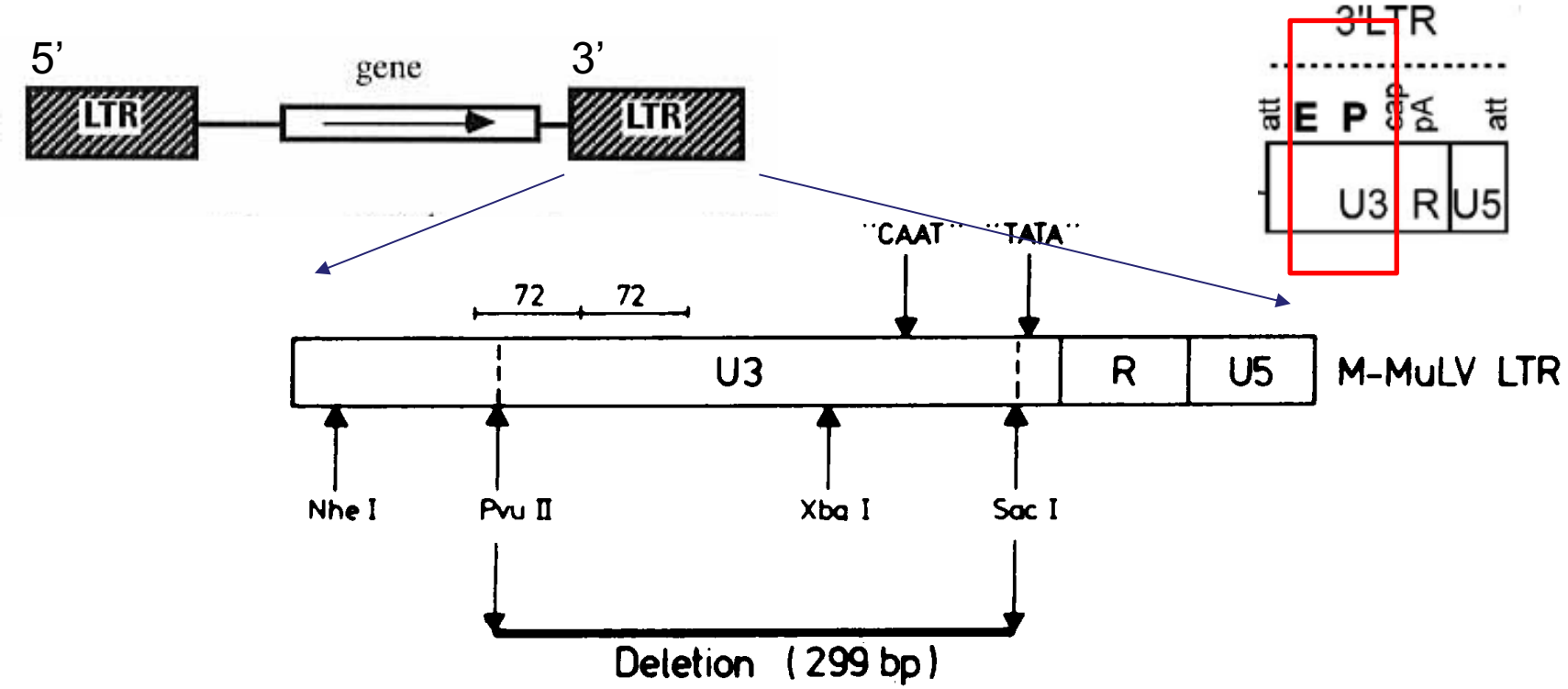
4-First jump: DNA hybridizes with the remaining R sequence at the 3' end.

5-A DNA strand is extended from the 3' end.

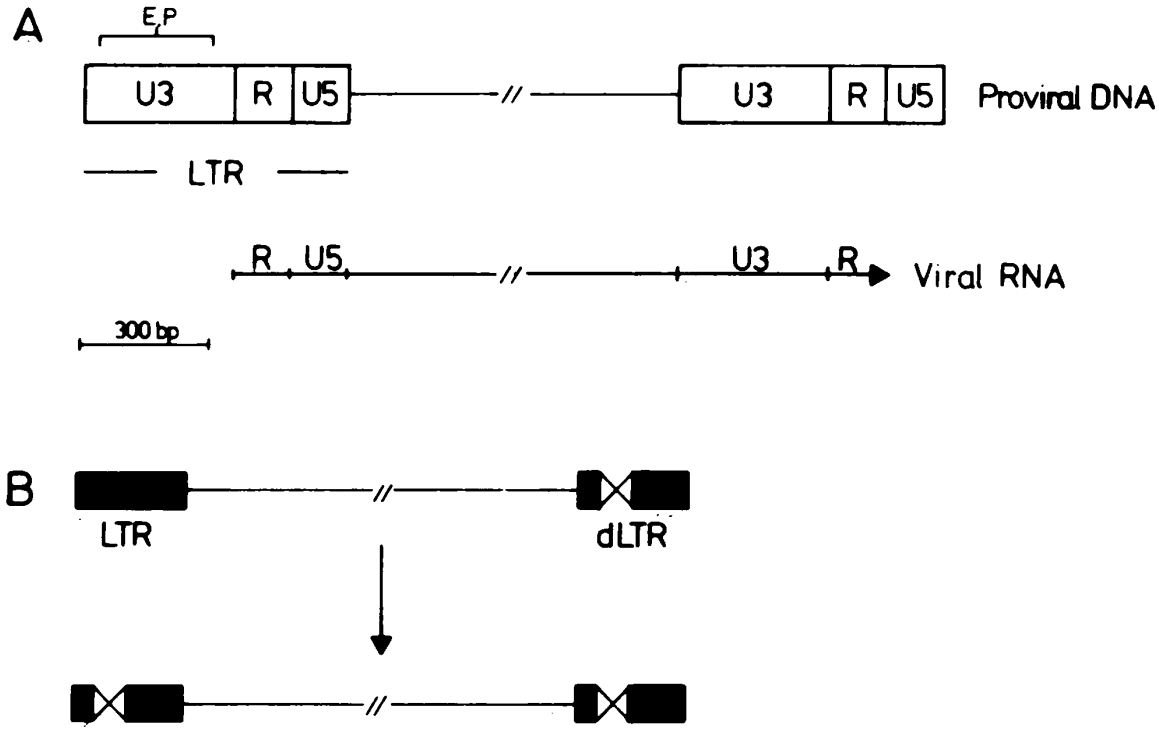
6- Most viral RNA is removed by RNase H.

SIN (self inactivating) vector development



The deletion introduced into the 3'LTR of SIN vectors. The 299-bp deleted segment includes most of the two72-bp repeats associated with the viral enhancer and the presumptive promoter region, which contains the canonical "CAAT" box.

SIN (self inactivating) vector development



-structure of the proviral DNA (integrated into the genome)

-structure of the viral RNA (incorporated into virions)

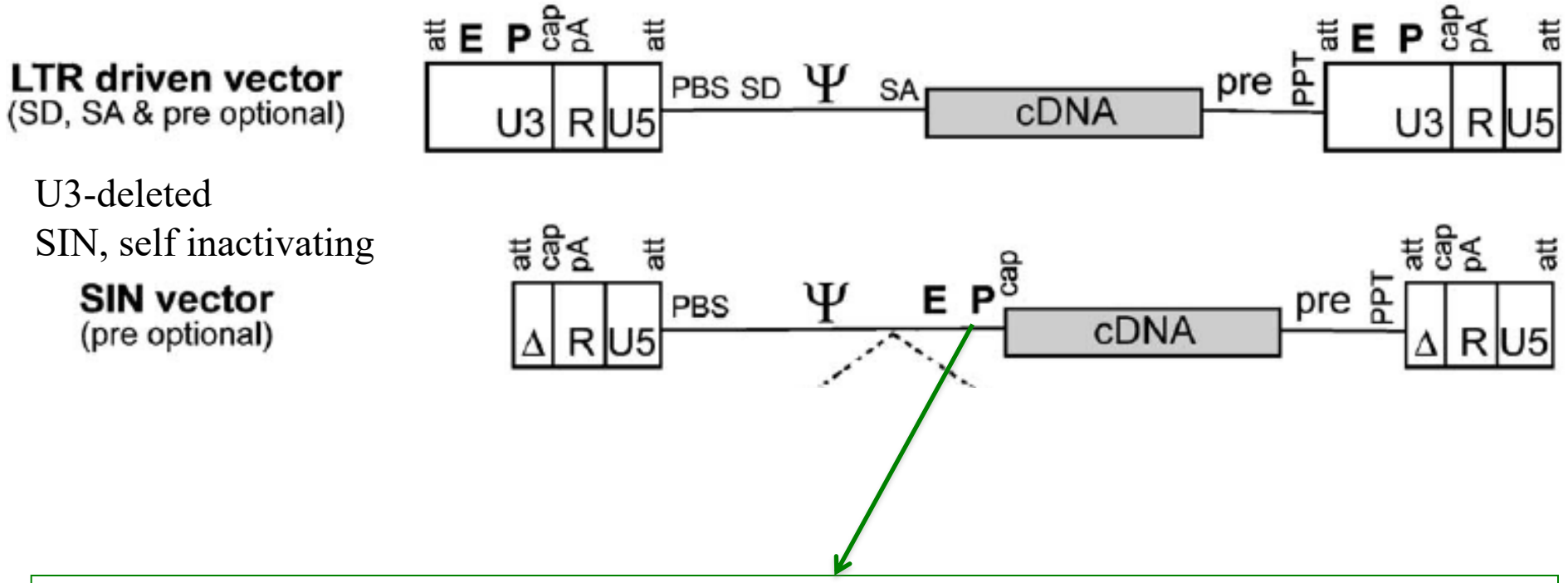
B - retrotranscription of a 3' ΔU3 LTR viral RNA, produce a ΔLTR provirus

A deletion in the U3 region of the 3'LTR of the proviral DNA is transferred to the 5' LTR of the progeny proviral DNA. As a consequence of the removal of the enhancer and promoter sequences from both the 5' and 3' LTR in the progeny provirus, the viral transcriptional unit is eliminated).

E, enhancer; P,promoter; dLTR, LTR containing the deletion shown in C.

from the archetypal vector to the SIN vector

(LTR can induce expression of flanked genes)

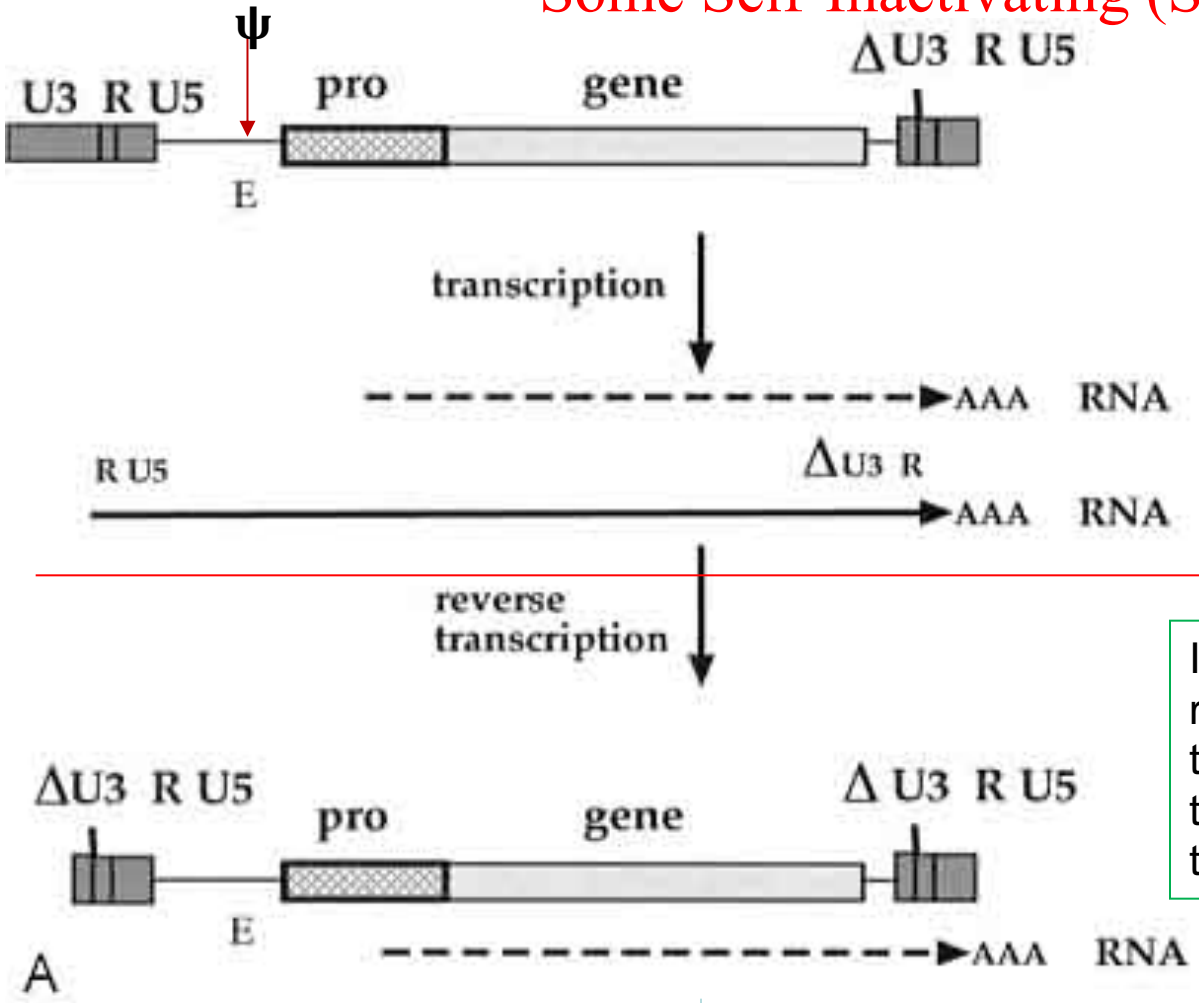


the gene cloned in the SIN vector must be equipped with a promoter, since the LTRΔU3 is without the promoter.

SIN vectors are expected to:

- *reduce host gene-activation following insertion into the host genome*
- *to reduce promoter interference*

Some Self-Inactivating (SIN) vectors



In packaging cells two transcripts are expected :
1-transgene mRNA (not required in packaging cells)
2- the viral genome RNA, which is the only one that can be inserted into the viral particle

In target cells, when the proviral recombinant DNA is inserted into the host genome, only one transcript is expected: the transgene transcript.

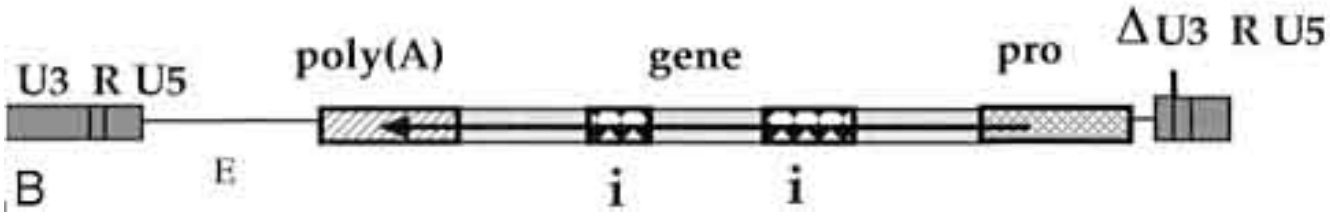
from this recombinant SIN provirus only one transcript, the transgene transcript, is expected

So-called self-inactivating (SIN) vectors are obtained by deleting enhancer–promoter sequences from the U3 region of the 3’ long terminal repeats (LTRs).

Initial MLV- based SIN vectors had poor titers, but recent data suggest that modifications of the 5’ promoter and the 3’ untranslated region allow the production of MLV SIN vectors with titers that are equivalent to those of their LTR-driven counterparts.

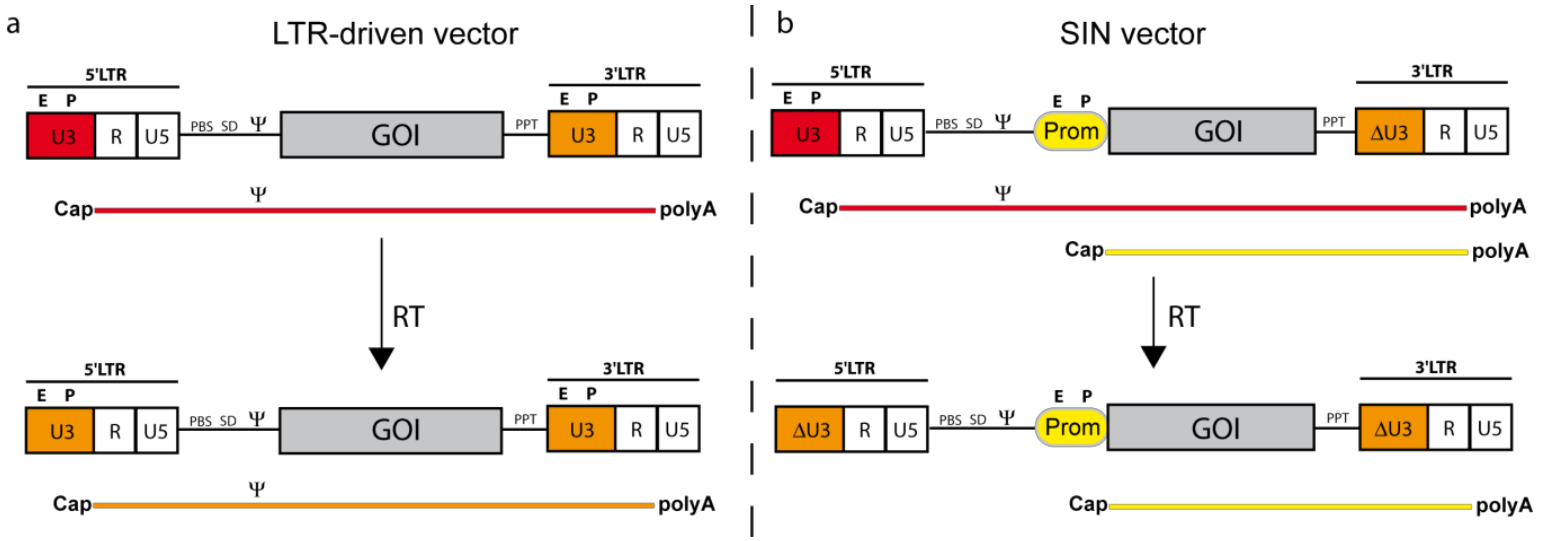
SIN vectors

SIN vector with an expression cassette containing introns (**i**)



transgene expression is improved by the presence of introns

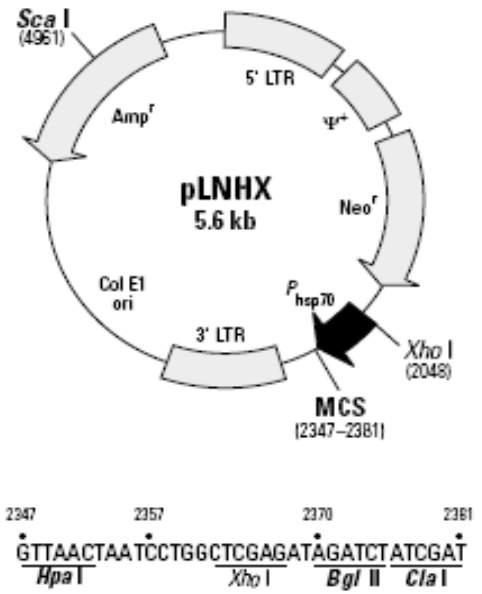
LTR-driven *versus* SIN



Maetzig T, Galla M, Baum C, Schambach A. Gammaretroviral vectors: biology, technology and application. Viruses. 2011 Jun;3(6):677-713. doi: 10.3390/v3060677. Epub 2011 Jun 3. PMID: 21994751; PMCID: PMC3185771.

commercial retroviral vectors

Commercial retrovirus vectors (Clontech)



Location of Features

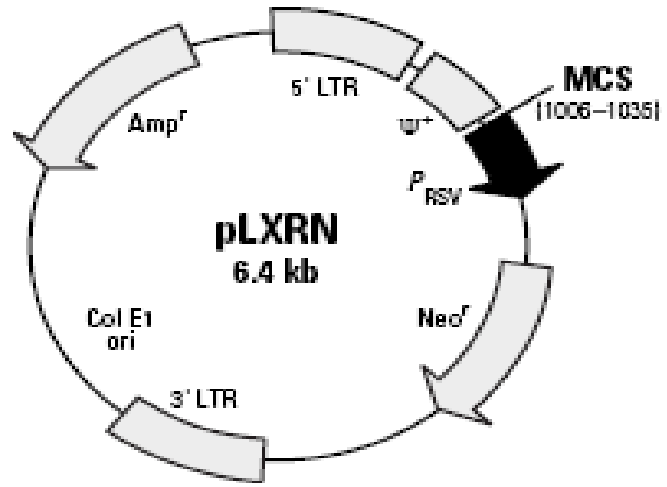
- 5' MoMuSV LTR: 1–588
- Ψ+ (packaging signal): 657–988
- Neomycin resistance gene (Neo^r):
  Start codon: 1067–1069; stop codon: 1859–1861
- *Drosophila* hsp70 promoter (P_{hsp70}): 2047–2330
- Multiple Cloning Site (MCS): 2347–2381
- 3' MoMuLV LTR: 2518–3111
- Col E1 origin of replication:
  Site of replication initiation: 3647
- Ampicillin resistance gene (β-lactamase):
  Start codon: 5267–5265; stop codon: 4409–4407

pLNHX contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV), and is designed for retroviral gene delivery and expression. Upon transfection into a packaging cell line, pLNHX can transiently express, or integrate and stably express, a transcript containing the viral packaging signal Ψ+, the neomycin selection marker (Neor), and a target gene.

The 5' viral LTR in this vector contains promoter/enhancer sequences that control expression of the Neor gene, which allows antibiotic selection in eukaryotic cells. A target gene can be cloned into the multiple cloning site downstream of the Drosophila heat shock protein 70 (hsp70) promoter (Phsp70).

pLNHX also includes the Col E1 origin of replication and E. coli Ampr gene for propagation and antibiotic selection in bacteria.

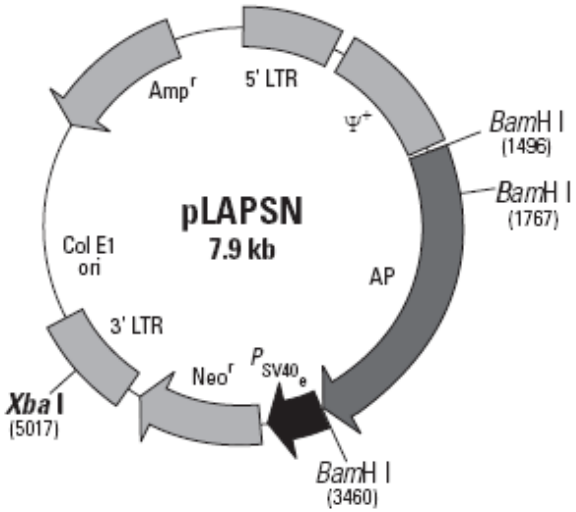
changing the MCS position respect to viral LTR



Location of Features

- 5' MoMuSV LTR: 1–589
- Ψ+ (packaging signal): 659–989
- Multiple Cloning Site (MCS): 1006–1035
- Rous sarcoma virus (RSV) promoter (P_{RSV}): 1039–1350
- Neomycin resistance gene (Neo^r):
  Start codon: 1704–1706; stop codon: 2496–2498
- 3' MoMuLV LTR: 3290–3883
- Col E1 origin of replication
  Site of replication initiation: 4419
- Ampicillin resistance gene (β-lactamase):
  Start codon: 6039–6037; stop codon: 5181–5179

Commercial retrovirus vectors
Control vector with Alkaline Posphatase gene



Location of Features

- 5′ MoMuSV LTR: 1–589
- Ψ+ (extended packaging signal): 659–1468
  Mutated *gag* (ATG->TAG): 1049–1051
- Fragment containing the alkaline phosphatase (AP) gene: 1558–3150
- Early SV40 promoter (P_{SV40e}): 3467–3819
- Neomycin resistance gene (Neo^r):
  Start codon: 3865–3867; stop codon: 4657–4659
- 3′ MoMuLV LTR: 4719–5312
- Col E1 origin of replication:
  Site of replication initiation: 5848
- Ampicillin resistance gene (β-lactamase):
  Start codon: 7468–7466; stop codon: 6610–6608

Alkaline Phosphatase assay: p-nitrophenyl phosphate (pNPP) turns yellow when dephosphorylated by AP.

pLAPSN contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV), and is a control vector for establishing a retroviral gene delivery and expression system (1–3).This vector was created by cloning the gene for human placenta alkaline phosphatase (AP) into the Xho I site in the multiple cloning site of the pLXSN Retroviral Vector (Cat. No. 631509). Upon transfection into a packaging cell line, pLAPSN can transiently express, or integrate and stably express, a transcript containing Ψ+ (the extended viral packaging signal) **the human placenta alkaline phosphatase gene, and a selectable marker**.The 5’ viral LTR in this vector contains promoter/enhancer sequences that control expression of the alkaline phosphatase gene. The SV40 early promoter (PSV40e) con- trols expression of the neomycin resistance gene (Neor), which allows antibiotic selection in eukaryotic cells. pLAPSN also includes the Col E1 origin of replication and E. coli Ampr gene for propagation and antibiotic selection in bacteria.

pLAPSN can be used in control experiments to establish retroviral gene transduction proce- dures. After being transfected into a packaging cell line (such as the RetroPack PT67 Cell Line, Cat. No. 631510), RNA from the vector is packaged into infectious, replication-incompetent retroviral particles. pLAPSN does not contain the structural genes (gag, pol, and env) necessary for particle formation and replication; however, these genes are stably integrated into PT67 (4–7). Subsequent introduction of pLAPSN, containing Ψ+ (psi), transcription and processing elements, and the alkaline phosphatase gene, produces high-titer, replication-incompetent infectious virus. That is, these retroviral particles can infect target cells and transmit the al- kaline phosphatase gene, but cannot replicate within these cells since the cells lack the viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation. The expression of alkaline phosphatase can be confirmed using a standard assay.

packaging

Certificate of Analysis

Clontech

GP2-293 Packaging Cell Line

Catalog No.
631458

Lot Number
Specified on product label.

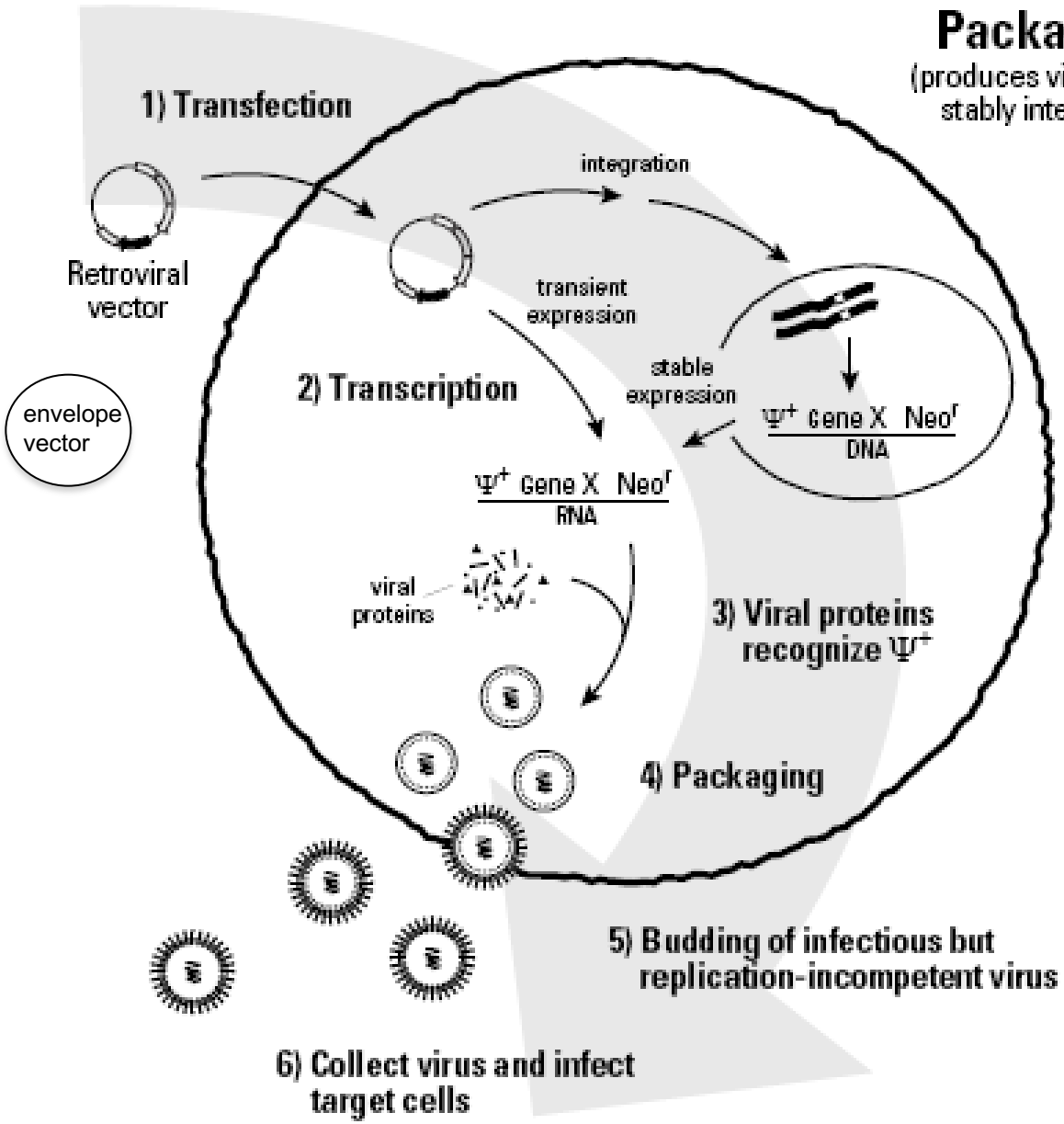
Description

GP2-293 is a HEK 293-based retroviral packaging cell line. The essential viral packaging genes *gag* and *pol* are stably integrated, the viral envelope must be supplied in trans. High titer retrovirus is produced by transient co-transfection of an MMLV- or MSCV-based retroviral expression vector and a plasmid that expresses a viral envelope, such as pVSV-G..

Package Contents

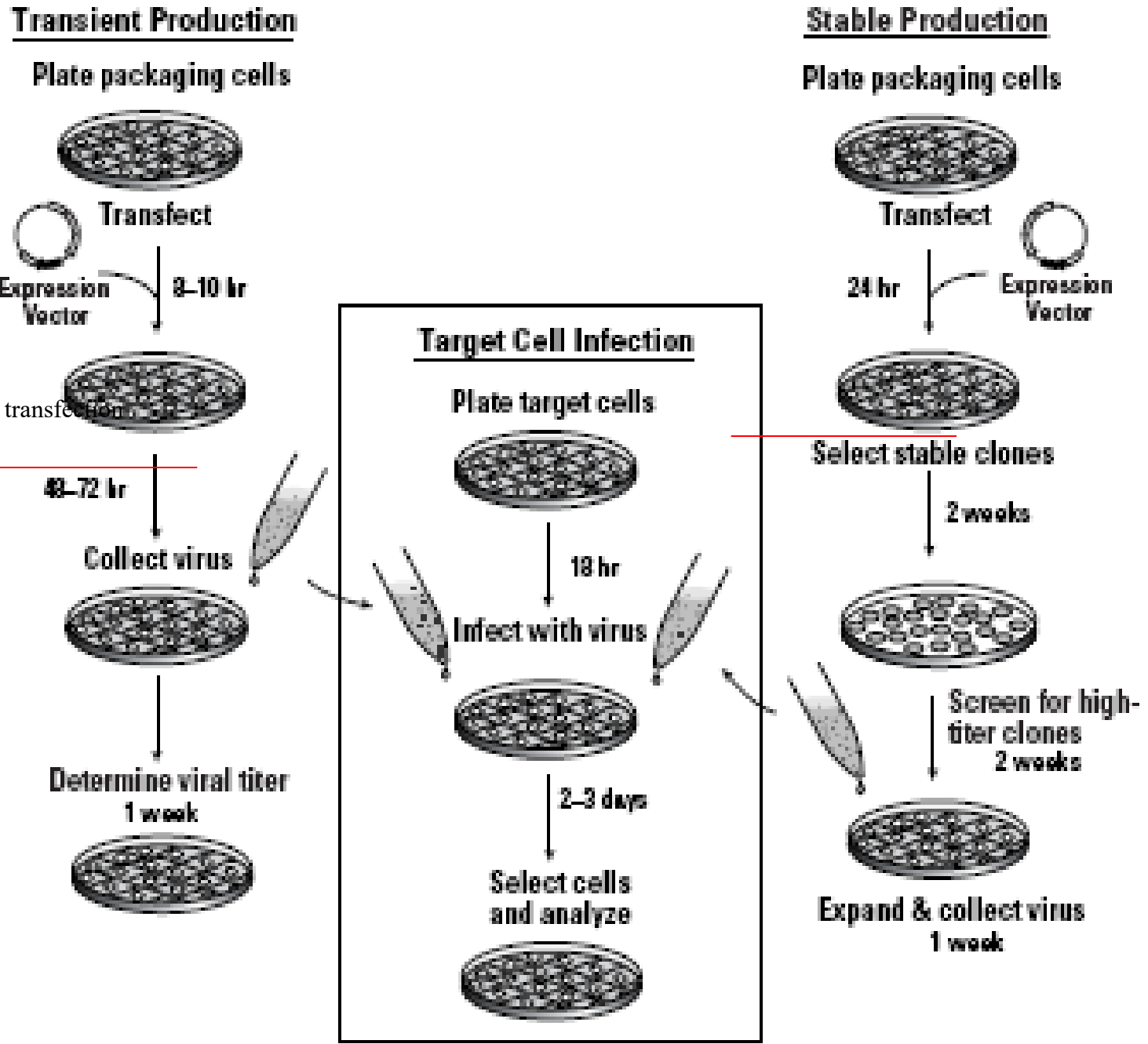
As part of the Pantropic Retroviral Expression System, pLNHX can be co-transfected with pVSV-G into the GP-293 Packaging Cell Line to produce infectious, replication-incompetent retrovirus

pLNHX does not contain the structural genes necessary for viral particle formation and replication.The genes encoding the viral gag and pol proteins are stably integrated into GP-293. Because the VSV-G envelope protein is toxic, this protein is expressed transiently from pVSV-G.
Although the virus can infect target cell lines and transmit a target gene, it cannot replicate because the target cell lines lack the viral structural genes. By using the minimal viral sequences and separately introducing the structural genes into the packaging cell line, the chance of producing replication-competent virus due to recombination events is minimized.



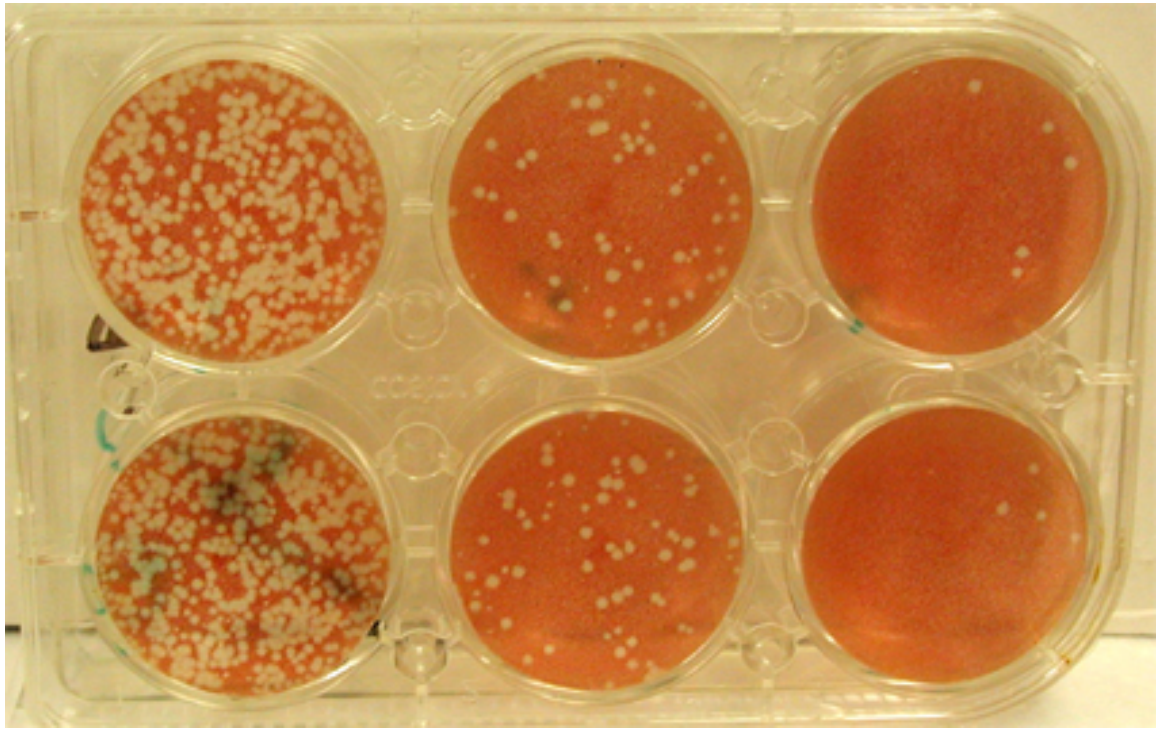
Le cellule packaging esprimono stabilmente i geni virali necessari per produrre il virus ricombinate

Produzione del virus trasducente



Transient transfection

virus titration by plaque assay



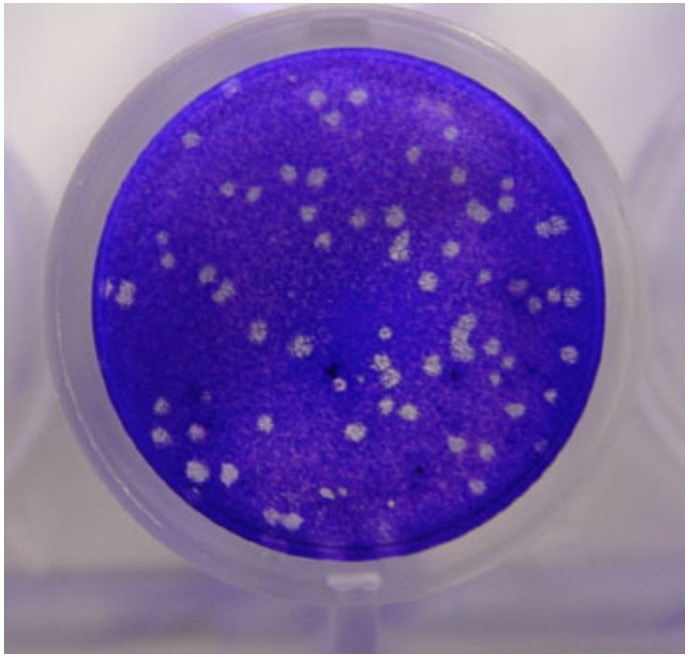
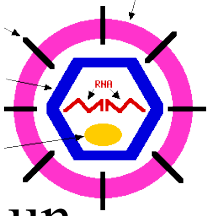


Figure 5. A plaque assay. Serial dilutions of virus have been plated on confluent monolayer cultures of cells (packaging cells must be used). The cells are stained after a period of time in which a single virus infects a cell, produces new virus particles and infects surrounding cells. The white areas show areas of the culture in which the cells have been infected. Each "plaque" is the result of the presence of one original infectious virus particle.

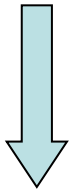


aspetti positivi e negativi del trasferimento genico mediato da un vettore retrovirale

- Il recettore per env di Mo-MLV è ubiquitario in cellule di mammiferi (*ecotropici*, envelope di virus che infettano solo cellule murine; *anfotropici*, a più ampio spettro d'ospite)
- L'infezione non è patogena
- L'integrazione è random in alcuni casi sono stati individuati siti preferenziali e/o siti di cromatina aperta/*positivi o negativo?*
- Possono essere inseriti fino ad 8 kb di DNA
- LTR contengono un promotore ed un enhancer efficienti in molti tipi cellula/*positivi o negativo?*

I vettori retrovirali sono limitati dal fatto che l'integrazione ed espressione genica richiede cellule che si dividono attivamente.

Questo limite può essere superato lavorando ex vivo ed inducendo le cellule a proliferare



Superare questo limite cambiando virus

Da consultare

Baum et al 2006, Retrovirus Vectors: Toward the Plentivirus? MOLECULAR THERAPY Vol. 13, No. 6, June 2006

Kurian et al 2000, Retroviral vectors, J Clin Pathol: Mol Pathol 2000;53:173–176

Dornburg R. The history and principles of retroviral vectors. Front Biosci. 2003 May 1;8:d818-35. doi: 10.2741/967. PMID: 12700110.