

# Ретровирусы и ретровирусные векторы МГУ

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# Открытие вирусов

1892 год

Д.И.Ивановский – инфекционный фильтрующийся агент, вызывающий табачную мозаику.

1898 год

M.Beijerinck - “contagium vivum fluidum”.

Loeffler & Frosch – foot-and- mouth disease

1908 год

Ellerman & Bang – ALV

1911 год

P.Rous - RSV

# Ретровирусы

## *Retroviridae*

From *reverse transcription*, characteristic of these viruses

### VIRION

Spherical enveloped particle.

Diameter 100 nm.

Envelope assembled at plasma membrane.

Icosahedral or conical capsid.

Contains reverse transcriptase.

### GENOME

Linear ss RNA, positive sense.

Two identical molecules, 7–10 Kb, packaged in virions.

5' cap, 3' poly(A) tail.

### GENES AND PROTEINS

mRNAs transcribed from integrated provirus DNA by RNA polymerase II.

Three major genes, each translated as a polyprotein and cleaved into mature proteins:

- *gag*: nucleocapsid proteins
- *pol*: reverse transcriptase, protease, and integrase
- *env*: envelope proteins

Gag and Pol proteins made on unspliced mRNA.

Env proteins made on singly spliced mRNA.

Lentiviruses and human T-cell leukemia viruses have additional regulatory genes expressed on multiply spliced mRNAs.

### VIRUSES AND HOSTS

Seven genera are recognized: *Alpha-* to *Epsilonretrovirus*, *Lentivirus*, *Spumavirus*.

Simple retroviruses: Rous sarcoma virus (chickens), mouse mammary tumor virus (mice), Jaagsiekte sheep retrovirus.

Lentiviruses: human, feline, bovine, and simian immunodeficiency viruses

Spumaviruses: simian foamy virus.

### DISEASES

Humans: AIDS, leukemia.

A variety of cancers in monkeys, mice, cats, sheep, birds, etc.

### DISTINCTIVE CHARACTERISTICS

Two identical copies of genome per virion.

Genome RNA converted to DNA by reverse transcription.

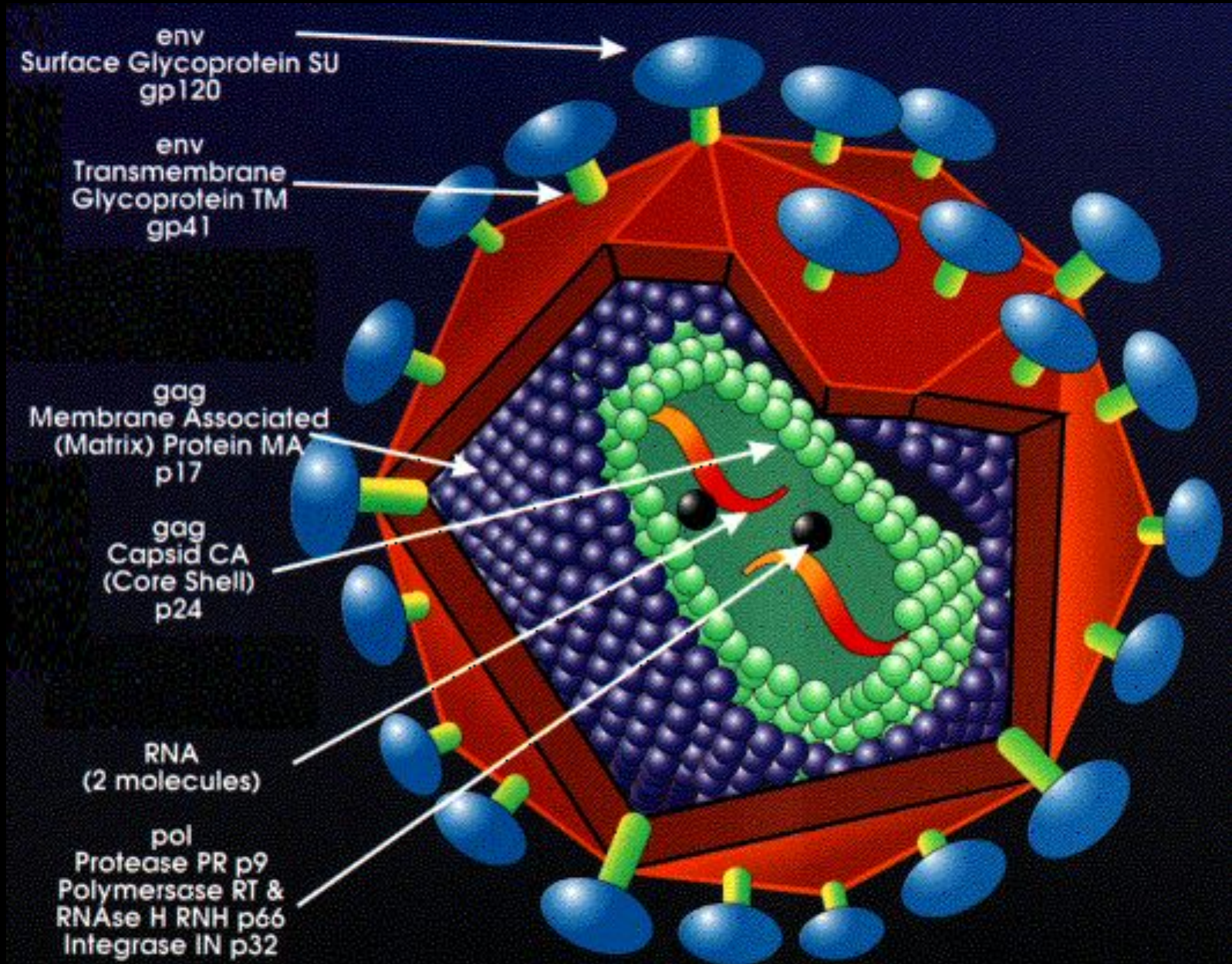
A cellular tRNA used as primer to initiate reverse transcription.

# Классификация ретровирусов

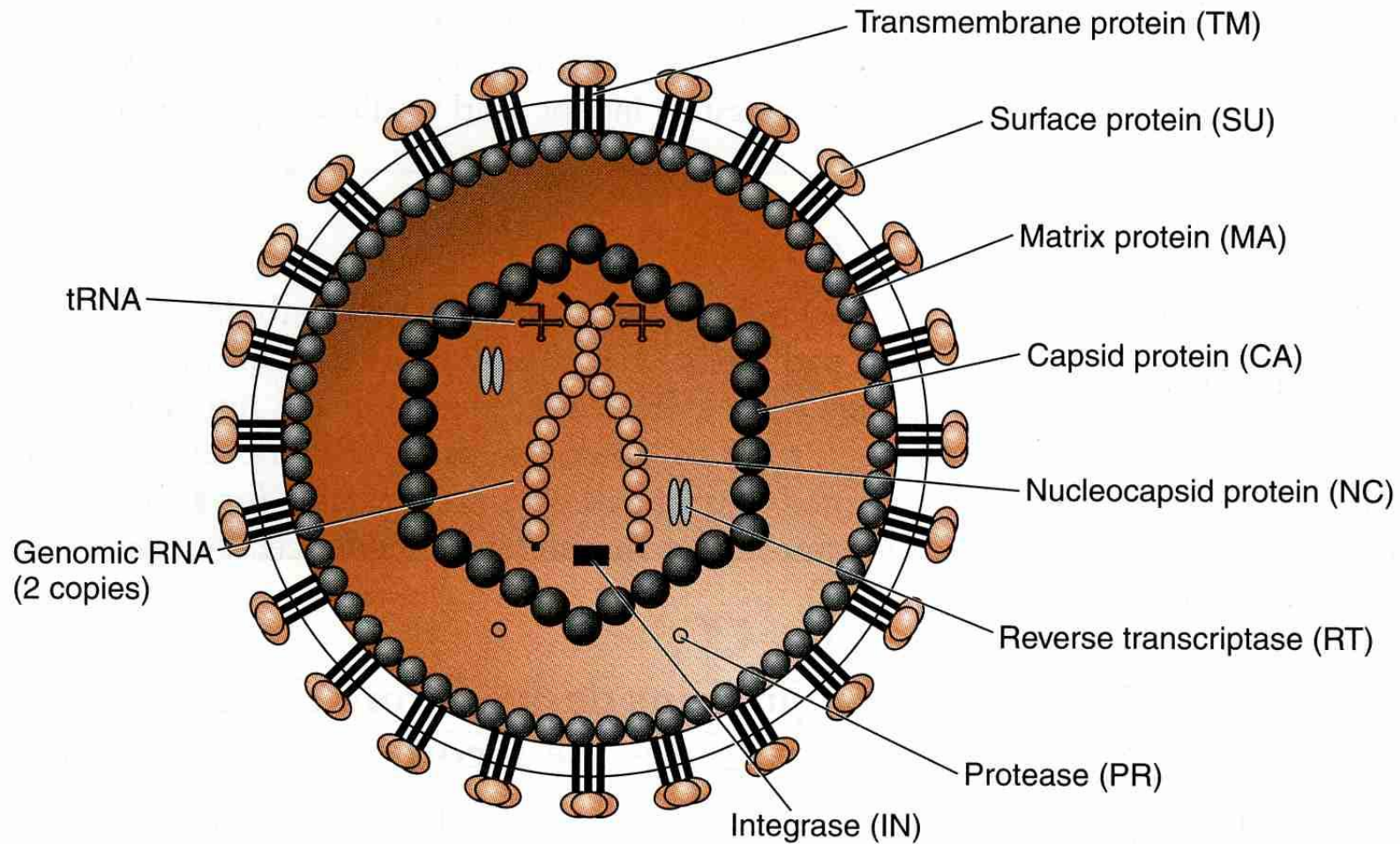
## Retrovirus genera

Genus	Examples of virus	Host
<i>Alpharetrovirus</i>	Rous sarcoma virus	Chickens
<i>Betaretrovirus</i>	Mouse mammary tumor virus	Mice
<i>Gammaretrovirus</i>	Murine leukemia virus	Mice
<i>Deltaretrovirus</i>	Human T cell leukemia virus type 1	Humans
<i>Epsilonretrovirus</i>	Walleye dermal sarcoma virus	Fish
<i>Lentivirus</i>	Human immunodeficiency virus type 1	Humans
	Simian immunodeficiency virus	Monkeys
	Feline immunodeficiency virus	Cats
<i>Spumavirus</i>	Simian foamy virus	Monkeys

# morphology of a retrovirus (HIV1, a *lentivirus*)



# Схема строения ретровирусного вириона (MuLV)



# Гены простых и сложных ретровирусов

Gene	Properties/function of protein
<b>Common to All Retroviruses</b>	
<i>gag</i>	precursor to internal structural proteins
<i>pro</i>	PR enzyme
<i>pol</i>	precursor to RT and IN enzymes
<i>env</i>	precursor to envelope glycoproteins
<b>Accessory Genes</b>	
HTLV/BLV (e.g., HTLV-1)	
<i>tax</i>	transcription activator
<i>rex</i>	splicing/RNA transport regulator
Lentiviruses (for primate lenti, e.g., HIV-1)	
<i>tat</i>	activates transcription
<i>rev</i>	regulates splicing/RNA transport
<i>vif</i>	affects infectivity of viral particles
<i>vpr and/or vpx</i>	is present in virion; has nuclear localization signal; facilitates infectivity
<i>nef</i>	in quiescent cells; triggers CD4 endocytosis, alters signal transduction in T cells; enhances virion infectivity
<i>vpu</i>	integral membrane protein; triggers CD4 degradation; enhances virion release
<i>dut</i>	dUTPase (only in nonprimate lentiviruses); facilitates replication in certain cell types
Type B (e.g., MMTV)	
<i>sag</i>	superantigen
<i>du</i>	dUTPase (NC-DU fusion)
Type D (e.g., M-PMV)	
<i>du</i>	DU enzyme (NC-DU fusion)
Spumaviruses (e.g., HSRV)	
<i>bell</i>	activates transcription
<i>bel2</i>	?
<i>bet</i>	?
Piscine retroviruses (e.g., WDSV)	
<i>orf A</i>	?
<i>orf B</i>	?
<i>orf C</i>	?

# Величина геномов различных ретровирусов

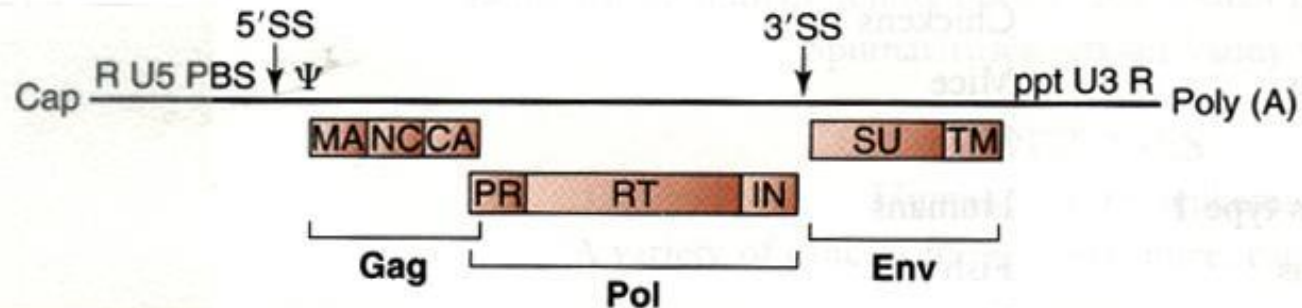
Approximate Size of Genome and LTR Elements in Prototype Retroviruses

Genus	Prototype virus	Genome (RNA)	U3	R	U5	tRNA primer	Notes
ASLV	RSV	9,300	230	20	80	Trp	includes v- <i>src</i> gene
	ALV	7,300	230	20	80	Trp	
Mammalian C-type	MLV	8,300	450	70	80	Pro, Glu	
Lenti	HIV-1	9,200	450	100	80	Lys-1,2,3	
Type D	M-PMV	7,900	220	30	100	Lys-1,2	
Type B	MMTV	8,600	1,200			Lys-3	U3 contains <i>sag</i> gene
HTLV/BLV	HTLV-1	8,500	350	230	220	Pro	
Fish	WDSV	12,300	440	80	70	His	
Spuma	HFV	11,200	910	190	160	Lys-1,2	
Fly		7,000	190	50	240	*	

The number of nucleotides is presented to the nearest hundred for the genomic RNA and to the nearest ten for the LTR elements.

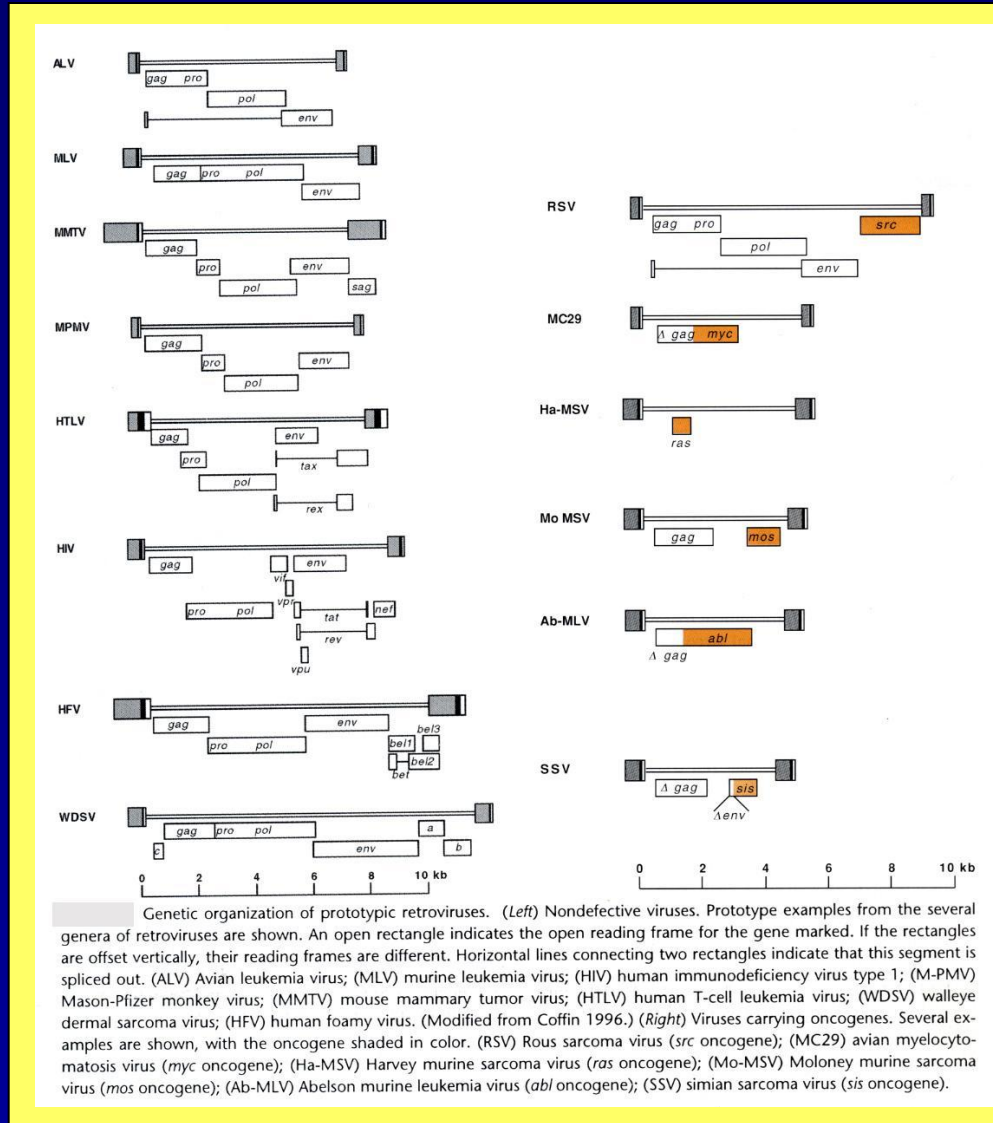


# Структура геномной РНК простого ретровируса

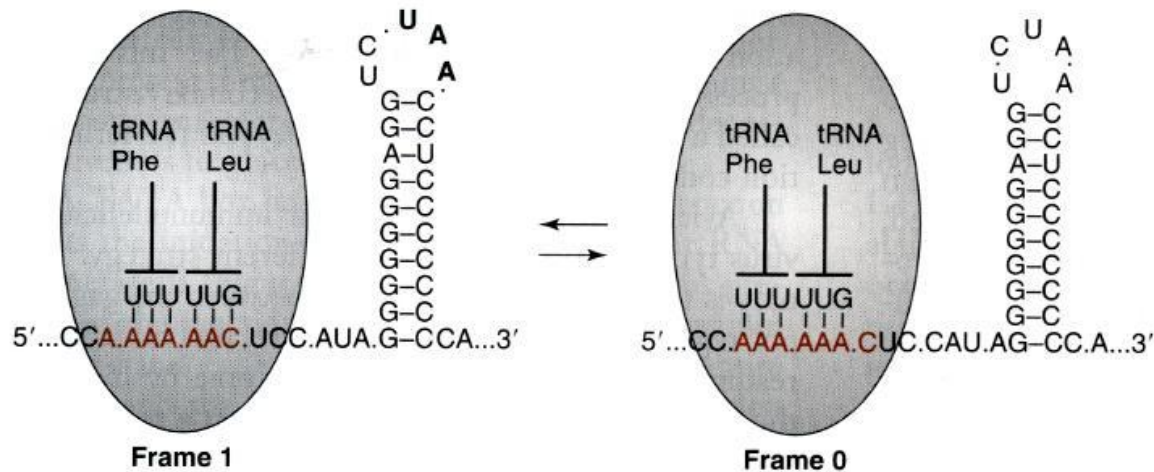


**Structure of retrovirus RNA.** Beginning at the left end, features are: methylated cap; repeat region (R); untranslated 5' sequence (U5); primer binding sequence (PBS); 5' splice site (5' ss); psi ( $\psi$ ) packaging sequence; gag, pol, and env reading frames for viral structural genes; 3' splice site (3' ss); polypurine tract (ppt) used during reverse transcription; untranslated 3' sequence (U3); repeat region (R), poly(A) tail.

# Геномная организация различных ретровирусов



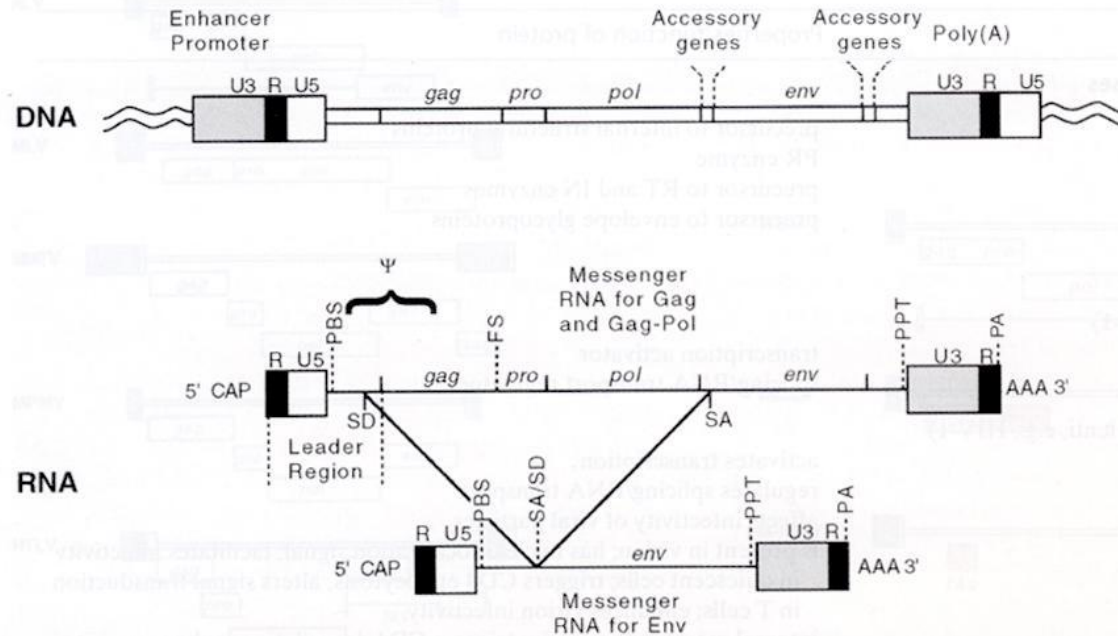
# Смена рамки считывания, реализуемая при трансляции РНК



## Ribosome

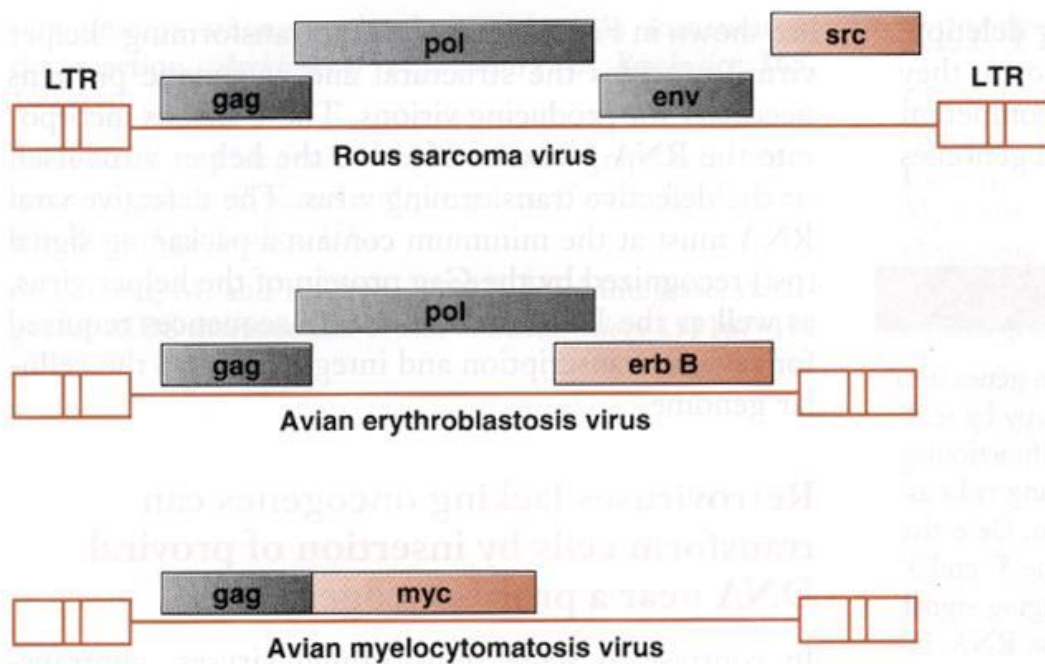
**frameshifting.** A base-paired RNA stem induces stalling upstream by the translating ribosome (orange ellipse) at the heptamer sequence. While stalled, the ribosome can shift from reading frame 1 to frame 0. Translation in frame 0 does not recognize the UAA termination codon (bold letters).

# Образование двух форм ретровирусной РНК



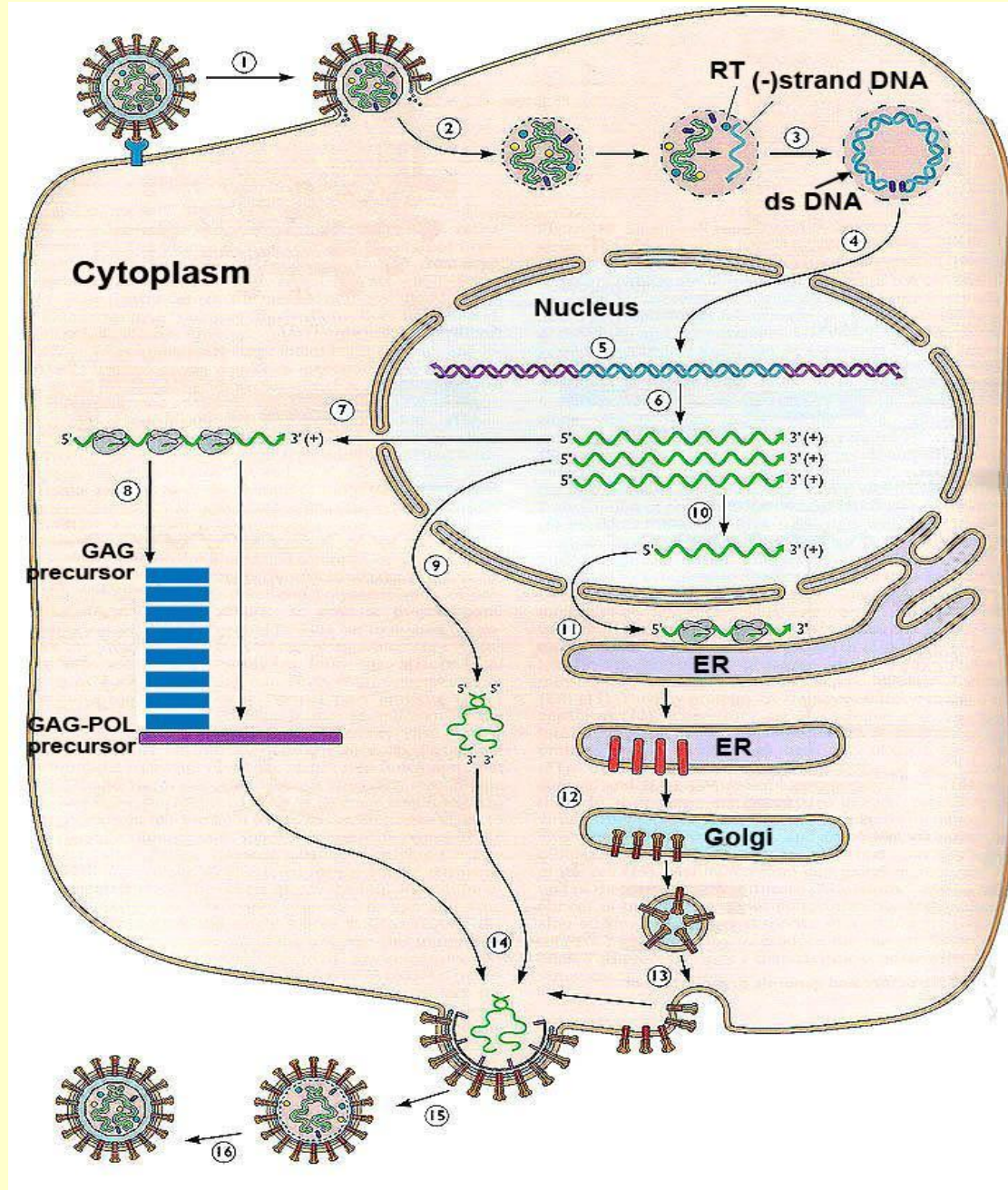
Genetic organization of generalized provirus. The proviral DNA as it is inserted into host DNA is shown at the top, with the long terminal repeats (LTRs) composed of U3, R, and U5 elements at each end abutting cellular sequences. Sequences in the LTR that are important for transcription, for example, enhancers, the promoter, and the poly(A) addition signal, are marked. The *gag*, *pro*, *pol*, and *env* sequences are located invariably in the positions shown in all retroviruses. Accessory genes are located as shown, and also overlapping *env* and U3 and each other, and occasionally in other locations. The RNA that is the primary transcriptional product is shown on the second line. Sequences that are important for replication and gene expression are shown in the approximate locations in which they are typically found. (PBS) Primer-binding site; ( $\psi$ ) encapsidation sequence; (SD) splice donor site; (FS) frameshift site; (SA) splice acceptor site; (PPT) polypurine tract; (PA) polyadenylation signal; (AAA) poly(A) tail. The spliced messenger RNA for the Env protein is shown on the third line. Retroviruses with accessory genes have other spliced mRNAs and thus other splice donor and splice acceptor sites as well.

# Геномная организация РНК трансформирующих вирусов

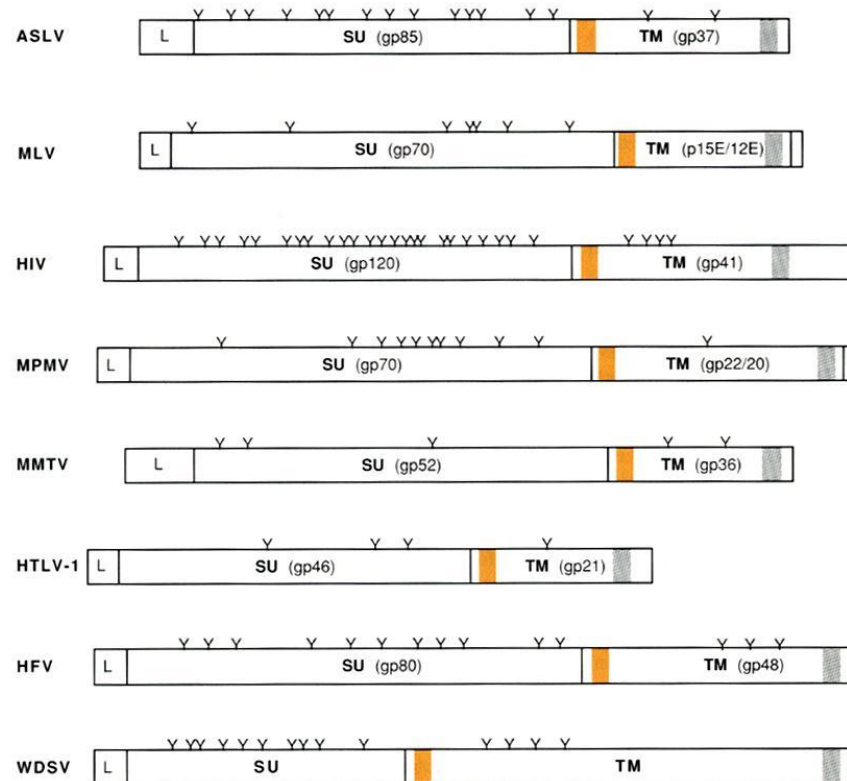


**Genomes of acute transforming retroviruses.** Oncogenes are indicated in color. Rous sarcoma virus is replication-competent because it contains all structural genes; however, most transducing retroviruses have deletions of structural genes and often express their oncogenes as fusion proteins.

# SINGLE-CELL REPRODUCTIVE CYCLE OF MuLV



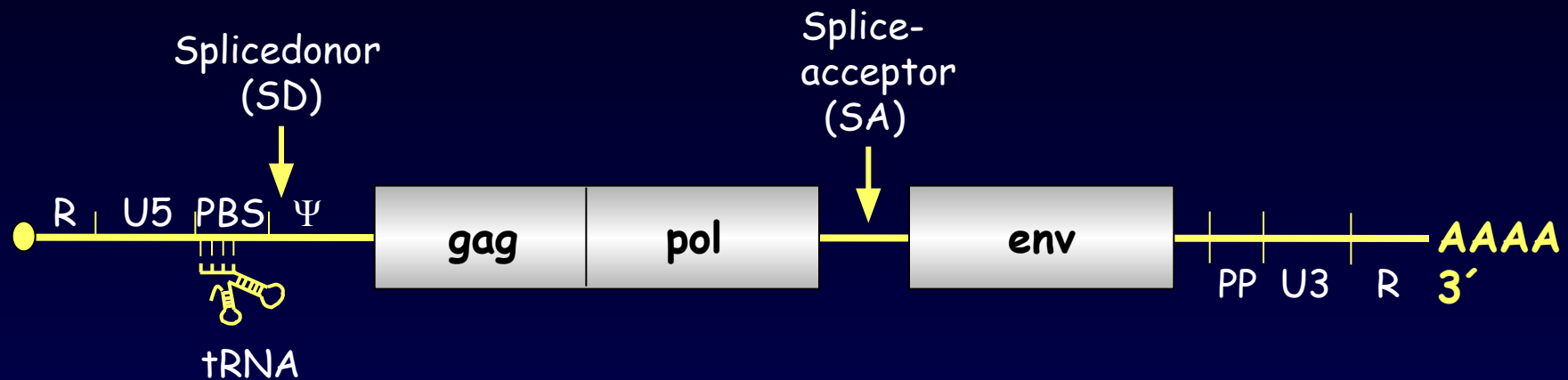
# Организация белков оболочки различных ретровирусов



Organization of Env proteins. Schematic representations of Env proteins are drawn for examples from each retroviral genus. The sequences representing the mature proteins SU and TM are indicated, along with the alternative older naming of these proteins based on their approximate molecular weights. Vertical lines, separating the leader peptide (L) and SU and separating SU and TM, mark cleavage sites for cellular proteases. Vertical lines mark cleavage sites for the viral protease near the carboxyl terminus of TM in MLV and M-PMV. The hydrophobic fusion domain of TM is shown in color and the membrane anchor is shown in gray. The Ys indicate sites of predicted N-linked carbohydrate addition.

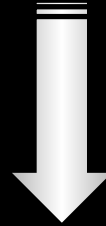
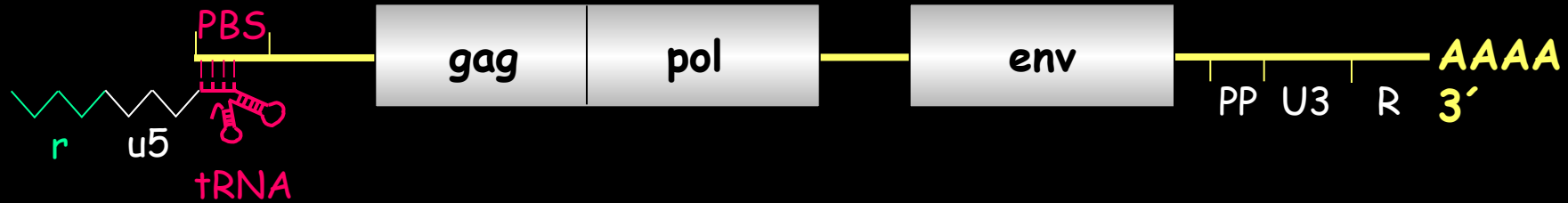
# The process of reverse transcription

## RNA-genome of an infectious retrovirus



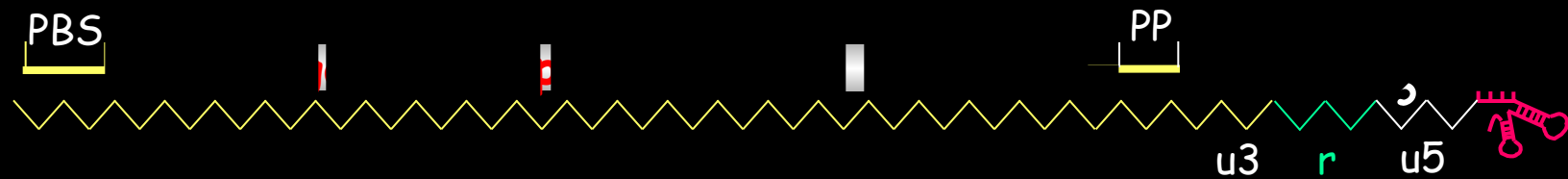


# The process of reverse transcription



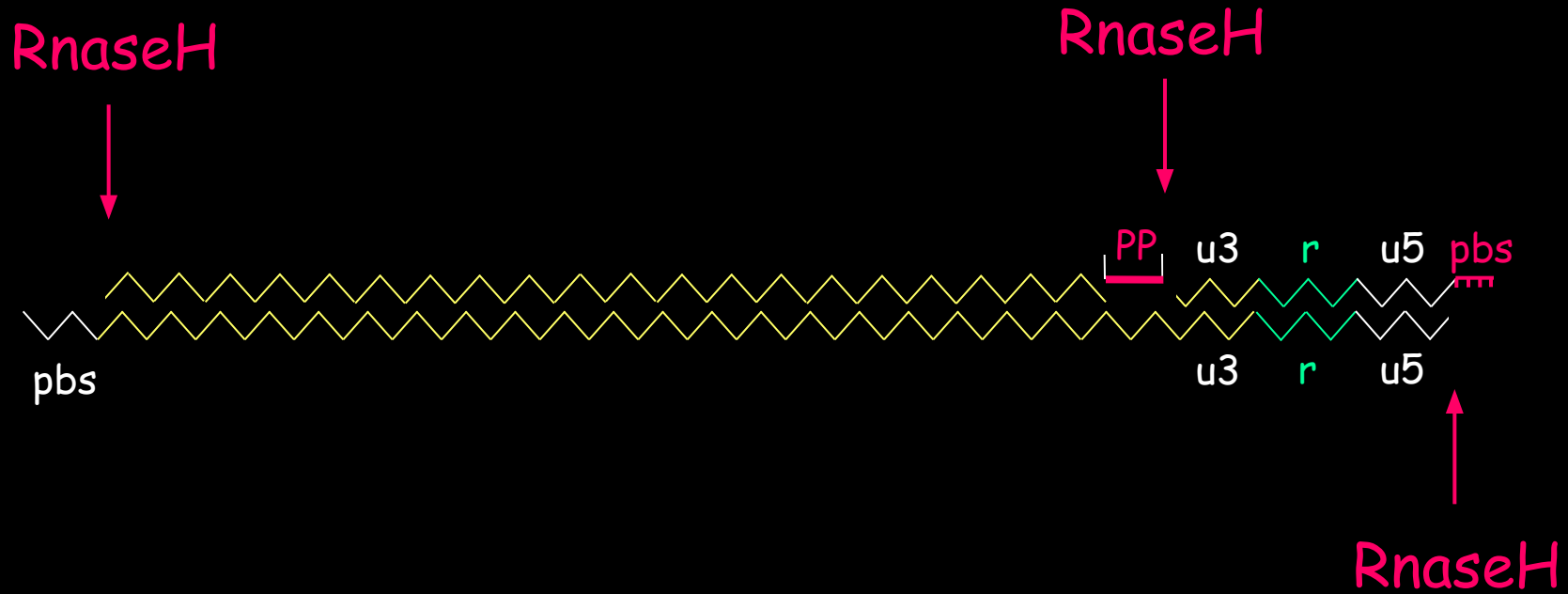
**first strand** exchange (inter- or intramolecular)

# The process of reverse transcription

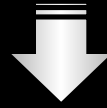
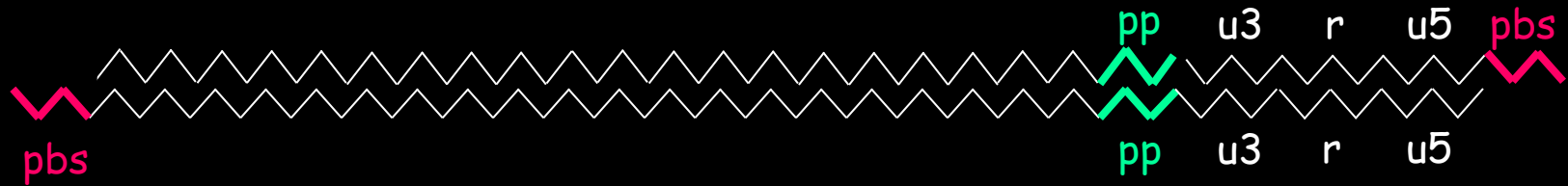


strand displacement synthesis

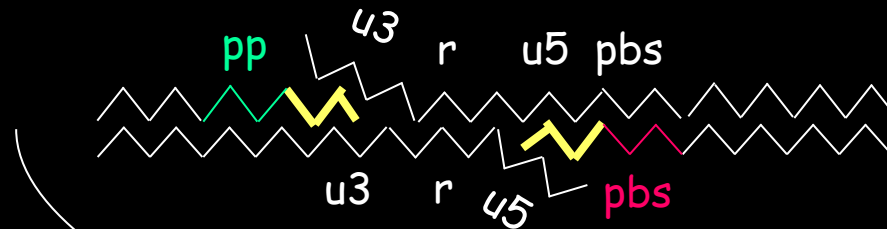
# The process of reverse transcription



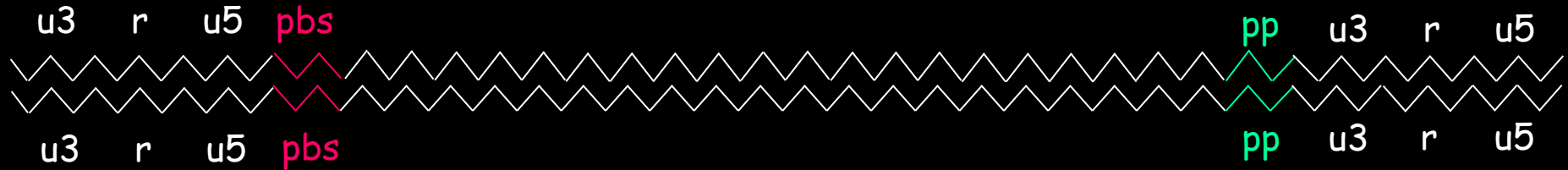
# The process of reverse transcription



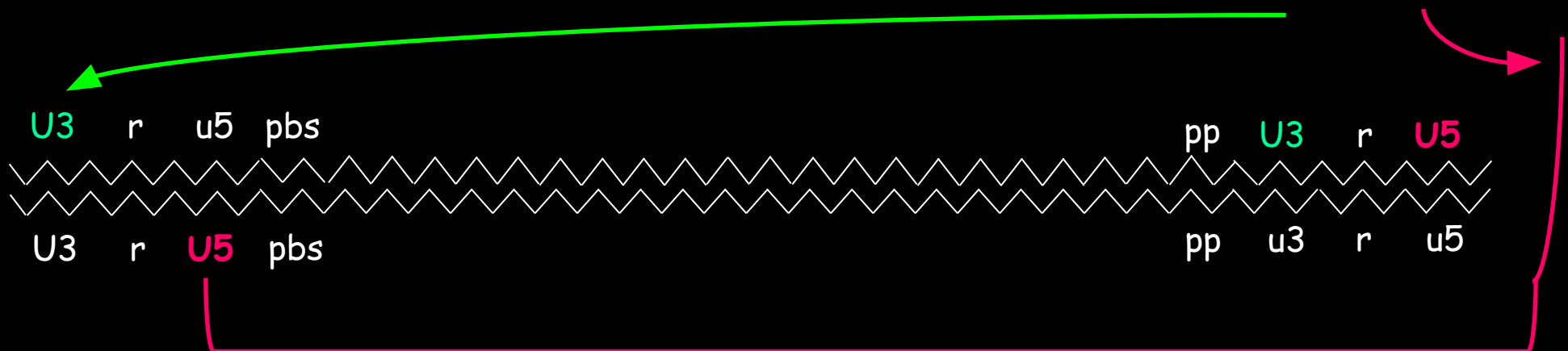
second template exchange (intramolecular)



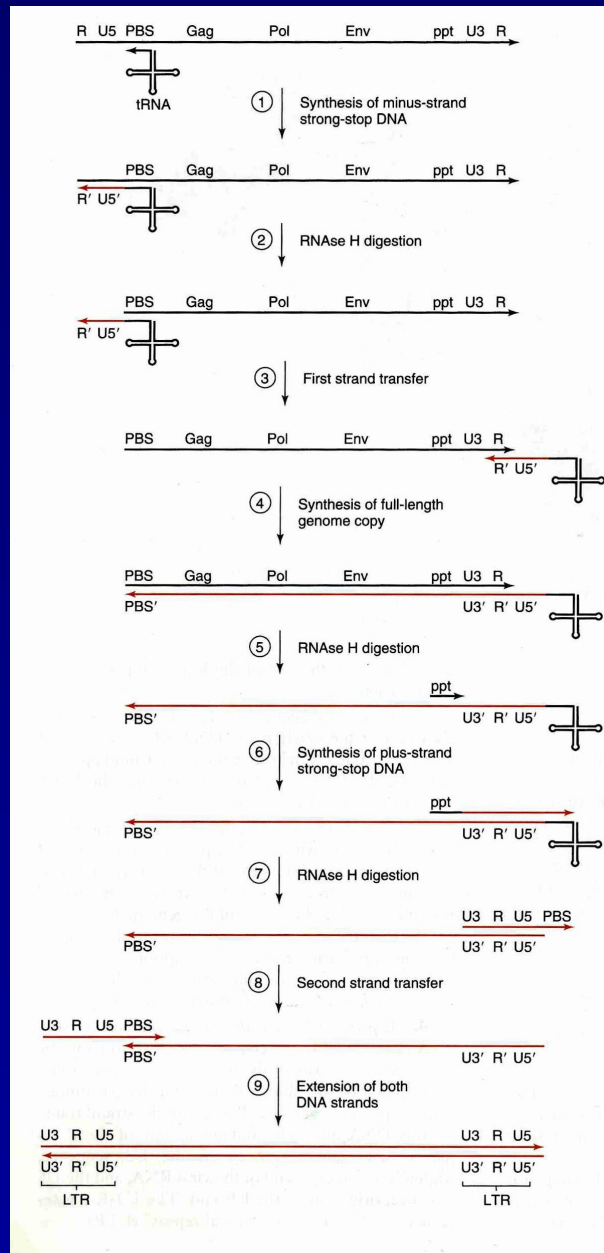
# The process of reverse transcription



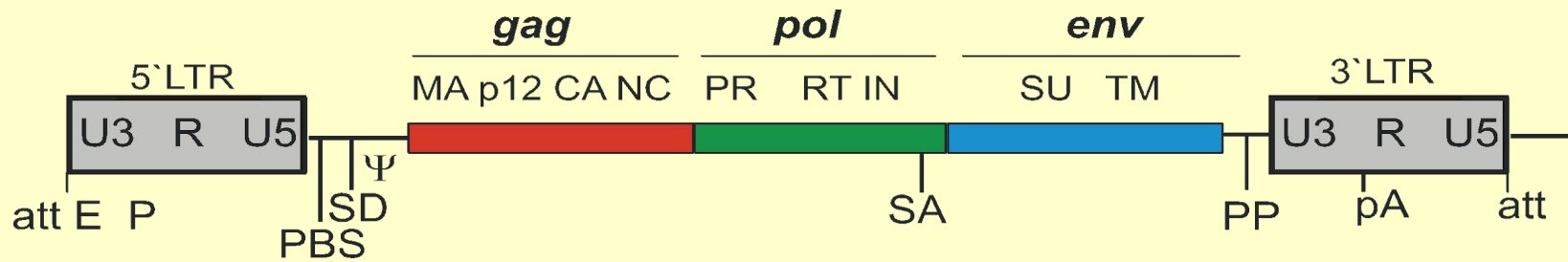
who was copied to where ?



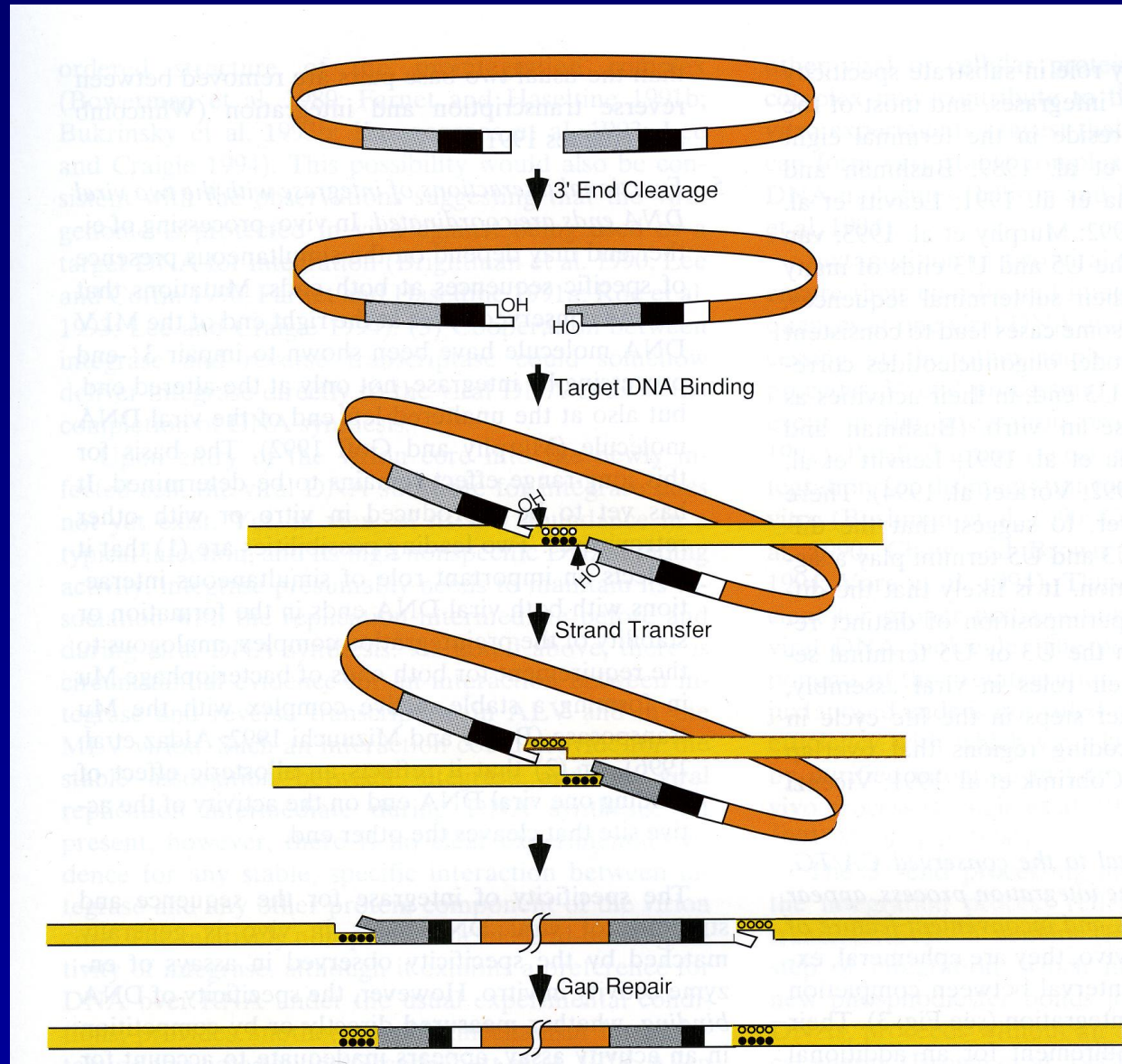
# Схема обратной транскрипции



## Genomic organization of murine retroviruses

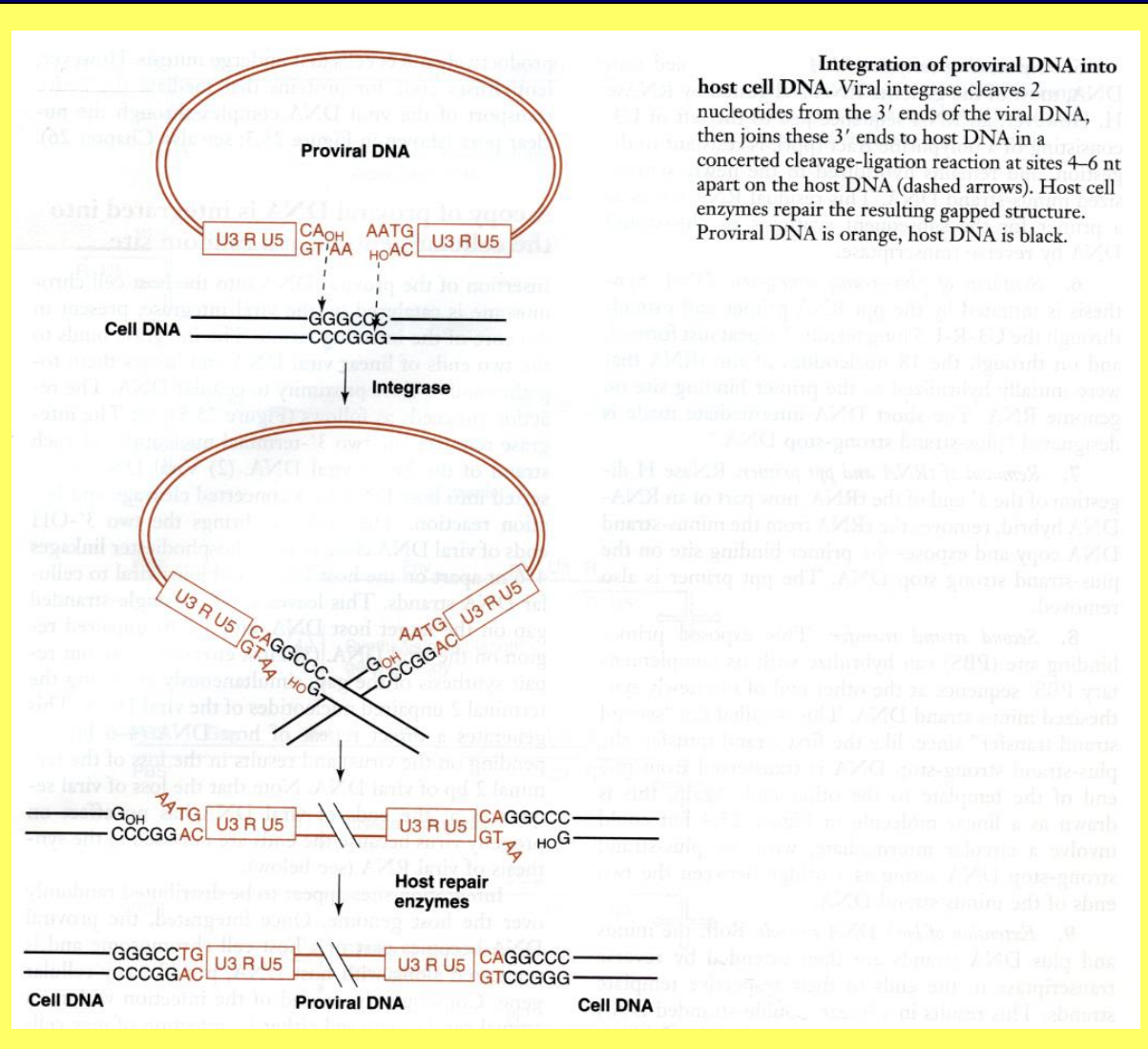


# Схема интеграции ДНК провируса в геном клетки хозяина

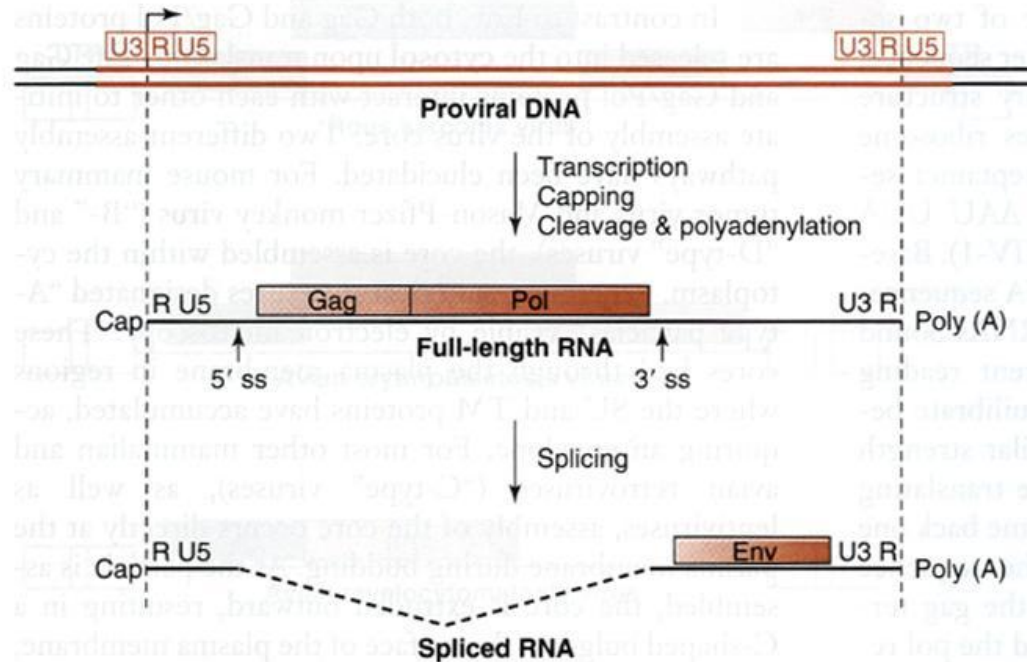




# Схема интеграции провируса в геномную ДНК клетки-хозяина



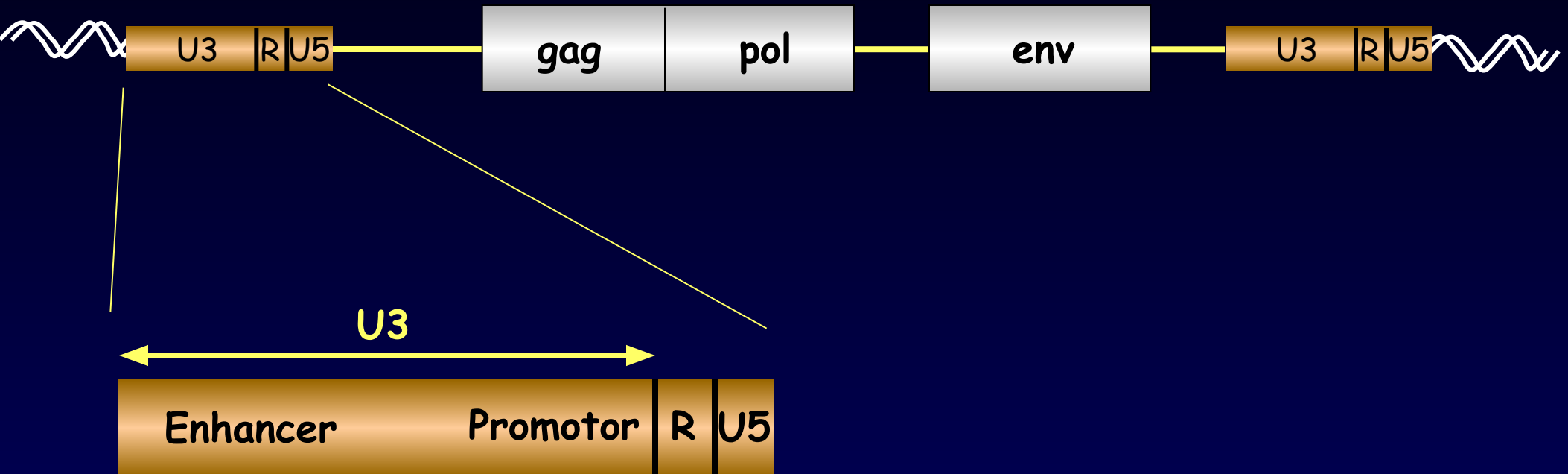
# Образование молекул РНК простого ретровируса



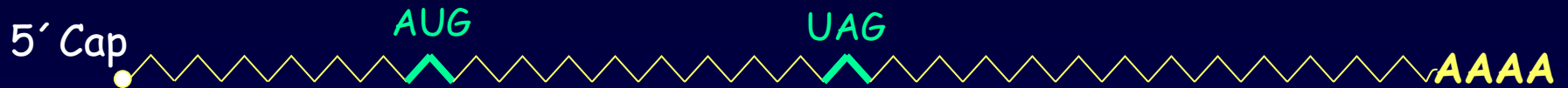
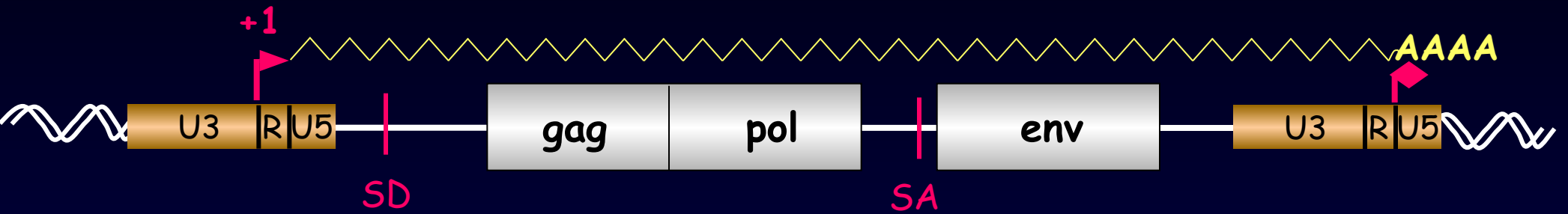
**Production of retrovirus RNAs.** Transcription initiates at junction of U3 and R, and RNA is cleaved and polyadenylated at junction of R and U5. Full-length RNA serves as mRNA for Gag and Gag/Pol and is packaged in virions. A fraction of genome-length RNA is spliced, removing the Gag/Pol reading frames, and serves as mRNA for Env.

# Transcriptional control of MoMLV

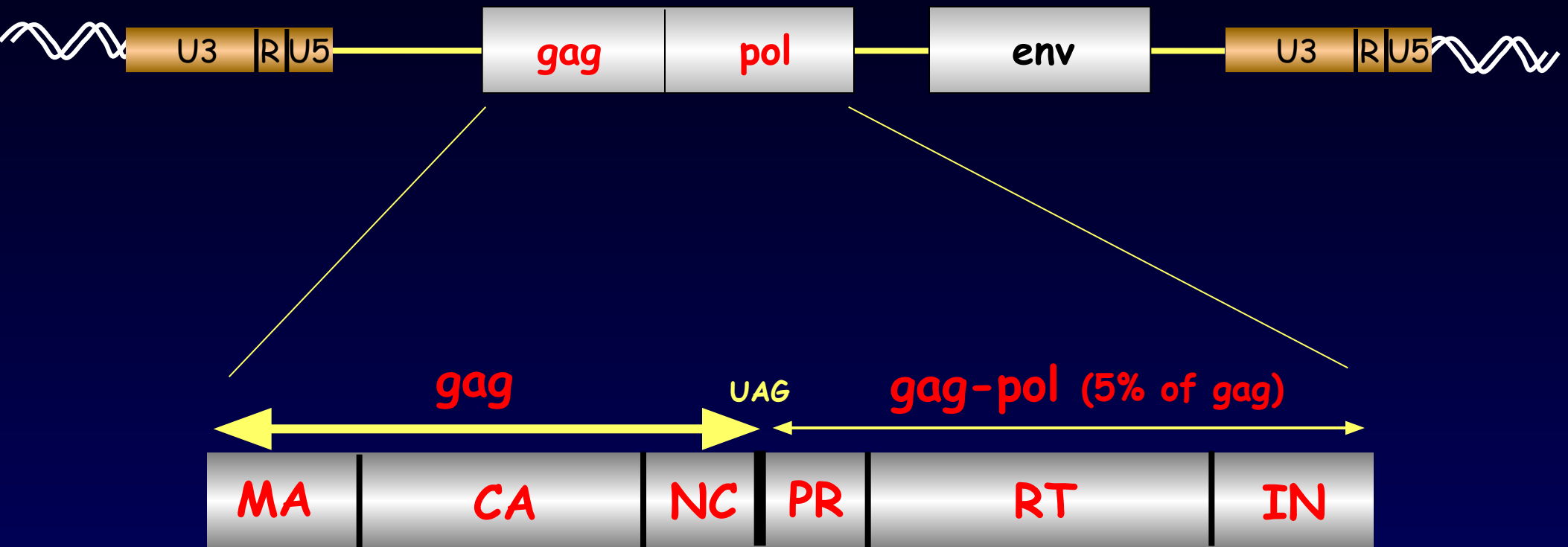
integrated provirus  
(Moloney Murine Leukemia Virus, MoMLV)



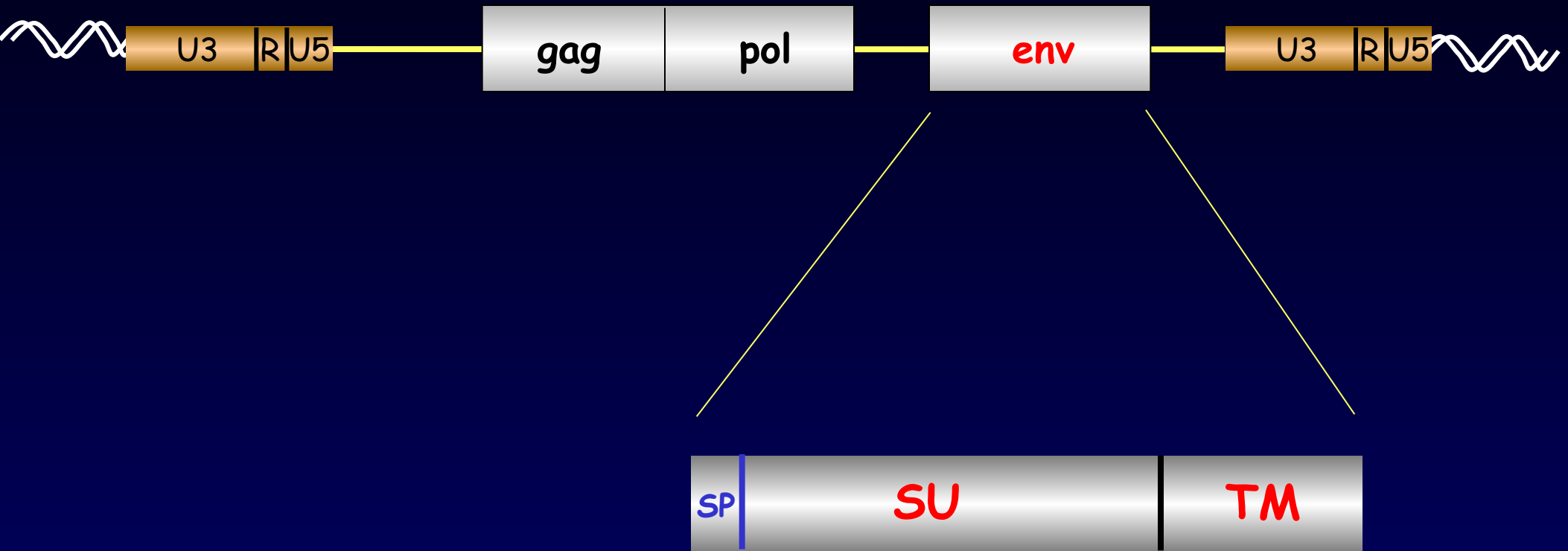
# Gene expression of simple retroviruses



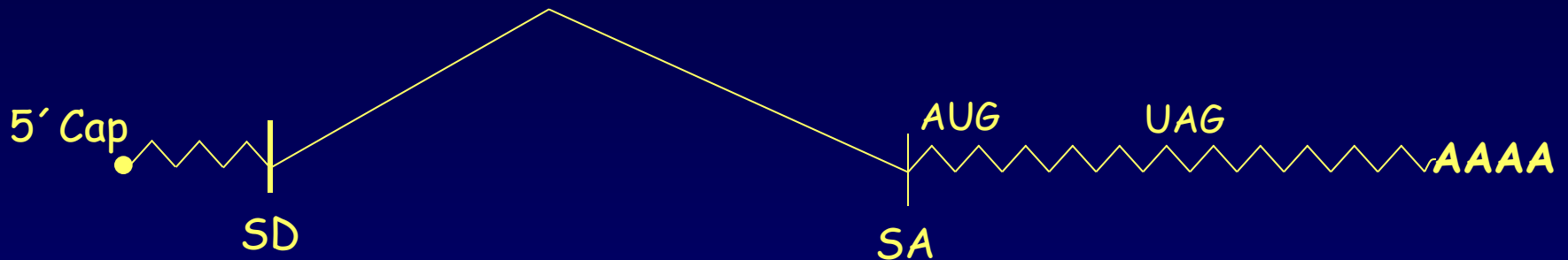
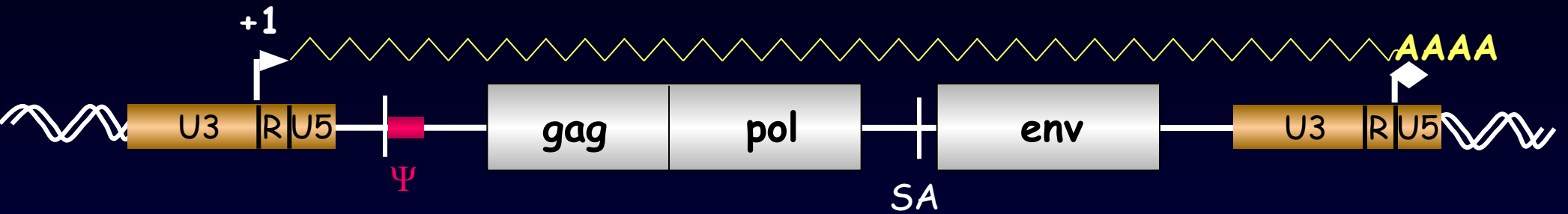
# gag-pol region of simple retroviruses



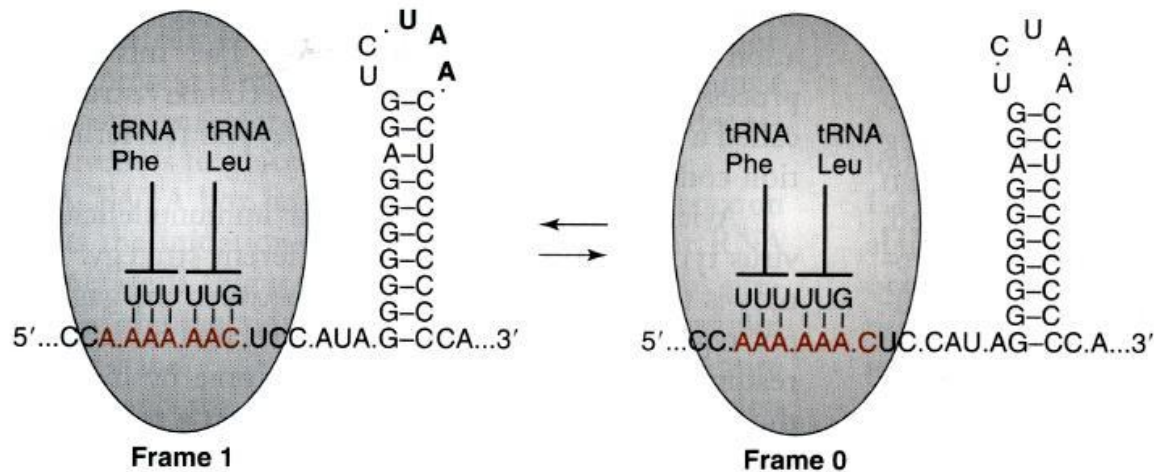
# env-region of retroviruses



# Packaging signal of retroviruses



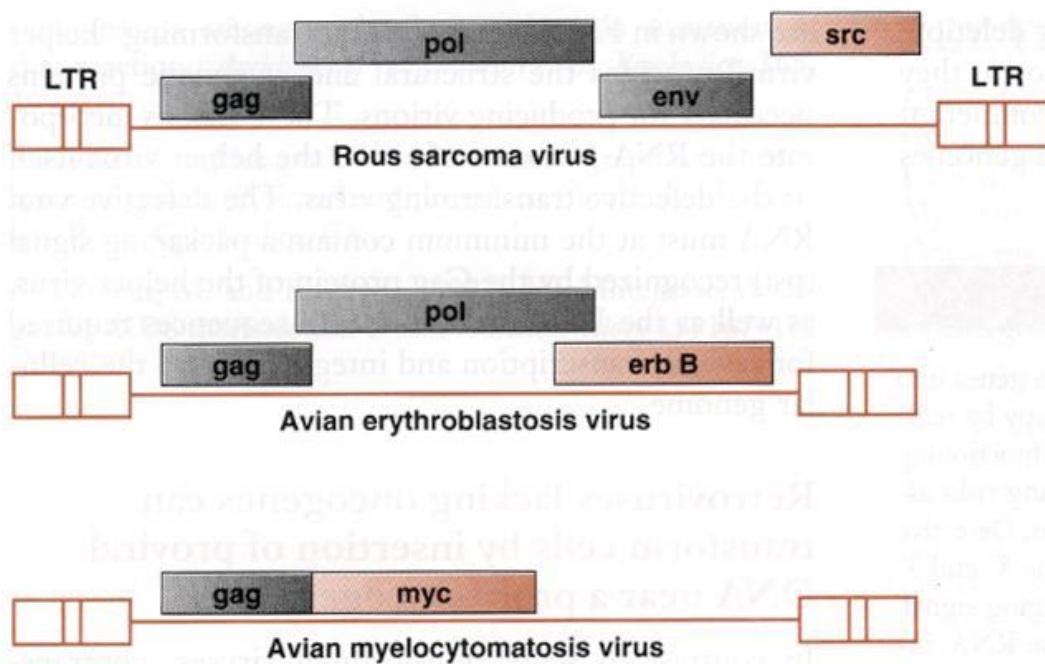
# Смена рамки считывания, реализуемая при трансляции РНК



**Ribosome frameshifting.** A base-paired RNA stem induces stalling upstream by the translating ribosome (orange ellipse) at the heptamer sequence. While stalled, the ribosome can shift from reading frame 1 to frame 0. Translation in frame 0 does not recognize the UAA termination codon (bold letters).



# Геномная организация РНК трансформирующих вирусов



**Genomes of acute transforming retroviruses.** Oncogenes are indicated in color. Rous sarcoma virus is replication-competent because it contains all structural genes; however, most transducing retroviruses have deletions of structural genes and often express their oncogenes as fusion proteins.

# Лентивирусы – возбудители медленных инфекций

## *Lentivirus*

From Latin *lentis* (slow), for slow progression of disease

### VIRION

Spherical enveloped particle.  
Diameter 100 nm.  
Conical capsid.

### GENOME

Linear ss RNA, positive sense.  
Two identical segments, each 9.3 Kb.  
Cellular tRNA<sup>lys3</sup> molecules packaged in virions used as primers for reverse transcription.

### GENES AND PROTEINS

Four capsid proteins: MA (p17), CA (p24), NC (p7), p6.  
Three enzymes: PR (p10), RT (p51/66), IN (p32).  
Two envelope proteins: SU (gp120) and TM (gp41).  
Six regulatory proteins: Vif, Vpu, Vpr, Tat, Rev, Nef.

### VIRUSES AND HOSTS

Human immunodeficiency virus types 1 and 2 (HIV-1, HIV-2).  
Simian immunodeficiency virus.  
Equine, bovine, feline immunodeficiency viruses.

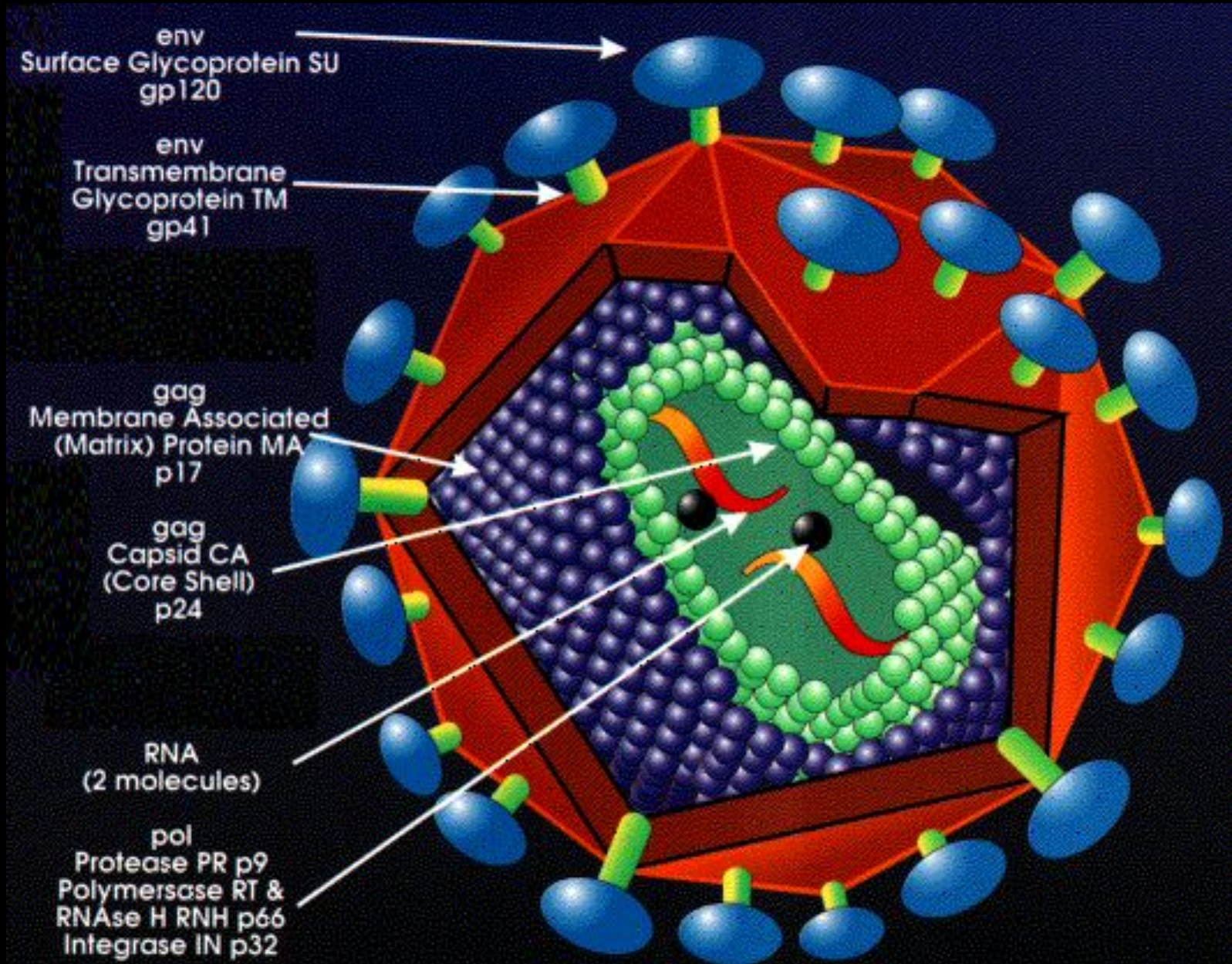
### DISEASES

Acquired immune deficiency syndrome (AIDS) first described in 1981.  
A major global pandemic today (more than 30 million people infected).  
HIV replicates in and kills lymphocytes and macrophages.  
Results in depletion of CD4+ T cells to render host immune-incompetent.  
As a result, opportunistic infections by other pathogens are often fatal.  
HIV is transmitted through sexual contact and blood exchange.

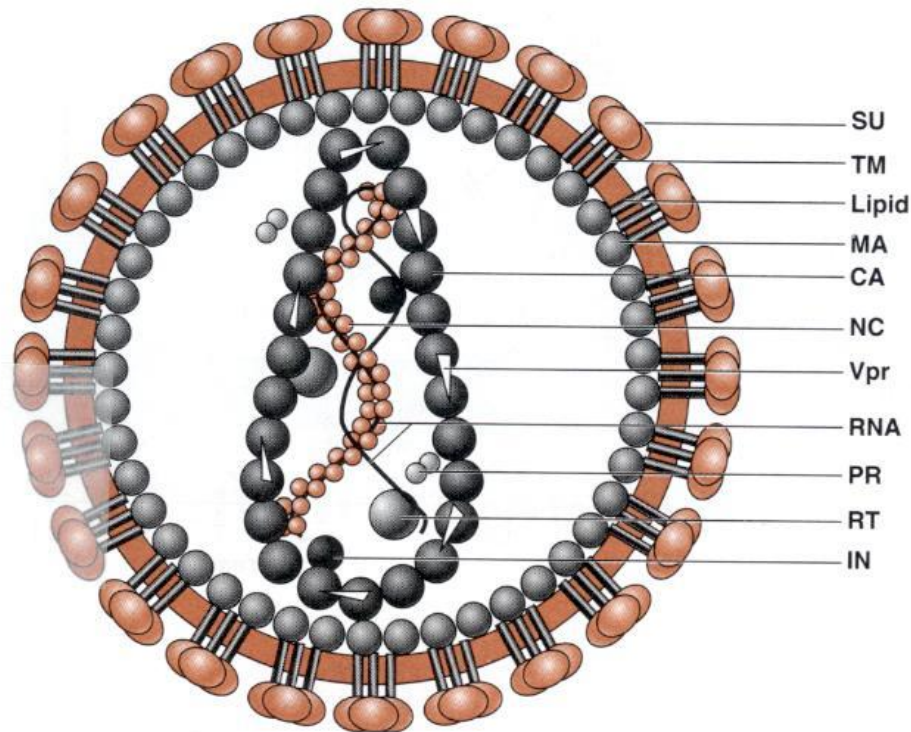
### DISTINCTIVE CHARACTERISTICS

Proviral DNA can enter nucleus without requirement for cell division.  
Lentiviruses make a complex set of singly and doubly spliced mRNAs.

# morphology of a retrovirus (HIV1, a *lentivirus*)

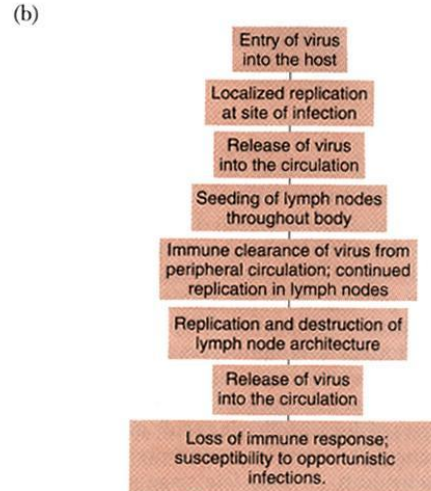
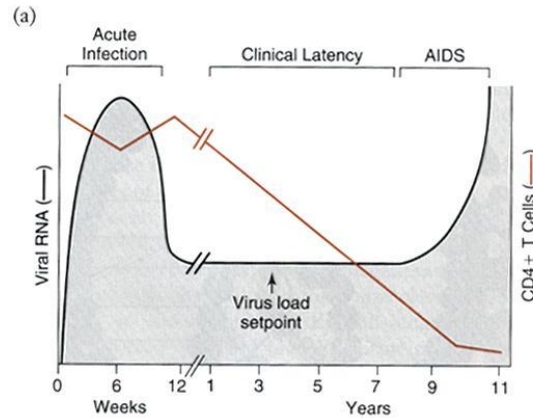


# Схематическое изображение вирусной частицы HIV-1



**Diagram of structure of HIV-1 virion.** Components of the virion are labeled; see Table 26.2. For the sake of clarity, only one of the two genome RNA molecules is shown covered by NC protein.

# Этапы развития СПИДа



## Events associated with progression to AIDS.

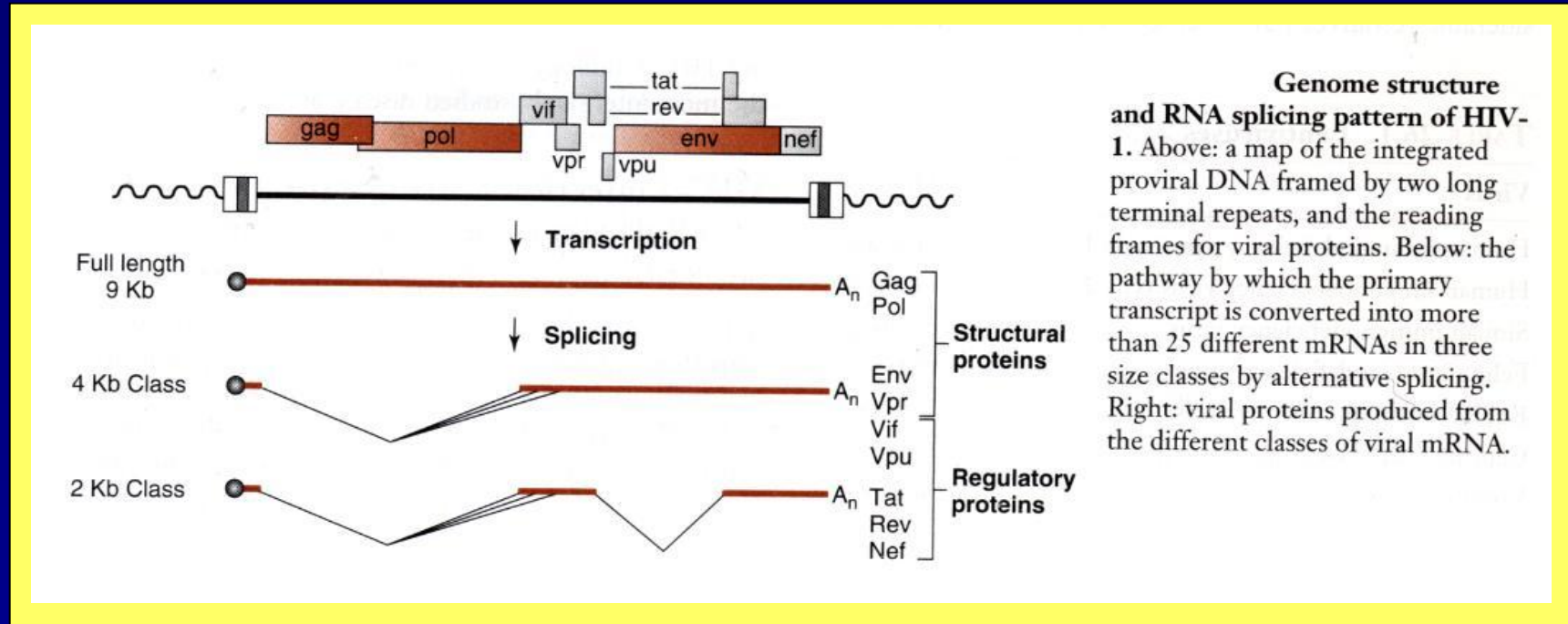
(a) Changes in viral RNA and CD4-positive T cell levels in the peripheral circulation from acute infection to end-stage disease. (b) Stages of HIV-1 infection.

# Лентивирусы млекопитающих

## Lentiviruses

<b>Virus</b>	<b>Host</b>
Human immunodeficiency virus type 1	Humans
Human immunodeficiency virus type 2	Humans
Simian immunodeficiency virus	Monkeys
Feline immunodeficiency virus	Cats
Equine infectious anemia virus	Horses
Caprine arthritis-encephalitis virus	Goats
Visna-maedi virus	Sheep

# Структура генома и схема сплайсинга РНК HIV-1



# Структурные белки HIV-1

## HIV-1 structural proteins

Name	Abbreviation	M. Wt.
Matrix	MA	p17
Capsid	CA	p24
Nucleocapsid	NC	p7
Protease	PR	p14
Reverse transcriptase	RT	p66/51
Integrase	IN	p32
Surface protein	SU	gp120
Transmembrane protein	TM	gp41
Virion protein R	Vpr	p15

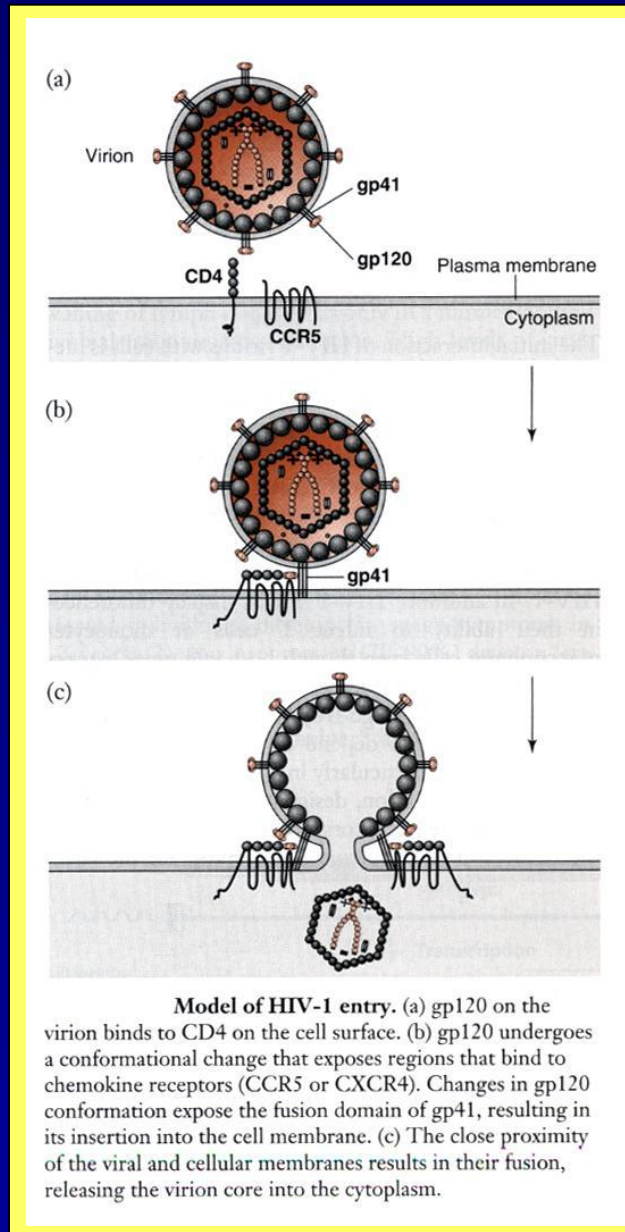


# Неструктурные белки HIV-1

## HIV-1 nonstructural proteins

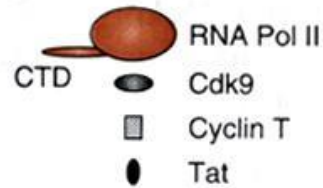
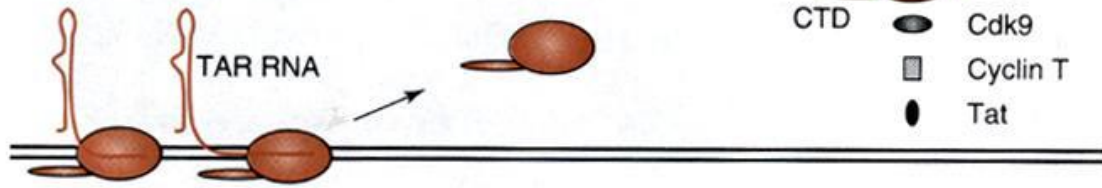
Name	Abbreviation	M. Wt.
Viral infectivity factor	Vif	p23
Virion protein unique to HIV-1	Vpu	p16
Transactivator of transcription	Tat	p15
Regulator of expression of virion proteins	Rev	p19
Negative effector	Nef	p27

# Проникновение НІV-1 в клетку

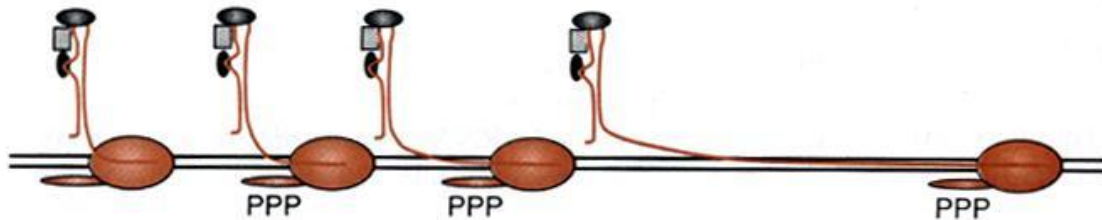


# Что делает белок Tat

(a) No Tat



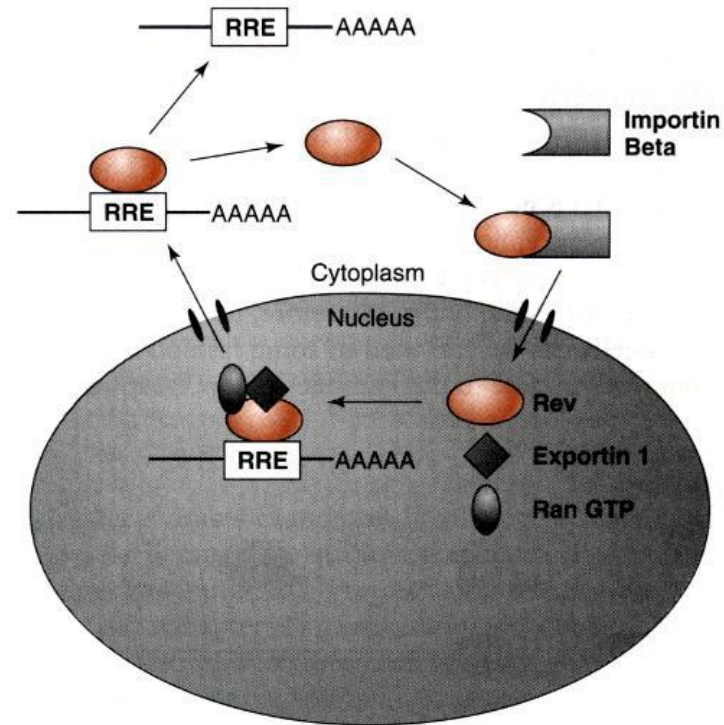
(b) Tat



## Mechanism of Tat

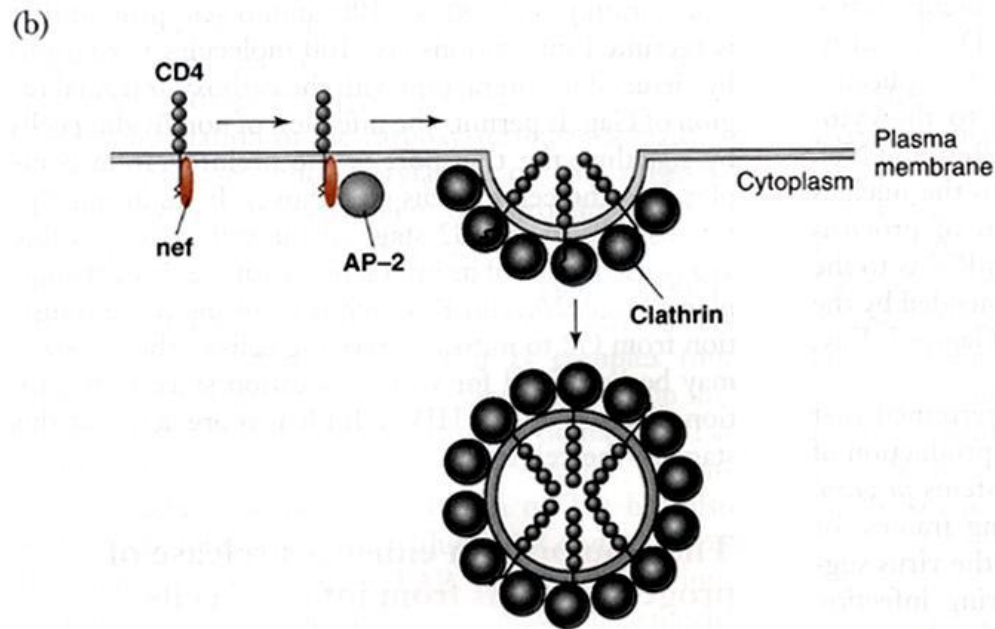
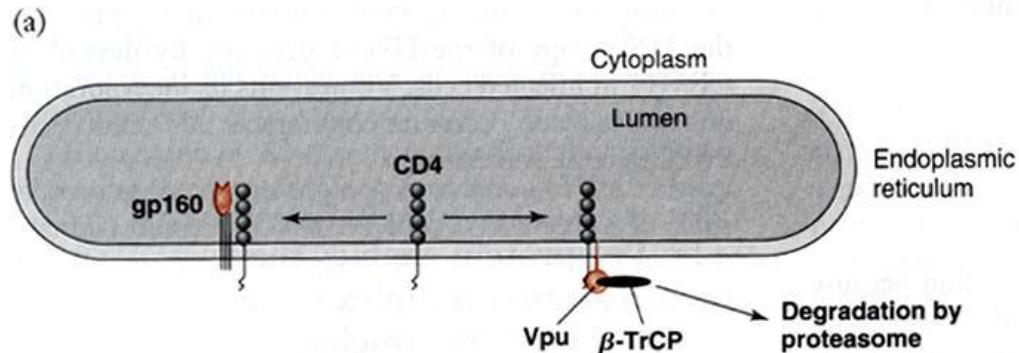
**function.** (a) In the absence of Tat, RNA polymerase II molecules that initiate transcription at the HIV-1 promoter lack processivity and most are released from the template before reaching the polyadenylation site. (b) Tat recruits cyclin T and cyclin-dependent kinase-9 (Cdk9) to the transcription complex shortly after initiation, resulting in hyperphosphorylation (P) of the carboxy-terminal domain of RNA polymerase II. This increases processivity of the polymerase and generates increased levels of full-length transcripts. CTD: carboxy terminal domain of RNA polymerase II.

# Зачем нужен белок Rev



**Mechanism of Rev function.** Rev is transported into the nucleus via nuclear pores by direct interaction with importin beta. Within the nucleus, Rev binds to the Rev-responsive element (RRE) in viral RNAs, and recruits exportin 1 and Ran GTP to generate a complex capable of exporting the RNA to the cytoplasm. The complex dissociates in the cytoplasm, releasing the mRNA and allowing Rev to return to the nucleus and repeat the cycle.

# Модуляция экспрессии поверхностного антигена CD4



**Down-regulation of CD4 expression.** (a) In the absence of Vpu, CD4 interacts with gp160 in the endoplasmic reticulum, resulting in formation of a complex that blocks transport to the cell surface. Vpu binds to the cytoplasmic domain of CD4 and through interaction with  $\beta$ -TrCP directs CD4 to the proteasome degradation pathway. (b) Nef binds to the cytoplasmic tail of CD4 at the inner surface of the plasma membrane, and via interaction with AP-2 increases uptake of CD4 into clathrin-coated pits, lowering its concentration on the cell surface.

# Функции вирусных белков

1. Белок Tat увеличивает транскрипцию провирусного генома HIV-1, стимулируя элонгаторную активность РНК-полимеразы II.
2. Белок Rev1 способствует транспорту в цитоплазму вирусных информационных РНК, кодирующих структурные белки HIV-1. Tat и Rev белки в значительной степени стимулируют синтез вирусных белков.
3. Белок Vif увеличивает инфекционность вируса HIV-1, взаимодействуя с клеточной дезоксицитидин дезаминазой. (*Vif, присоединяясь к клеточной дезаминазе CEM15 индуцируя убиквитинизацию и последующую деградацию этого фермента протеосомами*).
4. Белок Vpr важен для переноса преинтеграционного комплекса HIV-1 из цитоплазмы в ядро.
5. Белок Vpr усиливает выход вирусного потомства из заражённой клетки.
6. Белок Nef – важный медиатор патогенеза (Nef снижает уровень экспрессии белков CD4 и МНС на поверхности клетки. Влияет на инфекционность вируса и изменяет сигнальные пути: взаимодействие Nef с src-родственными киназами lyn и hck, приводит к их активации. А взаимодействие Nef с lyp и fyn приводит к их подавлению. Активация hck приводит к увеличению экспрессии в Т клетках ряда цитокинов и хемокинов, что в свою очередь увеличивает репликацию HIV-1 и привлекает больше Т-клеток к очагу заражения)

# Вирус Т-клеточного лейкоза 1 типа человека

## *Deltaretrovirus*

From Greek letter *delta*

### VIRION

Spherical enveloped particle.

Diameter 100 nm.

Icosahedral capsid.

### GENOME

Linear ssRNA, positive sense.

Two identical segments, each 8.7 Kb.

Cellular tRNA<sup>pro</sup> molecules packaged in virions used as primers for reverse transcription.

### GENES AND PROTEINS

Gag, Pol, and Env proteins packaged in virion.

Also codes for regulatory proteins Rex, Tax, p12<sup>I</sup>, p13, and p30.

### VIRUSES AND HOSTS

A human retrovirus, member of genus *Deltaretrovirus*.

Related viruses: bovine leukemia virus, simian T-lymphotropic viruses.

### DISEASES

Adult T-cell leukemia, which affects CD4<sup>+</sup> T cells.

Tropical spastic paraparesis, a neurological demyelinating disease.

### DISTINCTIVE CHARACTERISTICS

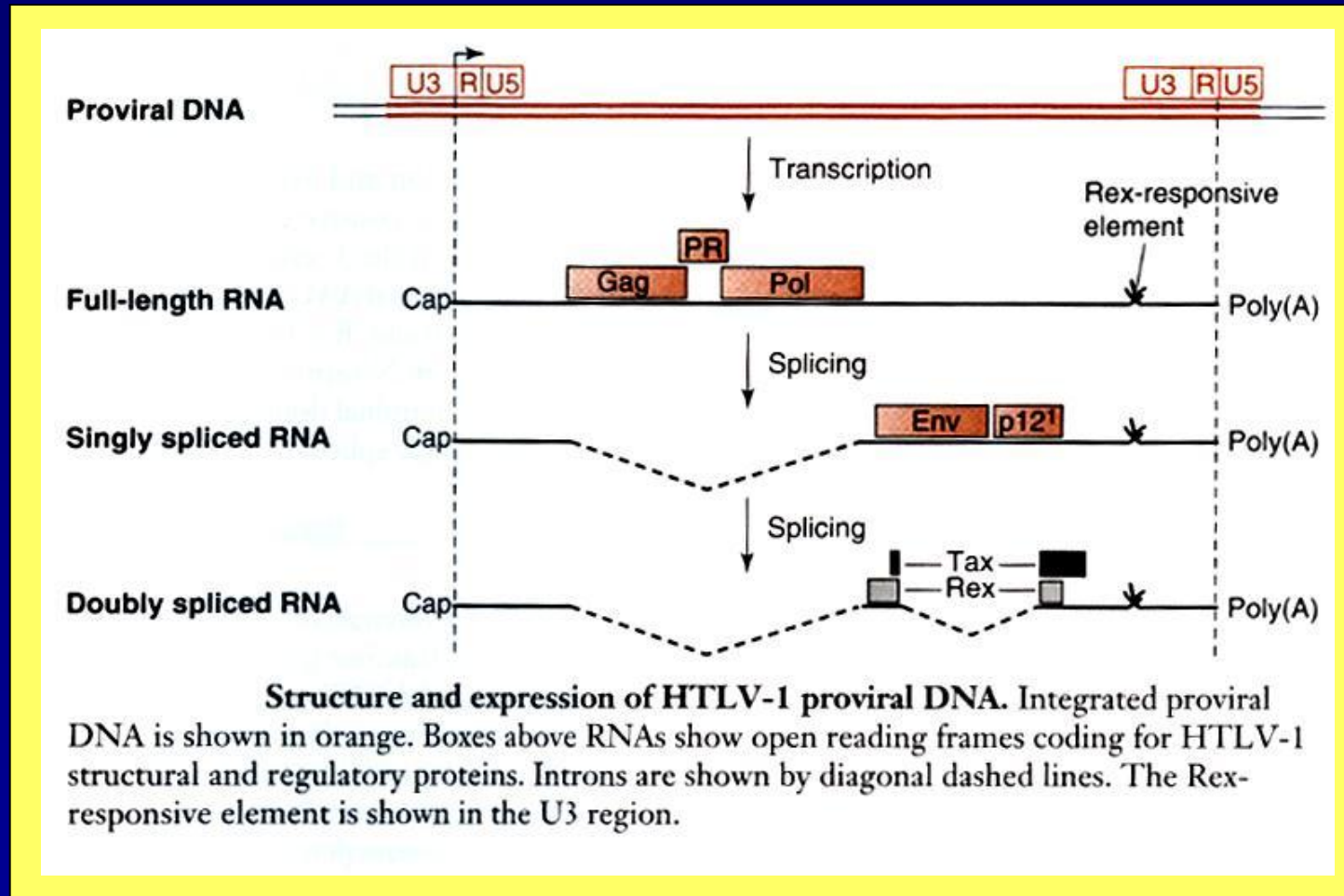
Infects human T lymphocytes.

Tax protein regulates gene expression and leads to oncogenic transformation.

Causes T-cell proliferation and transformation by multiple pathways.

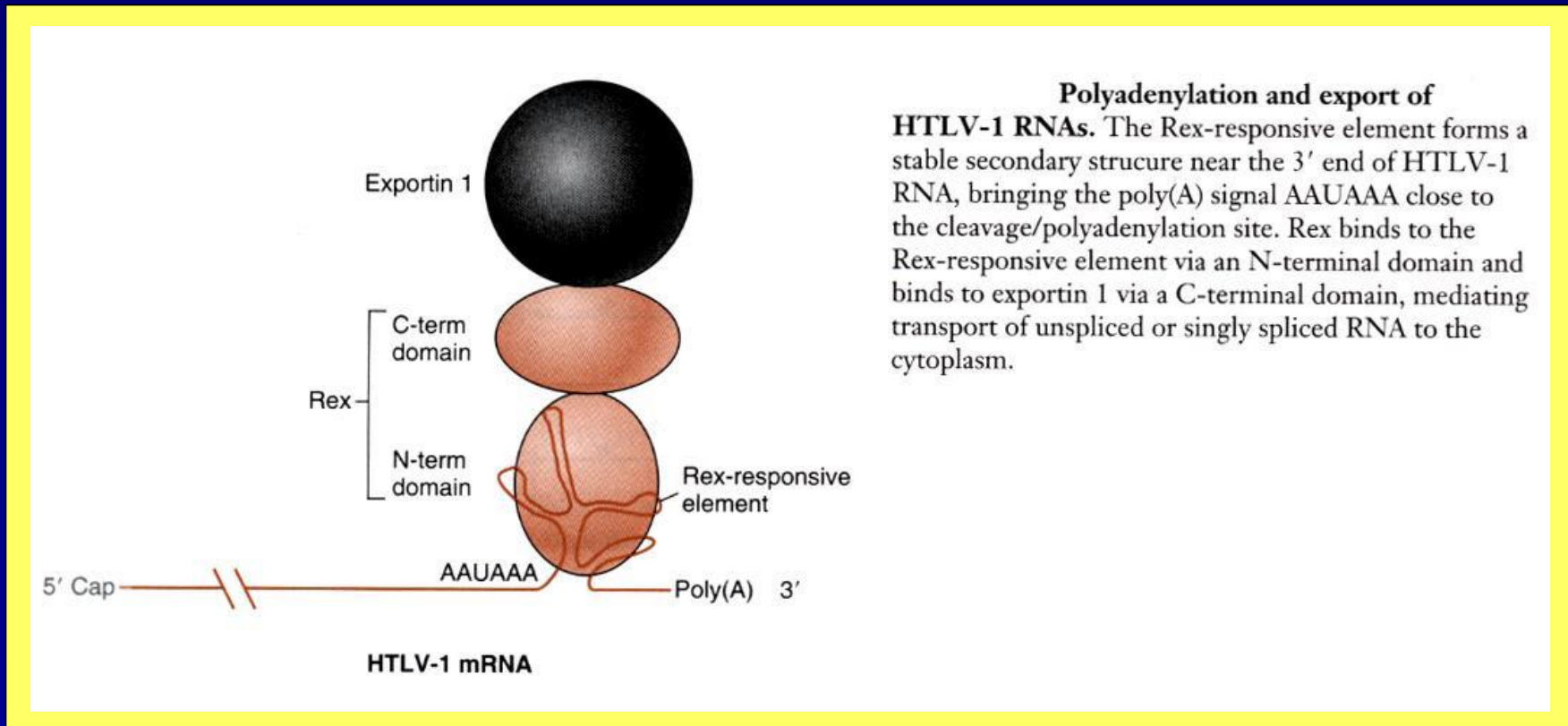
A human tumor virus.

# Синтез регуляторных белков HTLV-1 направляется дважды сплайсированными РНК

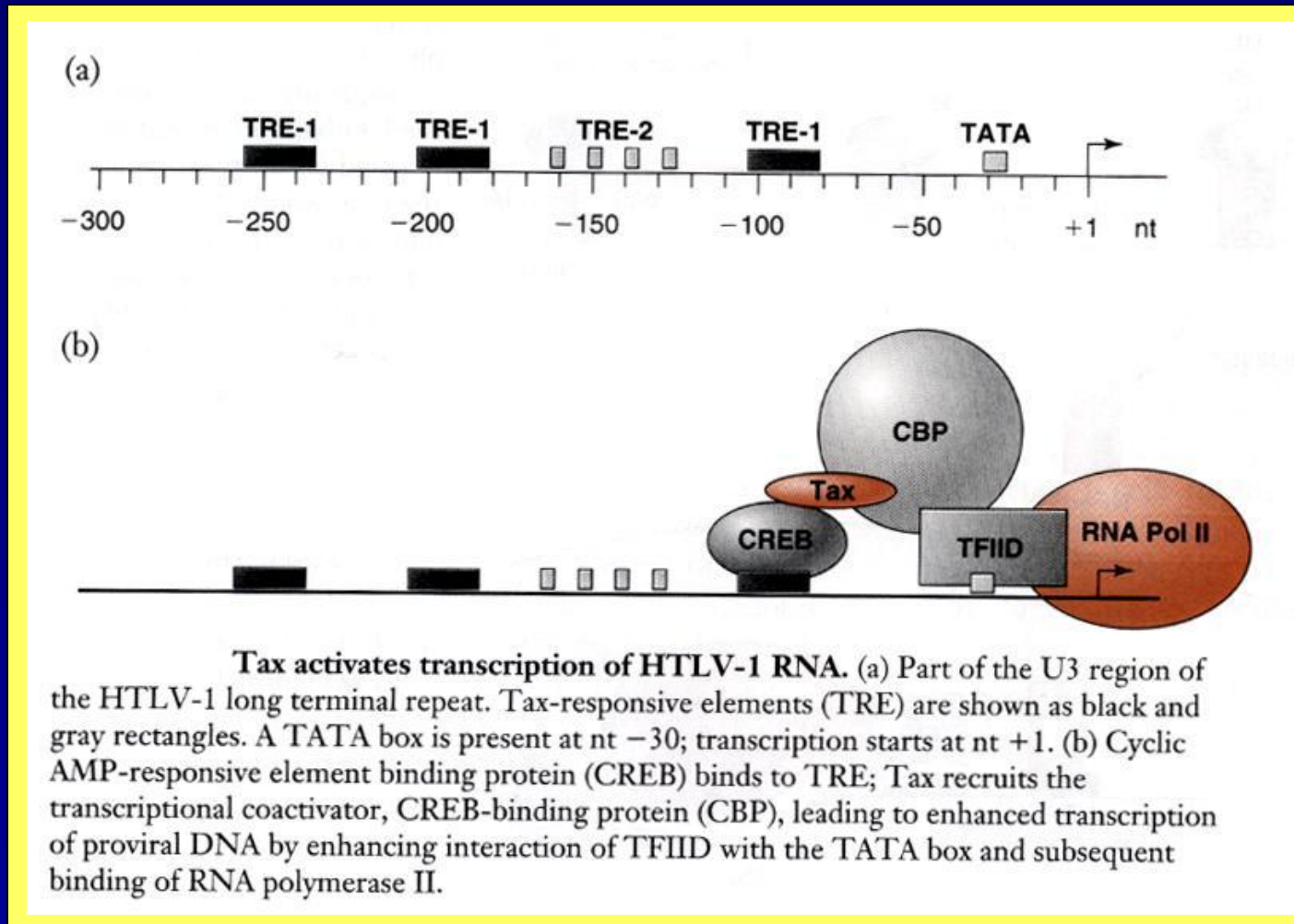




# Полиаденилирование и экспорт РНК HTLV-1



# Белок Tax активирует транскрипцию РНК HTLV-1



# Клеточные гены, активируемые вирусным белком Tax

## Activation of gene expression by HTLV-1 Tax

Genes induced by Tax	Examples	Transcription factor used <sup>a</sup>
Viral genes	HTLV-1 HIV-1 Cytomegalovirus	CREB/ATF NF- $\kappa$ B NF- $\kappa$ B
Cytokines and receptors	Interleukin-1, 2, 6, 8, 15 Tumor necrosis factor- $\alpha$ , $\beta$ Interferon- $\beta$ Interleukin-2 receptor $\alpha$ T-cell receptor MHC class I	NF- $\kappa$ B
Transcription factors and proto-oncogenes	c-fos, erg-1 fra-1 c-myc, c-rel, c-sis	SRF NF- $\kappa$ B EGR-1
Cell adhesion molecules	Endothelial leukocyte adhesion molecule-1 (ELAM-1) Intracellular cell adhesion molecule-1 (ICAM-1) Vimentin	NF- $\kappa$ B NF- $\kappa$ B ?
Acute phase proteins	Angiotensinogen Serum amyloid A precursor Complement factors	NF- $\kappa$ B

<sup>a</sup>CREB/ATF: cAMP responsive element binding/activation transcription factor; NF- $\kappa$ B: nuclear factor- $\kappa$ B; SRF: serum response factor; EGR-1: early growth response factor-1.

# Human T-Cell Leukemia Virus Type 1

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## *Deltaretrovirus*

From Greek letter *delta*

### VIRION

Spherical enveloped particle.

Diameter 100 nm.

Icosahedral capsid.

### GENOME

Linear ssRNA, positive sense.

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Also codes for regulatory proteins Rex, Tax, p12<sup>l</sup>, p13, and p30.

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A human retrovirus, member of genus *Deltaretrovirus*.

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### DISEASES

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Tropical spastic paraparesis, a neurological demyelinating disease.

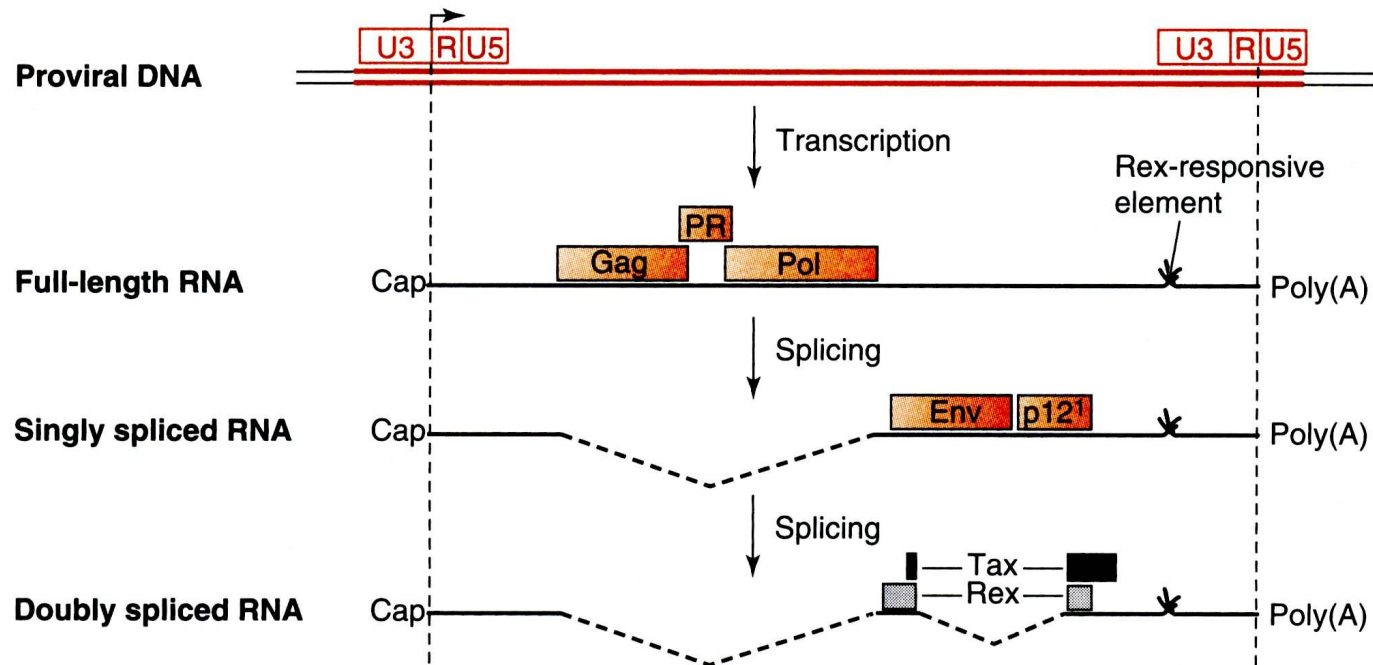
### DISTINCTIVE CHARACTERISTICS

Infects human T lymphocytes.

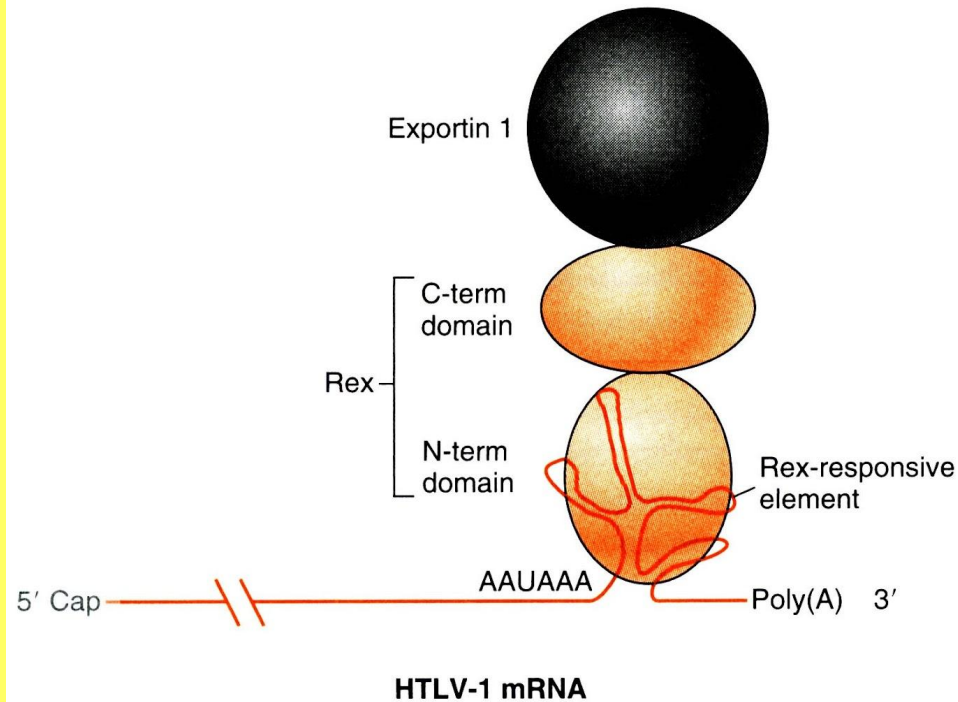
Tax protein regulates gene expression and leads to oncogenic transformation.

Causes T-cell proliferation and transformation by multiple pathways.

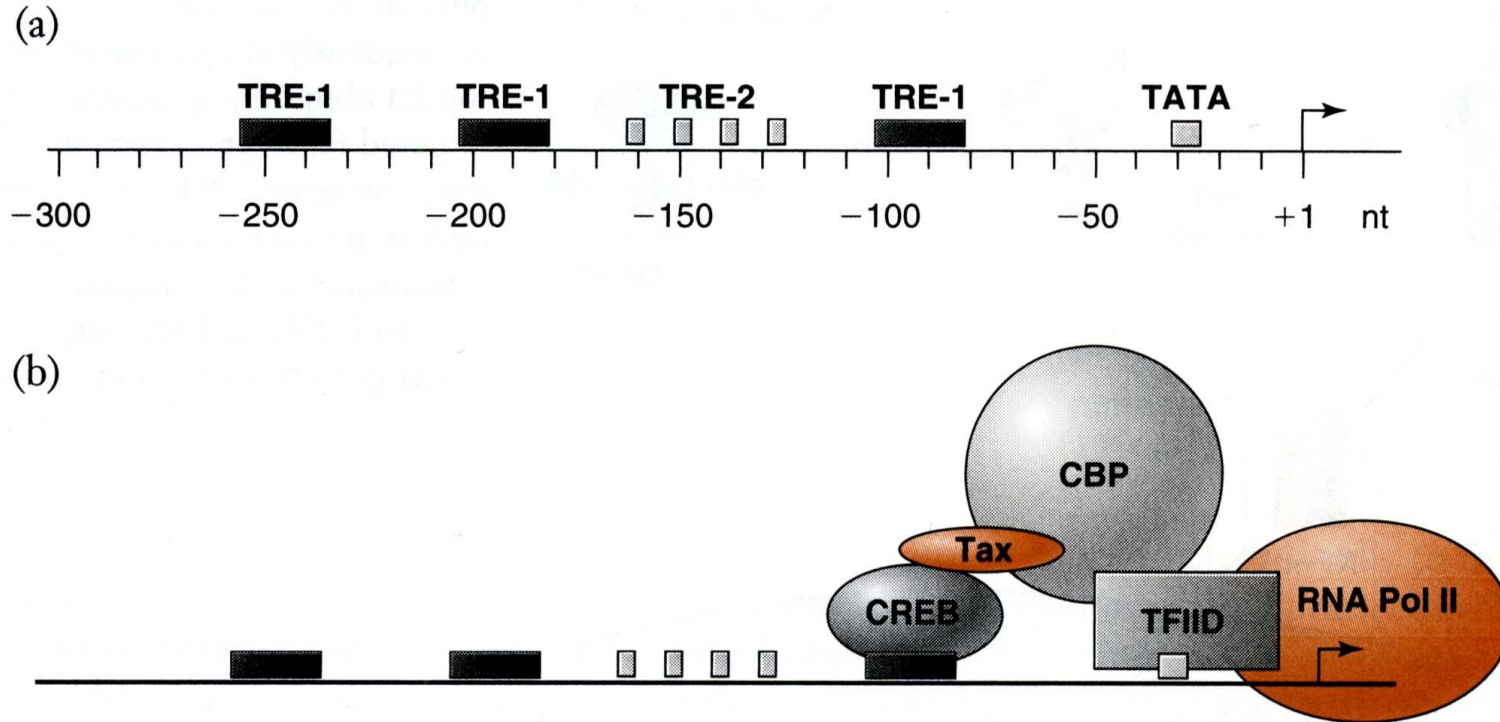
A human tumor virus.



**FIGURE 27.1 Structure and expression of HTLV-1 proviral DNA.** Integrated proviral DNA is shown in orange. Boxes above RNAs show open reading frames coding for HTLV-1 structural and regulatory proteins. Introns are shown by diagonal dashed lines. The Rex-responsive element is shown in the U3 region.



**FIGURE 27.2 Polyadenylation and export of HTLV-1 RNAs.** The Rex-responsive element forms a stable secondary structure near the 3' end of HTLV-1 RNA, bringing the poly(A) signal AAUAAA close to the cleavage/polyadenylation site. Rex binds to the Rex-responsive element via an N-terminal domain and binds to exportin 1 via a C-terminal domain, mediating transport of unspliced or singly spliced RNA to the cytoplasm.



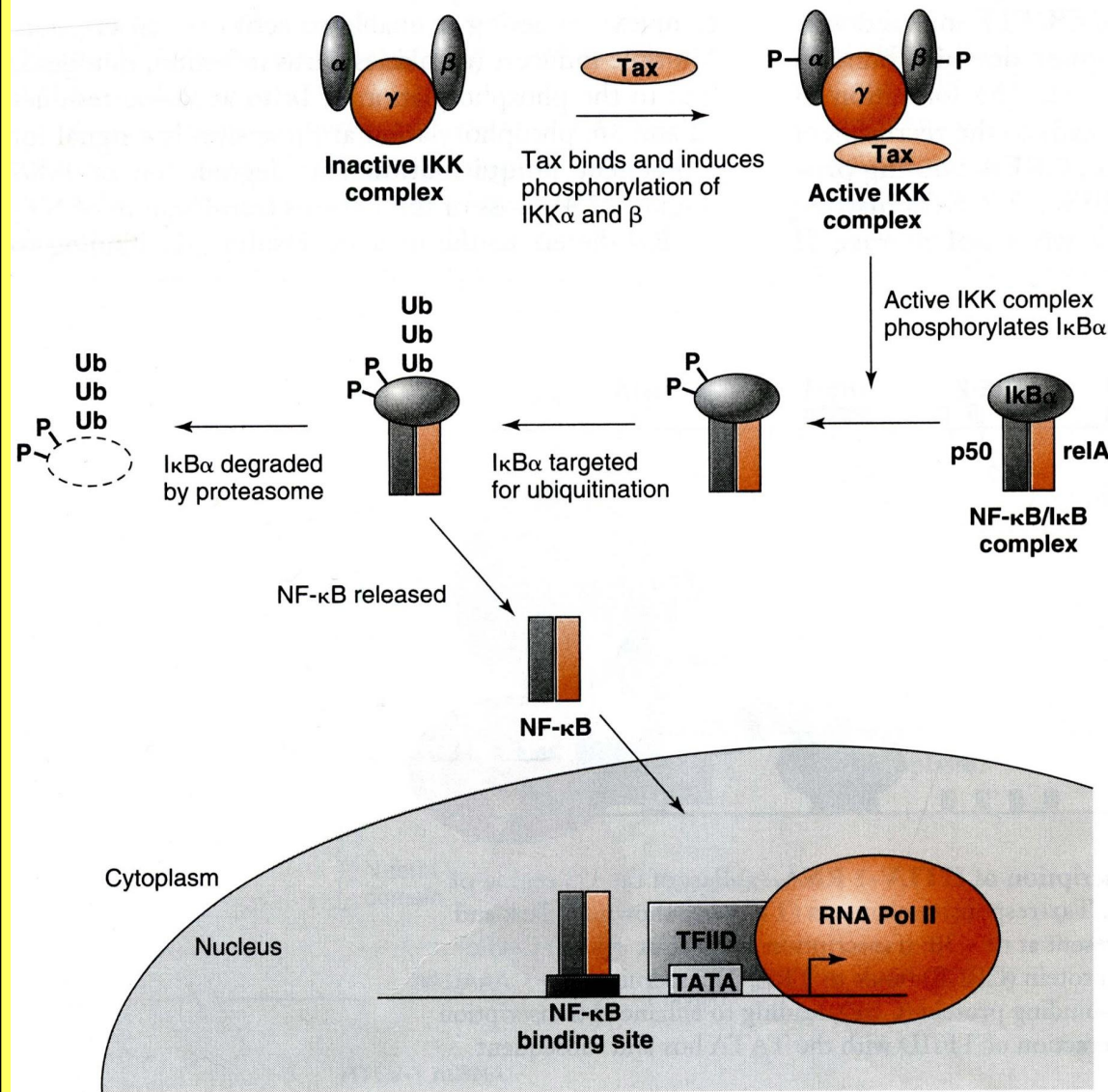
**FIGURE 27.3** Tax activates transcription of HTLV-1 RNA. (a) Part of the U3 region of the HTLV-1 long terminal repeat. Tax-responsive elements (TRE) are shown as black and gray rectangles. A TATA box is present at nt  $-30$ ; transcription starts at nt  $+1$ . (b) Cyclic AMP-responsive element binding protein (CREB) binds to TRE; Tax recruits the transcriptional coactivator, CREB-binding protein (CBP), leading to enhanced transcription of proviral DNA by enhancing interaction of TFIID with the TATA box and subsequent binding of RNA polymerase II.

**TABLE 27.1** Activation of gene expression by HTLV-1 Tax

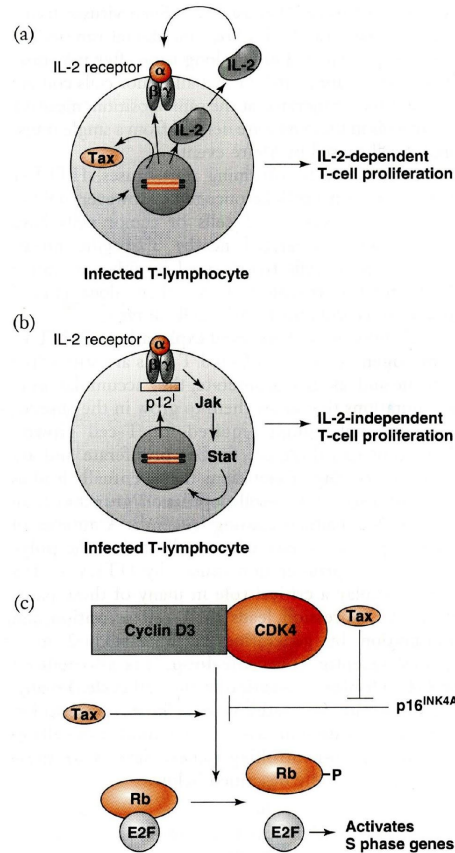
Genes induced by Tax	Examples	Transcription factor used <sup>a</sup>
Viral genes	HTLV-1	CREB/ATF
	HIV-1	NF- $\kappa$ B
	Cytomegalovirus	NF- $\kappa$ B
Cytokines and receptors	Interleukin-1, 2, 6, 8, 15	
	Tumor necrosis factor- $\alpha$ , $\beta$	
	Interferon- $\beta$	NF- $\kappa$ B
	Interleukin-2 receptor $\alpha$	
	T-cell receptor MHC class I	
Transcription factors and proto-oncogenes	c-fos, erg-1	SRF
	fra-1	NF- $\kappa$ B
	c-myc, c-rel, c-sis	EGR-1
Cell adhesion molecules	Endothelial leukocyte adhesion molecule-1 (ELAM-1)	NF- $\kappa$ B
	Intracellular cell adhesion molecule-1 (ICAM-1)	NF- $\kappa$ B
	Vimentin	?
Acute phase proteins	Angiotensinogen	
	Serum amyloid A precursor	NF- $\kappa$ B
	Complement factors	

<sup>a</sup>CREB/ATF: cAMP responsive element binding/activation transcription factor; NF- $\kappa$ B: nuclear factor- $\kappa$ B; SRF: serum response factor; EGR-1: early growth response factor-1.





**FIGURE 27.4 NF- $\kappa$ B signaling cascade.** The I $\kappa$  kinase (IKK) complex, composed of  $\text{IKK}\alpha$ ,  $\text{IKK}\beta$ , and  $\text{IKK}\gamma$ , is activated by phosphorylation induced by Tax binding. The active complex phosphorylates I $\kappa$ B $\alpha$  at Ser-32 and Ser-36. The phosphorylated I $\kappa$ B $\alpha$  is subsequently ubiquitinated by the E3 ubiquitin ligase and targeted for degradation by the proteasome. NF- $\kappa$ B dimers are then free to translocate to the nucleus, bind to DNA, and activate target gene transcription.

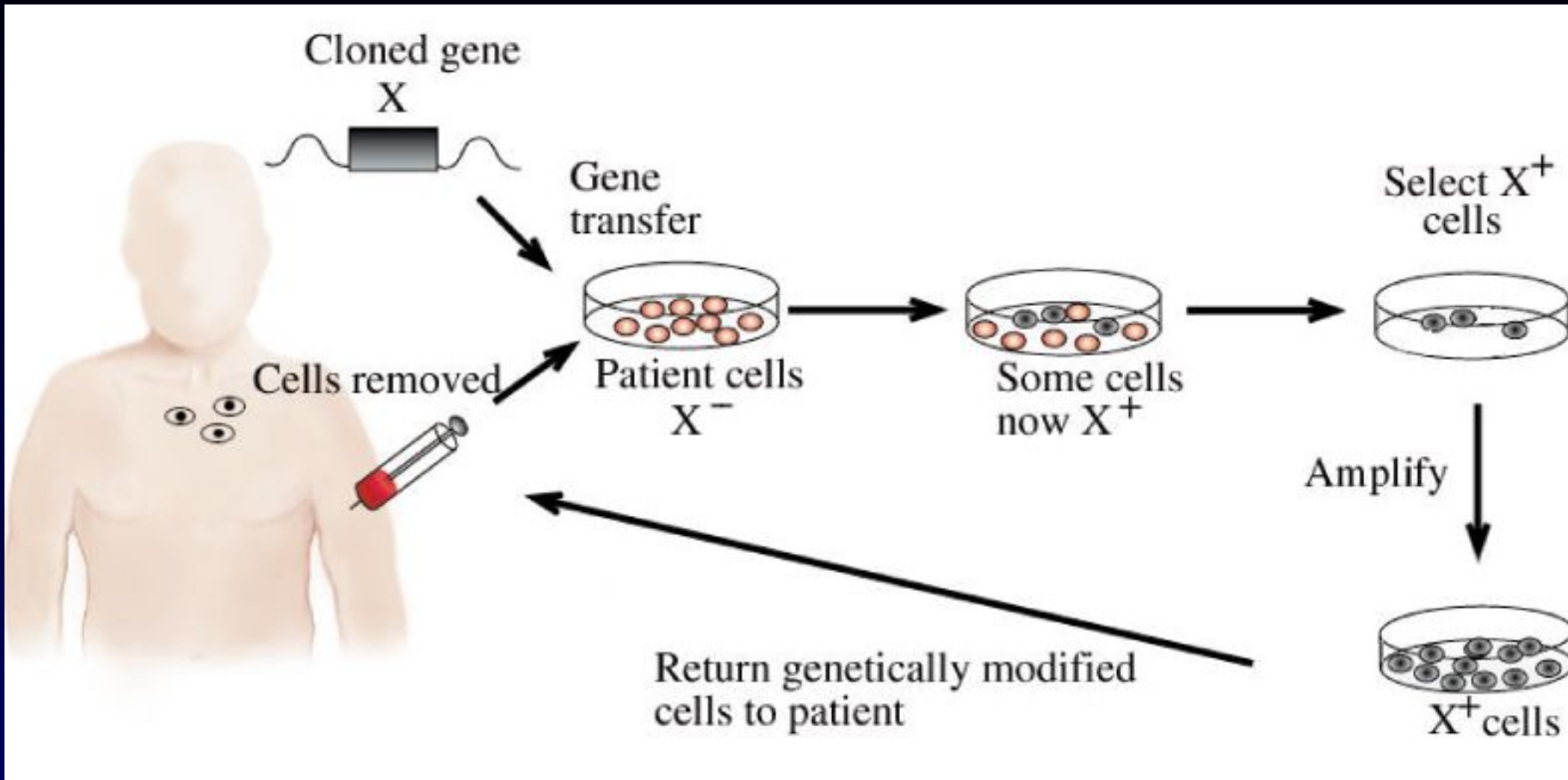


**FIGURE 27.5** Possible mechanisms of HTLV-1 induced T-cell proliferation and leukemogenesis. (a) HTLV-1 infected T cells that express Tax from an integrated proviral DNA (in orange) secrete interleukin 2 (IL-2) and up-regulate its receptor, causing polyclonal IL-2 dependent proliferation. (b) p12<sup>I</sup> can bind to and dimerize  $\alpha_c$  and  $\beta_c$  subunits of the IL-2 receptor, leading to activation of the Jak-Stat pathway in absence of IL-2 and resulting in IL-2 independent proliferation. (c) Tax blocks p16<sup>INK4A</sup> inhibition of cyclin-dependent kinase activity, and binds to cyclin D3 to increase cyclin-dependent kinase activity, resulting in increased phosphorylation of Rb protein and subsequent activation of S phase genes by free E2F transcription factor.

**TABLE 27.2** Clinical phases of adult T-cell leukemia

Disease phase	Clinical signs	Survival
Pre-ATL	<ul style="list-style-type: none"><li>• No clinical signs for decades</li></ul>	
Smoldering	<ul style="list-style-type: none"><li>• Less aggressive</li><li>• Normal white blood cell count</li><li>• Skin lesions</li><li>• 5% abnormal T lymphocytes</li></ul>	More than 10 months
Chronic	<ul style="list-style-type: none"><li>• Less aggressive</li><li>• Normal white blood cell count</li><li>• Prominent skin lesions</li><li>• 5% abnormal T lymphocytes</li><li>• Modest bone marrow and visceral involvement</li></ul>	More than 10 months
Lymphoma	<ul style="list-style-type: none"><li>• Skin lesions</li><li>• 5% abnormal T lymphocytes</li><li>• Liver and spleen pathology</li></ul>	10 months
Acute	<ul style="list-style-type: none"><li>• Very aggressive</li><li>• Elevated white blood cell count</li><li>• Prominent skin lesions</li><li>• &gt;5% abnormal T cells</li><li>• Liver and spleen pathology</li></ul>	Less than 6 months

# The concept of Gene Therapy



# Необходимые свойства эффективной системы переноса и экспрессии гена

- Высокая эффективность переноса выбранного генетического материала в клетки-мишени (*in vivo* и *in vitro*)
- Простота и высокая воспроизводимость метода трансдукции
- Стабильная экспрессия внесённого гена
- Возможность направленной регуляции экспрессии
- Регулируемый тропизм (способность избирательно вносить экспрессируемые гены в клетки определённых типов)

# Выбор метода переноса и экспрессии целевых генов

<p>1. Са-фосфатная трансфекция</p>	<p><b>Простота выполнения, быстрота и низкая стоимость (не требует специального оборудования)</b> Применима лишь к ограниченному ряду клеток, возможность использования только прикрепленных клеток, низкая эффективность (0,01-2%) Неконтролируемое число копий внесённого в геном генетического материала</p>
<p>2. Липосомы (искусственные мембраны)</p>	<p><b>Удобный и быстрый и достаточно “мягкий” способ</b> Высокая стоимость, для многих типов клеток – низкая эффективность. Неконтролируемое число копий внесённого в геном генетического материала</p>
<p>3. Электропорация (электропробой)</p>	<p><b>Простой и быстрый метод. Возможность использовать различные типы клеток.</b> Высокая гибель клеток при электропробое (30-50%) Требует специального дорогостоящего оборудования. Не может быть использован в генотерапевтических целях. Возможны нарушения структуры хромосом.</p>
<p>4. Баллистический метод (shortgun)</p>	<p><b>Быстрый метод, требующий опыта и не являющийся универсальным. Требует специального оборудования. Низкая воспроизводимость.</b> Сопровождается разрушением клеточных органелл и хромосом, что может приводить к злокачественным перерождениям клеток</p>

# Выбор метода переноса и экспрессии целевых генов (продолжение)

## 5. Перенос и экспрессия генов с помощью вирусных векторов

### Ретровирусные векторы:

*Векторы на основе простых ретровирусов*

*Векторы на основе лентивирусов*

*Векторы на основе спумавирусов (пенящих вирусов)*

Высокая эффективность переноса (до 95-100%)

Широкий спектр действия.

Высокая пластичность. Отсутствие цитотоксичности. Возможность избирательного переноса и экспрессии в клетках различных типов и различных организмов. Простота, высокая воспроизводимость и низкая стоимость. Отсутствие иммуногенности. Контролируемое число копий интегрированных в геном клеток-мишеней.

Вероятность повреждения клеточных генов в месте интеграции векторов в геном

Векторы на основе аденовирусов

Векторы на основе адено-ассоциированного вируса

Векторы на основе вируса простого герпеса

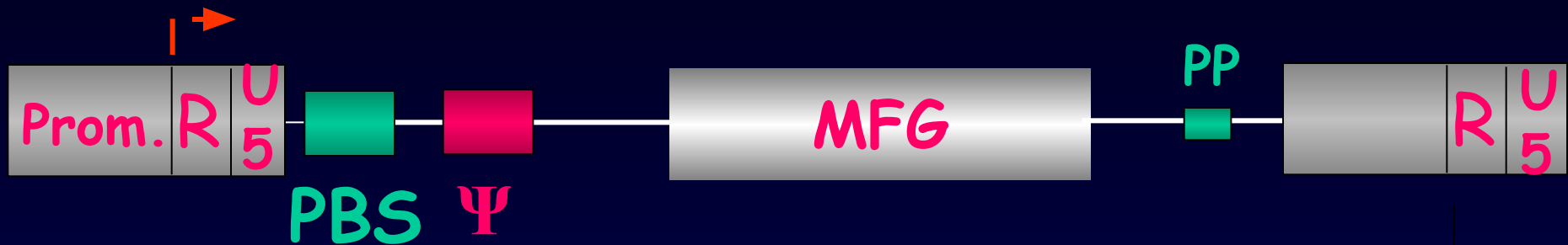
Высокая эффективность переноса. Низкая цитотоксичность. Отсутствие интеграции в геном трансдуцированной клетки. Нежелательная иммуногенность. Невысокая пластичность.

Интеграция в определённый участок 19-ой хромосомы человека. Низкая эффективность.

Способность заражать неделящиеся клетки (нервные). Невысокая воспроизводимость и низкая эффективность

# retroviral vectors

components needed *in cis*



\* My Favorite Gene

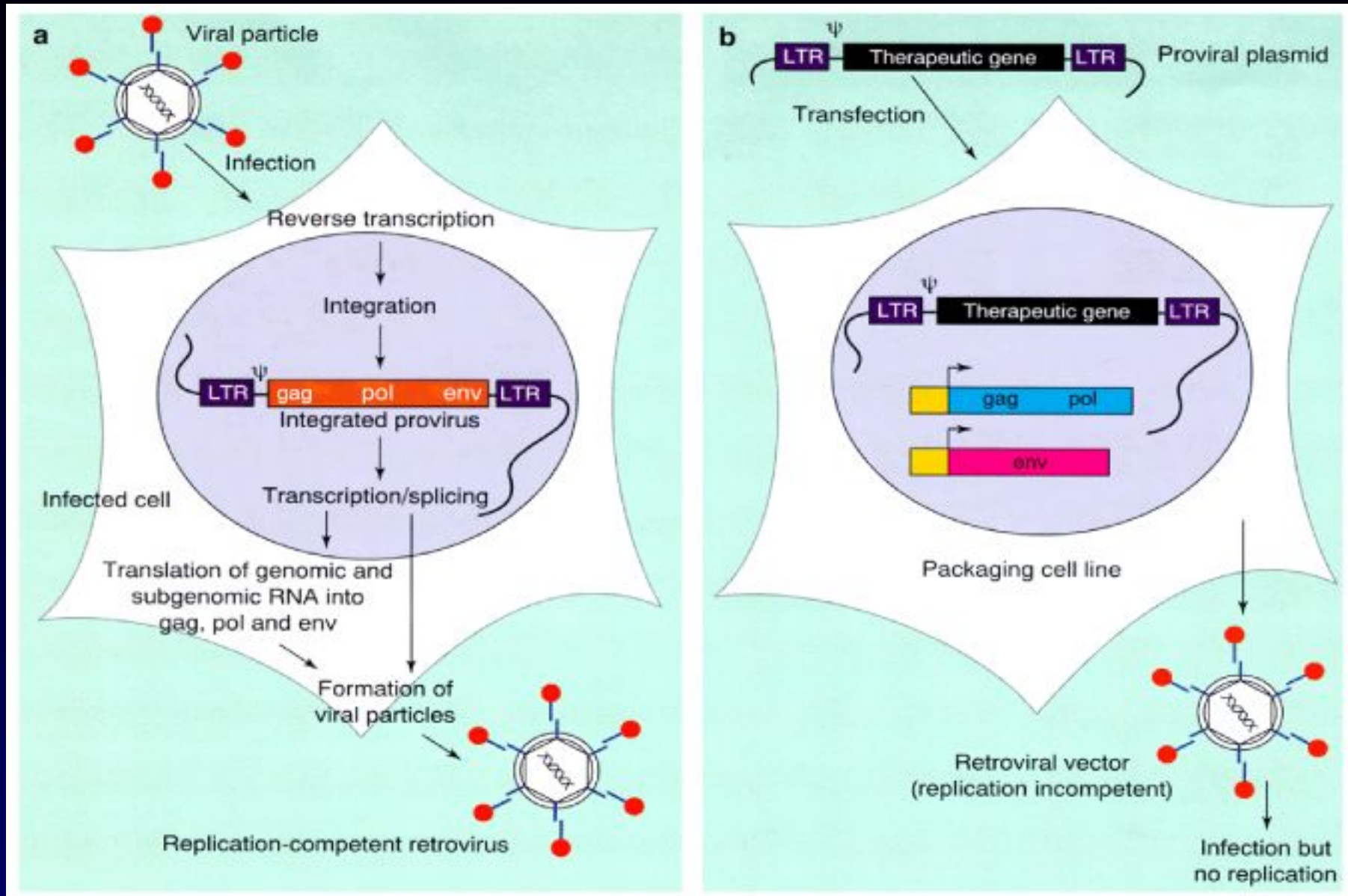


# retroviral vectors

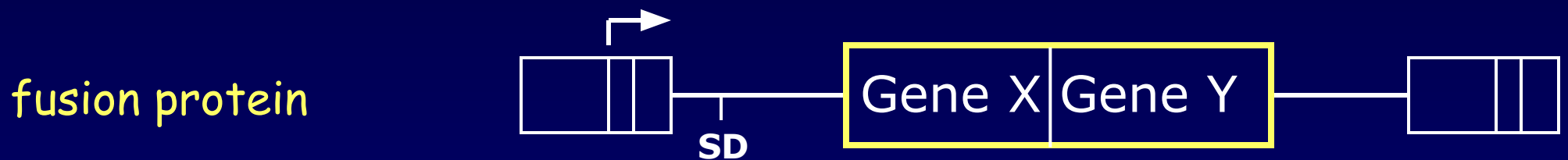
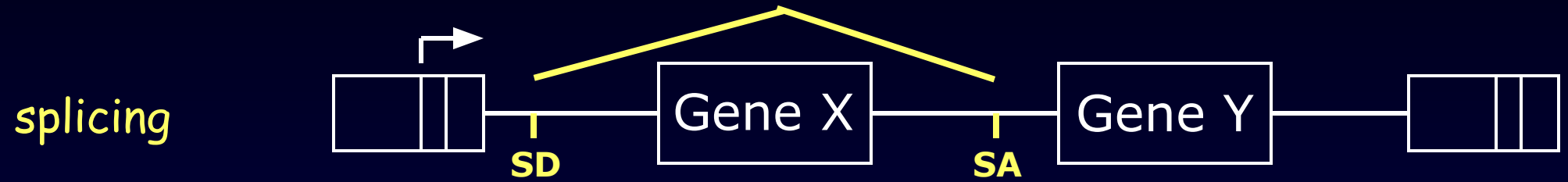
components needed *in trans*



# retroviral replication / packaging of defective particles



# Coexpression strategies using retroviral vectors



# Utilized coexpression strategies

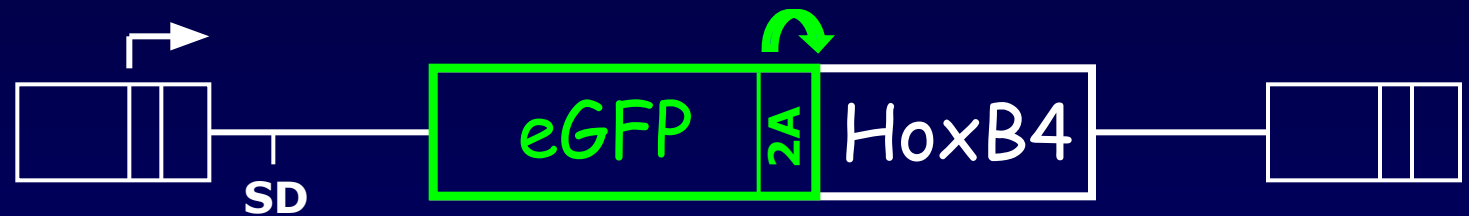
internal  
ribosomal  
entry



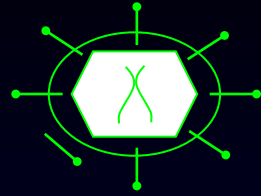
fusion protein



cotranslational  
separation



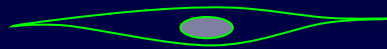
# Calculation of retroviral titers („GFP-Transfer Units“)



X **GTUs** infect X cells



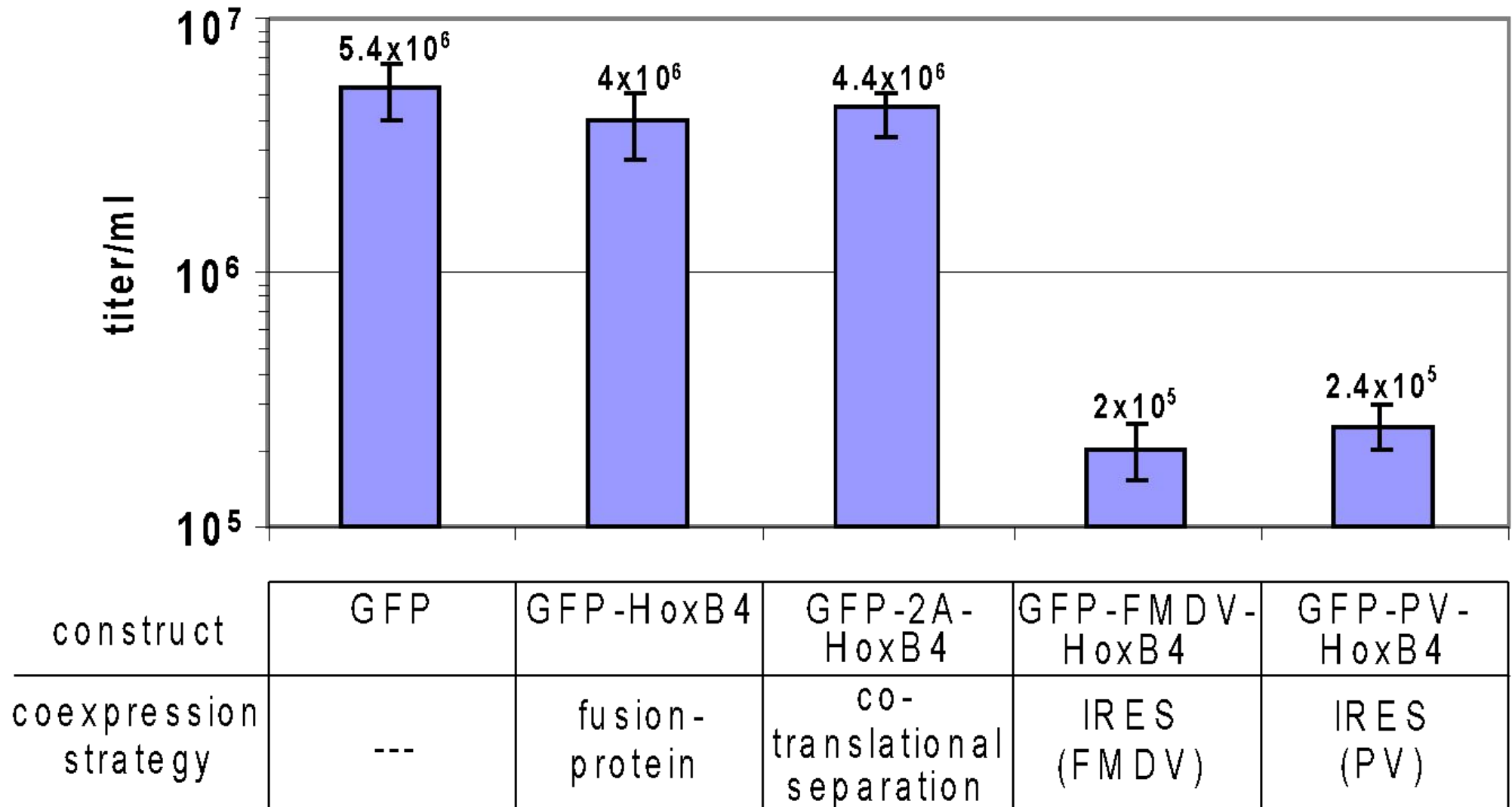
first cell division,  
integration between G2 and S



outcome after 24-48 hours:  
original cell number x2  
infected: only half of the cells

-> % GFP positive cells x original cell number x 2 (x dilution)

# Titers of retroviral expression vectors



# Why do IRES containing vectors show reduced titers?

vector production

transduction

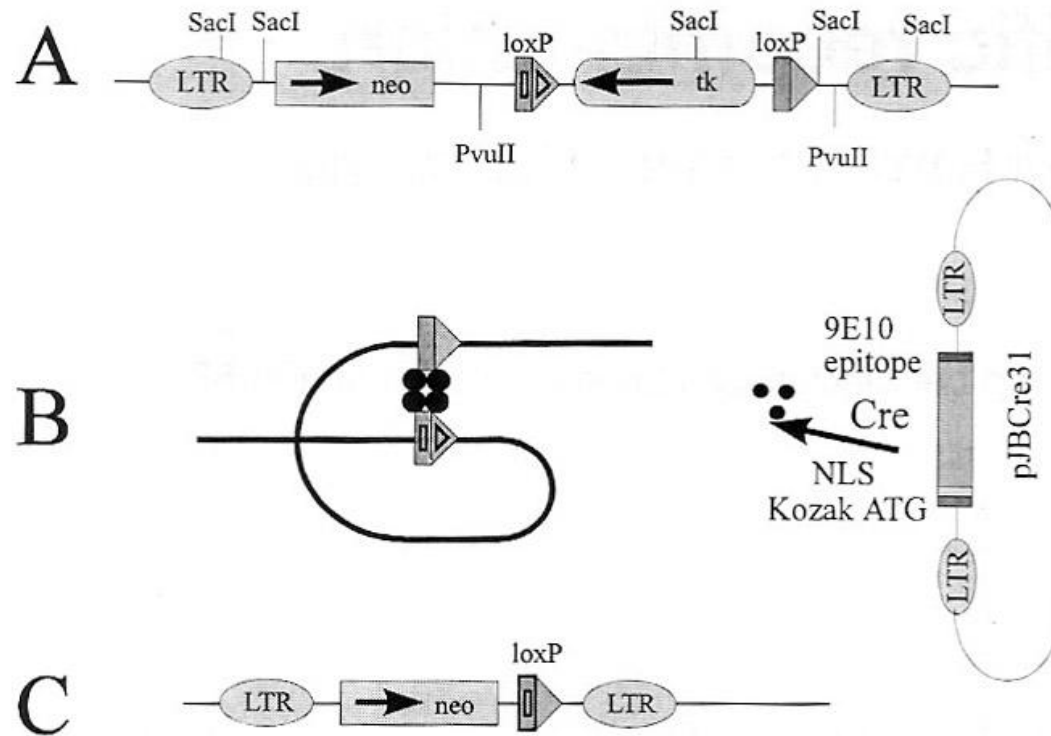
splicing / nuclear export

reverse transcription

packaging



pJBlox7: G418 resistant, BrdU sensitive



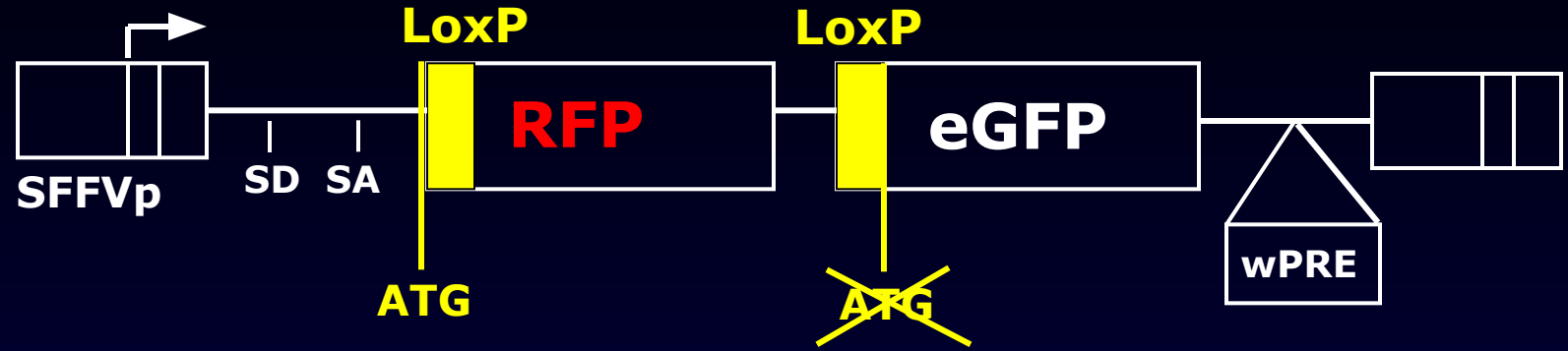
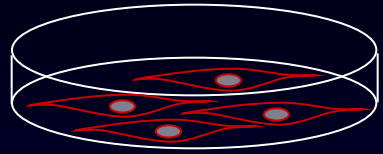
pJBlox7-Cre1: G418 resistant, BrdU resistant

**Figure 1.** Experimental design for a Cre mediated excision of DNA from integrated retroviral vectors. Schematic representation of an integrated retroviral vector with the neomycin resistance gene (*neo*) as a positive selectable marker and the herpes simplex virus type 1 thymidine kinase gene (HSV-1 TK) as a negative selectable marker. The HSV-1 TK gene is flanked by two *lox* sites in a head to tail orientation (A). The recombination event is drawn schematically in (B). The modified ORF for the Cre recombinase in the expression plasmid pJBCre31 is indicated by different rectangles. The integrated retroviral vector after site-specific excision of DNA is shown in (C).



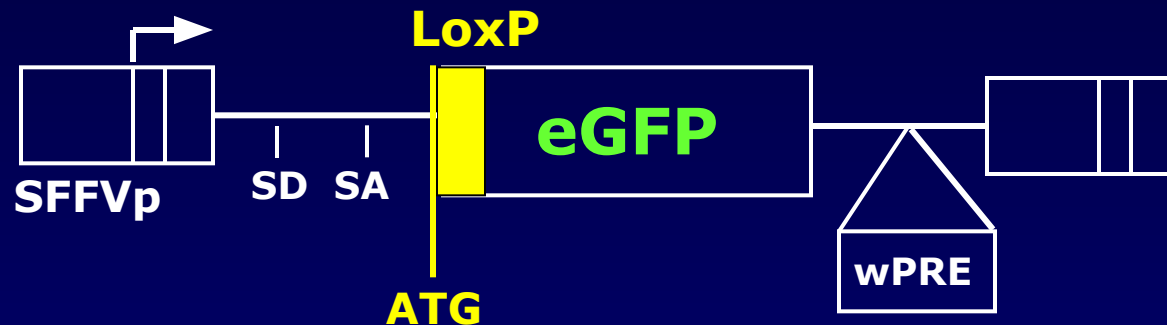
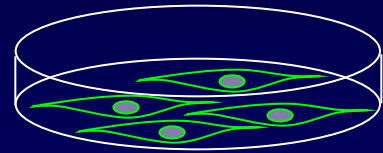
# Excision of genes via direct application of Cre-recombinase

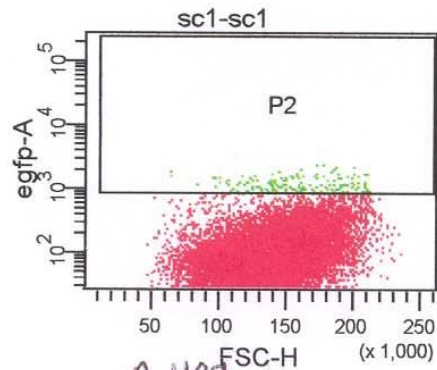
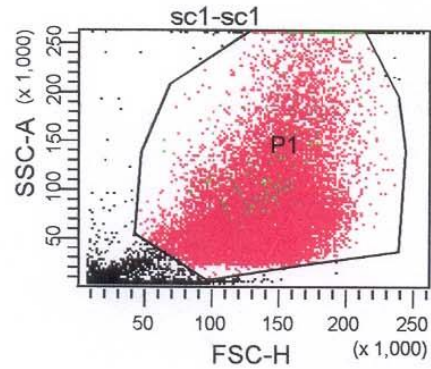
reporter  
cell line



Internalization of

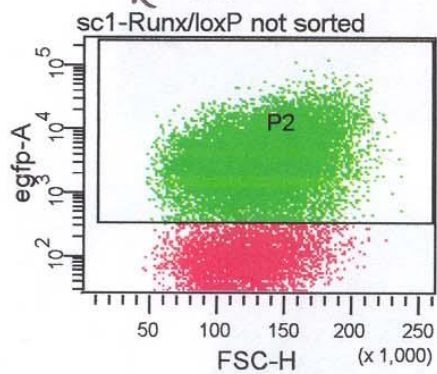
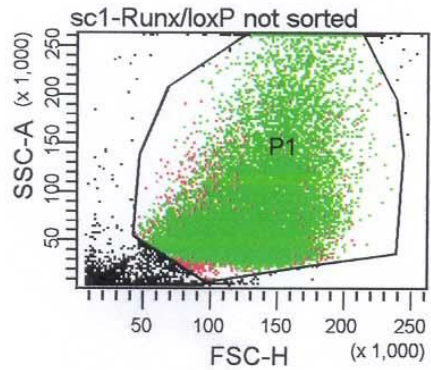
**PTD** Cre-recombinase





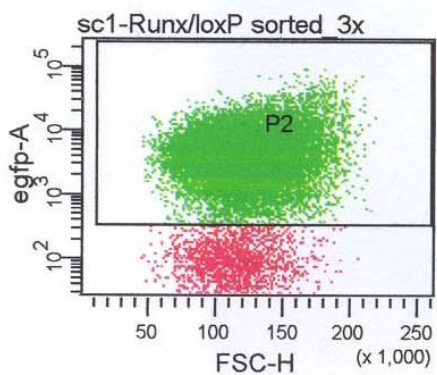
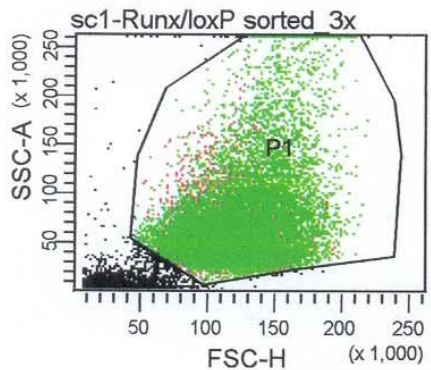
Tube: sc1

Population	#Events	%Parent	%Total
All Events	30,000		100.0
P1	22,949	76.5	76.5
P2	153	0.7	0.5



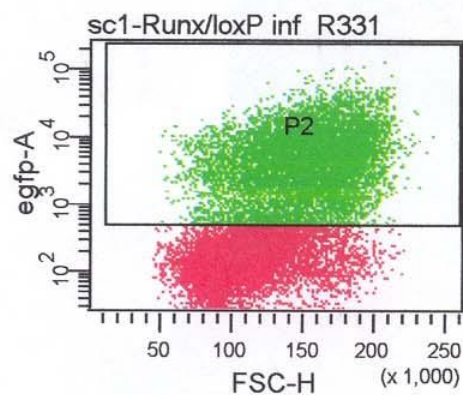
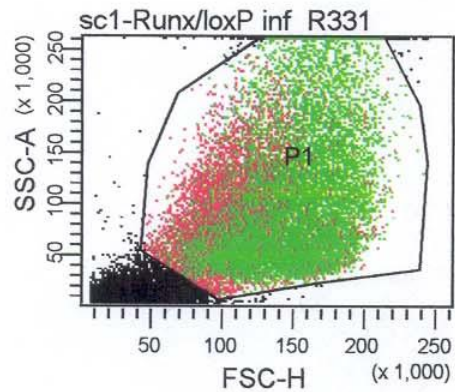
Tube: Runx/loxP not sorted

Population	#Events	%Parent	%Total
All Events	30,000		100.0
P1	25,463	84.9	84.9
P2	19,605	77.0	65.4



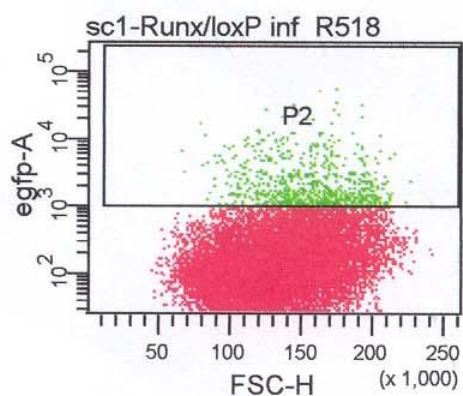
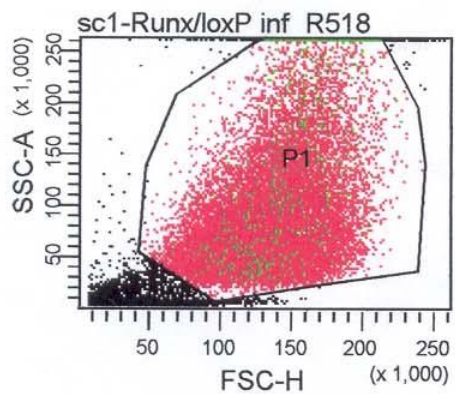
Tube: Runx/loxP sorted\_3x

Population	#Events	%Parent	%Total
All Events	30,000		100.0
P1	23,606	78.7	78.7
P2	21,696	91.9	72.3



Tube: Runx/loxP inf R331

Population	#Events	%Parent	%Total
All Events	30,000		100.0
P1	20,023	66.7	66.7
P2	9,487	47.4	31.6

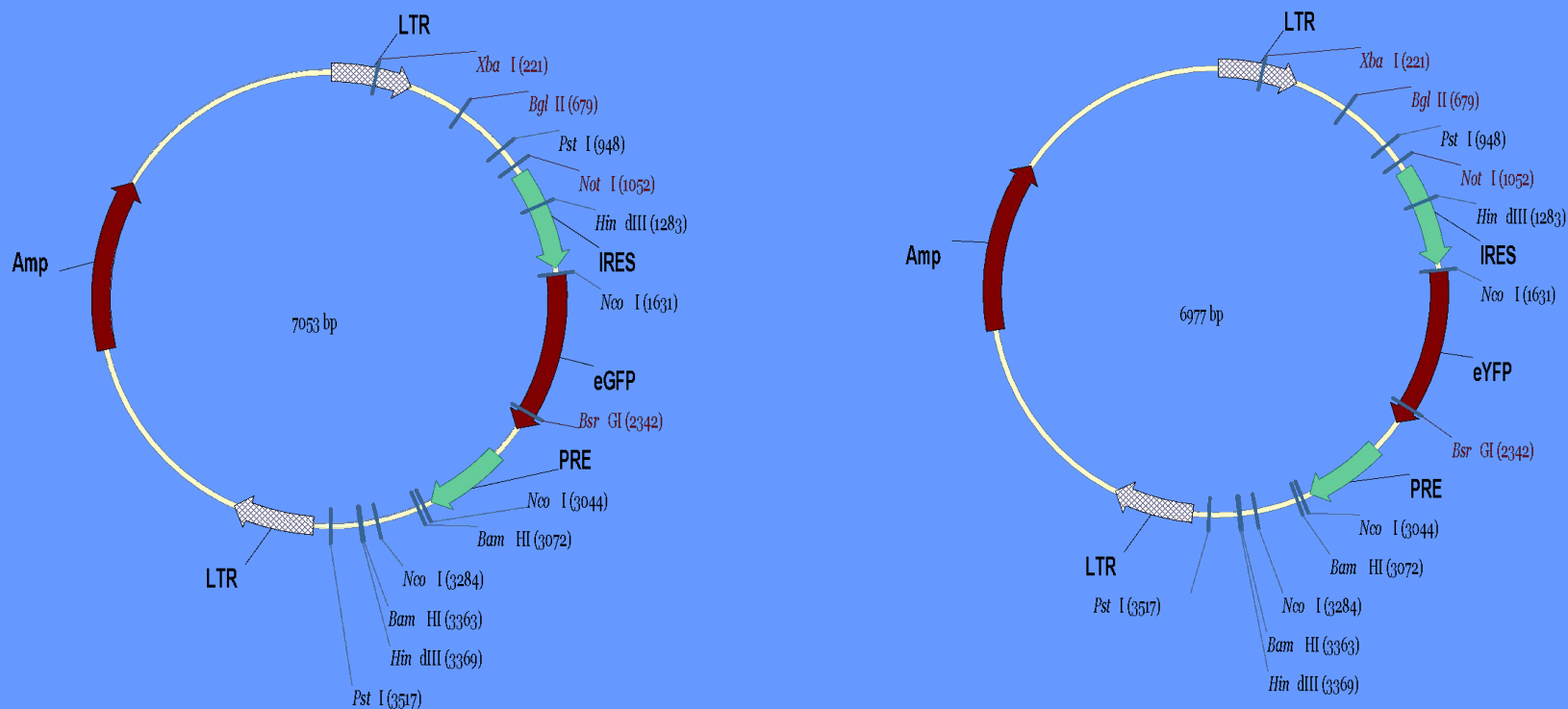


Tube: Runx/loxP inf R518

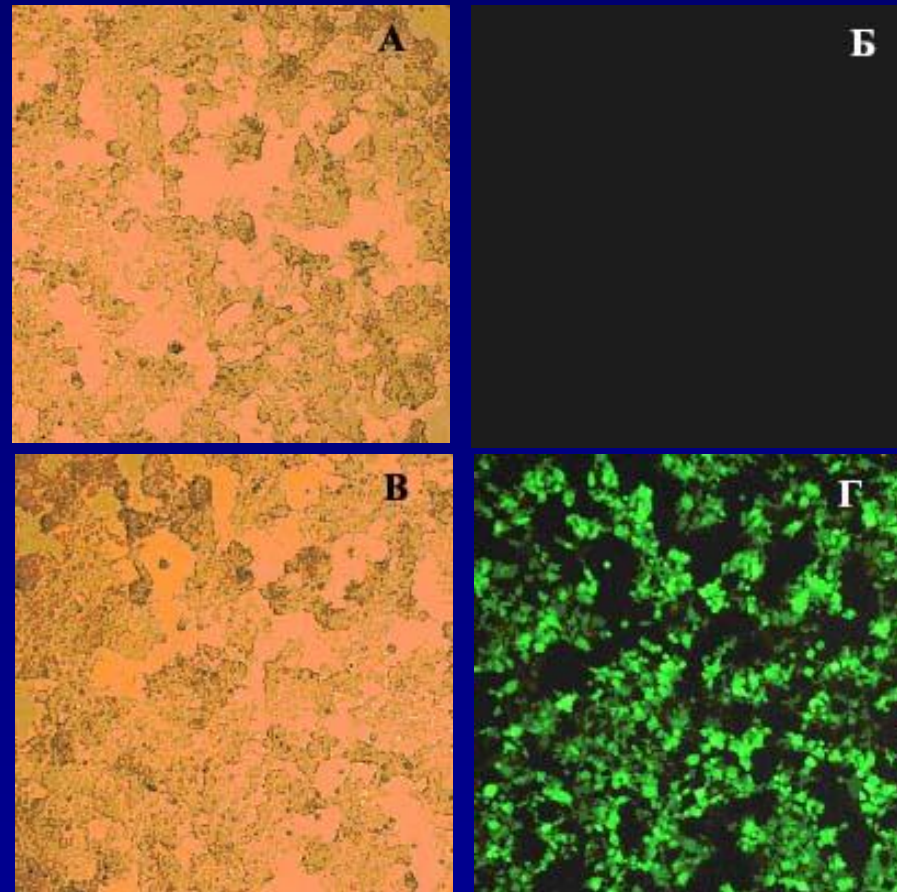
Population	#Events	%Parent	%Total
All Events	30,000		100.0
P1	24,044	80.1	80.1
P2	738	3.1	2.5

В процессе работы были получены ретровирусные векторы содержащие в своем составе в качестве маркерного гена ген зеленого флуоресцирующего белка или ген желтого флуоресцирующего белка, RV-eGFP/07-07 и Rv-tYFP/07-07, соответственно, и лентивирусные векторы, несущие в своем составе маркерный ген зеленого флуоресцирующего белка, по своим свойствам не уступающие лучшим зарубежным образцам, а по некоторым параметрам и превосходящие их.

Была разработана методика получения рекомбинантных ретровирусных частиц, обеспечивающая получение стоковых растворов с высоким титром (до  $10^6$  ИВЧ/мл).

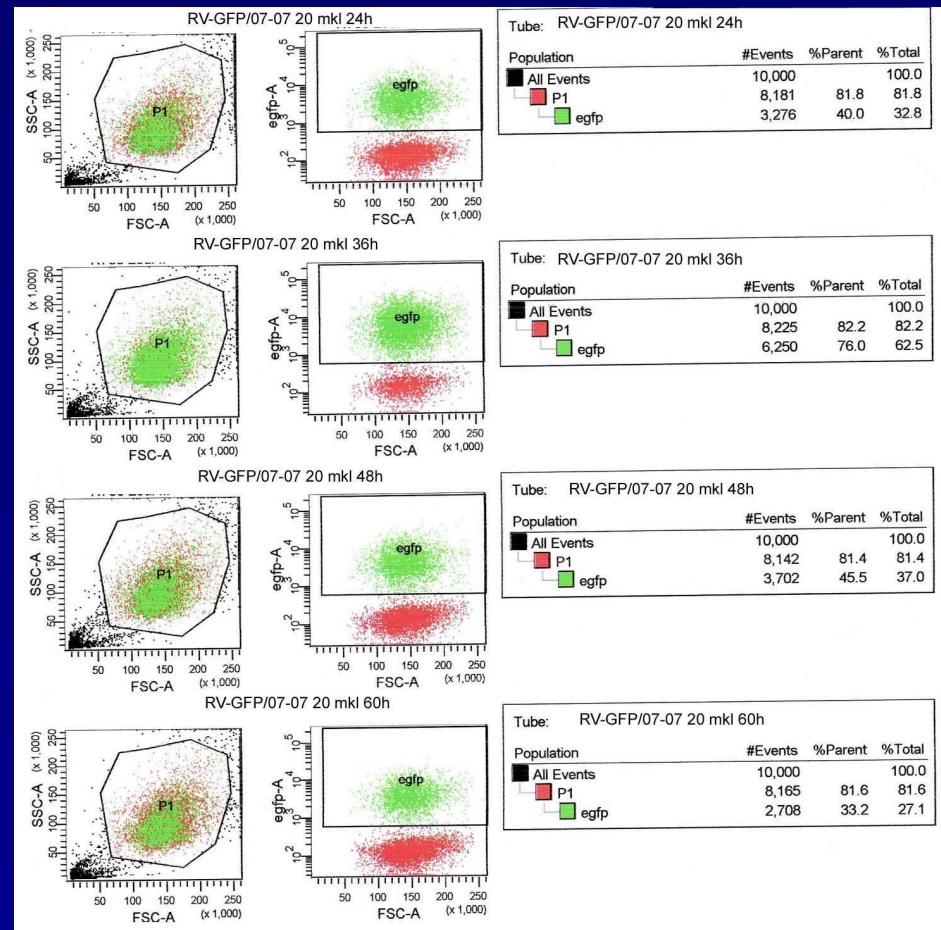


Экспрессия гена GFP (зелёного флуоресцирующего белка, внесённого в клетки) почки эмбриона человека линии 293, внесённого в составе ретровирусного вектора RV-GFP/07-07



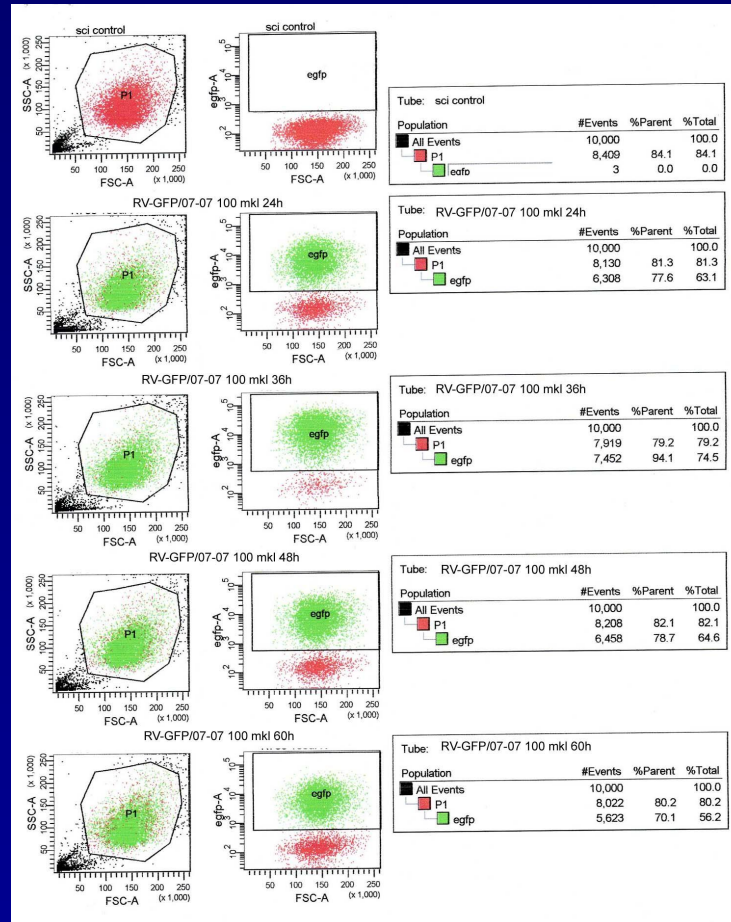
(A) и трансфицированные (B) клетки 293 при микроскопии в видимом свете. Исходные (B) и трансфицированные (Г) клетки 293 при микроскопии в ультрафиолетовом свете ( $\lambda_{\text{макс}} = 488 \text{ нм}$ ).

# Кинетика продукции ретровирусного вектора RV-GFP/07-07 упаковывающими клетками НЕК293gr.



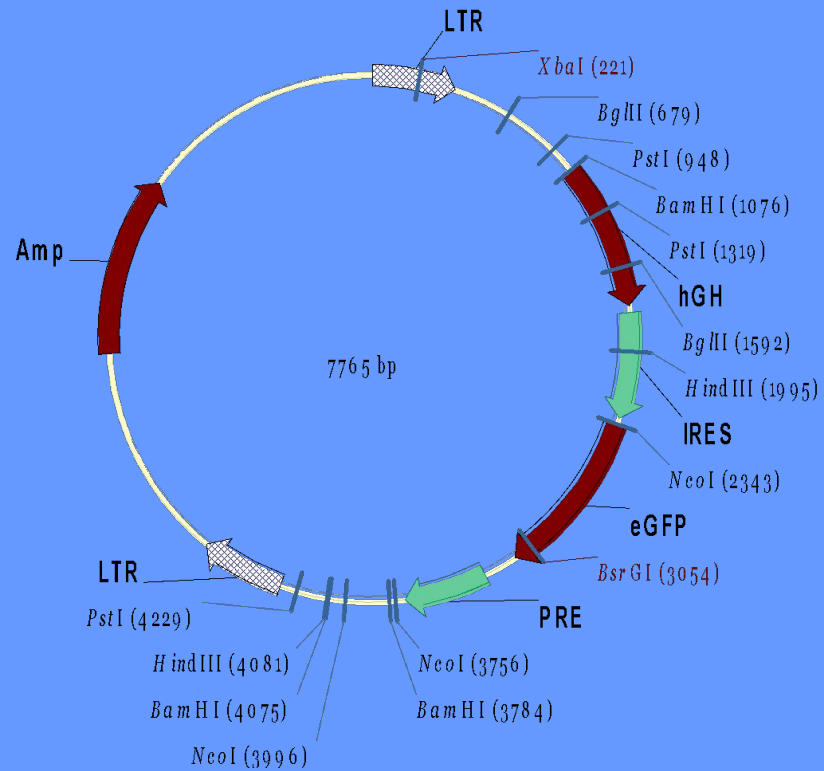
Сбор вируса осуществлялся через 24 часа после трансфекции клеток НЕК293gr с интервалом 10-12 часов. Титрование осуществляли на клетках мишенях SC-1. Для переноса в клетки SC-1 использовали 20 мкл культуральной среды трансфицированных упаковывающих клеток.

# Кинетика продукции ретровирусного вектора RV-GFP/07-07 упаковывающими клетками НЕК293gp.



Для переноса использовали 100 мкл культуральной среды трансфицированных упаковывающих клеток.

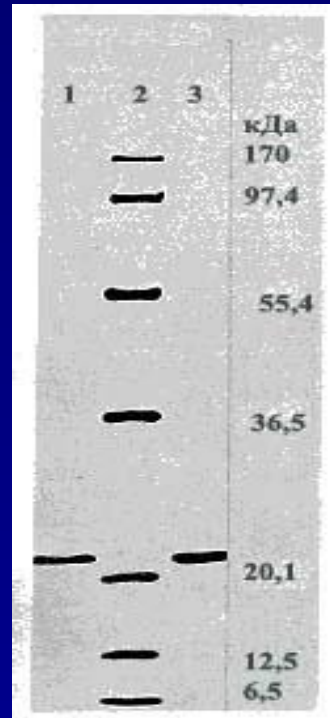
Создание клеток продуцентов (перевиваемых фибробластов мыши) секретируемого гормона роста человека с помощью ретровирусного вектора RV-GFP/ 07-07.



Карта ретровирусного вектора R-hGH-IRES-eGFP

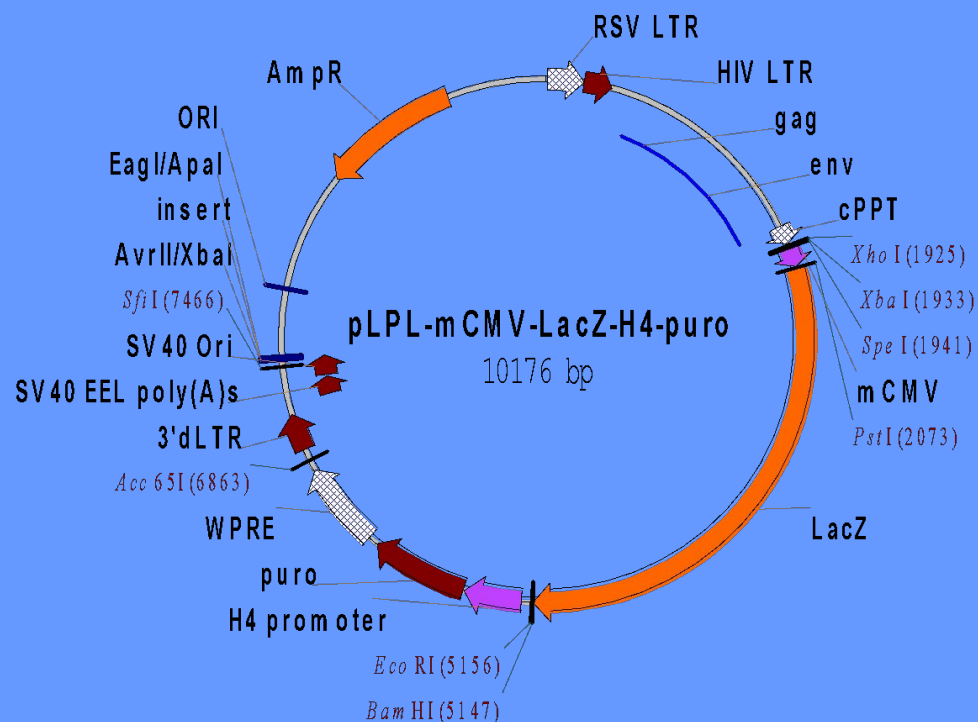


# Продукция гормона роста человека перевиваемыми клетками мышы линии SC1, трансдуцированных ретровирусным вектором R-hGH-IRES-eGFP

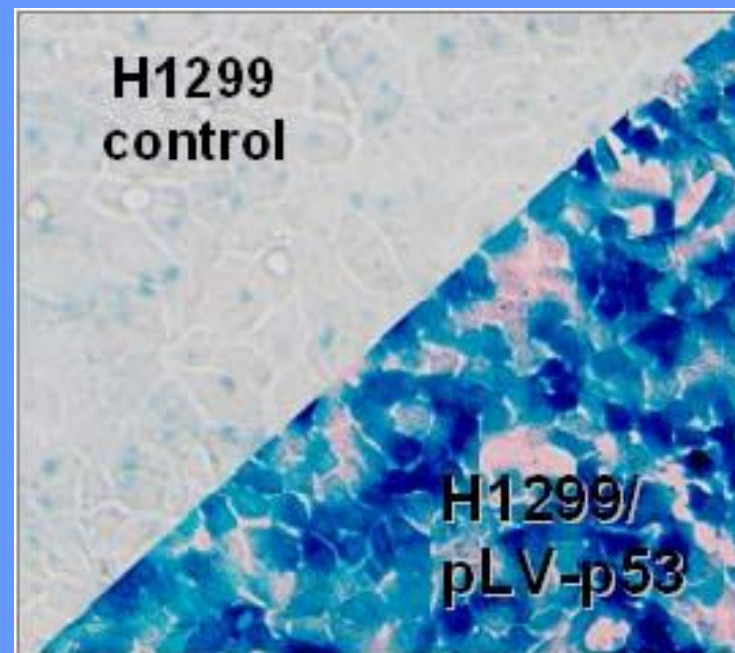


Электрофоретическое разделение в полиакриламидном геле в денатурирующих условиях зрелого гормона роста человека, полученного из культуральной среды трансгенных клеток, геном которых содержит интегрированный рекомбинантный вектор R-hGH-IRES-eGFP при помощи иммуносорбентной колонки (1) и зрелого гормона роста человека, полученного из клеток *E. coli* (3), (2)-маркерные белки. Концентрация зрелого гормона роста человека, в кондиционированной среде трансгенных клеток SC1 составляла 5-8 мкг/мл.

# Перенос целевых генов в клетки человека с помощью лентивирусного вектора pLPL-mCMV-H4puro



Физическая карта модульного лентивирусного вектора pLPL-mCMV-H4puro



Индукция синтеза бета-галактозидазы в перевиваемых клетках рака лёгкого человека H1299, имеющих делецию гена p53 с помощью лентивирусного вектора pLPL-mCMV-H4puro, несущего ген p53 дикого типа.

# Компартмент адресованная экспрессия генов целевых белков, направляемая лентивирусным вектором.

Дополнительно, для направления продуктов в определенные структуры клетки в область, примыкающую к промотору CMV и участку клонирования введены последовательности, кодирующие N-концевые пептиды – сигналы внутриклеточной локализации. Эти сигналы включают: пептид кальретикулина, отвечающий за локализацию в эндоплазматическом ретикулуме, пептид нейромодулина, направляющий белки в плазматическую мембрану, и пептид цитохром С оксидазы, направляющий белок в митохондрии.

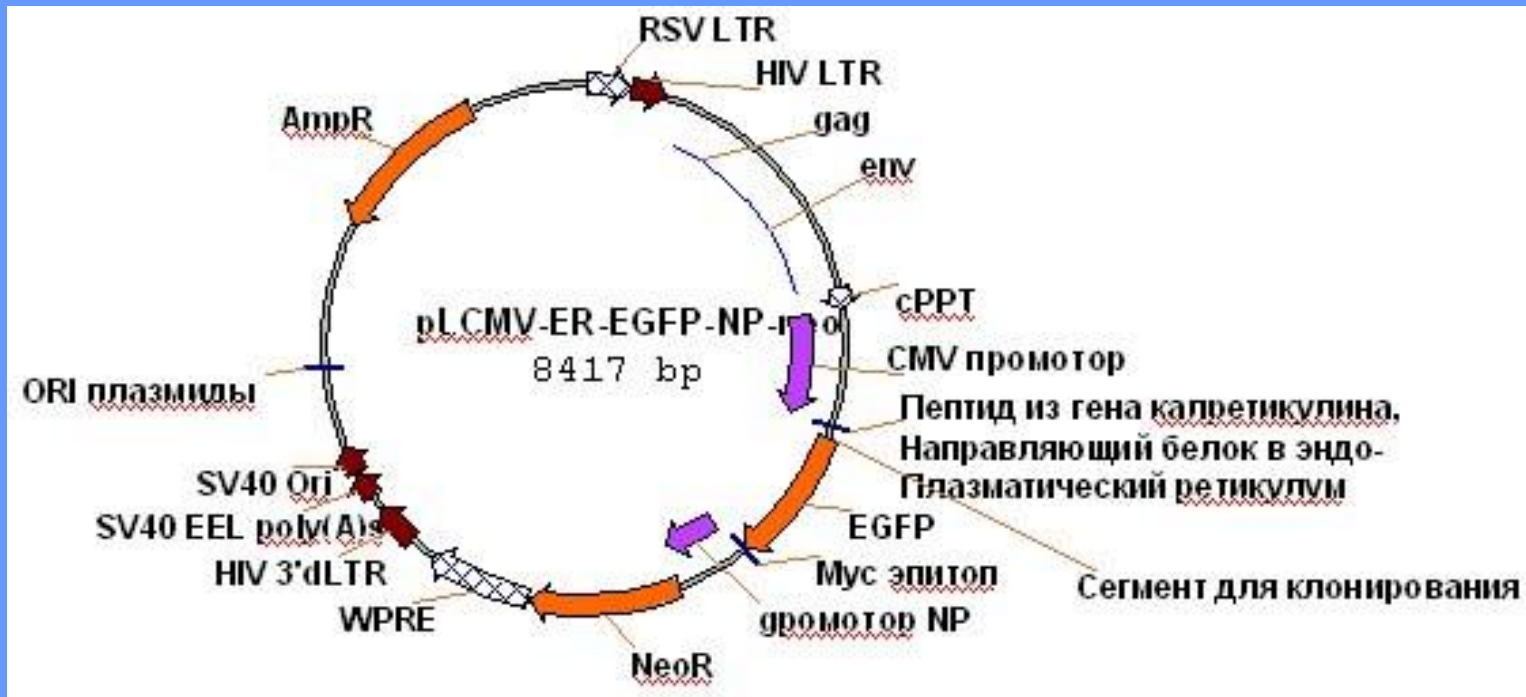
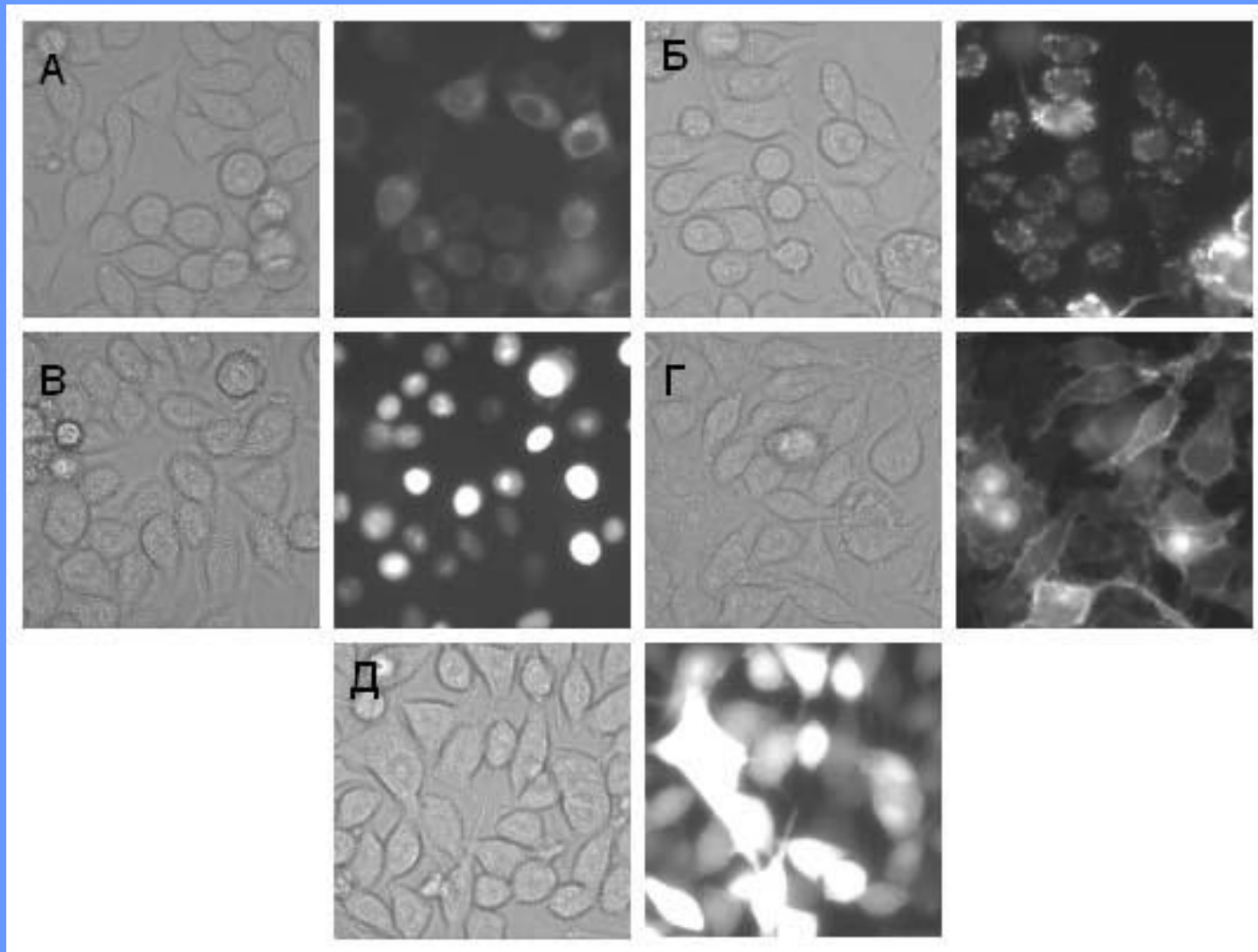


Схема конструкции для экспрессии зеленого флуоресцентного белка с локализацией в эндоплазматическом ретикулуме, на основе нового лентивирусного вектора pLCMV-NP-neo

Испытание лентивирусного вектора pLCMV для стабильной экспрессии зеленого флуоресцентного белка, направляемого в различные структуры клетки



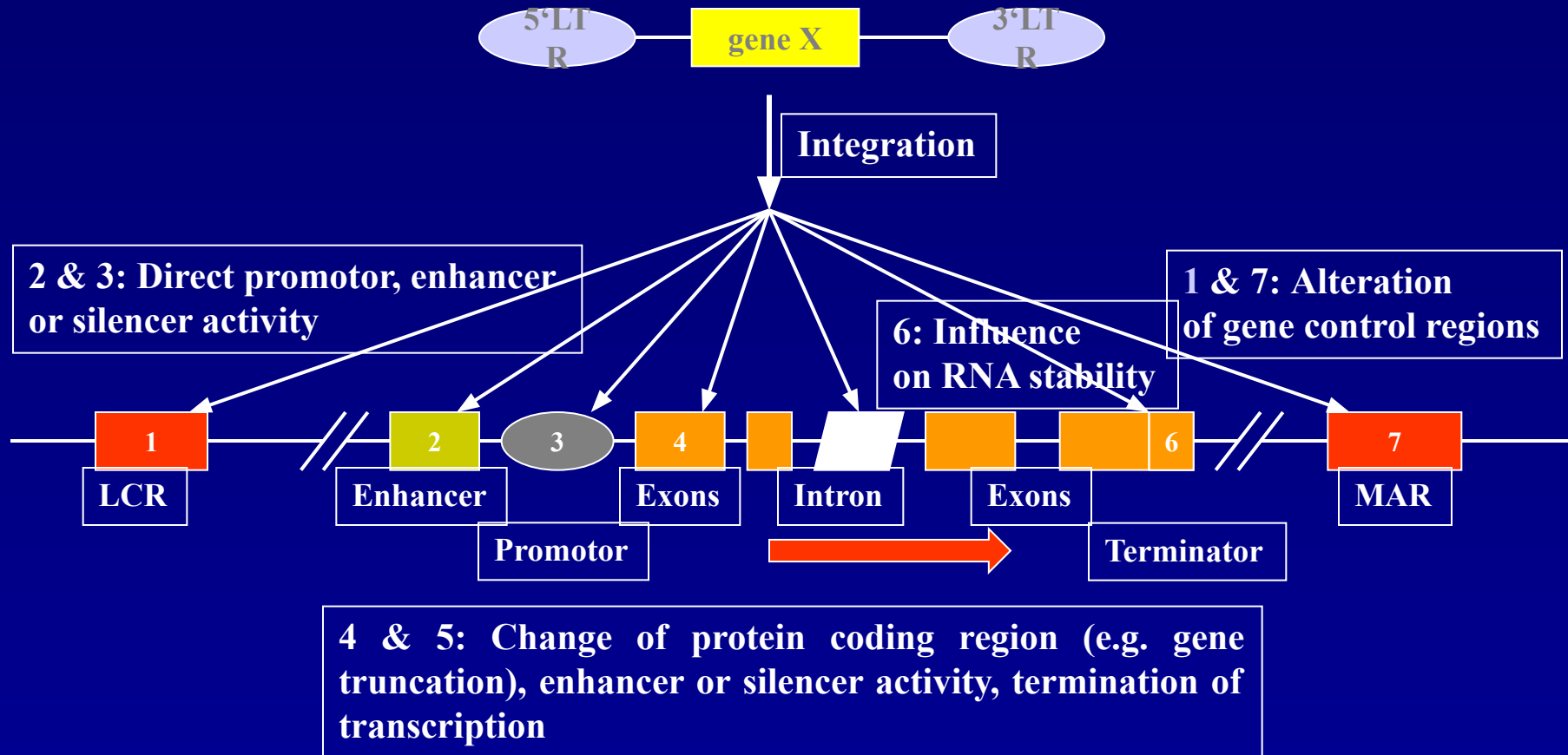
# *Retroviral vectors*

- most commonly used gene transfer system in gene therapy
- genome integration ensures stable long-term expression

***BUT***

- any genome integration may be associated with insertional mutagenesis  
(... which may in the worst case lead to malignant transformation)

# *Possible mechanisms of (retroviral) insertional mutagenesis*



**... at least some of these mechanisms do occur !!!**

*... theoretical risk assessment*

Target cell number:  $1 \times 10^9$  ( $1 \times 10^7$  per kg)

Starting cell number:  $10^8$

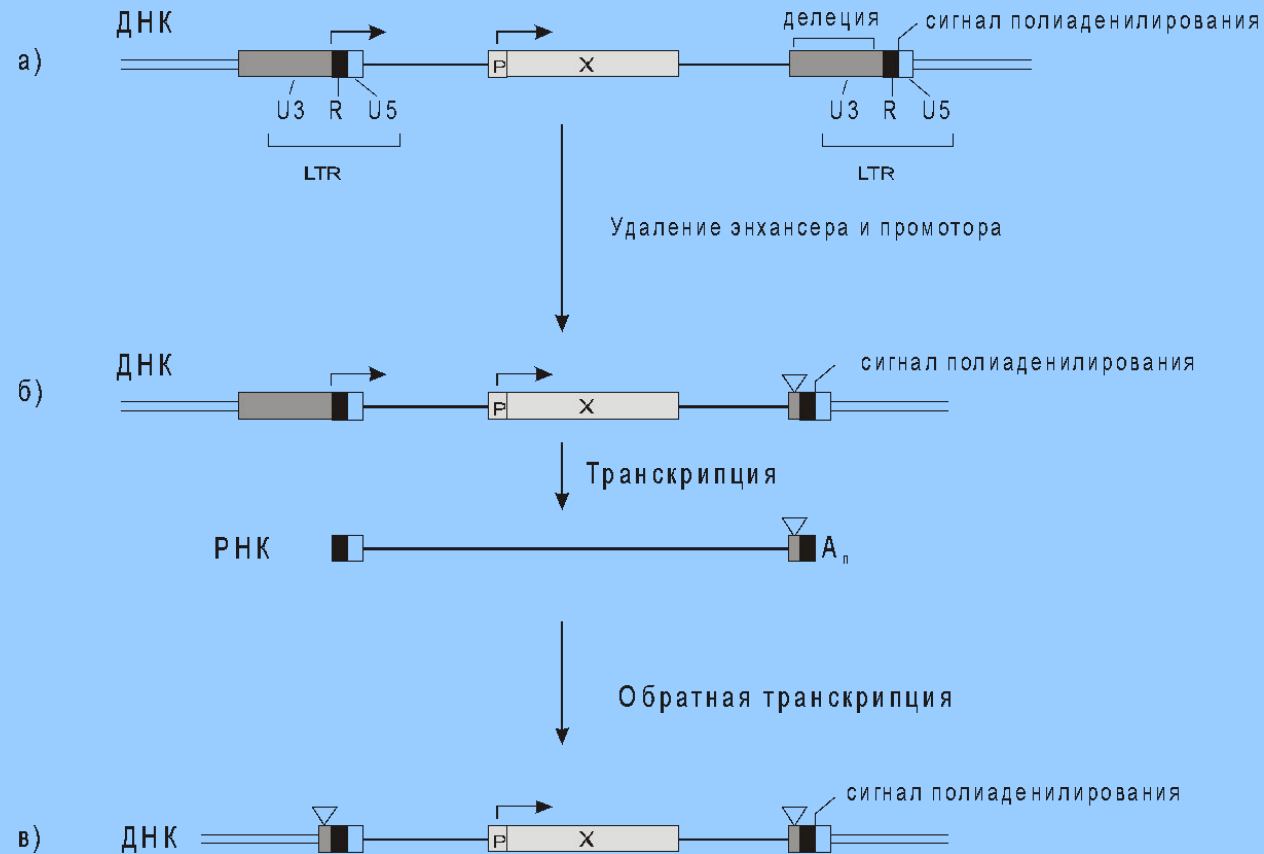
Transduction efficiency: 25-30%  $\square$   $3 \times 10^7$  insertions

Genome size:  $3 \times 10^9$  bp



**Random integration would statistically result  
in one insertion **every 100 bp!!!****

# Схематическое изображение структурно-функциональных изменений ретровирусного вектора, приводящих к делециям в промоторно-энхансерных областях обоих LTR



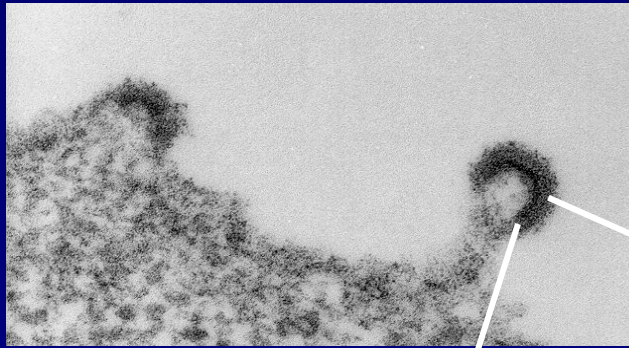
а) - ретровирусный вектор (оба LTR содержат энхансерно-промоторную область)

б) - самоинактивирующийся вектор в виде провируса в геноме упаковывающей клетки

в) - самоинактивирующийся вектор в виде провируса, интегрированного в геном клетки-мишени

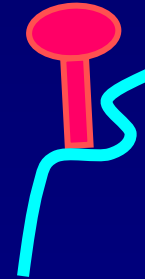


# MuLV Infection



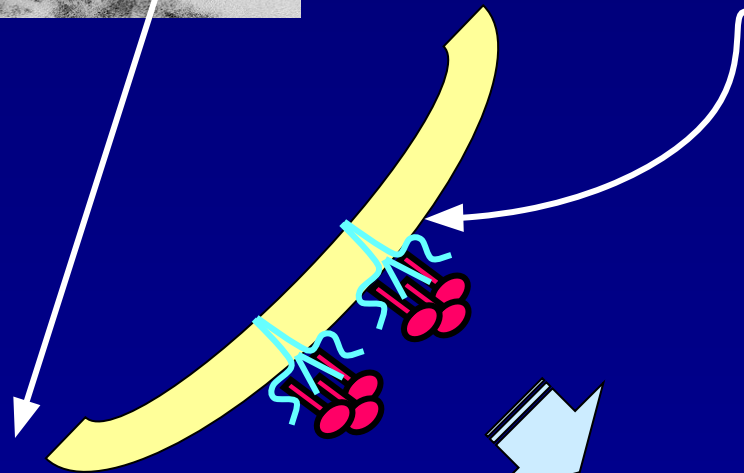
Env protein

Surface Protein  
SU

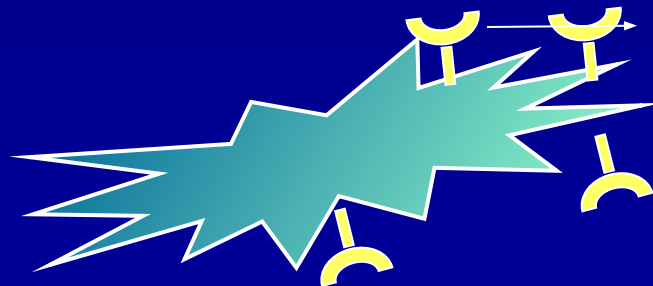


Transmembrane  
Domain TM

Trimer-Formation

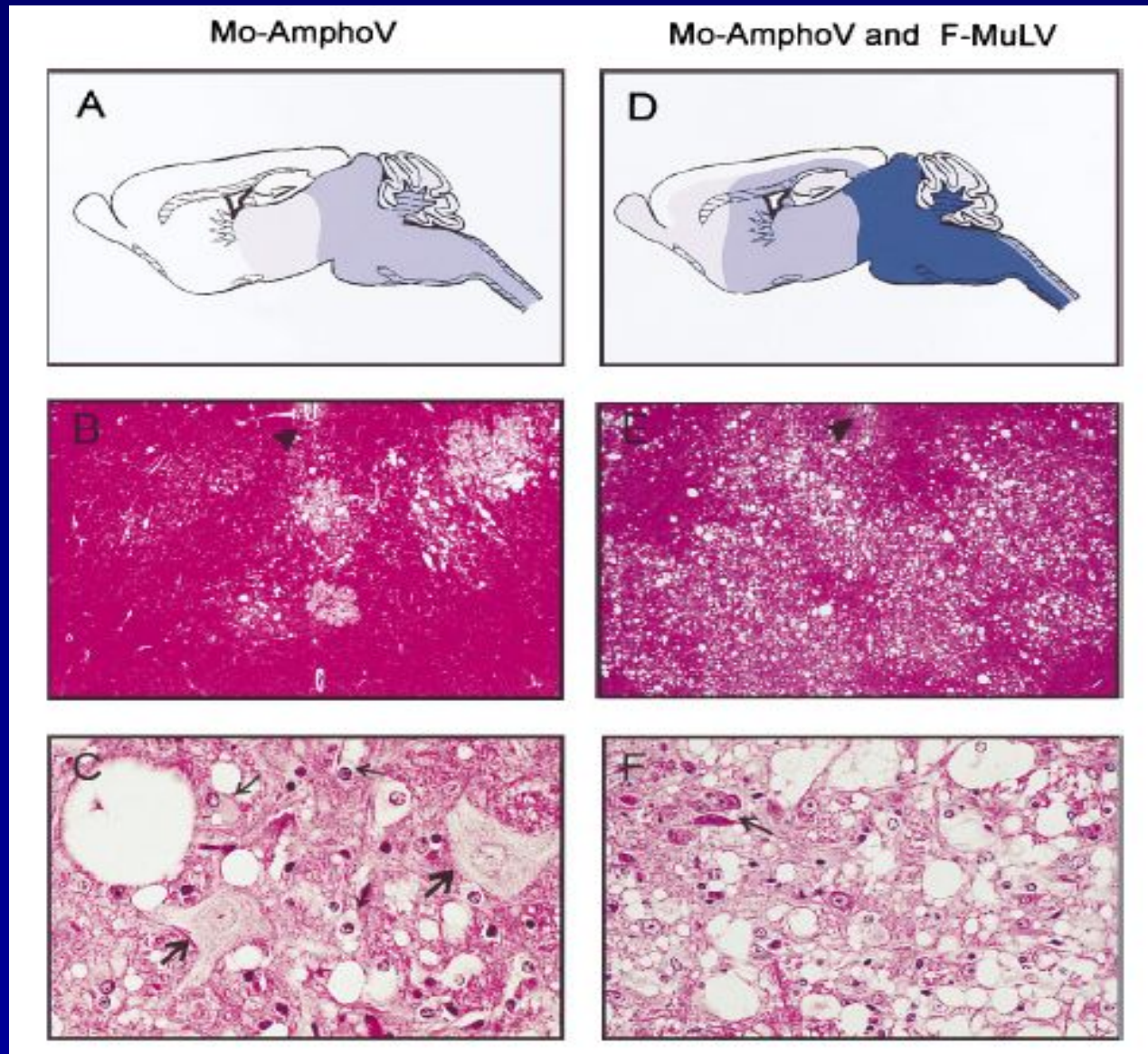


Binding



Cell Surface Protein  
( Receptor)

**Neuropathology of DDD mice infected with Mo-AmphoV alone (A–C),  
or coinfecting with Mo-AmphoV and F-MuLV (D–F).**



## Classification of MuLVs Based on Receptor Usage or Homology

Interference Group	Prototype	Receptor	Chromosome Location	
			Mouse	Human
<b><i>Mus musculus</i></b>				
Ecotropic	Mo-MuLV	m-CAT1	5	13
Xenotropic	NZB	h-XPR	1	1
Polytropic	Mo-MCFV	XPR	1	1
Amphitropic	4070A	Pit2	8	8
10A1	10A1	Pit1 / Pit2	2 / 8	2 / 8
<b><i>Mus dunni</i></b>				
Multitropic	MDEV	?	?	?
<b><i>Mus cervicolor</i></b>				
C I	CERV C1	?	?	?
CII	M813	?	2	?
<b><i>Mus caroli</i></b>				
CI		?	?	?

## Host Spectrum: M813 infects only murine cells and, less efficiently, rat cells

Species	Cell Line	Titer	
		M813neo	10A1neo
Mouse	SC-1	$3.5 \times 10^4$	$2.6 \times 10^5$
	NIH 3T3	$8.0 \times 10^4$	$3.0 \times 10^5$
Rat	Rat1	$3.7 \times 10^1$	$5.6 \times 10^4$
	NRK	$3.0 \times 10^1$	$1.8 \times 10^5$
	REF52	$1.3 \times 10^2$	$1.7 \times 10^5$
Human	HeLa	0	$8.3 \times 10^5$
	Ht1080	0	$4.3 \times 10^4$
	TE671	0	$1.4 \times 10^5$

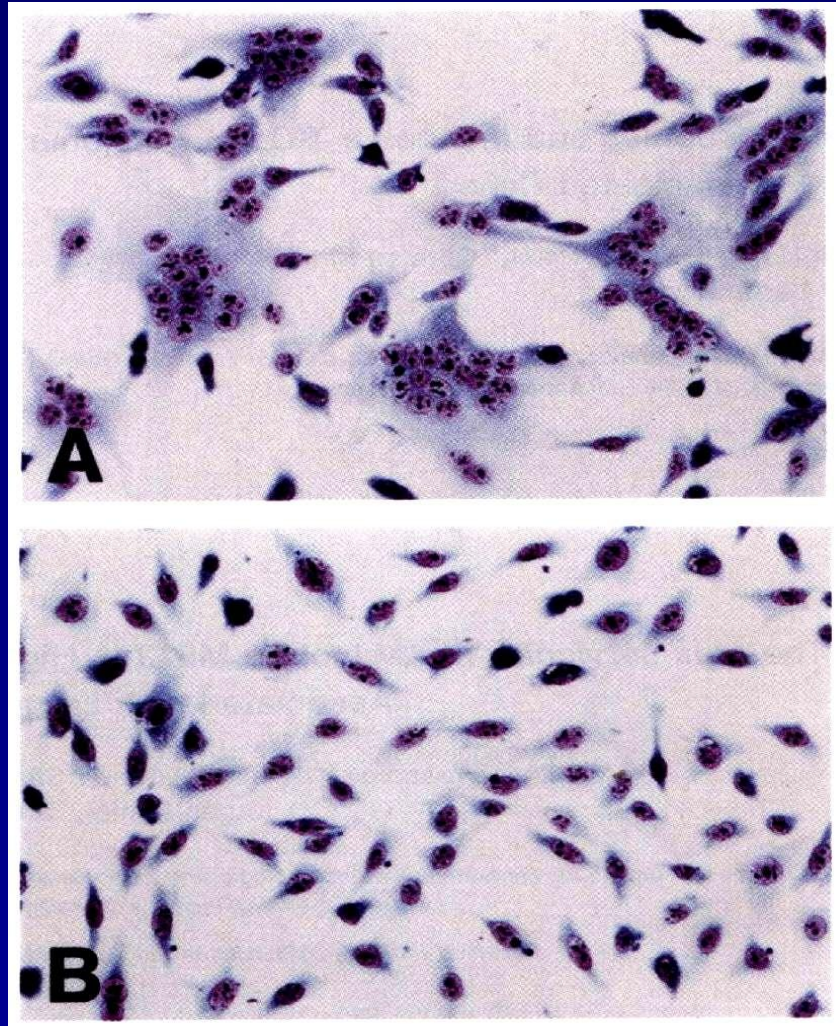
**M813 Infection Is Not Blocked  
by Virus of Any of the *M. musculus* Interference Groups**

Target Cells	Virus	
	M813-neo	10A1-neo
SC1	3.4 x 10e4	7.6 x 10e4
SC1 i M813	1.5	7.4 x 10e4
SC1 i MoMuLV	3.5 x 10e4	7.6 x 10e4
SC1 i MoAmphoV	5.7 x 10e4	8.8 x 10e4
SC1 i 10A1V	8.8 x 10e3	2.0
SC1 i MoMCFV	4.3 x 10e4	2.0 x 10e5

# M813 expression slightly interferences with ecotropic MuLV infection but not with other MuLVs

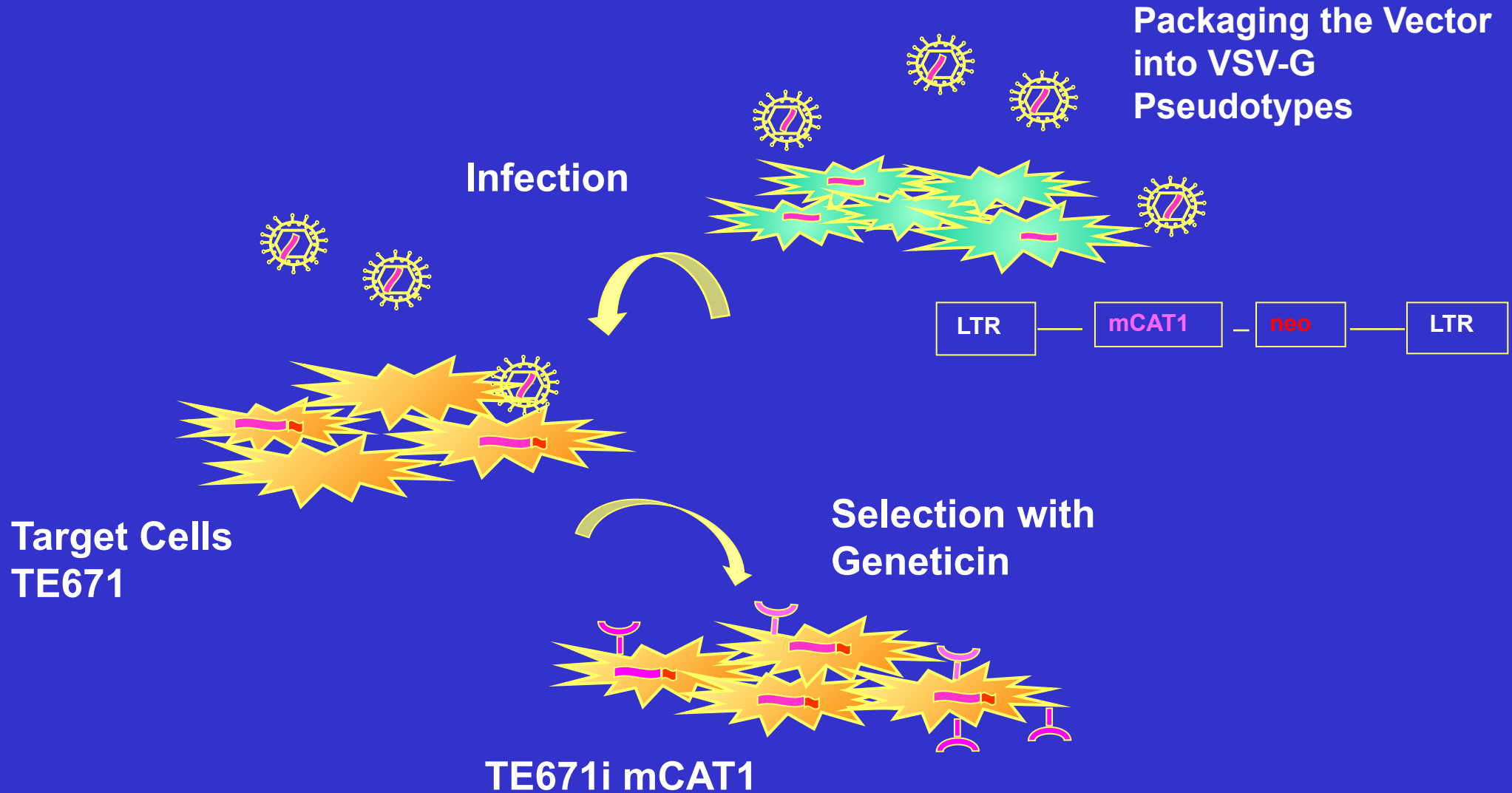
Pseudotypes of MPEVneo	Titer on target cells (GTU/ml)	
	SC-1	SC-1 M813
M813	$5,4 \times 10^4$	0
Mo-MuLV	$1,8 \times 10^5$	$5,1 \times 10^3$
Mo-AmphoV	$1,2 \times 10^5$	$1,2 \times 10^5$
10A1	$7,8 \times 10^4$	$8,2 \times 10^4$
Mo-MCF	$2,6 \times 10^3$	$3,0 \times 10^3$

## Syncytia formation induced by M813



A) PA 317 cells incubated with M813 for 4h  
B) Uninfected PA317

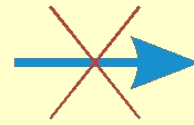
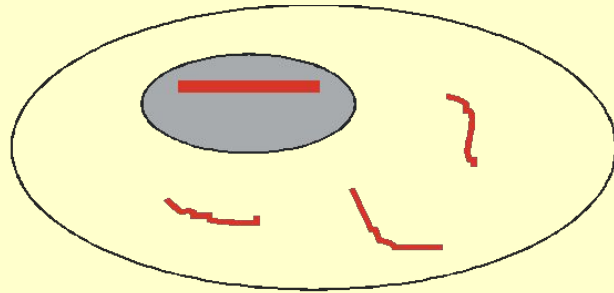
# Establishing Human Cells that Express Murine CAT1 Receptor





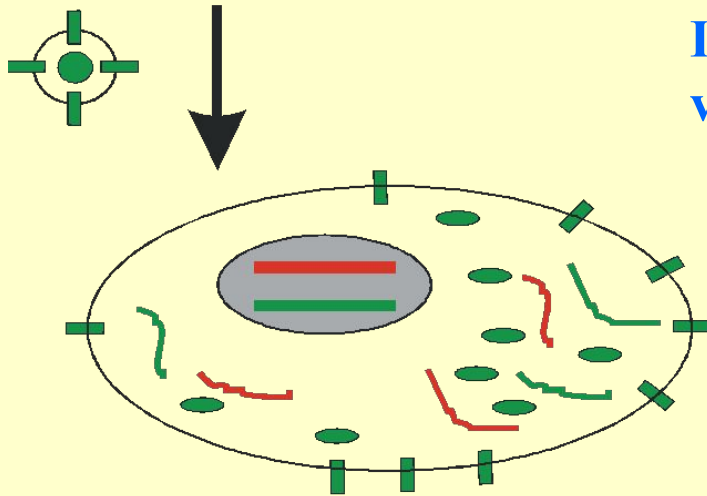
# Marker-rescue assay (A)

Cell with integrated provirus of replication defective virus with marker gene

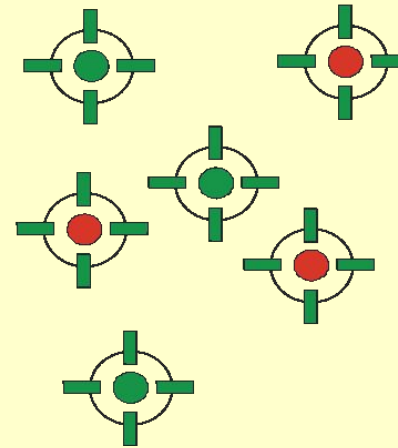


No virus production

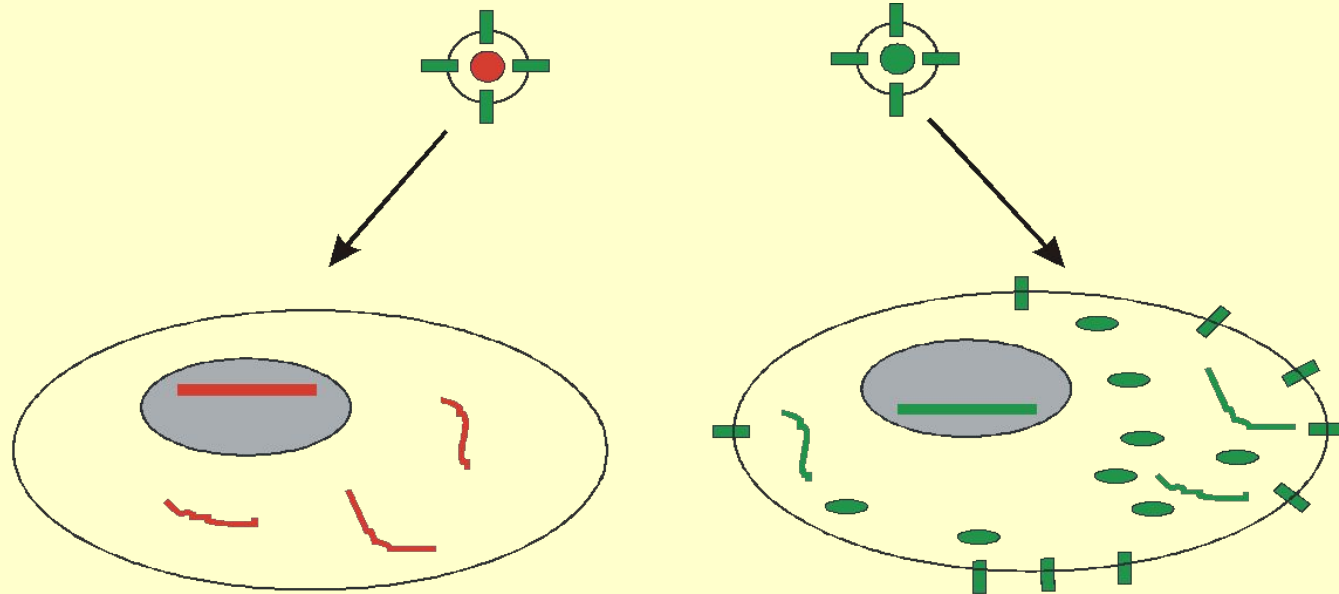
Infection by replication-competent virus



Virus production



## Marker-rescue assay (B)

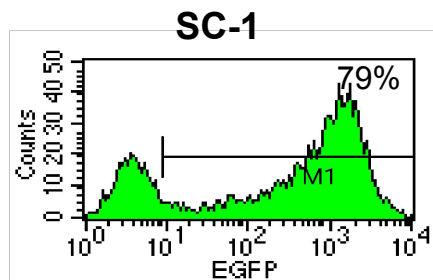


No viral production  
Marker gene expression

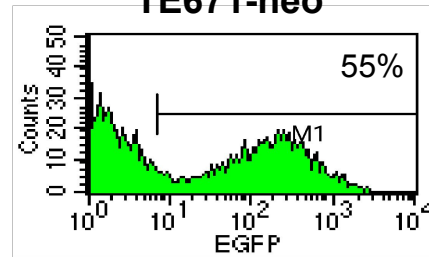
Viral production  
No marker gene expression

# M813 Does Not Use mCAT1 for Infection

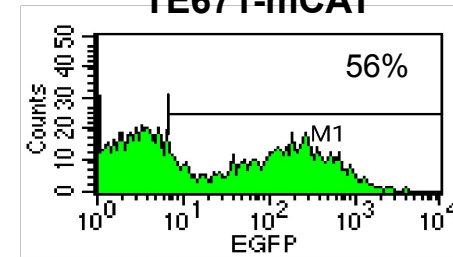
10A1



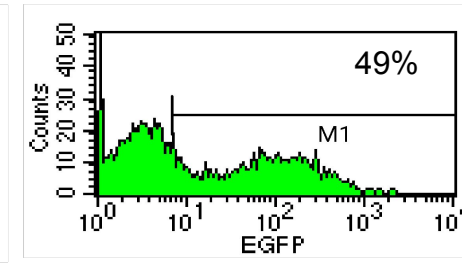
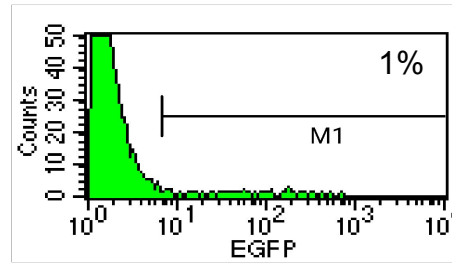
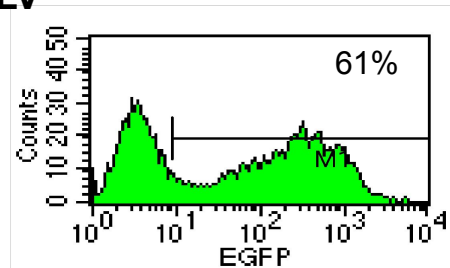
TE671-neo



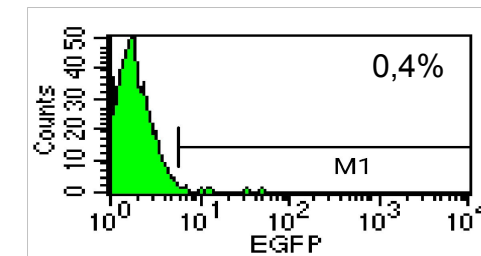
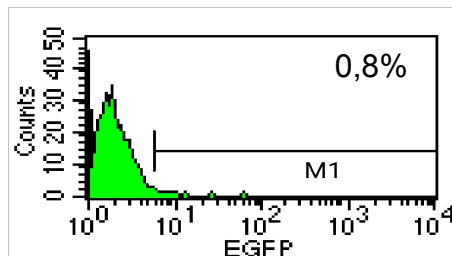
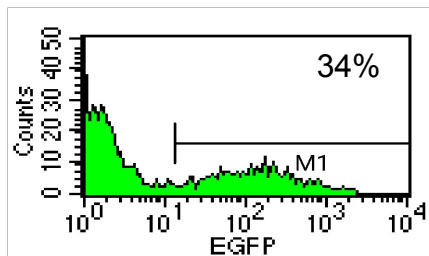
TE671-mCAT



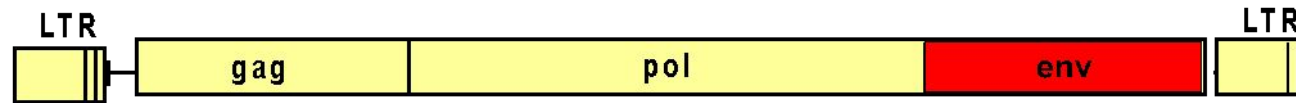
Mo-MuLV



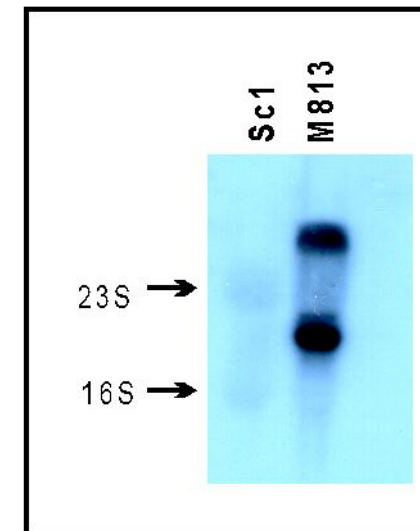
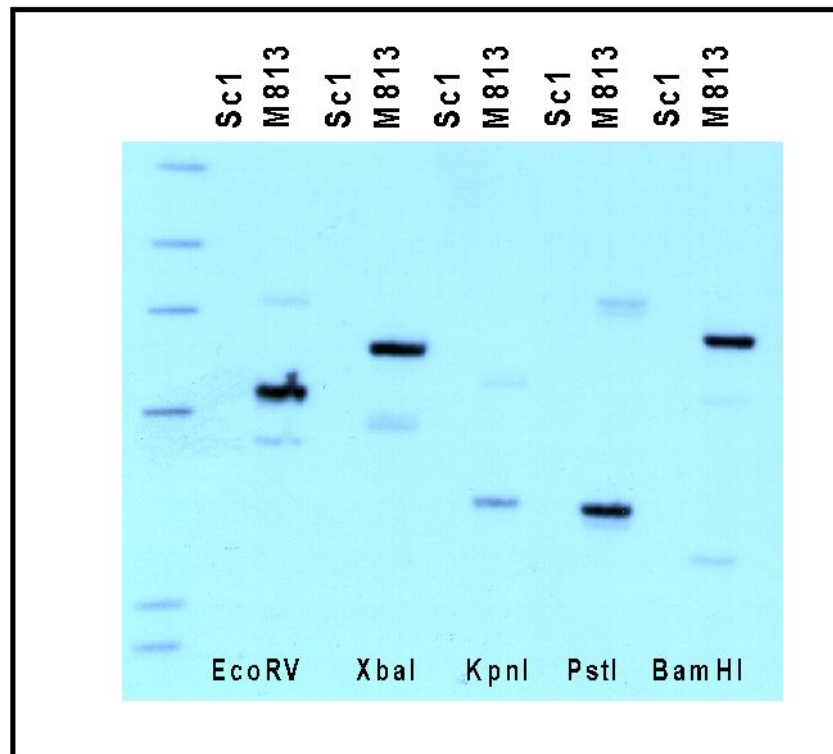
M813



# Molecular Cloning of Env-Region of M813



RT-PCR using oligos  
from conserved sequences  
template: viral RNA

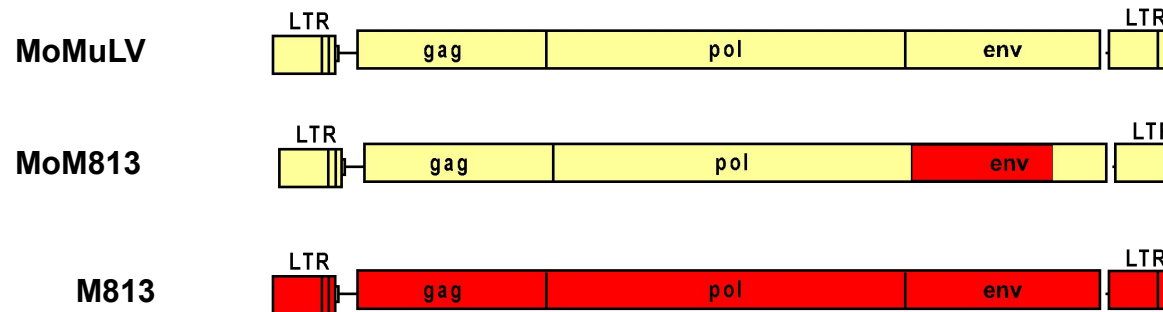


Genomic DNA

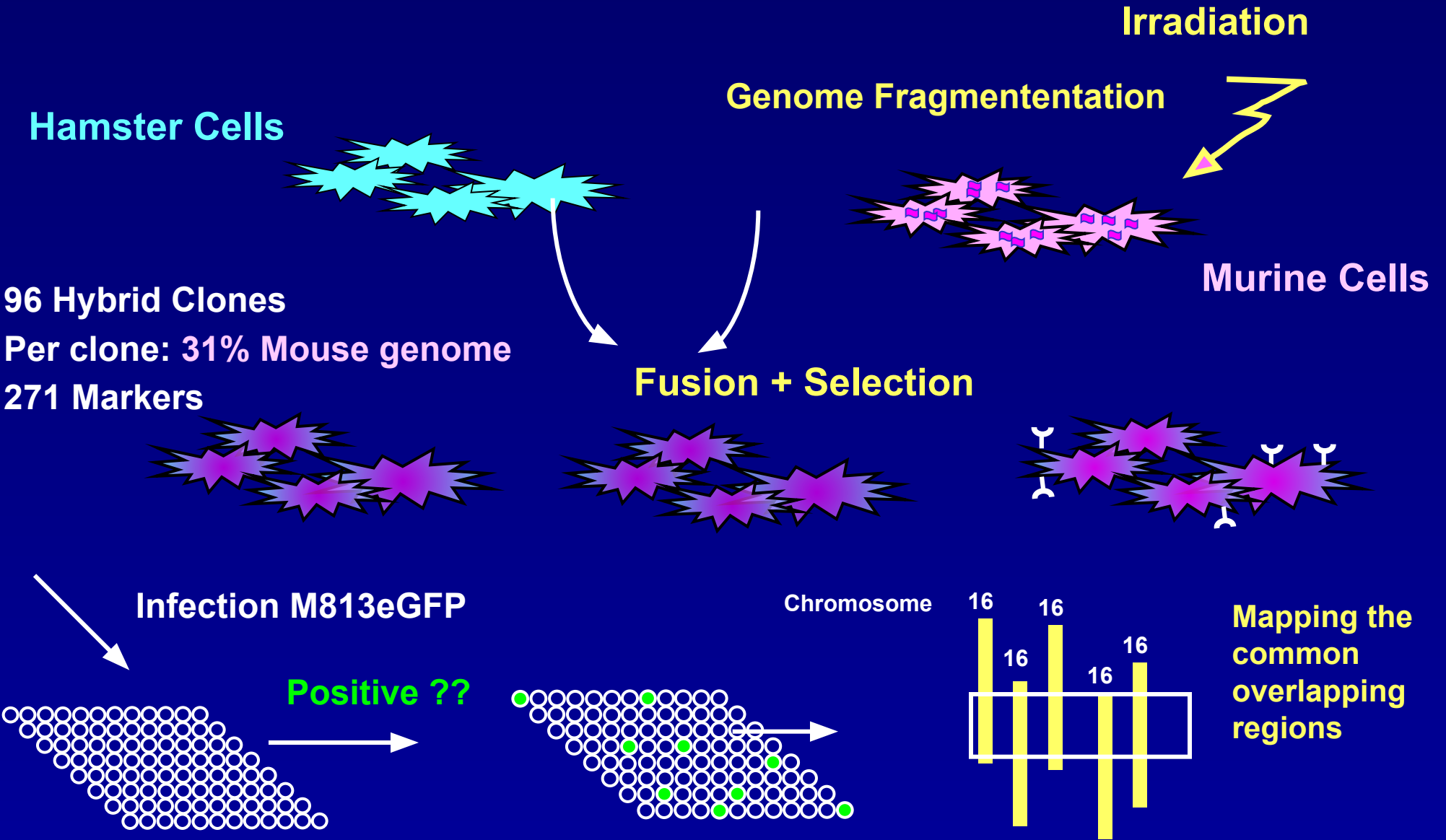
RNA

# M813 SU Sequences Dictate Receptor Usage

Target Cells	Titer of MPEVneo pseudotyped with	
	MoM813	MoMuLV
SC1	1.0 x 10 <sup>5</sup>	5.2 x 10 <sup>5</sup>
SC1 i MoM813	0	7.0 x 10 <sup>5</sup>
SC1 i MoMuLV	1.0 x 10 <sup>5</sup>	nd
SC1 i MoAmphoV	1.5 x 10 <sup>5</sup>	nd
SC1 i M813	0	nd

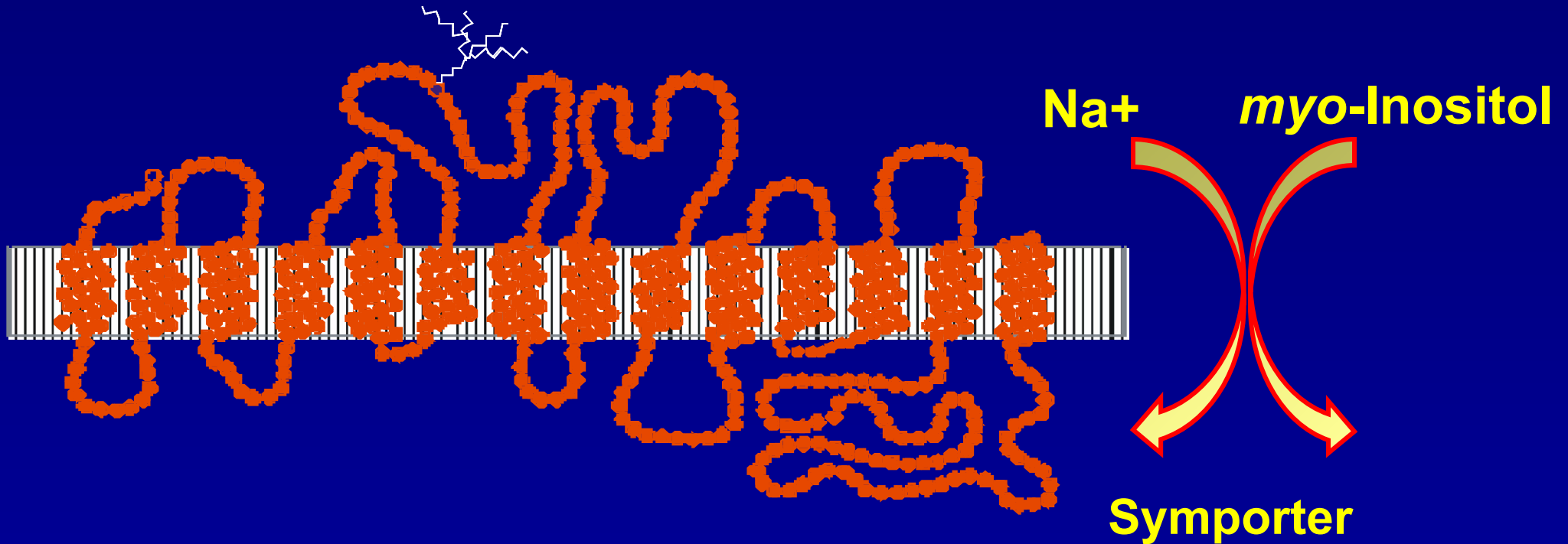


# Localisation of the M813 Receptor Gene by Hybrid Cell Lines



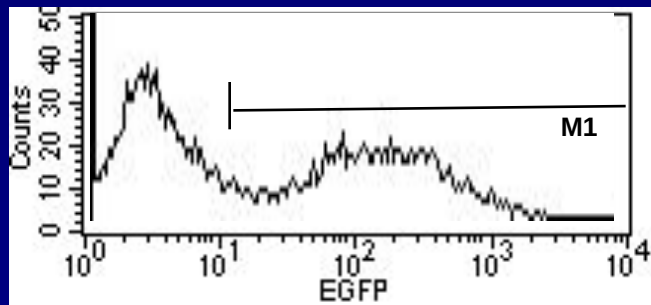
promising candidate gene on Chr. 16:

► *Sc/5A3* : multiple membrane spanning surface protein encoding SMIT1: Sodium myo-Inositol Transporter 1



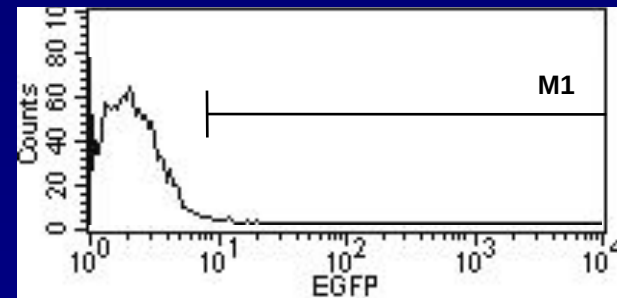
# Expression of mSMIT1 in Human Cells Imparts Susceptibility to M813 Infection

**SC1**



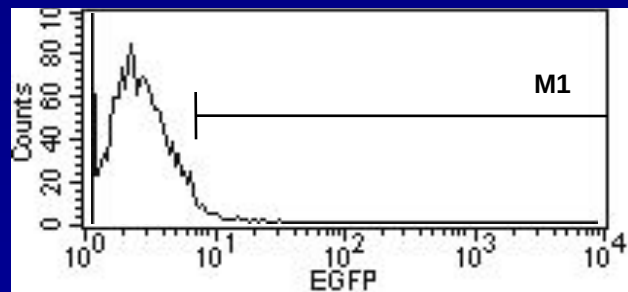
**29%**

**TE671**



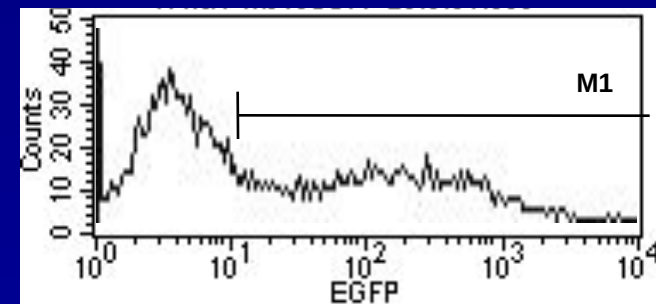
**1%**

**TE671-neo**



**1%**

**TE671-mSMIT1**



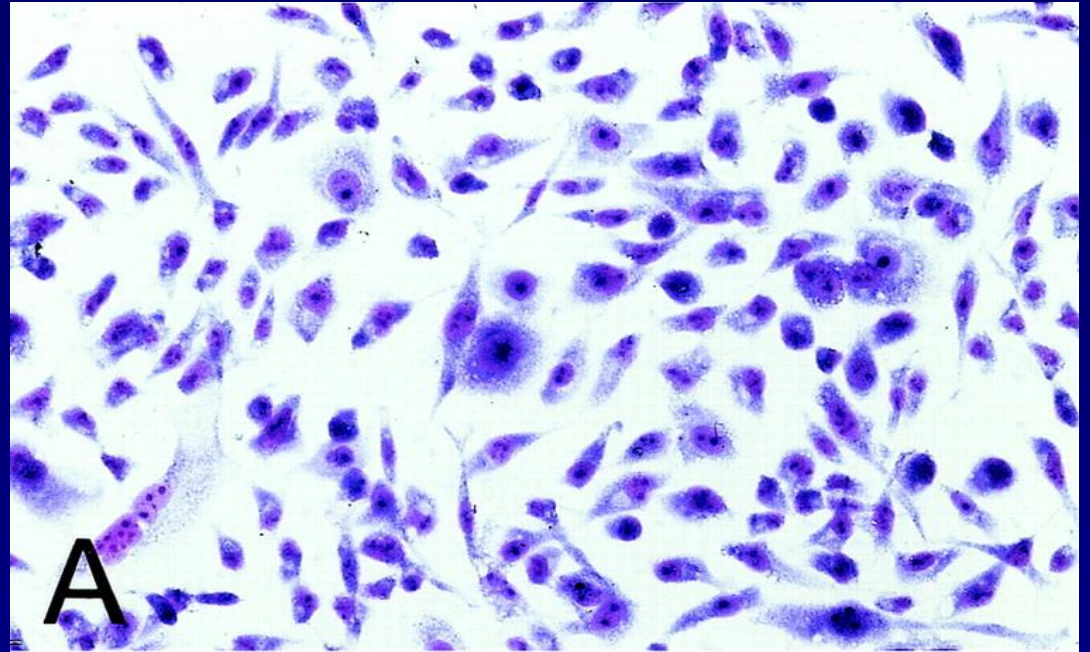
**52%**

**Infection with MP-eGFP pseudotyped with M813**

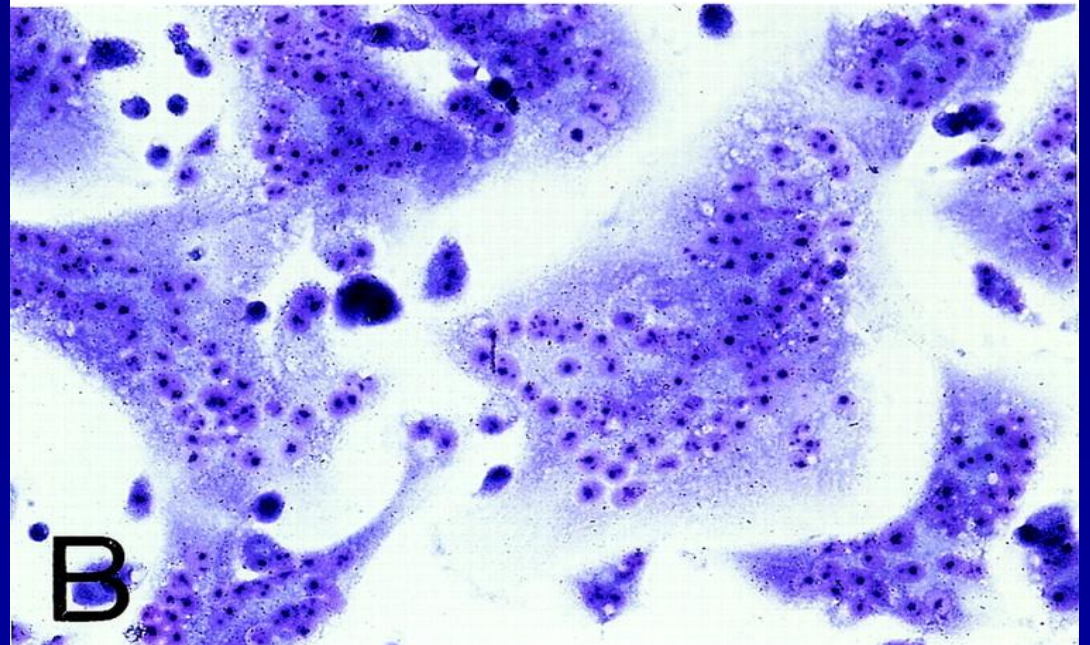


# M813 Induces Syncytia Formation in Cells Expressing Its Receptor

Te671 + neo

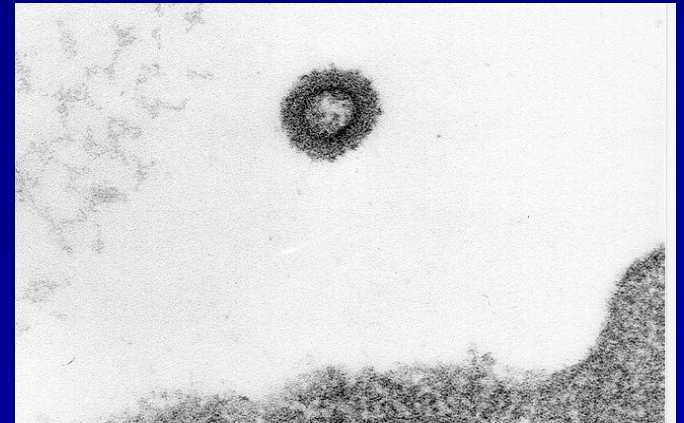
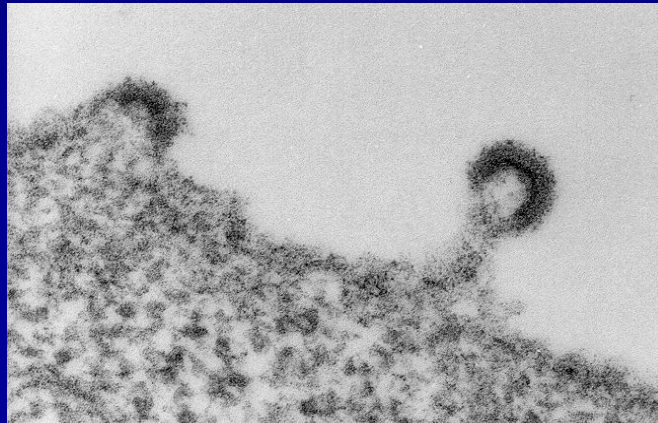
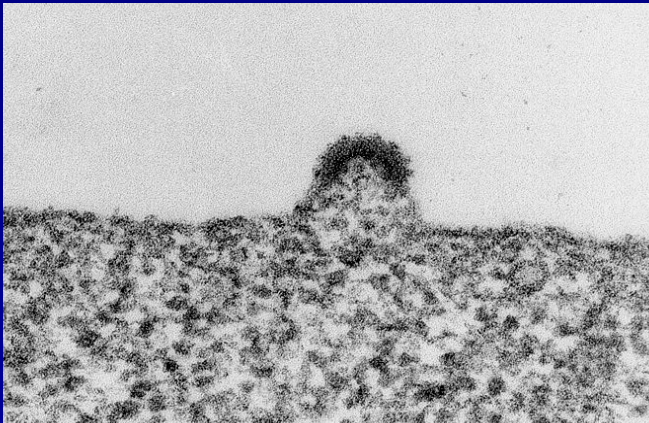


Te671 + mSmit1



# Summary

- ❖ M813 belongs to a unique receptor interference group
- ❖ M813 is highly fusogenic in vitro and in vivo
- ❖ M813 uses the m SMIT1 protein as a receptor
- ❖ M813 induces T-cell lymphoma associated with large multinucleated cells



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## **MuLV EXPRESSION IN SC-1 CELLS DETERMINES THE FUSOGENIC PROPERTIES OF SC-1 CELLS**

<b>Target Cell</b>	<b>Fusion Index (%)</b>	
	<b>uninfected</b>	<b>M813-infected</b>
<b>SC-1</b>	<b>2.4</b>	<b>5.4</b>
<b>SC-1+MoMuLV</b>	<b>0.8</b>	<b>94</b>
<b>SC-1+MoMCF</b>	<b>1.6</b>	<b>73.2</b>
<b>SC-1+MoAmphoV</b>	<b>2.0</b>	<b>70.1</b>
<b>SC-1+Mo10A1</b>	<b>2.8</b>	<b>77.4</b>

## **M813 INDUCES SYNCYTIA FORMATION MORE EFFECTIVE THAN OTHER MURINE LEUKEMIA RETROVIRUSES**

	<b>Virus</b>			
<b>Target cells</b>	<b>M813</b>	<b>Mo-MuL V</b>	<b>Mo-MC FV</b>	<b>Mo-Ampho V</b>
<b>PA317</b>	<b>80.1%</b>	<b>10.2%</b>	<b>6.1%</b>	<b>2.4%</b>
<b>SC1-Mo-MuLV</b>	<b>67.8%</b>	<b>1.9%</b>	<b>2.1%</b>	<b>2.5%</b>
<b>SC1-Mo-MCFV</b>	<b>68.5%</b>	<b>10.8%</b>	<b>4.1%</b>	<b>3.0%</b>
<b>SC1-Mo-AmphoV</b>	<b>68.2%</b>	<b>5.7%</b>	<b>1.1%</b>	<b>1.6%</b>
<b>SC1-M813</b>	<b>1.9%</b>	<b>4.2%</b>	<b>2.3%</b>	<b>2.4%</b>
<b>SC1</b>	<b>3.0%</b>	<b>3.2%</b>	<b>1.3%</b>	<b>2.2%</b>

$$\text{Fusion index (FI)} = (\text{N} - \text{S})/\text{T}$$

**N – number of nuclei in syncytium**

**S – number of syncytium**

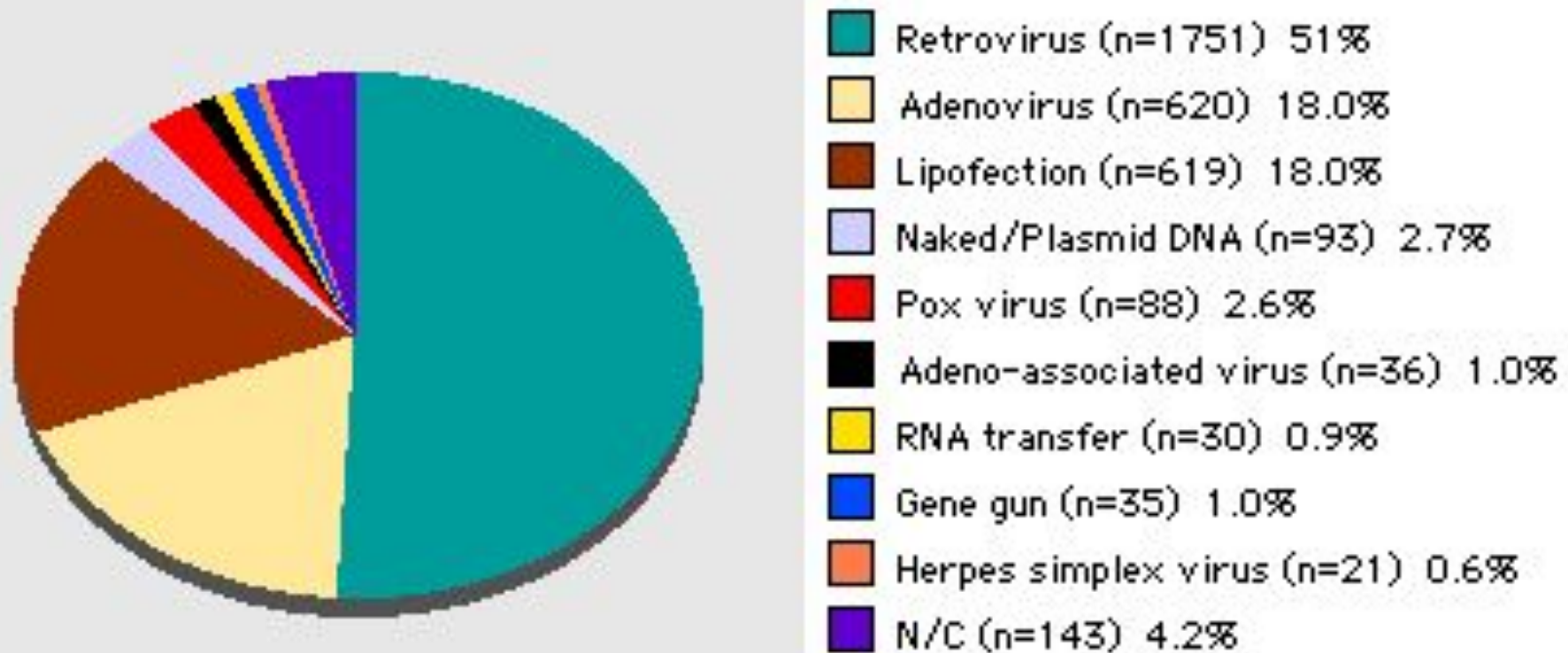
**T – total number of nuclei**

**Over 500 nuclei were counted in each  
experiment to obtain the FI value**

# Gene Therapy trials - overview

<http://www.wiley.co.uk/wileychi/genmed/clinical/>

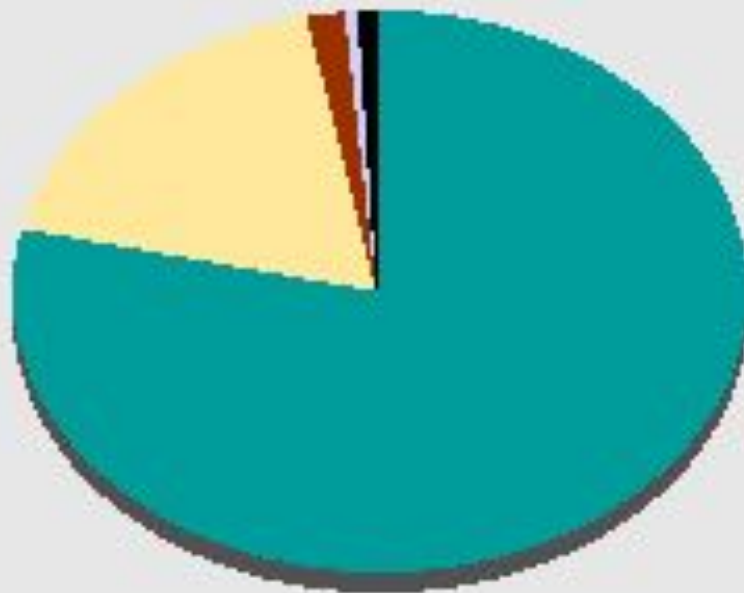
Patients by vector









# Gene Therapy trials - overview

<http://www.wiley.co.uk/wileychi/genmed/clinical/>

Protocols by continent



	America (n=418) 78.6%
	Europe (n=98) 18.4%
	Asia (n=8) 1.5%
	Australia (n=3) 0.6%
	Africa (n=1) 0.2%
	Multi-continent (n=4) 0.8%



# Gene Therapy trials - overview

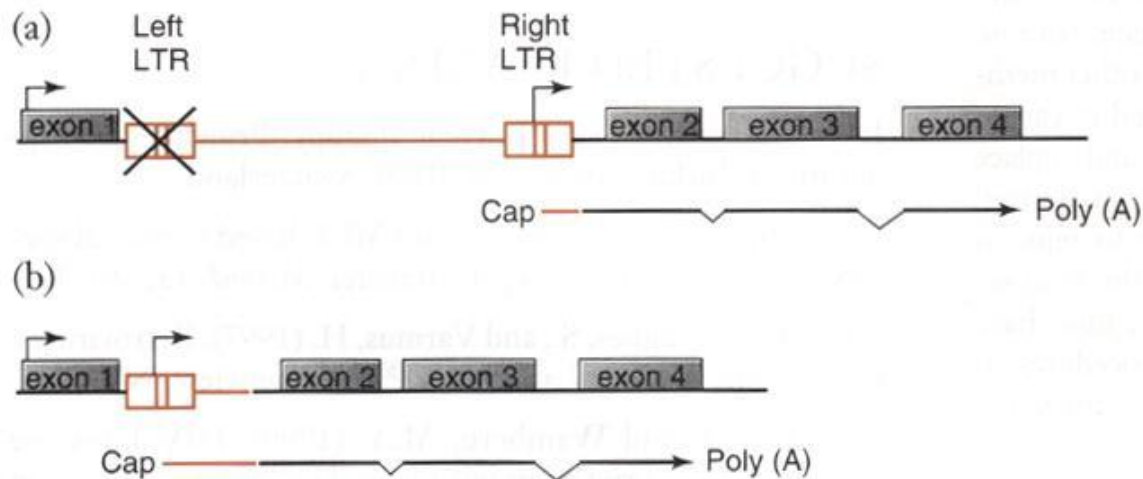
<http://www.wiley.co.uk/wileychi/genmed/clinical/>

Patients by phases



# Инсерционный мутагенез

Встраивание провируса может приводить к трансформации клетки



**Promoter insertion near proto-oncogenes can lead to cell transformation and tumor formation.**

Transcription of the proto-oncogene (exons shown as filled boxes) initiates within the viral LTR, either as a result of inactivation of the left-hand LTR (a) or deletion of the right-hand LTR (b). Result is a mixed viral/cellular mRNA that expresses the proto-oncogene under control of viral promoter/enhancer.

# Открытие вирусов

1892 год

Д.И.Ивановский – инфекционный фильтрующийся агент, вызывающий табачную мозаику.

1898 год

M.Beijerinck - “contagium vivum fluidum”.

Loeffler & Frosch – foot-and- mouth disease

1908 год

Ellerman & Bang – ALV

1911 год

P.Rous - RSV

