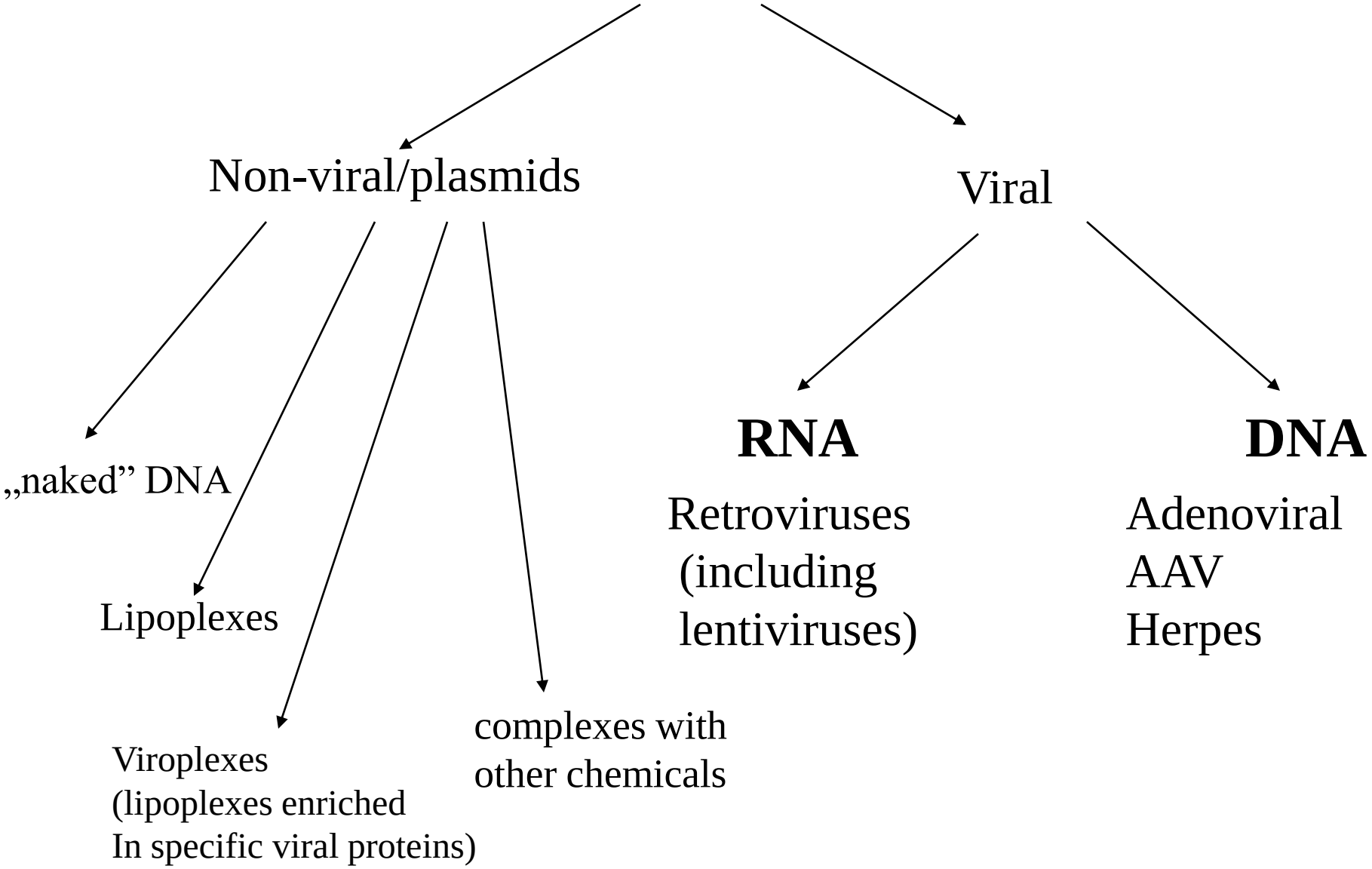
Lecture IV

Viral vectors

7th November 2011

Vectors


Non-viral/plasmids
Viral
„naked” DNA
Lipoplexes
Viroplexes
(lipoplexes enriched
In specific viral proteins)
complexes with
other chemicals
RNA
Retroviruses
(including
lentiviruses)
DNA
Adenoviral
AAV
Herpes

Viral vectors used in clinical trials of gene therapy

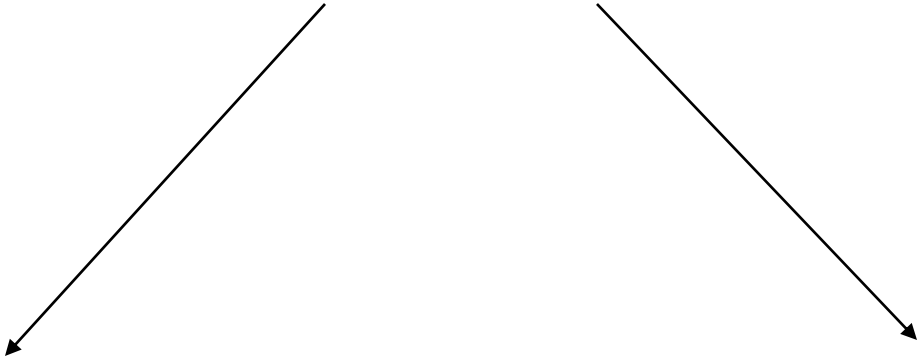
1. Adenoviruses

2. Retroviruses
    a) oncoretroviruses
    b) lentiviruses

3. Adeno-associated viruses

4. Herpes simplex virus

Viral vectors



Integrating

Lentiviral
-retroviral
-AAV (limited)

Non-integrating

Adenoviral
HSV
Baculoviral

Integration depends on:
-LTR sequences and integrase (retroviruses)
- ITR seqeuences and rep proteins (AAV)

Integrated transgene

Advantage:

- perpetual
- may provide a stable expression and a cure

Disadvantage:

- random insertion may lead to silencing of a transgene or inactivation or dysregulated activation of host genes

- unknown, long-term effects of the transgene

Episomal transgene

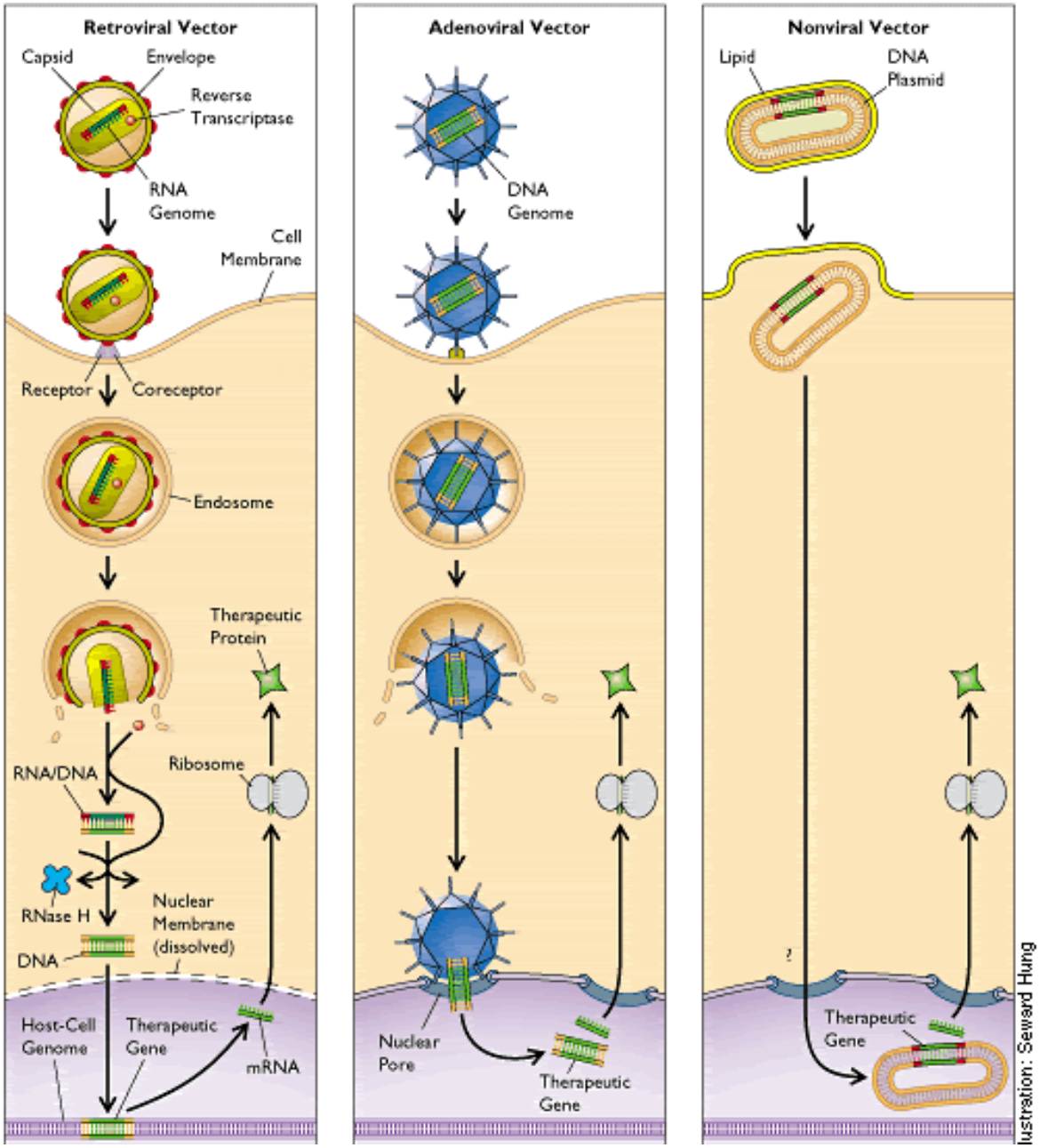
Advantage

- no risk of insertional mutagenesis

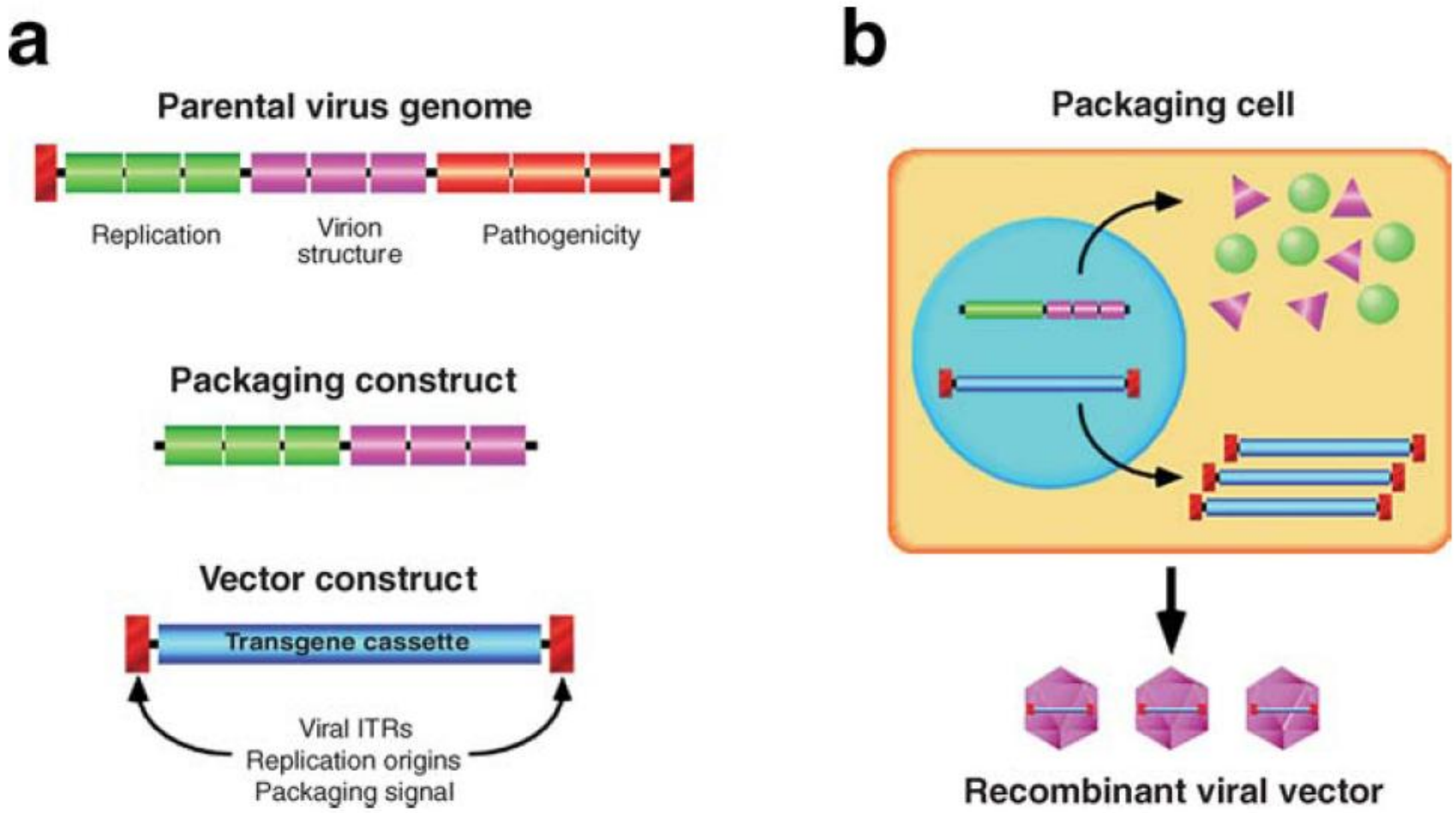
Disadvantage

- transient expression
- repeated treatments may be necessary

Viral vectors follow the rule of their parent viruses



Principle of generating viral vector

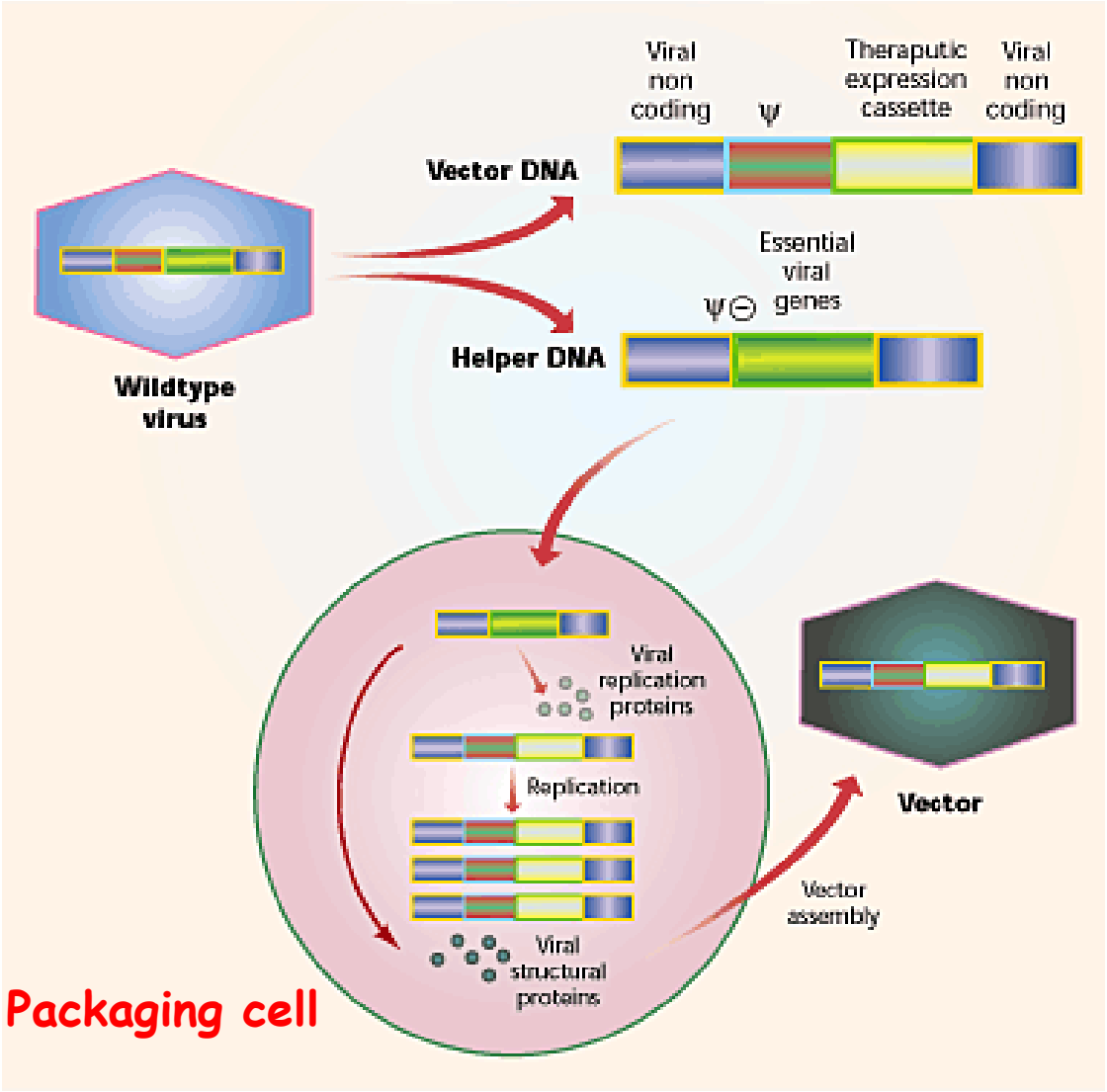


Cis- sequences

trans- sequences

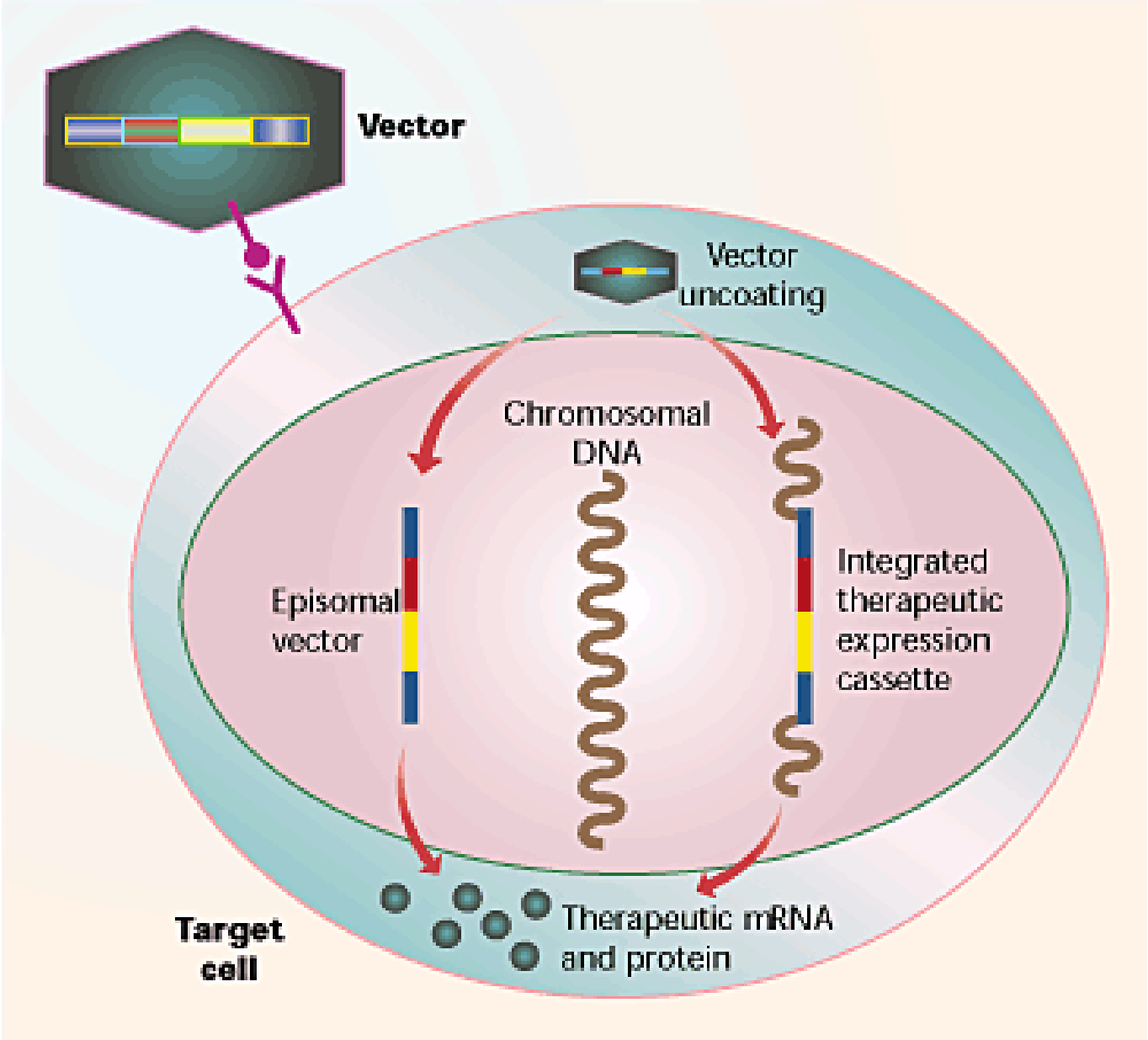
Verma & Weitzman, Ann Rev Biochem 2005

Genetic strategy for engineering a virus into a vector.



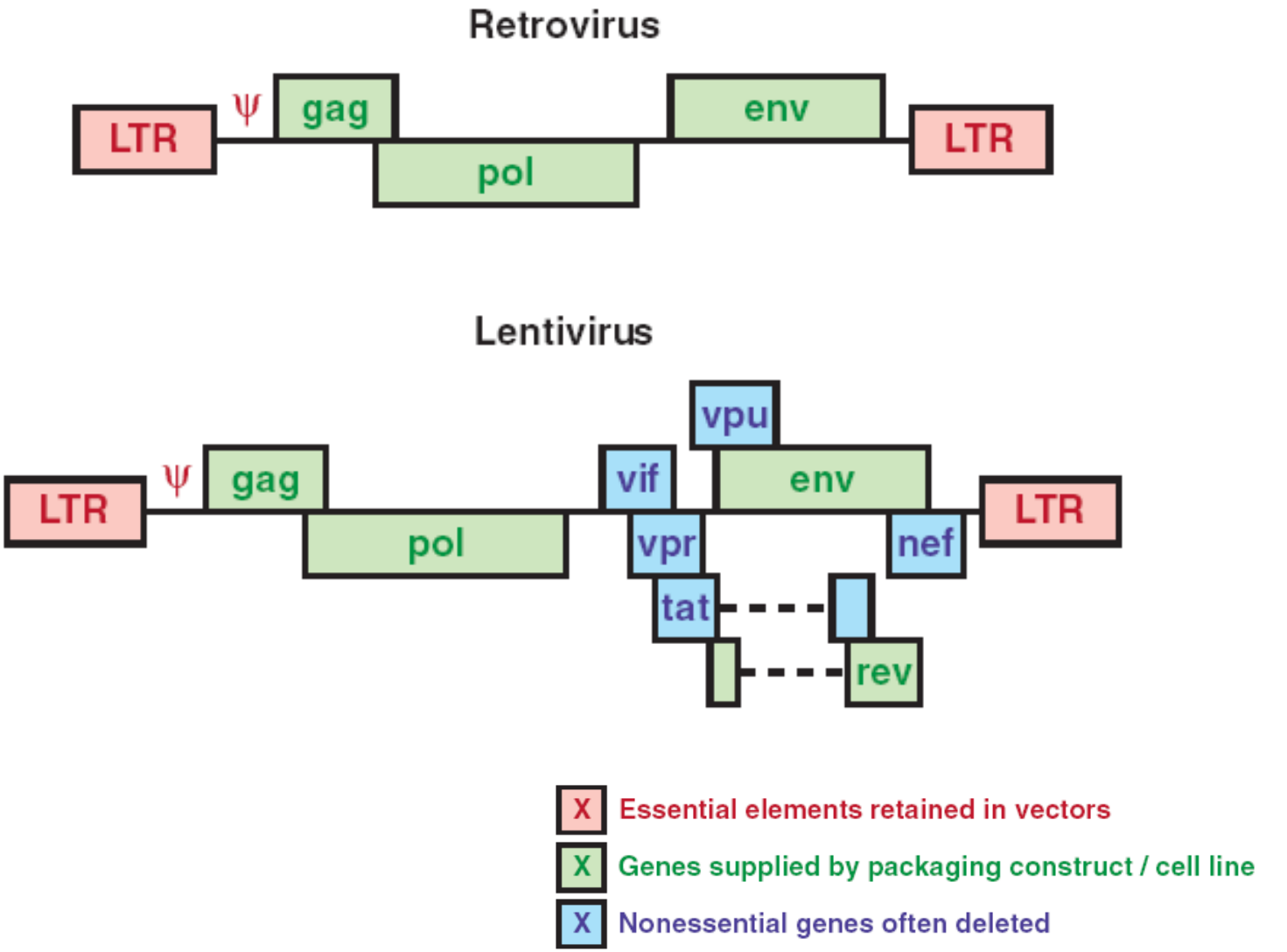
Kay et al., Nature Medicine **7**, 33 - 40 (2001)

Transduction of a target cell



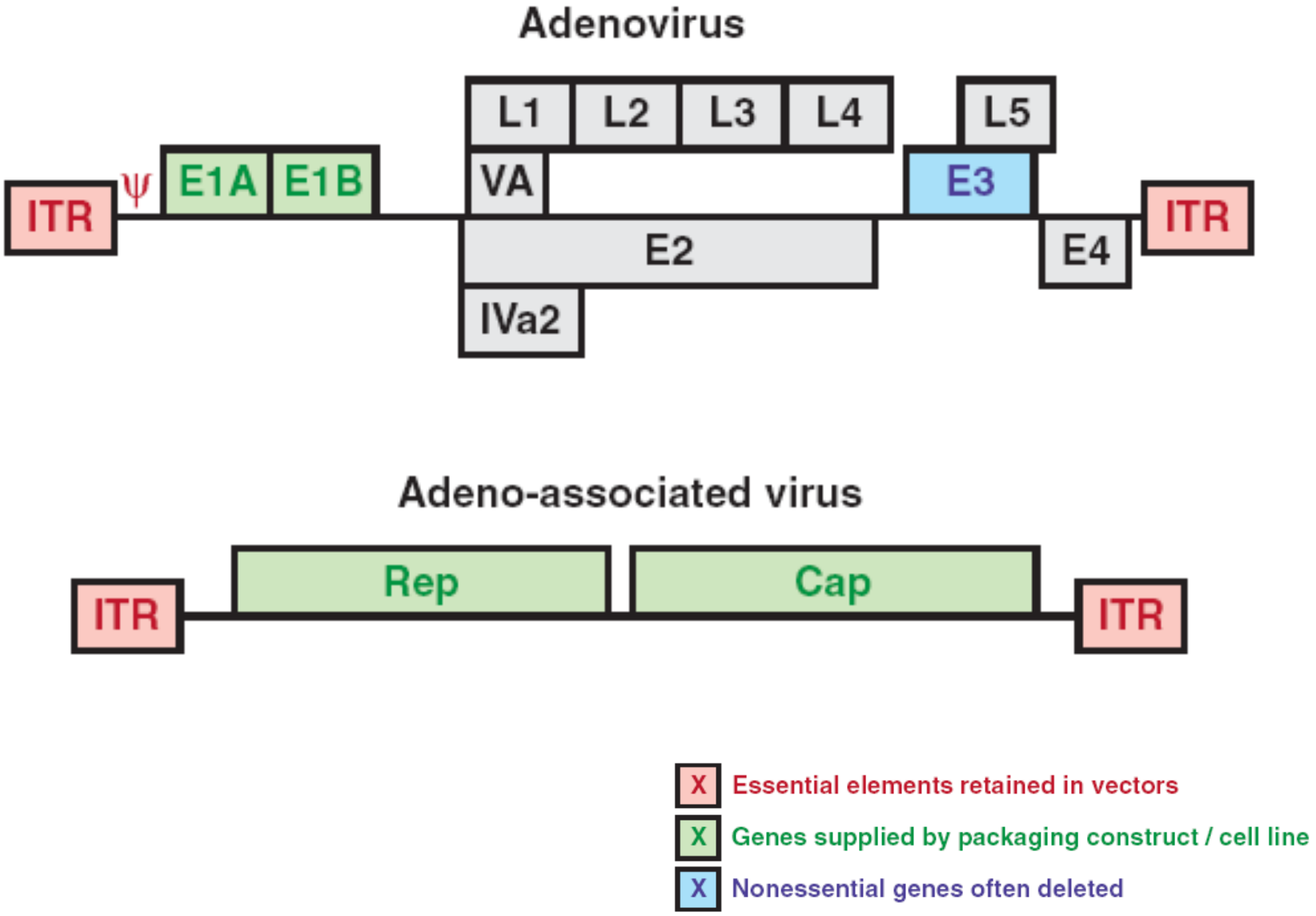
Kay et al., Nature Medicine **7**, 33 - 40 (2001)

Essential and non-essential elements in different viral vectors (1)



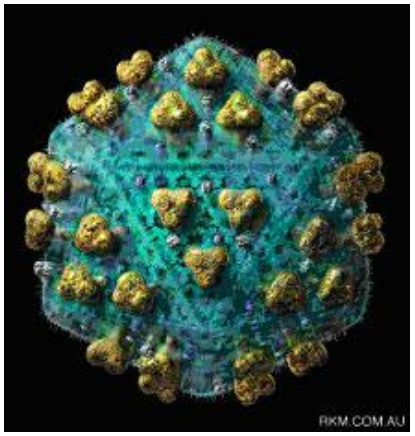
Verma & Weitzman, Ann Rev Biochem 2005

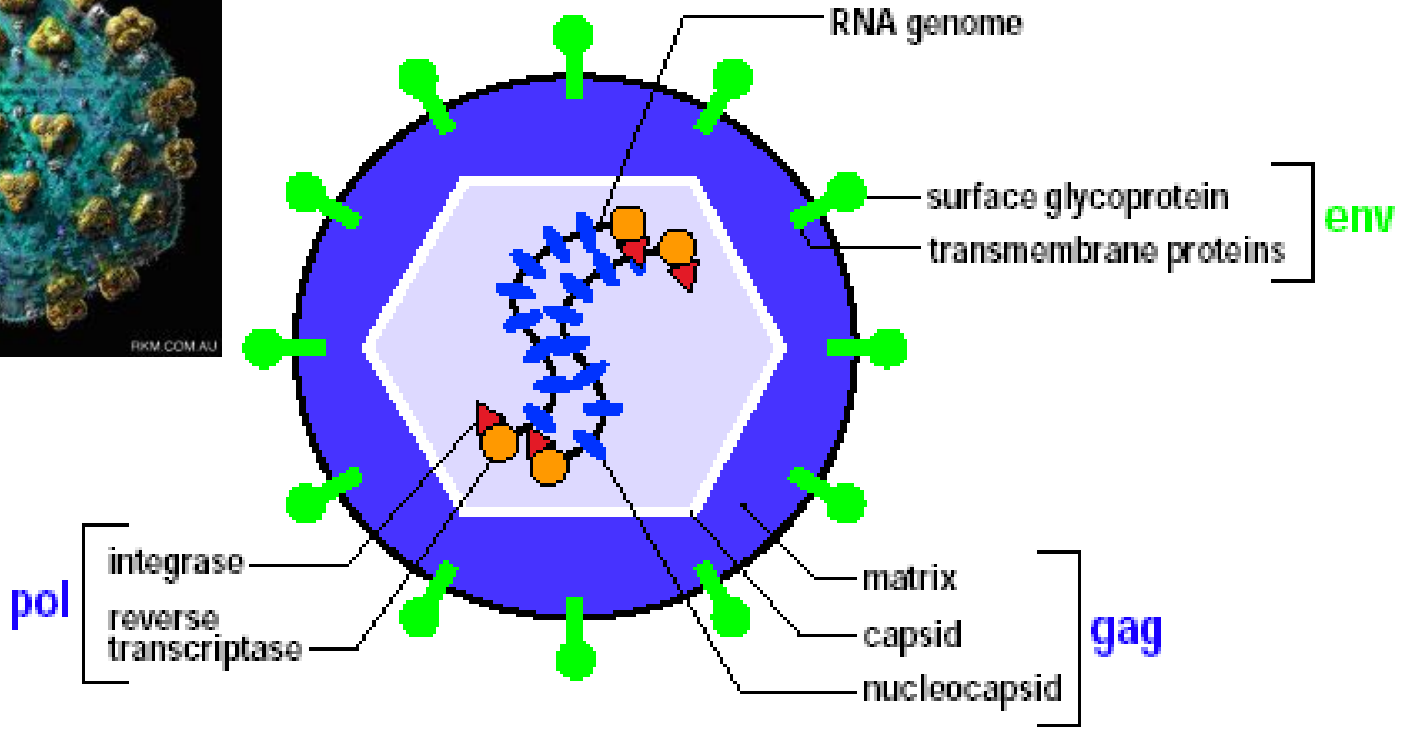
Essential and non-essential elements in different viral vectors (2)



Verma & Weitzman, Ann Rev Biochem 2005

Retroviral expression system





Gag – core proteins, matrix, nucleocapsid
Pol – reverse transcriptase and integrase
Env – envelope glycoproteins

Features of retroviruses

All retrovirus genomes consist of two molecules of RNA, which are s/s, (+)sense and have 5' cap and 3' poly-(A) (equivalent to mRNA). These vary in size from ~8-11kb. Retrovirus genomes have 4 unique features:

1. They are the only viruses which are truly diploid.
2. They are the only RNA viruses whose genome is produced by cellular transcriptional machinery (without any participation by a virus-encoded polymerase).
3. They are the only viruses whose genome requires a specific cellular RNA (tRNA) for replication.
4. They are the only (+)sense RNA viruses whose genome does **not** serve directly as mRNA immediately after infection.

Two RNA molecules are physically linked as a dimer by hydrogen bonds (co-sediment). In addition, there is a 3rd type of nucleic acid present in all particles, a specific type of tRNA (usually trp, pro or lys) - required for replication

Retroviral vectors

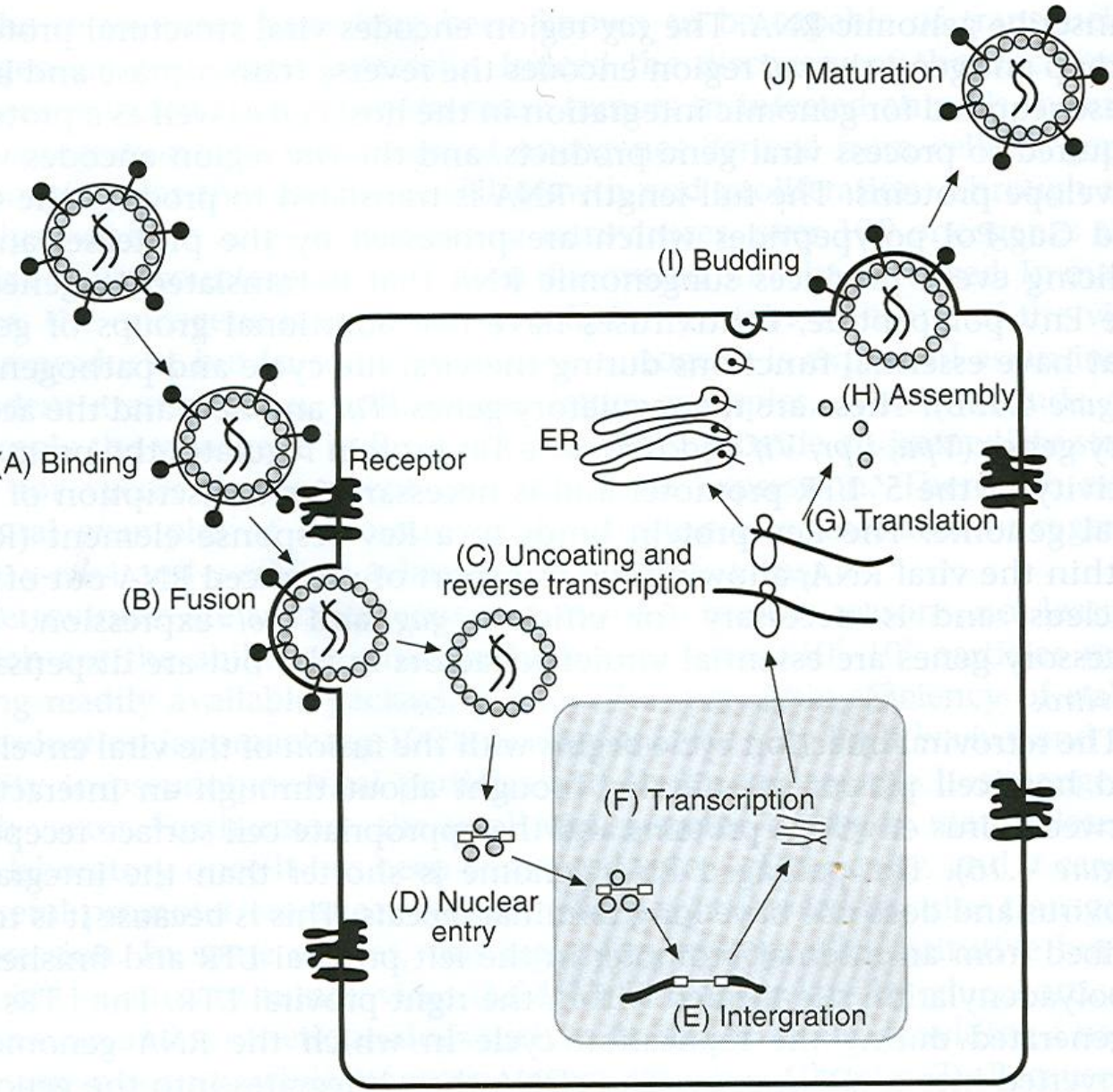
Family *Retroviridae*

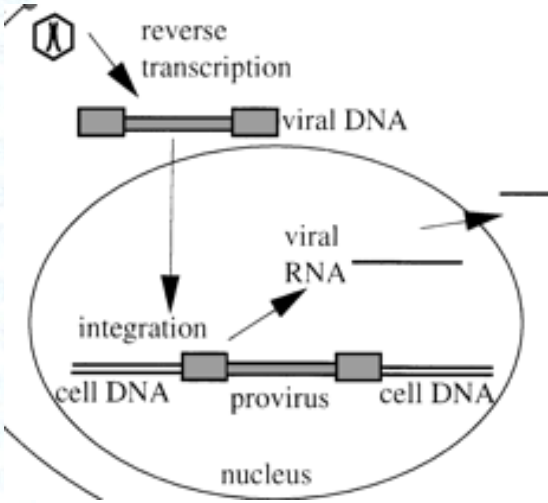
1. Oncoretroviruses:
   a) Alpharetrovirus – Avian leucosis virus (RSV)
   b) Betaretrovirus – Mouse mammary tumor virus (MMTV)
   c) Gammaretrovirus – Murine leukemia virus (Mo-MLV)
   d) Deltaretrovirus – Bovine leukemia virus
   e) Epsilonretrovirus – Walleye dermal sarcoma virus

2. Lentivirus – HIV, HTLV, BLV

3. Spumavirus – human spumavirus
   (human foamy virus – HMV)

Retroviral infectious cycle



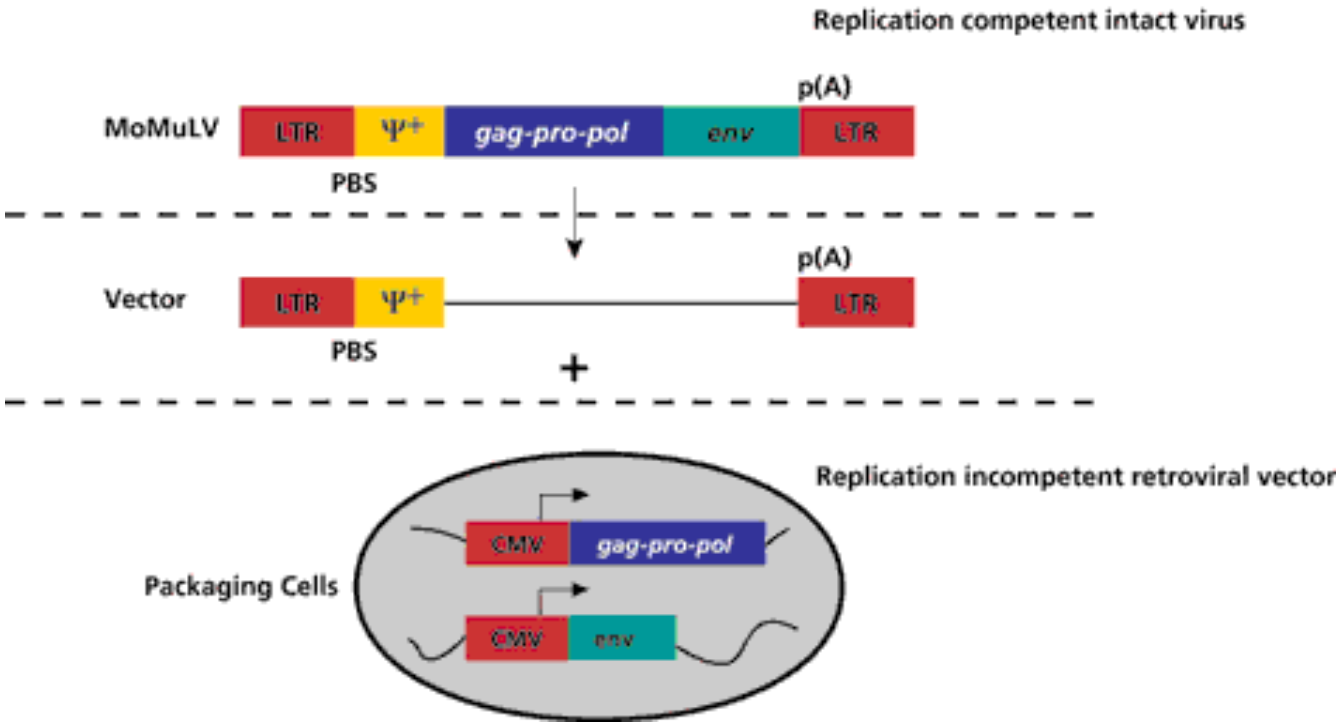


How to make a vector from a virus?

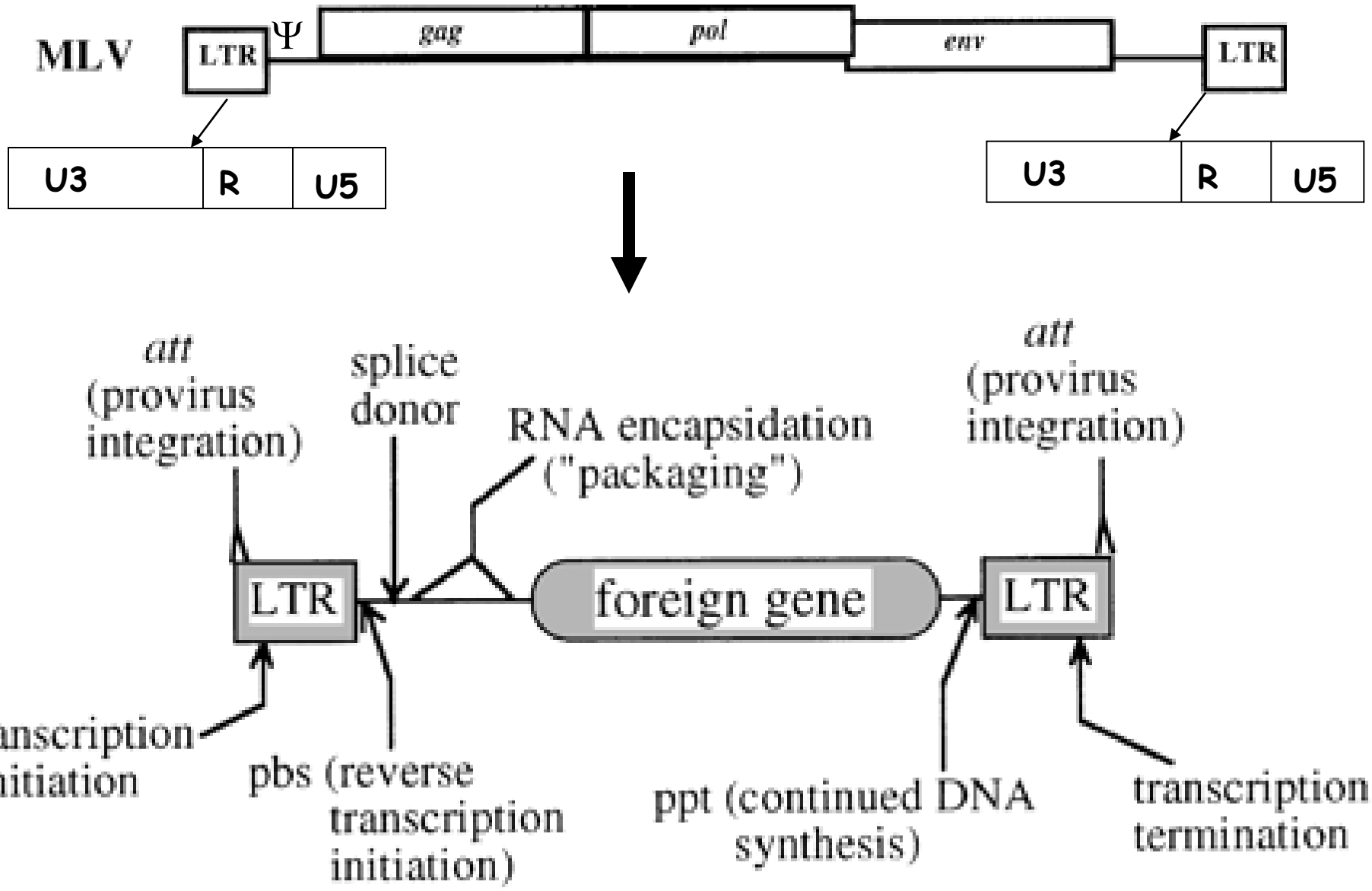
-replace the coding region of the virus with the therapeutic gene/reporter gene

-cist-acting sequences remain intact

- the construct is introduced into the packaging cell line, providing the structural viral proteins in trans



Structure of a retroviral vector



Retroviral vectors

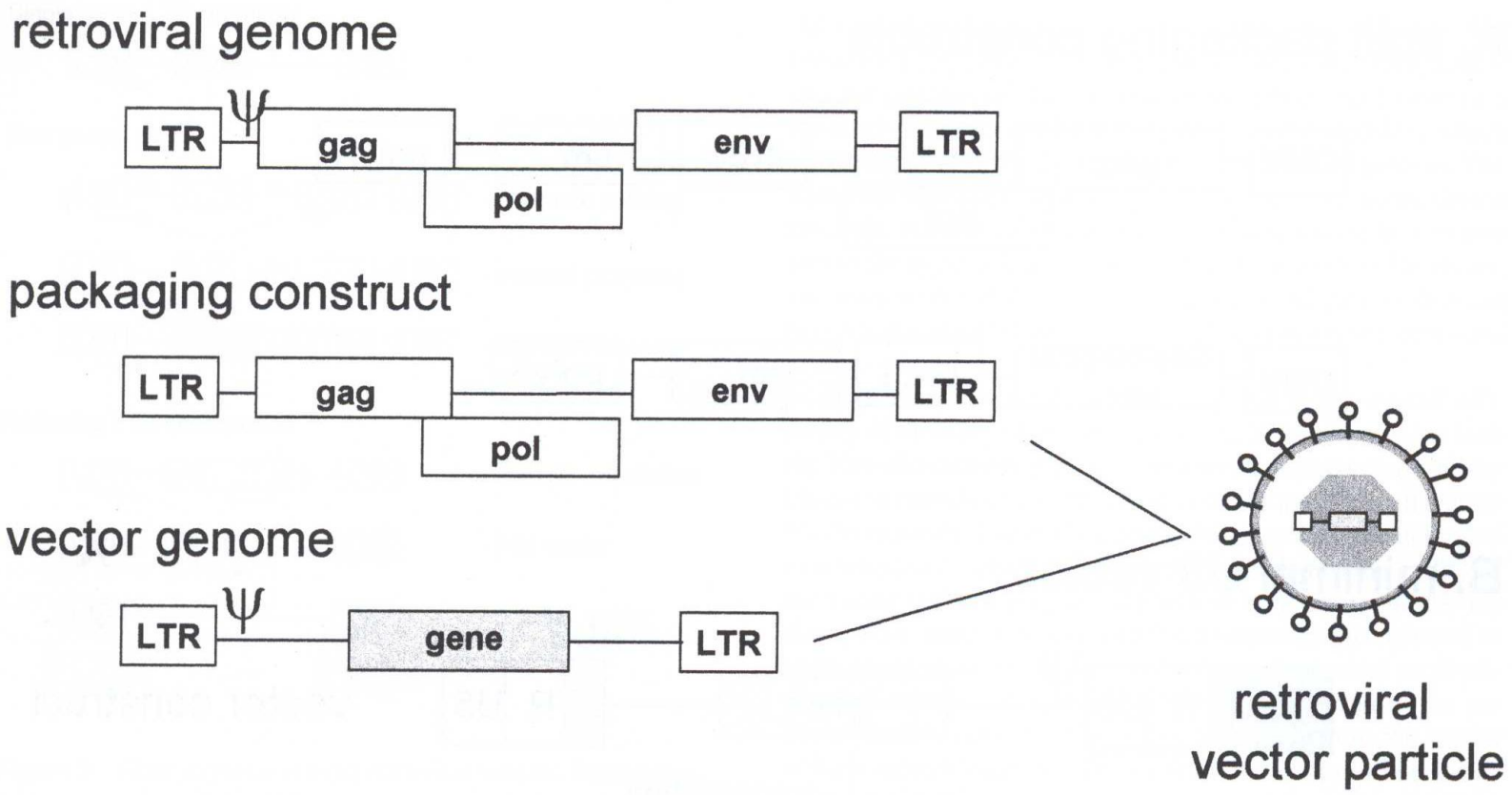


Figure 3 Basic retroviral vector design. The packaging construct provides all the viral proteins in *trans* to the vector genome, which codes for no viral proteins but retains all the necessary *cis* elements. The deletion of the packaging signal from the packaging construct prevents its incorporation into viral particles.

Construction of retrovirus vector

1. Construction of retrovirus vector as a recombinant plasmid in *E.coli*

2. Introduction of a plasmid into a packaging cell line

3. Incorporation of vector transcripts into transmissible virus particles

Important components of retroviral cloning strategy

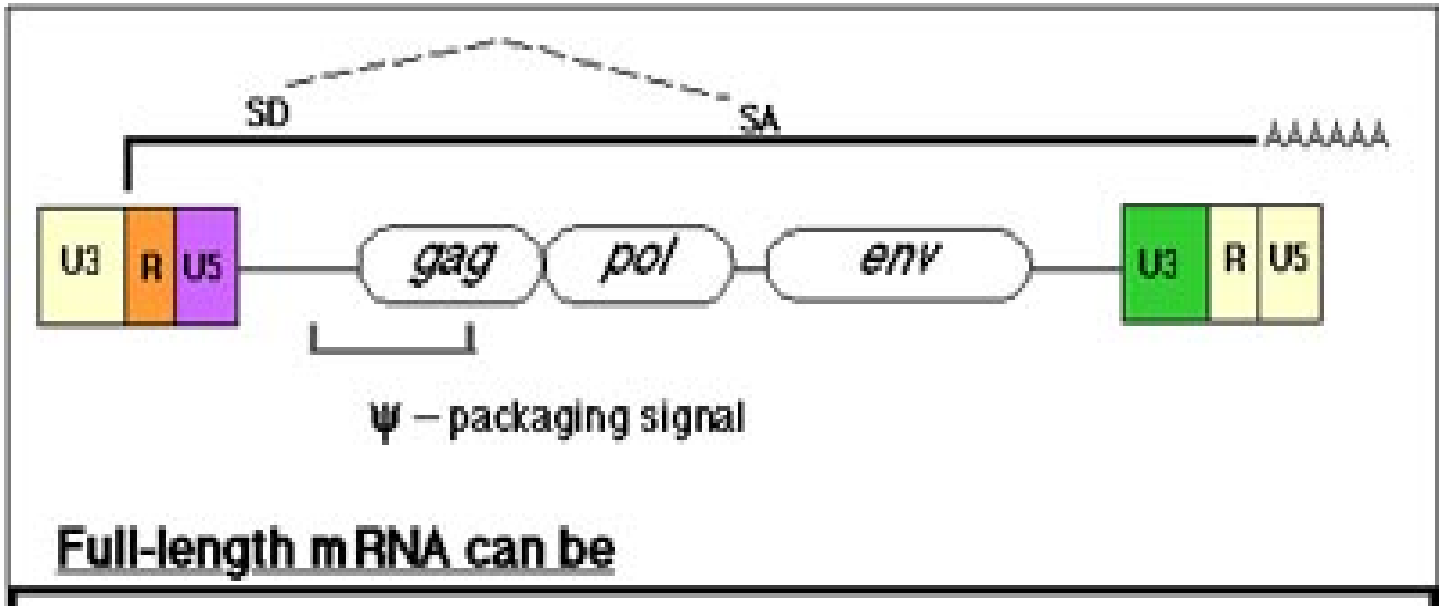
1. Separation of genes and sequences acting **in-cis** into different plasmid vectors in order to avoid viral reconstruction through recombinantion

   - coding sequences, required for formation of infectious particle act **in-trans**
   - in-trans sequences are delivered in separate plasmids and are present in genome of packaging cells

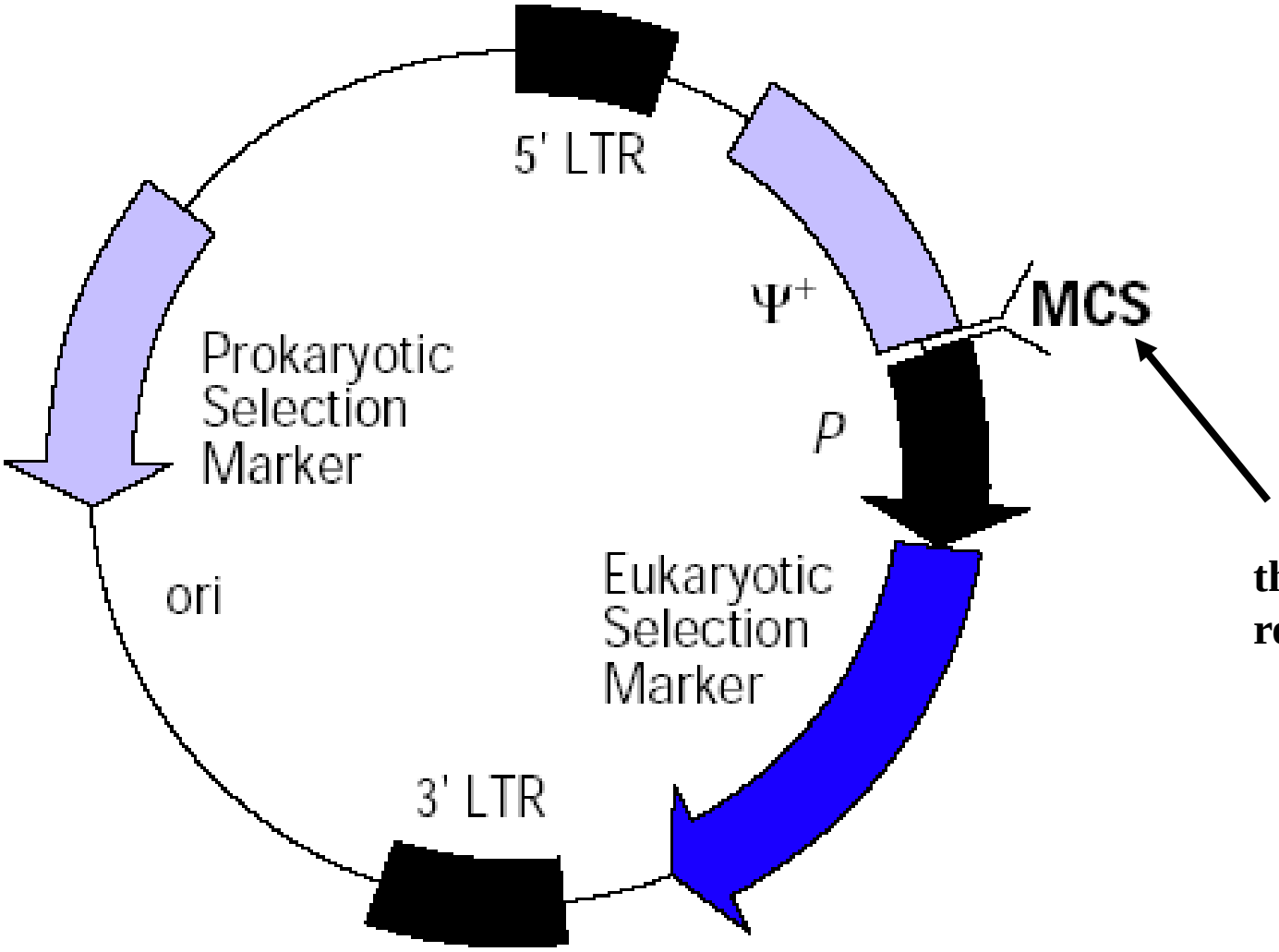
2. Construction of a plasmid containing in-cis required sequences plus a therapeutic gene

Cis-acting elements required for efficient gene transduction and integration

- *a promoter and a polyadenylation signal (poly A in retroviral vectors is already in LTR)*
- *a packaging signal*
- *a primer-binding site and a polypurine tract for initiation*
- *sequences at the termini of the viral LTR for integration*



Structure of a plasmid used for production of retroviral vectors



Production of retroviral vectors

Separate transfection of three vectors

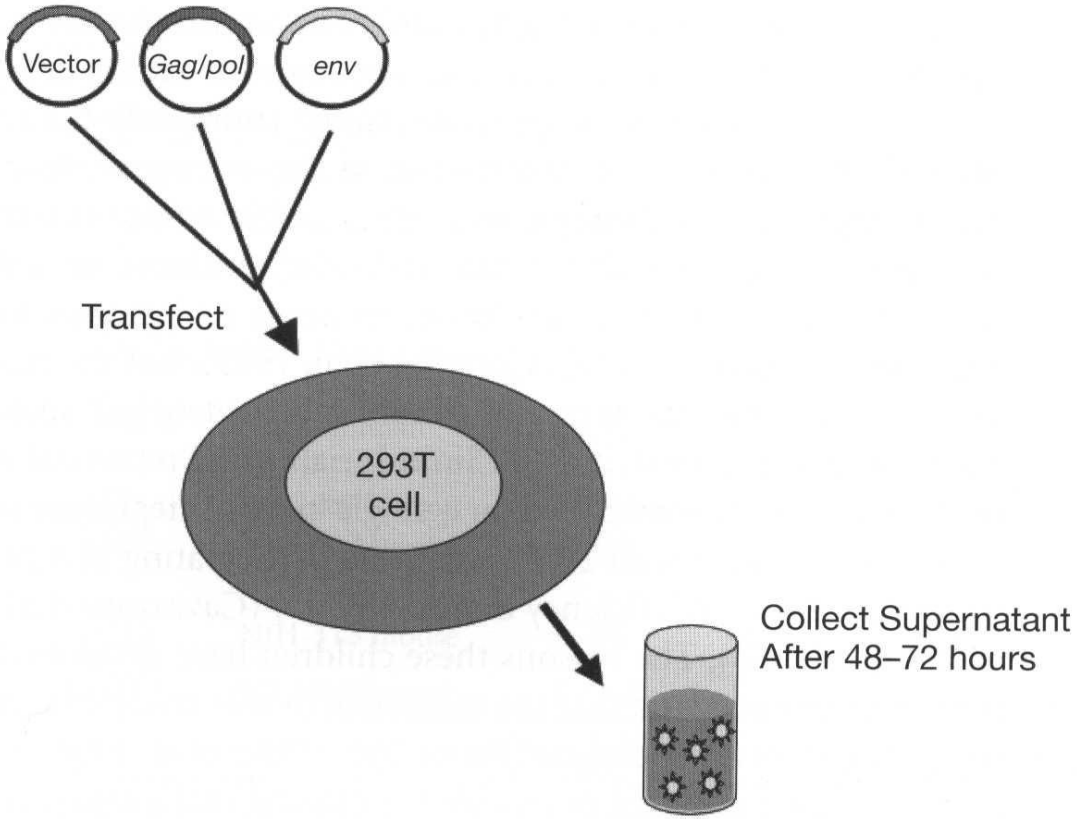
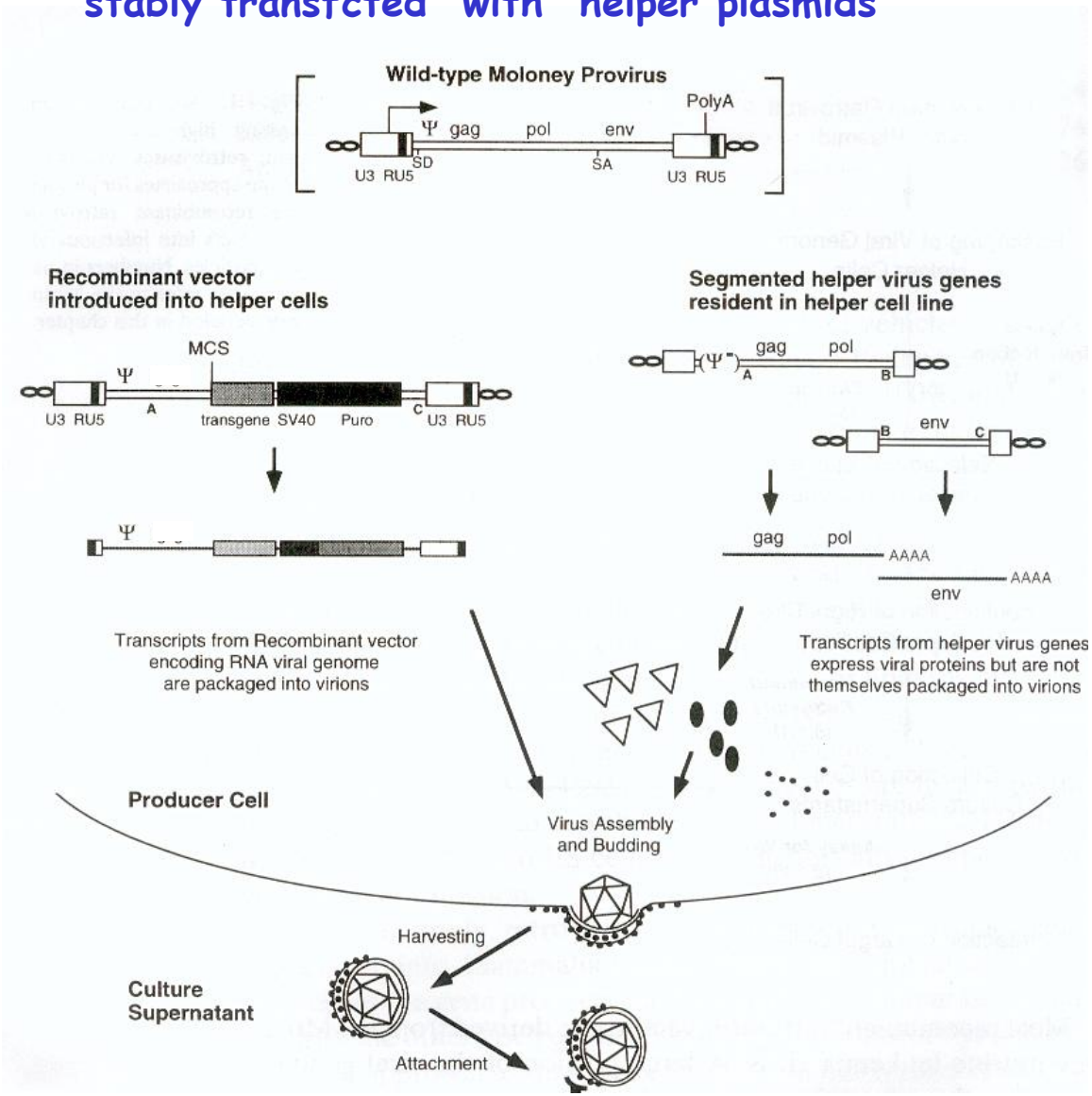


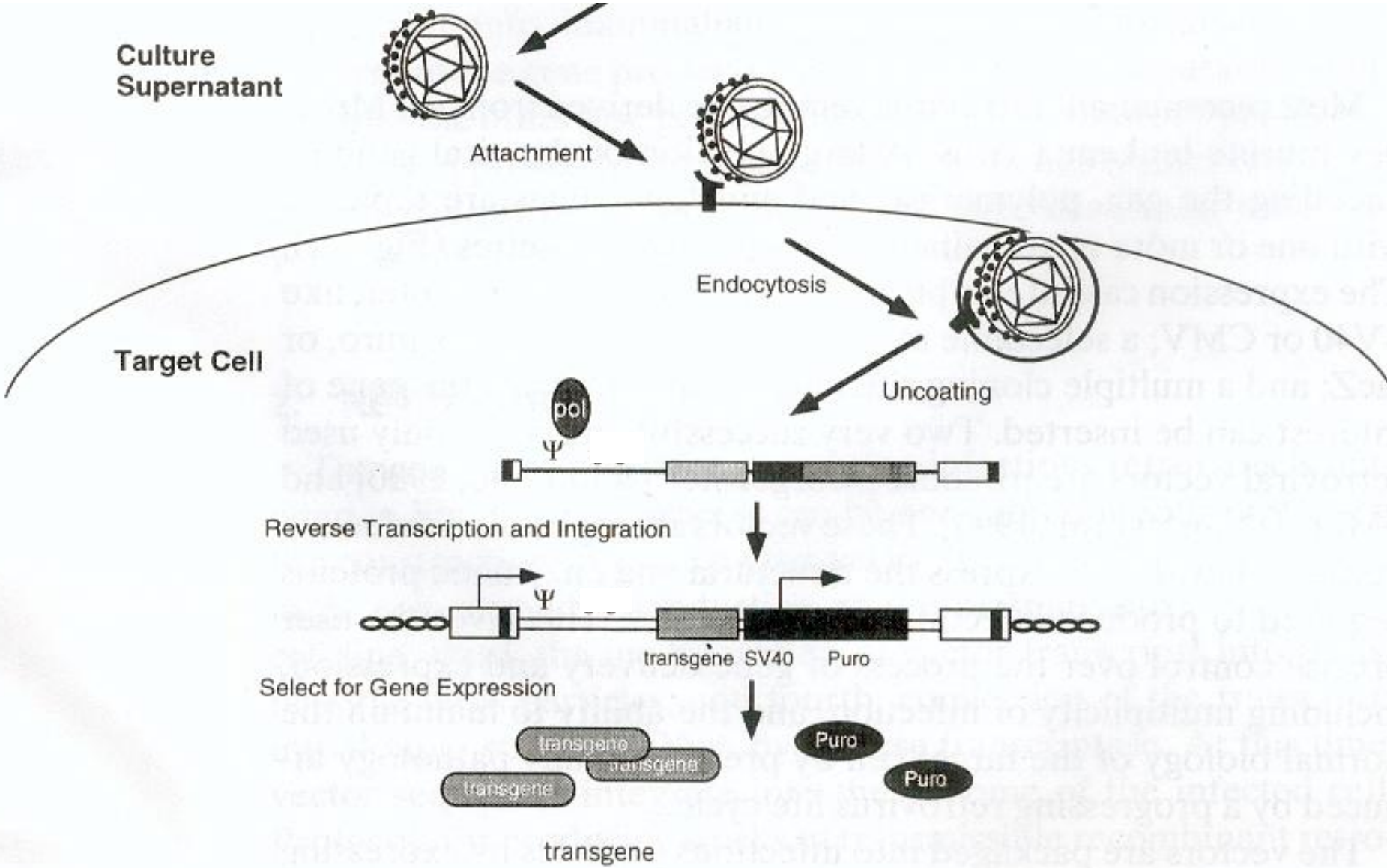
FIGURE 2. Vector production by transient transfection. Plasmids expressing the vector, the viral *gag* and *pol* genes, and the viral envelope are introduced into a cell that transfects with high efficiency (e.g., HEK 293T cells) using calcium phosphate, lipofection, or electroporation. Vector supernatant is harvested after 48–72 hours and can be used immediately or frozen at –70°C for later use.

Stages of retroviral construction (1)

Transfection of recombinant plasmid into the packaging cell previously stably transfcted with helper plasmids



Stages of retroviral construction (2)



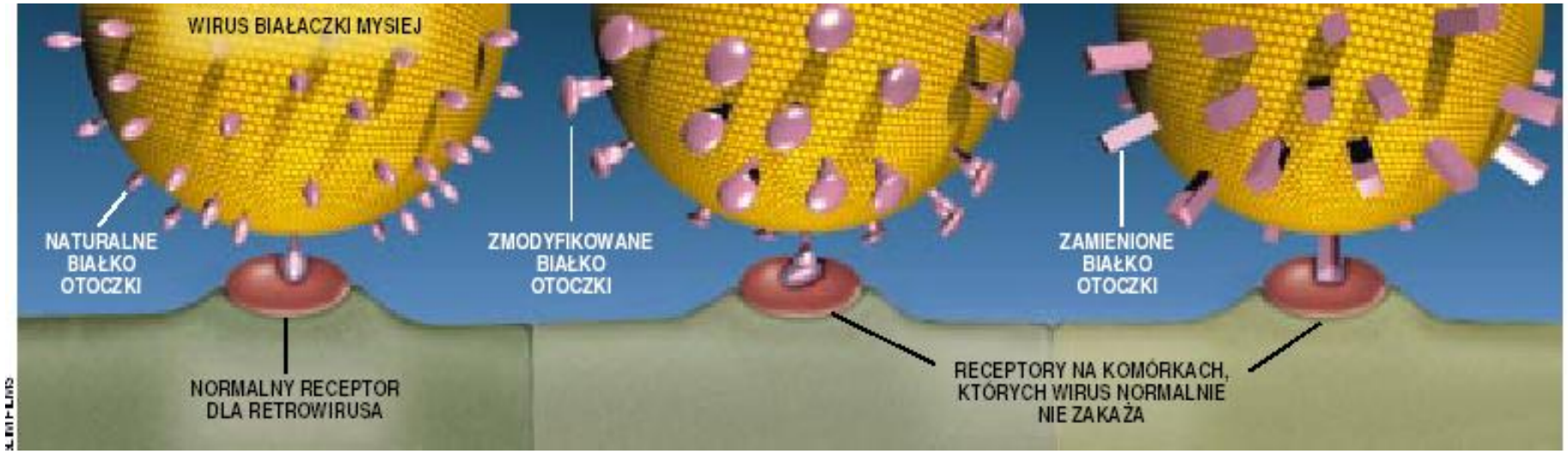
Features of retroviral vectors

Due to destruction of a natural, well-functioning gene composition, required for the formation of infectious virus, the resulting system of retroviral vector synthesis suffers from:

- low efficiency of packaging of vectors in comparison to wild type viruses

- formation of a large number of defective particles, which inhibit the transduction efficiency

Binding of retroviral vector to a cell surface



Pseudotyping

-one species of retrovirus is capable of incoporating the envelope protein of another species or a different virus

-the endogenous or heterologous envelope protein can be provided in trans to a replication-defective vector that lack the envelope coding sequence

- can be used to alter the tropism or increase the titer of the vectors

Types of retroviral vectors

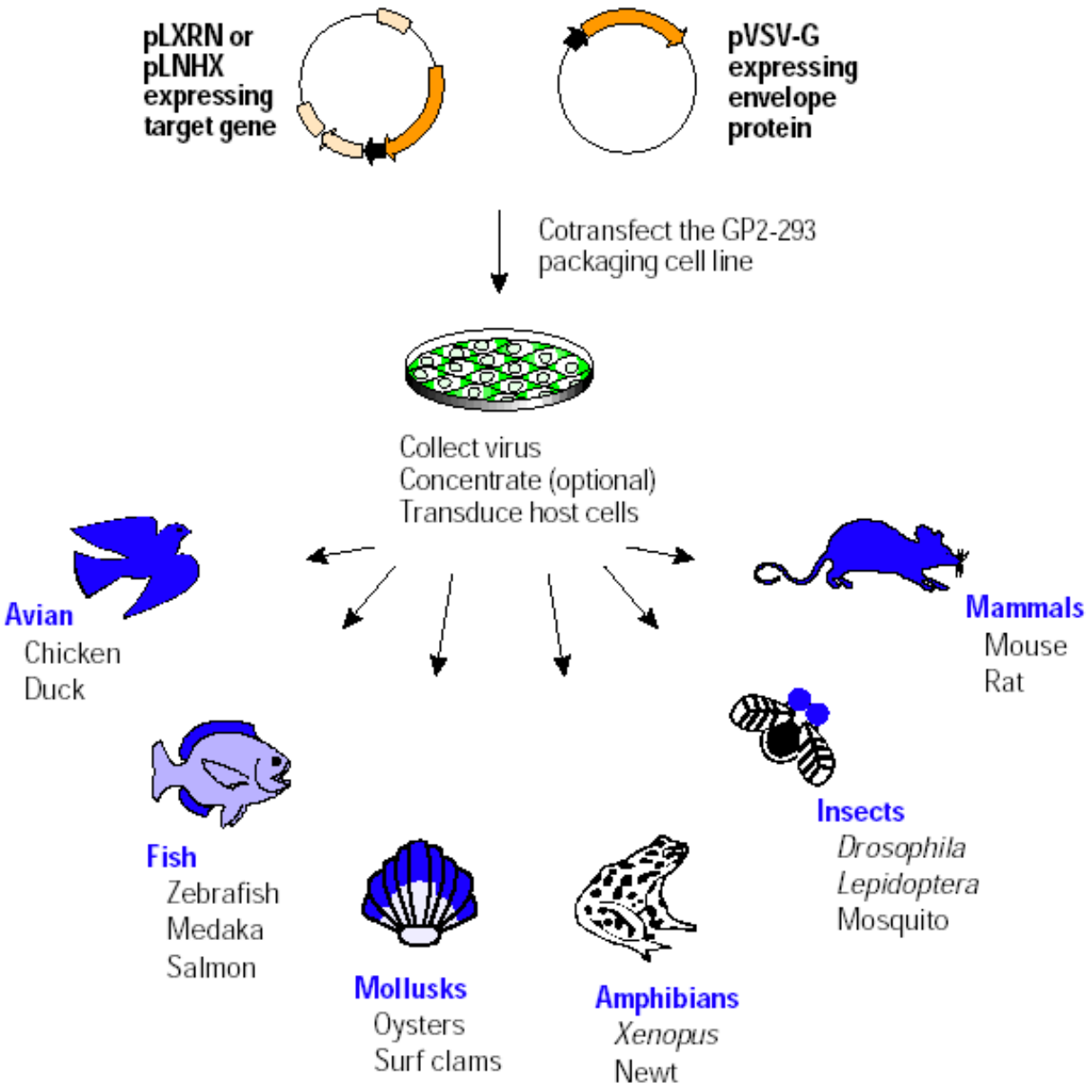
1. **Ecotropic -** infect only rodent cells (Eco-R receptor)

2. **Xenotropic -** infect most mammalian except rodent

3. **Amphotropic -** infect all mammalian (receptor Ram-1 and Glvr-1)

4. **Pantropic -** infect various species - VSV glicoprotein

Retroviral vectors can be used for transduction in many species



Titer (miano)

Concentration of viral particles and/or virions which are able to transduce the cells

The titer represents only a small fraction of a total number of viral particles

Significance of retroviral vectors

Retroviral gene delivery is the method of choice for gene expression in higher organisms because it is generally faster, more reliable, and has broader utility than alternative gene transfer methods.

Infection efficiencies of >90%

Efficient transduction of "difficult" cell types including primary, explant, embryonic stem (ES) and embryonic carcinoma (EC) cells

The host range of retroviruses has been expanded by *pseudotyping* the vectors with heterologous viral glycoproteins and receptor-specific ligands. This is possible because one species of retrovirus is capable of incorporating the envelope from another species or type of retrovirus. Therefore, the envelope protein can be provided in *trans* so that the virus produced can infect cells based on the tropism of that envelope protein

Transduction without variability or loss of expression

Retroviral Vectors as Gene Delivery Tools

The ability to transduce a variety of cell types

The ability to integrate efficiently into the genomic DNA of the dividing or mitotically active recipient cells

The ability to express the transduced gene at high levels

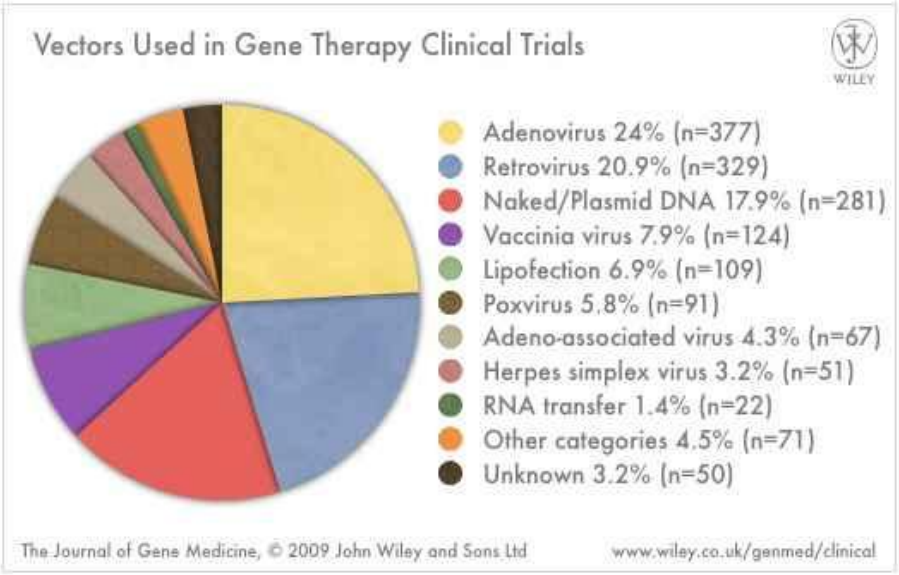
Capacity for long-term persistence and stable transmission of the gene to all future progeny of the transduced cell

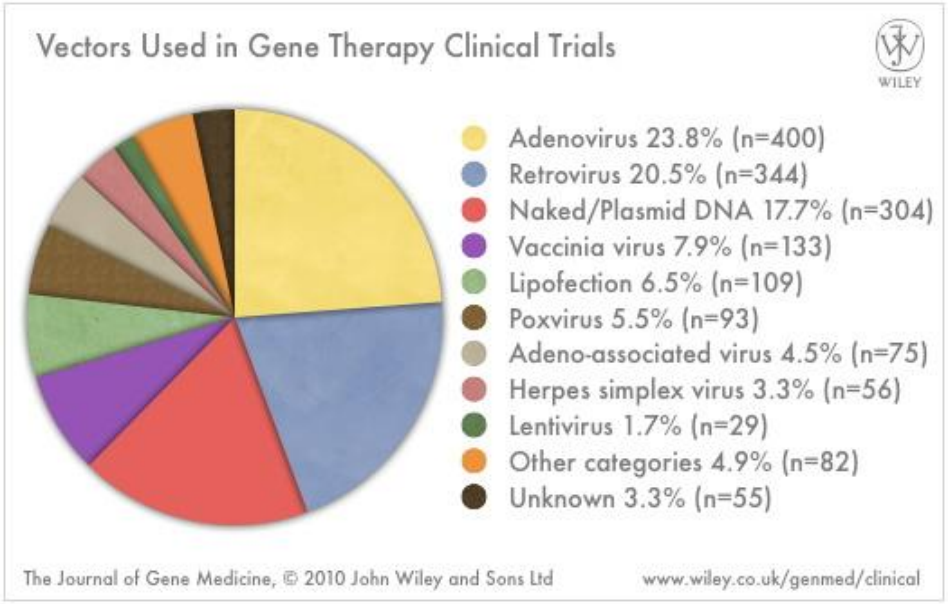
Up to 6.5 kb of foreign gene sequence can be packaged in a retroviral vector. This is adequate for most applications

Ability to be manufactured in large quantities to meet very stringent safety specifications

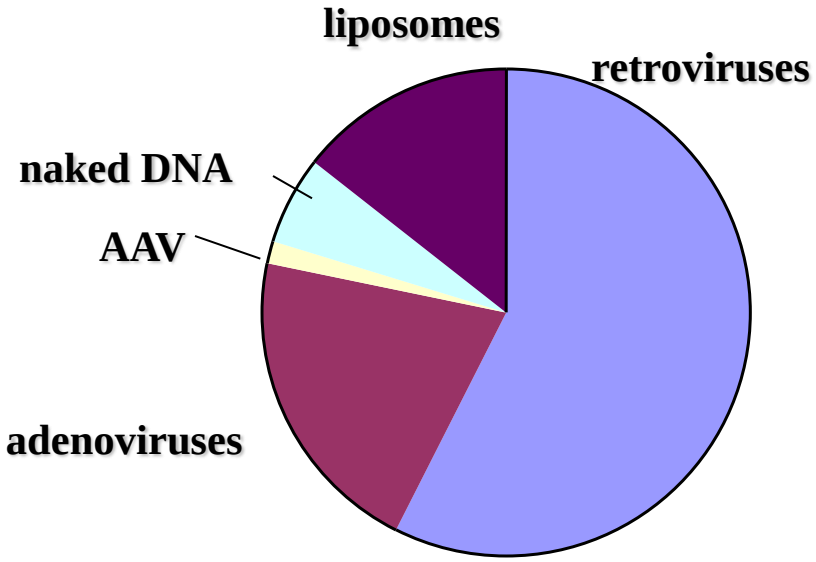
Retroviral vectors in clinical trials of gene therapy

Types of vectors used in clinical trials of gene therapy

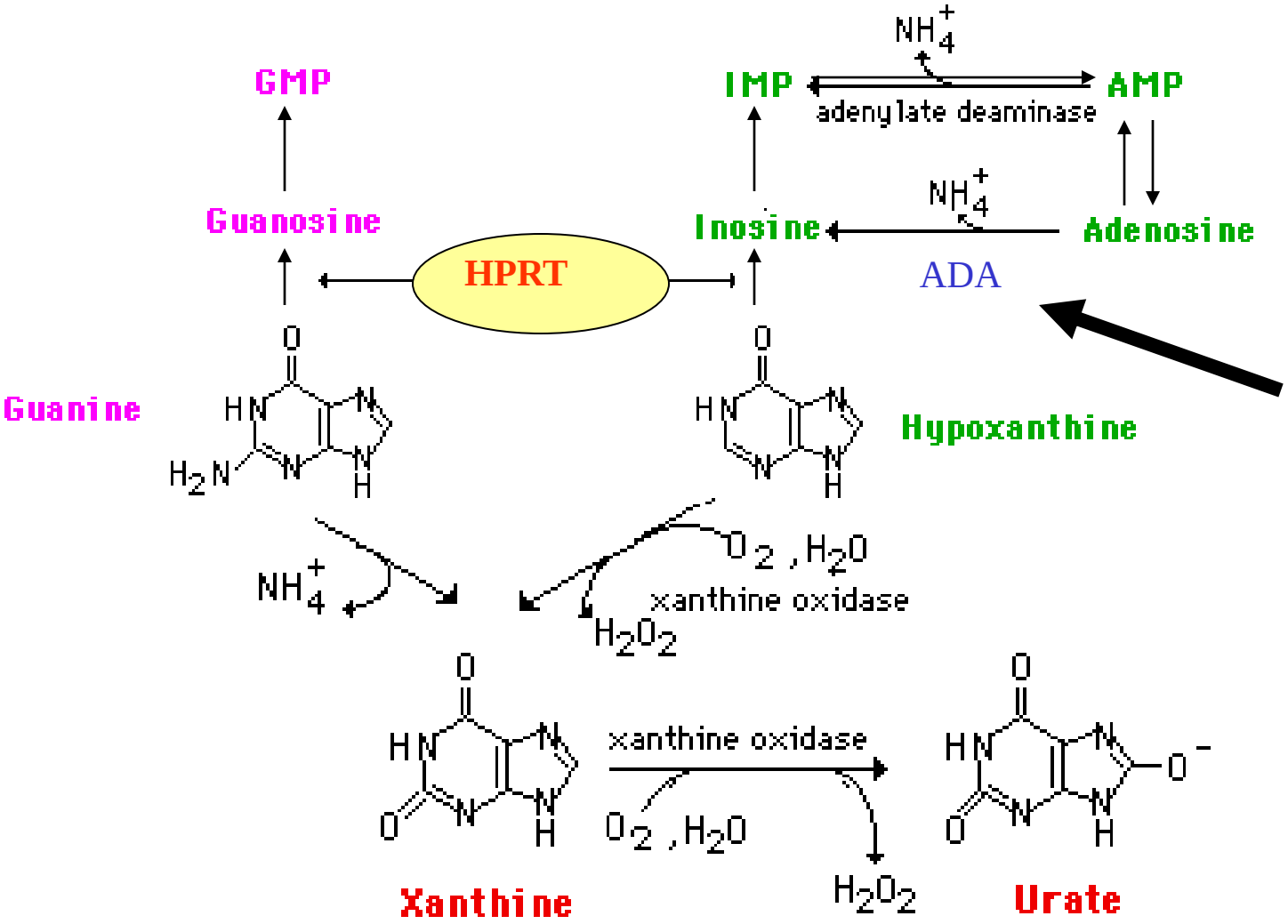




six years ago...

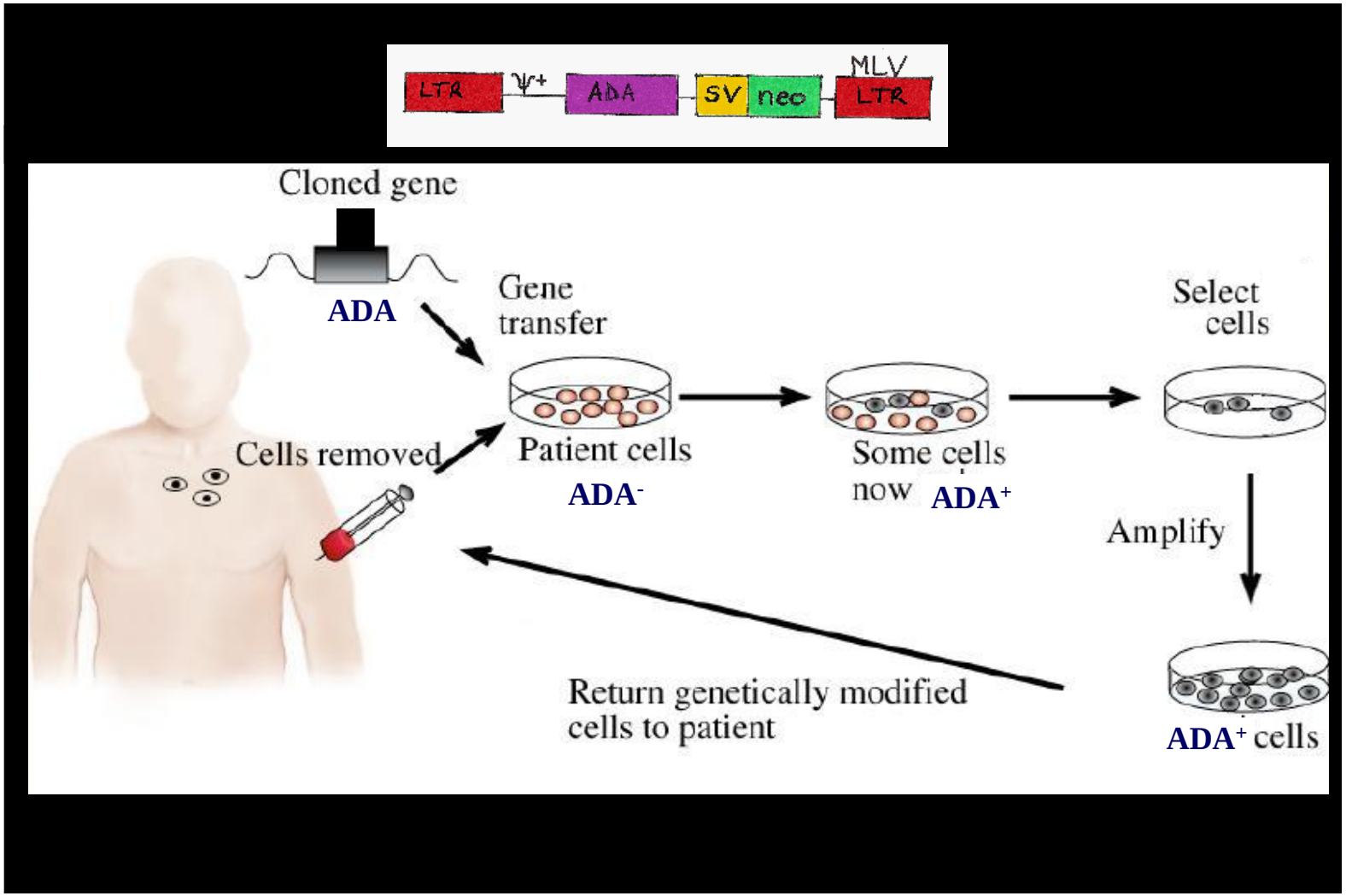


First controlled trial of gene therapy - 1990



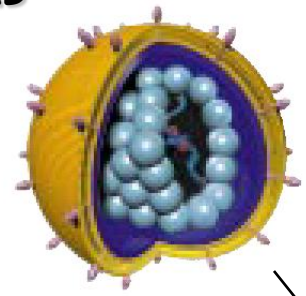
ADA deficiency– results in severe immunodeficiency syndrome

Gene therapy of ADA deficiency



First clinical trial of gene therapy - 1990

DMB



Retroviral vector containing correct ADA gene (cDNA) has been transduced into blood lymphocytes

Ashanti De Silva (patient)

This first clinical trial was not „pure" from the methodological point of view.

The patients have been treated concomitantly with enzyme injections – ADA-PEG.

Nevertheless, the marker transgene (neo) could be detected in the blood cells of the patients even more than 5 years after injection of modified cells.

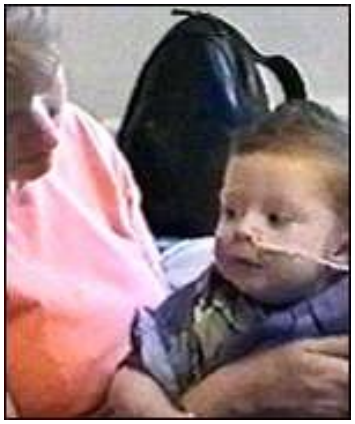
Succesful gene therapy

Cavazzana-Calvo M et al .

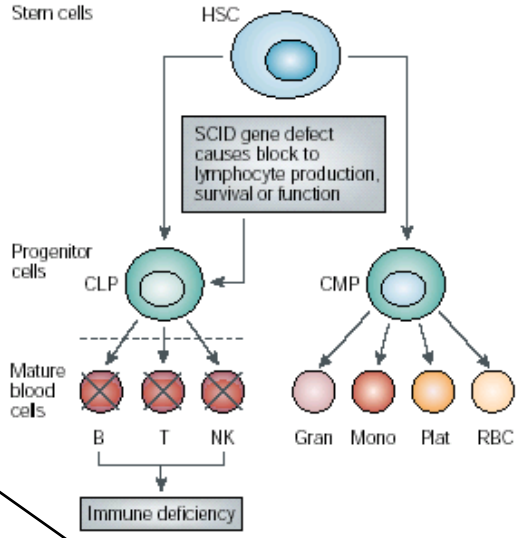
Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease

Science 2000: 28 April: 288: 669-672

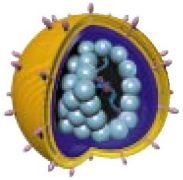
Gene therapy is efficient in treatment of X-SCID



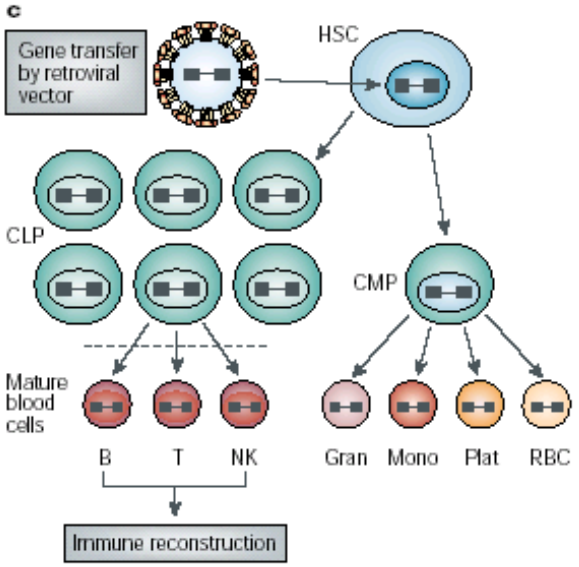
Stem cells without correct γc gene



Gene therapy



Retroviral vector with a correct γc gene



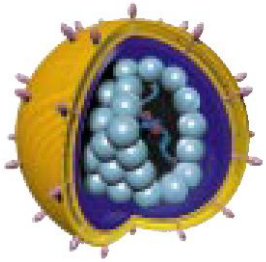
Gene therapy has been beneficial to most treated SCID-X1 patients!!!

- they can now cope with environment microorganisms and have a normal life in the absence of any specific therapy

- no evidence for γc transgene silencing has been observed

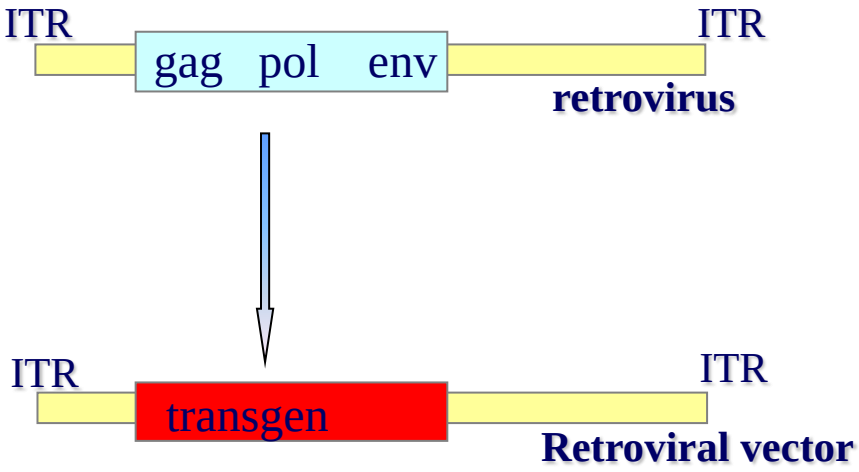
France – 10 boys treated, in 9 immune system corrected
UK – 10 boys treated, in 10 immune system corrected

Potential risk of application of retroviral vectors



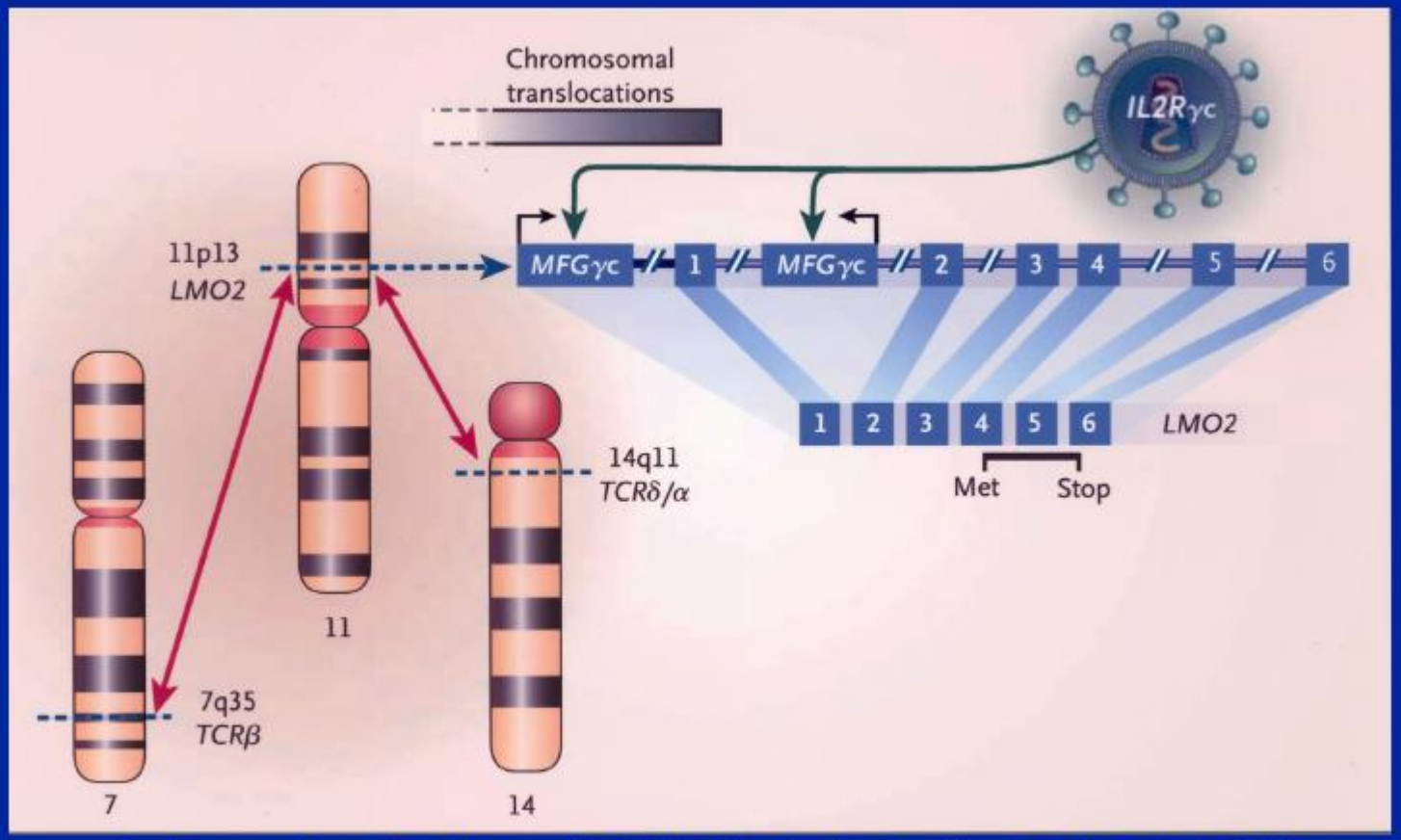
- *gag – structural proteins*
- *pol – reverse transcriptase*
- *env – envelope proteins*

- **long-term expression & integration into cellular genome**



random integration – risk of insertional mutagenesis

Integration of retroviral vector into the promoter of LMO2 gene



McCormack and Rabbitts (2004) *N. Engl. J. Med.* 350, 913-922

Serious side effects of SCID-X1 gene therapy

-development of uncontrolled clonal T lymphoproliferative syndrome, similar to acute lymphoblastic leukemia (ALL) in 4 out of 10 treated children in Paris

- due to the integration of a vector into an LMO2 gene either close to the promoter or in the first intron

Reasons: 1. LMO-2 locus is a frequent site for retroviral integration – **BUT NOT SUPPORTED BY DATA**

2. Cells with aberrant expression of LMO-2 could have been selected because the provide a clonal growth advantage

December 18, 2007 – a leukemia case has been reported in one of boys treated in hospital in London…

Gene therapy of ADA deficiency

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 29, 2009 VOL. 360 NO. 5

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

Alessandro Aiuti, M.D., Ph.D., Federica Cattaneo, M.D., Stefania Galimberti, Ph.D., Ulrike Benninghoff, M.D., Barbara Cassani, Ph.D., Luciano Callegaro, R.N., Samantha Scaramuzza, Ph.D., Grazia Andolfi, Massimiliano Mirolo, B.Sc., Immacolata Brigida, B.Sc., Antonella Tabucchi, Ph.D., Filippo Carlucci, Ph.D., Martha Eibl, M.D., Memet Aker, M.D., Shimon Slavin, M.D., Hamoud Al-Mousa, M.D., Abdulaziz Al Ghonaium, M.D., Alina Ferster, M.D., Andrea Duppenthaler, M.D., Luigi Notarangelo, M.D., Uwe Wintergerst, M.D., Rebecca H. Buckley, M.D., Marco Bregni, M.D., Sarah Marktel, M.D., Maria Grazia Valsecchi, Ph.D., Paolo Rossi, M.D., Fabio Ciceri, M.D., Roberto Miniero, M.D., Claudio Bordignon, M.D., and Maria-Grazia Roncarolo, M.D.

Gene therapy of ADA deficiency

BACKGROUND

We investigated the long-term outcome of gene therapy for severe combined immunodeficiency (SCID) due to the lack of adenosine deaminase (ADA), a fatal disorder of purine metabolism and immunodeficiency.

METHODS

We infused autologous CD34+ bone marrow cells transduced with a retroviral vector containing the *ADA* gene into 10 children with SCID due to ADA deficiency who lacked an HLA-identical sibling donor, after nonmyeloablative conditioning with busulfan. Enzyme-replacement therapy was not given after infusion of the cells.

RESULTS

All patients are alive after a median follow-up of 4.0 years (range, 1.8 to 8.0). Transduced hematopoietic stem cells have stably engrafted and differentiated into myeloid cells containing ADA (mean range at 1 year in bone marrow lineages, 3.5 to 8.9%) and lymphoid cells (mean range in peripheral blood, 52.4 to 88.0%). Eight patients do not require enzyme-replacement therapy, their blood cells continue to express ADA, and they have no signs of defective detoxification of purine metabolites. Nine patients had immune reconstitution with increases in T-cell counts (median count at 3 years, 1.07×10^9 per liter) and normalization of T-cell function. In the five patients in whom intravenous immune globulin replacement was discontinued, antigen-specific antibody responses were elicited after exposure to vaccines or viral antigens. Effective protection against infections and improvement in physical development made a normal lifestyle possible. Serious adverse events included prolonged neutropenia (in two patients), hypertension (in one), central-venous-catheter–related infections (in two), Epstein–Barr virus reactivation (in one), and autoimmune hepatitis (in one).

CONCLUSIONS

Gene therapy, combined with reduced-intensity conditioning, is a safe and effective treatment for SCID in patients with ADA deficiency. (ClinicalTrials.gov numbers, NCT00598481 and NCT00599781.)

Gene therapy of ADA deficiency

Table 2. Clinical Outcomes of the Study Patients.*

Patient No.	Clinical History before Gene Therapy	Years of Follow-up	Relevant Infections after Gene Therapy	Serious Adverse Events after Gene Therapy	PEG-ADA	Clinical Condition after Gene Therapy
1	Recurrent respiratory infection, failure to thrive	8.0	None	None	No	Well
2	Chronic diarrhea, recurrent respiratory infection, scabies, failure to thrive	7.5	Skin molluscum, urinary infection	None	Initiated 4.5 yr after gene therapy	Well
3	Recurrent respiratory infection, dermatitis, failure to thrive, eating disorder	6.3	None	Prolonged neutropenia and thrombocytopenia	No	Well
4	Recurrent respiratory infection, oral infection with *Candida albicans*, skin BCG and bacterial infections, chronic diarrhea, failure to thrive	5.9	Varicella	None	No	Well
5	Recurrent respiratory infection, aseptic meningitis, chronic diarrhea, failure to thrive	4.4	Varicella	None	No	Well
6	CMV lung infection, EBV infection, recurrent respiratory infection, hearing deficit, failure to thrive	3.8	CVC-related infection, EBV reactivation, varicella	None	No	Well
7	Facial dysmorphism, eating disorder, staphylococcal infection, oral and genital infection with *C. albicans*, failure to thrive	2.8	None	Autoimmune hepatitis	No	Well, but with eating disorder
8	Developmental delay, recurrent respiratory infection, autoimmune hemolytic anemia, macrophage activation syndrome, hearing deficit, failure to thrive	2.5	Recurrent respiratory infection, urinary infection	Hypertension, prolonged neutropenia, autoimmune thrombocytopenia	Restarted 0.4 yr after gene therapy	Mild symptoms
9	*Pneumocystis jiroveci* pneumonia	1.9	Gastroenteritis	None	No	Well
10	Postvaccinal BCG infection, recurrent respiratory infection, developmental delay, neurosensory deafness, genital ambiguity, congenital adrenal insufficiency, hypothyroidism, failure to thrive	1.8	CVC-related infection (two)	None	No	Well, but with developmental delay

* Serious adverse events were those other than serious infections during the follow-up period after gene therapy. BCG denotes bacille Calmette–Guérin, CMV cytomegalovirus, CVC central venous catheter, EBV Epstein–Barr virus, and PEG-ADA polyethylene glycol–modified bovine adenosine deaminase.

Gene therapy is succesful in treatment of diseases

Some Gene Therapy Successes

Disorder	Disease type	Patients benefiting	First publication
X-SCID	Immunodeficiency	17/20	2000
ADA-SCID	Immunodeficiency	26/37	2002
Adrenoleukodystrophy	Neurologic	2/4*	2009
Leber's congenital amaurosis	Blindness	28/30	2008
Wiskott-Aldrich syndrome	Immunodeficiency	8/10	2010
β-thalassemia	Hemoglobinopathy	1/1	2010
Hemophilia	Coagulation	6/6	2011?

*Includes a patient treated too recently to see benefit

Science, 7th October 2011

Retroviral vectors

Family *Retroviridae*

1. Oncoretroviruses:
   a) Alpharetrovirus – Avian leucosis virus (RSV)
   b) Betaretrovirus – Mouse mammary tumor virus (MMTV)
   c) Gammaretrovirus – Murine leukemia virus (Mo-MLV)
   d) Deltaretrovirus – Bovine leukemia virus
   e) Epsilonretrovirus – Walleye dermal sarcoma virus

2. Lentivirus – HIV, HTLV, BLV

3. Spumavirus – human spumavirus
   (human foamy virus – HMV)

Lentiviral vectors

- Transfect non-dividing cells

- Naturally infect cells expressing CD4 – change to a VSVG – results in a broad range of transfectable cell types

Lentiviral vectors are based on:

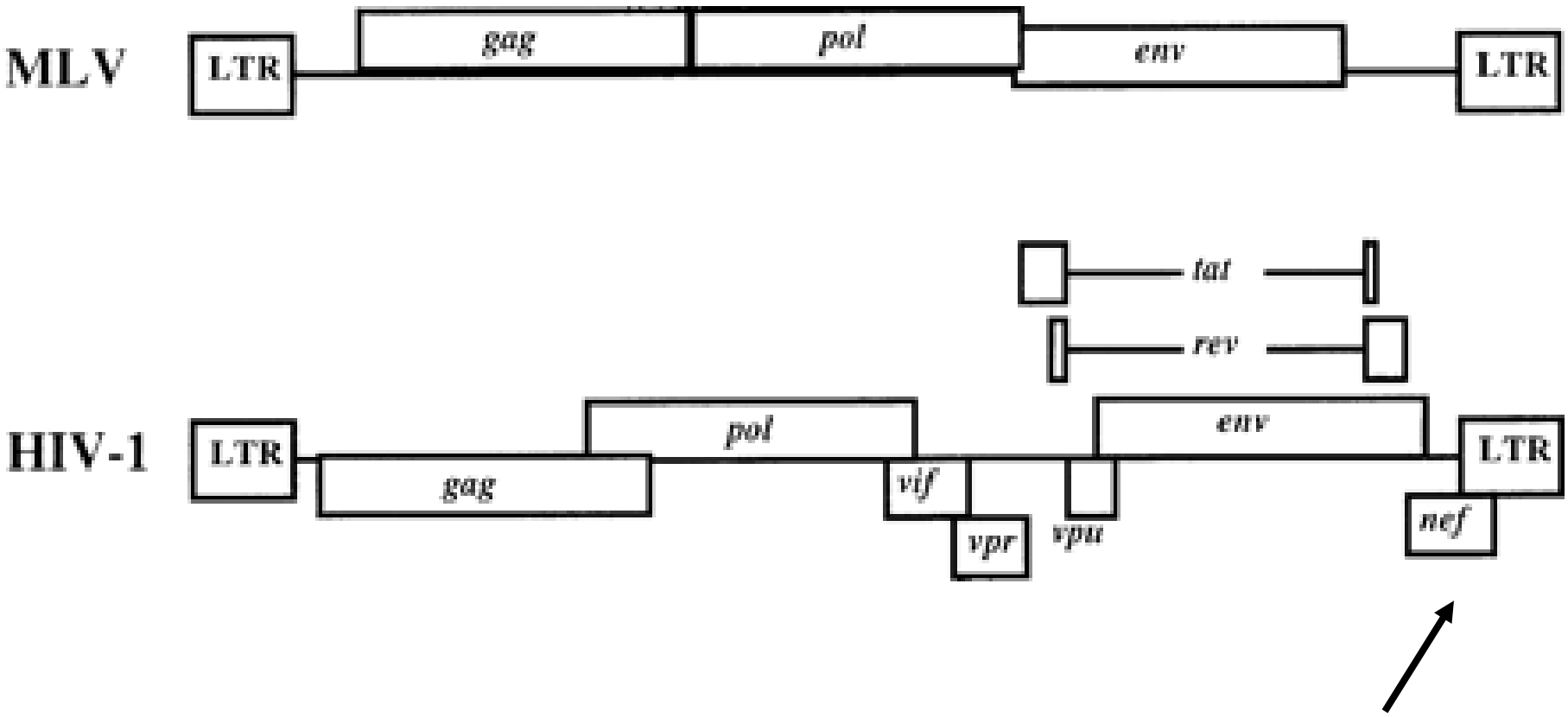
HIV-1, HIV-2

SIV

FIV

Self-inactivating lentiviruses: deletion of 299 bp in 3'LTR causes after transduction inactivation of 5'LTR, decreasing the risk of recombination and vector mobilisation

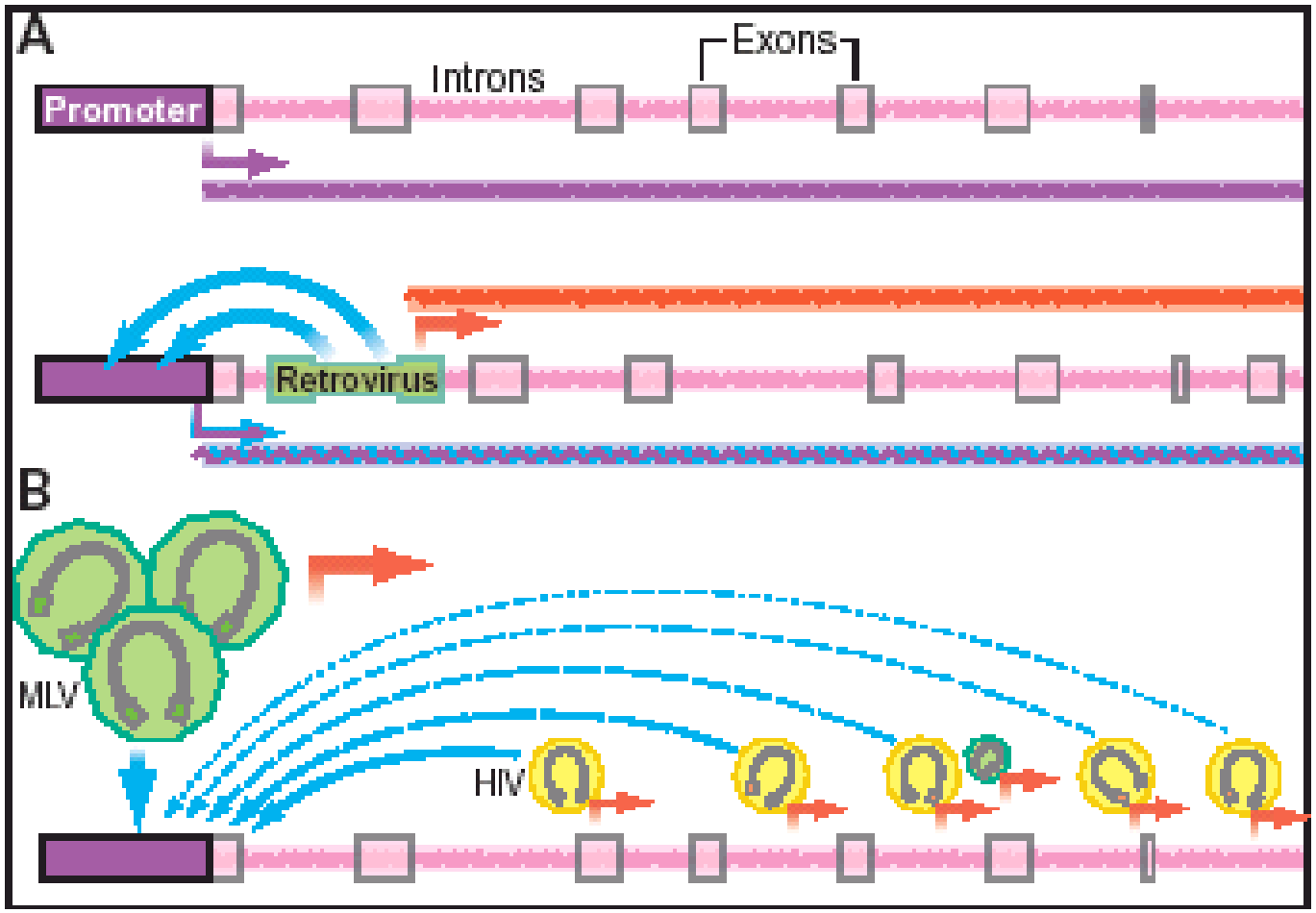
Comparison of oncoretroviruses and lentiviruses



Self-inactivating vectors have deletion in 3' LTR

Nuclear transport possible: proteins involved: integrase, one of Gag protein, Vpr

Sites of integration of retroviral vectors



D. Trono, Science 13 June 2003

GETTING INTEGRATED: Different host factors may affect a retroviral vector's pre-integration complex (PIC). The factors influencing the integrations are unknown

Gene therapy of adrenoleukodystrophy ALD

This X-linked recessive disease, with an estimated frequency of 1/20,000 men, presents in a variety of phenotypes [24]. Different phenotypes are commonly observed in the same family or the same kindred. In the most severe late infantile or juvenile cerebral form, which has a mean age of onset of about 7 years and constitutes 40 to 50% of the cases, neurological symptoms predominate. Initial behavioral and school problems are followed by gait disturbances, visual and hearing impairment, varying alterations of cognitive functions with progressive dementia and a devastating downhill course toward an apparent vegetative state in 3 to 5 years. Adrenal insufficiency can be demonstrated in 90% of cases. Most patients die in adolescence

People with ALD accumulate high levels of saturated, very long chain fatty acids in their brain and adrenal cortex because the fatty acids are not broken down by an enzyme in the normal manner. So, when the *ALD* gene was discovered in 1993, it was a surprise that the corresponding protein was in fact a member of a family of transporter proteins, not an enzyme. It is still a mystery as to how the transporter affects the function the fatty acid enzyme and, for that matter, how high levels of very long chain fatty acids cause the loss of myelin on nerve fibers.

Gene therapy of adrenoleukodystrophy

The most prominent biochemical finding is **increased concentrations of VLCFA ($>C_{22}$)** in the brain, adrenal, plasma, red cells and cultured fibroblasts. These fatty acids are present mostly in the forms of cholesterol esters, cerebrosides, gangliosides and sphingomyelin. There are no indications of other peroxisomal dysfunctions. The biochemical pathogenesis that leads to the massive demyelination is unclear because, even though the relative increase is large, the net concentrations of VLCFA in the tissue remain very low. Accumulation of VLCFA appears to be due to impaired activation of VLCFA-CoA, a reaction catalyzed by the peroxisomal enzyme VLCFA-CoA synthase. **The gene responsible for X-linked ALD has been cloned and shown to be an ABC transporter protein.** To date, the substrate transported by the ALD protein and the relationship between its transport function and VLCFA-CoA synthase activation are unknown. Elucidation of its precise physiological function should provide insight into the pathogenetic mechanism of this disorder. **More than 150 disease-causing mutations have been described.** Interestingly, many of them, including 60% of mis-sense mutations, lead to undetectable levels of ALD protein by either Western blot or immunofluorescence. A murine model has been generated.

Hematopoietic Stem Cell Gene Therapy with a Lentiviral Vector in X-Linked Adrenoleukodystrophy

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X-linked adrenoleukodystrophy (ALD) is a severe brain demyelinating disease in boys that is caused by a deficiency in ALD protein, an adenosine triphosphate–binding cassette transporter encoded by the *ABCD1* gene. ALD progression can be halted by allogeneic hematopoietic cell transplantation (HCT). We initiated a gene therapy trial in two ALD patients for whom there were no matched donors. Autologous $CD34^+$ cells were removed from the patients, genetically corrected ex vivo with a lentiviral vector encoding wild-type *ABCD1*, and then re-infused into the patients after they had received myeloablative treatment. Over a span of 24 to 30 months of follow-up, we detected polyclonal reconstitution, with 9 to 14% of granulocytes, monocytes, and T and B lymphocytes expressing the ALD protein. These results strongly suggest that hematopoietic stem cells were transduced in the patients. Beginning 14 to 16 months after infusion of the genetically corrected cells, progressive cerebral demyelination in the two patients stopped, a clinical outcome comparable to that achieved by allogeneic HCT. Thus, lentiviral-mediated gene therapy of hematopoietic stem cells can provide clinical benefits in ALD.

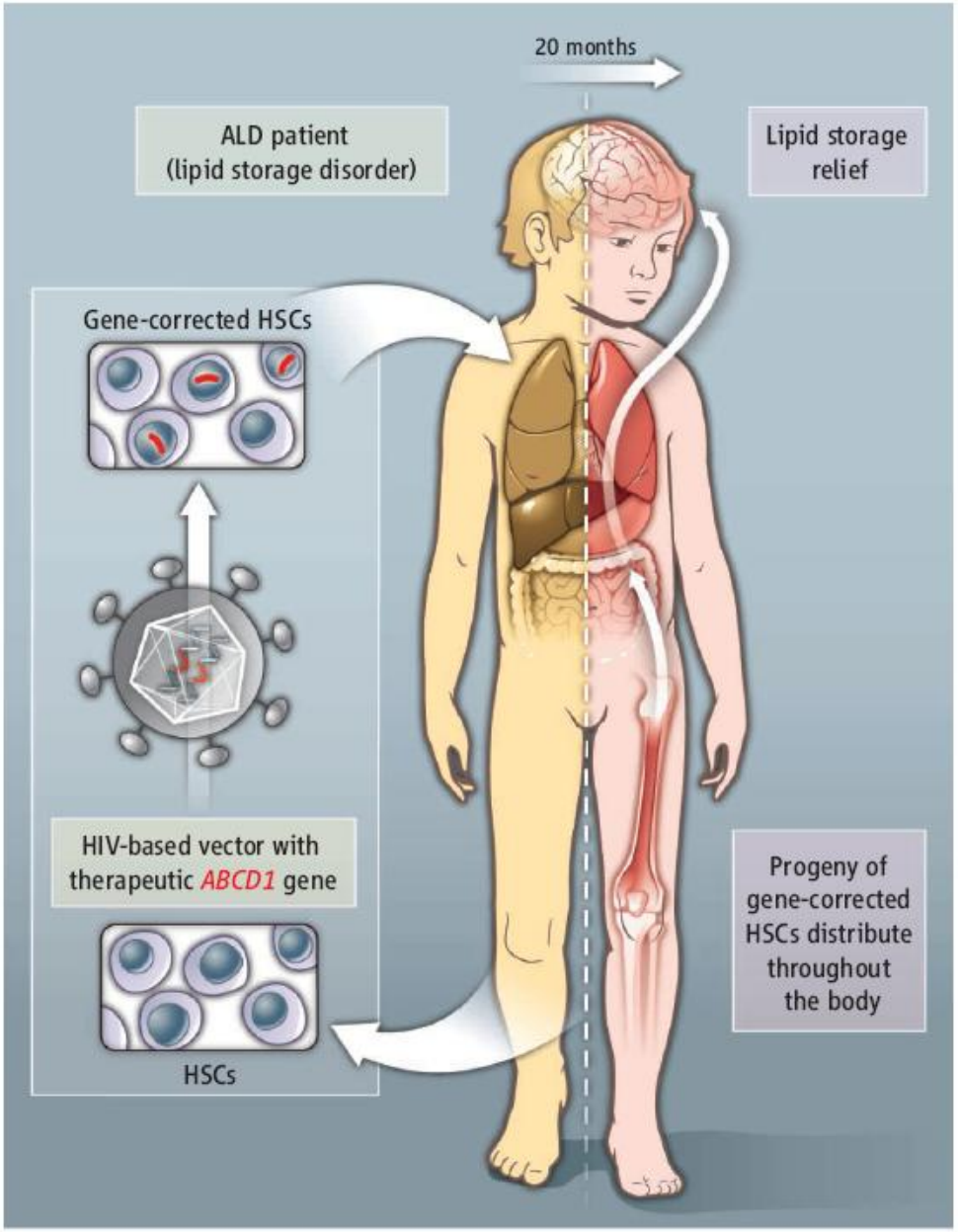
Science, 6th November 2009

Treatment of adrenoleukodystrophy

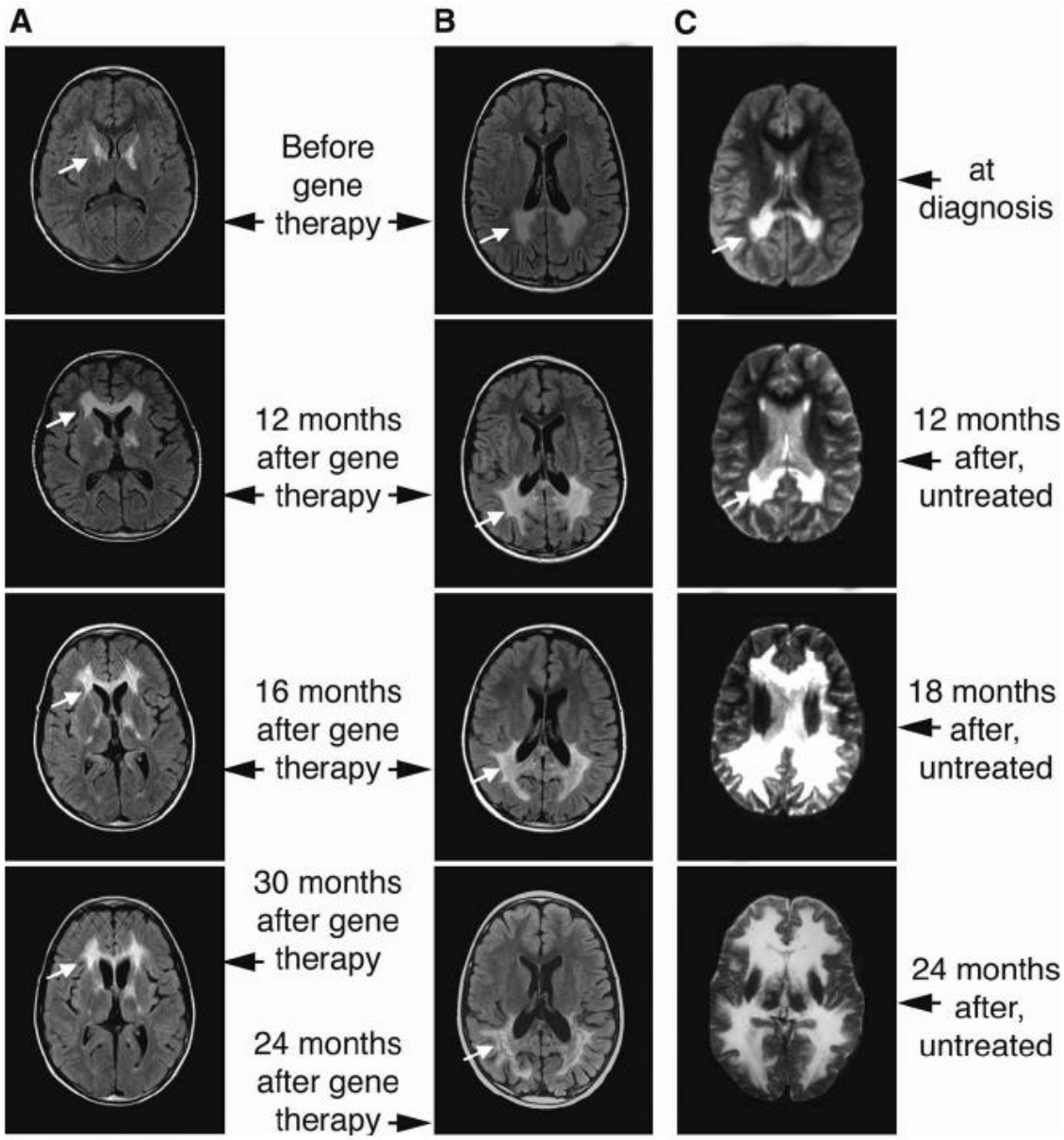
1. Bone marrow transplantation
2. Lorenzo's oil

1984 r. Michaela & Augusto Odone (in movie played by Susan Sarandon & Nick Nolte)

Gene therapy of adrenoleukodystrophy



Gene therapy of adrenoleukodystrophy



Pros and cons of lentiviral vectors

- Relatively high coding capacity (~ 9 kbp)
- Relatively low immunogenicity
- Host immunologically naïve to vector
- Highly efficient in dividing and non-dividing tissues
- Made into efficient non-integrating vectors

- Relatively low titres
- Difficult to scale up production

Retroviral vectors – properties

Capacity	4-6.5 kb – oncoretroviruses 8-9 kb - lentiviruses
Ability to integrate	yes
Tissue specificity	yes
Ability to transfect non-dividing cells	no – oncoretroviruses yes – lentiviruses
Duration of expression	long-term
Level of expression	moderate
Safety	risk of insertional mutagenesis

Application of retroviral vectors in gene therapy

1. Inherited diseases:
   a) metabolic disorders – eg. hypercholesterolemia
   b) haemophilia
   c) immunodeficiency diseases

2. Transfer of a suicide gene – thymidine kinase – prevention of graft versus host reaction GvH (*przeszczep przeciw gospodarzowi*) in patients after allogeneic bone marrow transplantation

3. Transfer of genes to stem cells

ABCD1

The *ABCD1* (*ALD*) gene maps to Xq28 and expresses a peroxisomally located half transporter that is mutated in adrenoleukodystrophy (ALD). X-ALD is an X-linked recessive disorder characterized by neurodegenerative phenotypes with onset typically in late childhood (189). Adrenal deficiency commonly occurs, and the presentation of ALD is highly variable. Childhood ALD, adrenomyeloneuropathy, and adult onset forms are recognized, but there is no apparent correlation to *ABCD1* alleles (190). Female heterozygotes can display symptoms including spastic paraparesis and peripheral neuropathy (191).

More than 406 mutations have been documented in the *ABCD1* gene and a database of ALD mutations has been created (190) (http://www.x-ald.nl). Although most mutations are point mutations, several large intragenic deletions have also been described (192). A contiguous gene syndrome, contiguous *ABCD1 DXS1357E* deletion syndrome (CADDS), has been described that includes *ABCD1* and the adjacent *DXS1357E* gene. These patients present with symptoms at birth, as opposed to X-ALD, which present after 3 years of age (193).

ALD patients have an accumulation of unbranched saturated fatty acids, with a chain length of 24–30 carbons, in the cholesterol esters of the brain and in adrenal cortex. The ALD protein is located in the peroxisome, where it is believed to be involved in the transport of very long chain fatty acids (VLCFAs). A treatment consisting of erucic acid, a C22 monounsaturated fat, and oleic acid, a C18 monounsaturated fat (Lorenzo's oil), was developed that results in a normalization of the VLCFA levels in the blood of patients but does not appear to dramatically slow the progression of the disease (194). This is probably because the treatment fails to lower fatty acid levels in the brain (195). An *Abcd1* –/– mouse has been generated, and the animals display accumulation of VLCFAs in kidney and brain; however, they do not show the severe neurological abnormalities of the childhood cerebral form of X-ALD (196, 197). The mice do show evidence of a late-onset neurological disorder characterized by slower nerve conduction and myelin and axonal anomalies detectable in the spinal cord and sciatic nerve (198).

ABCD1 is one of four related peroxisomal transporters that are found in the human genome, the others being ABCD2, ABCD3, and ABCD4. These genes are highly conserved in evolution, and a pair of homologous genes is present in the yeast genome, *PXA1* and *PXA2*. The *PXA2* gene has been demonstrated to transport long-chain fatty acids (199, 200). A defective *pxa1* gene in *Arabidopsis thaliana* results in defective import of fatty acids into the peroxisome (201).

X-linked adrenoleukodystrophy (ALD),

efore fatty acids can be degraded in the peroxisome, they must first be transported into the organelle from the cytosol. Mid-length fatty acids are esterified to coenzyme A in the cytosol; the resulting fatty acyl CoA is then transported into the peroxisome by a specific transporter. However, very long chain fatty acids enter the peroxisome by another transporter, and then are esterified to CoA once inside. In the human genetic disease *X-linked adrenoleukodystrophy (ALD),* peroxisomal oxidation of very long chain fatty acids is specifically defective, while the oxidation of mid-length fatty acids is normal. In ALD, very long chain fatty acids are transported normally into peroxisomes, but are not esterified to CoA and so cannot be oxidized. The enzyme that catalyzes this esterification is synthesized in the cytosol; as we discuss in Chapter 17, the *ADL* gene encodes the peroxisomal membrane protein required for uptake of this enzyme into peroxisomes. Patients with the severe form of ADL are unaffected until mid-childhood, when severe neurological disorders appear, followed by death within a few years

the peroxisomal oxidation of very-long-chain fatty acids is defective in *X-linked adrenoleukodystrophy (ALD)*, another genetic disease that affects peroxisome functioning. Peroxisomes from ALD patients lack long-chain fatty acyl CoA synthase, the matrix enzyme that normally links coenzyme A to very-long-chain fatty acids within the peroxisome. The protein encoded by the *ALD* gene has a structure similar to that of the CFTR (cystic fibrosis transmembrane regulator) membrane transport protein and of multidrug resistance proteins (see Figure 15-16). Apparently the ALD protein is the peroxisomal membrane transporter specific for uptake of long-chain fatty acyl CoA synthase from the cytosol