Gene therapy

- **Gene therapy:**
  - **to correct a genetic defect by transferring of a functional normal copy of the gene into cells**

- **Examples of diseases caused by genetic defect**
  - **Ornithine transcarbamylase (OTC deficiency)**
  - **Hemophilia (blood coagulation factors VIII or IX)**
  - **SCID(severe combined immunodeficiency)**
  - **Muscular dystrophy**
  - **Cystic fibrosis**
  - **Sickle cell anemia**

Application of gene therapy

- Genetic disorder (deficiency): OTC
- Cancer
  - Genetic predisposition
  - Mutation in oncogene or tumor suppressor gene
- Autoimmunity diseases: rheumatoid arthritis
  - Delivery of counteracting gene
- Diseases involve several genes and the environmental interact: diabetes

Factors to be considered in Gene therapy

- **How to deliver genes to specific cells, tissue and whole animals? (methods of delivery)**
- **How much and how long the introduced gene will be expressed?**
- **The site and dose of gene delivery**
- **Is there any adverse immunological consequence of both delivery vehicle (Virus) and the gene in animals?**
- **Is there any toxic effects?**

Methods of gene delivery

- Viral Vectors:
  - Adenovirus
  - Retrovirus
  - Lentivirus
  - Adeno-associated virus (AAV)
  - Herpes simplex virus (HSV)
- Non-viral vector based
  - Naked DNA (plasmid DNA): injection or genegun
  - Liposomes (cationic lipids): mix with genes
- Ex-vivo
- In vivo

Why use viral vectors

- **Virus are obligate intracellular parasites**
- **Very efficient at transferring viral DNA into host cells**
- **Specific target cells: depending on the viral attachment proteins (capsid or glycoproteins)**
- **Gene replacement: non-essential genes of virus are deleted and exogenous genes are inserted**

Generation of viral vector for gene therapy

- **Replication-competent virus**
- **Replication-defective virus**
  - **Amplicon: doesn't encode structural proteins**
  - **Can't replicate beyond the first cycle of infection**
- **Elements needed to generate amplicon**
  - **Transfer Vector: plasmid (promoter, gene of interest, ori, packaging signal)**
  - **Packaging vector (cosmid or cell lines): provide the viral structural proteins for packaging of transfer vector**
  - **Helper virus (packaging of transfer vector): deleted Packaging signal sequence**

Viruses as Vectors

Any virus can potentially be used to express foreign genes

Different viruses are better suited for different kinds of uses

Integration may be important, such as in many gene therapy uses

Larger viruses can express more and larger foreign genes but are more difficult to manipulate

The cis-acting promoters for genome replication and packaging must be understood

Potential Uses of Viral Vectors

Gene therapy to replace a missing or inadequate gene

Cure of illness by expressing a reagent to, for example, kill cancer cells

Immunization by expressing an antigen from a pathogen

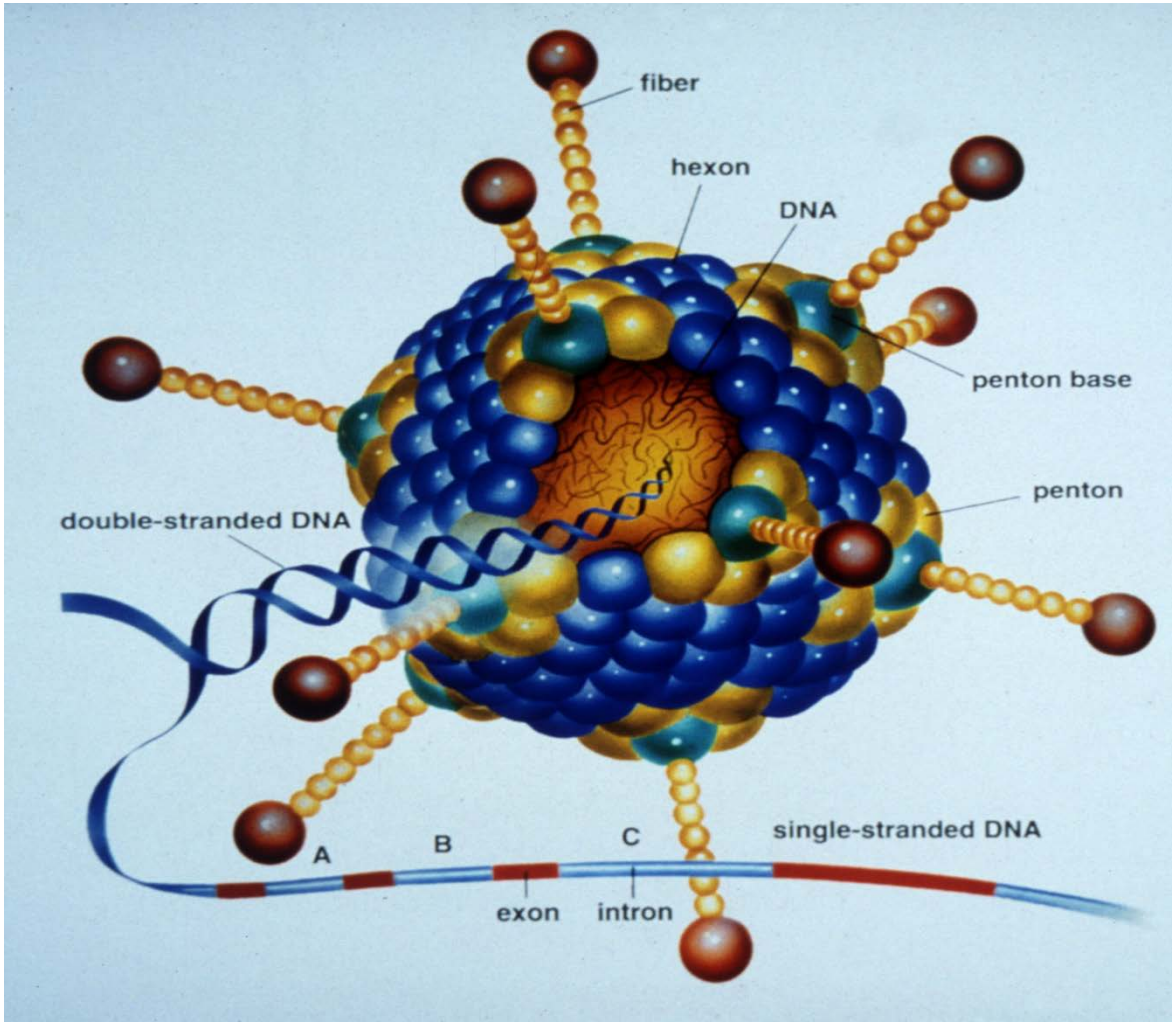
Expression of genes in cultured cells for scientific study

The Ideal Vector for Gene Transfer

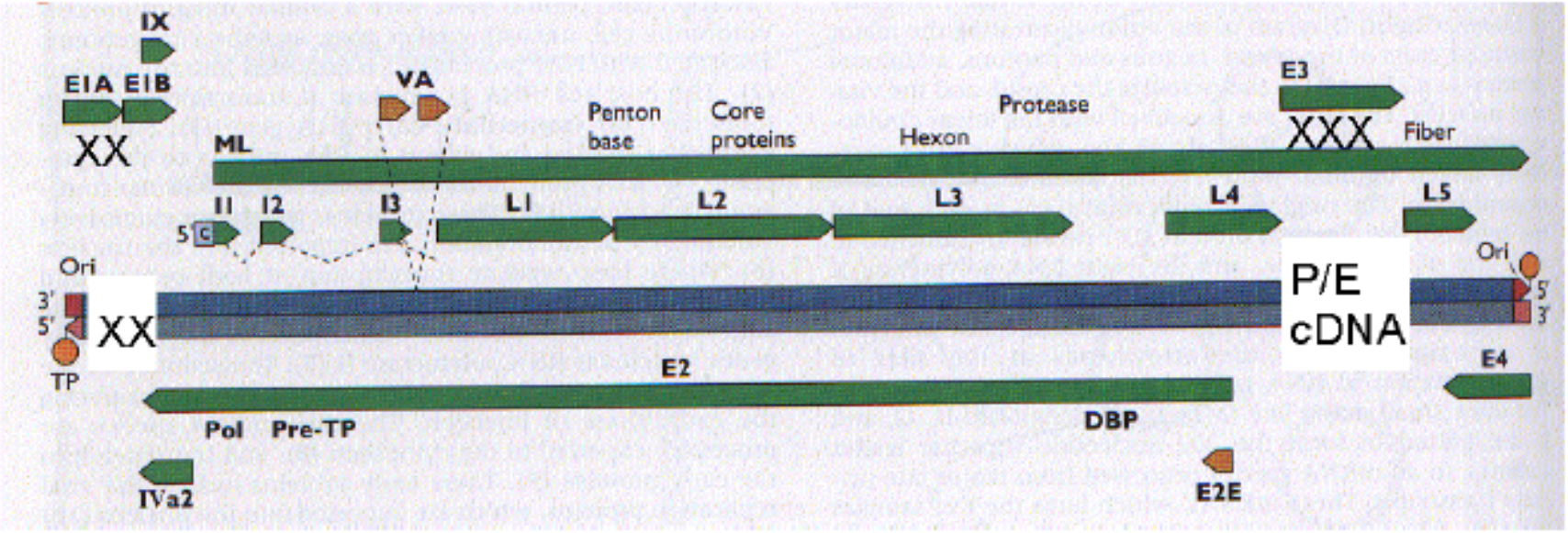
- High concentration of virus allowing many cells to be infected or transduced
- Convenience and reproducibility of production
- Ability to transduce dividing and non-dividing cells
- Ability to integrate into a site-specific location in the host chromosome, or to be successfully maintained as stable episome
- A transcriptional unit that can respond to manipulation of its regulatory elements
- Ability to target the desired type of cell
- No components that elicit an immune response

Adenovirus particle structure:

- Nonenveloped particle
- Contains linear double stranded DNA
- Does not integrate into the host genome
- Replicates as an episomal element in the nucleus



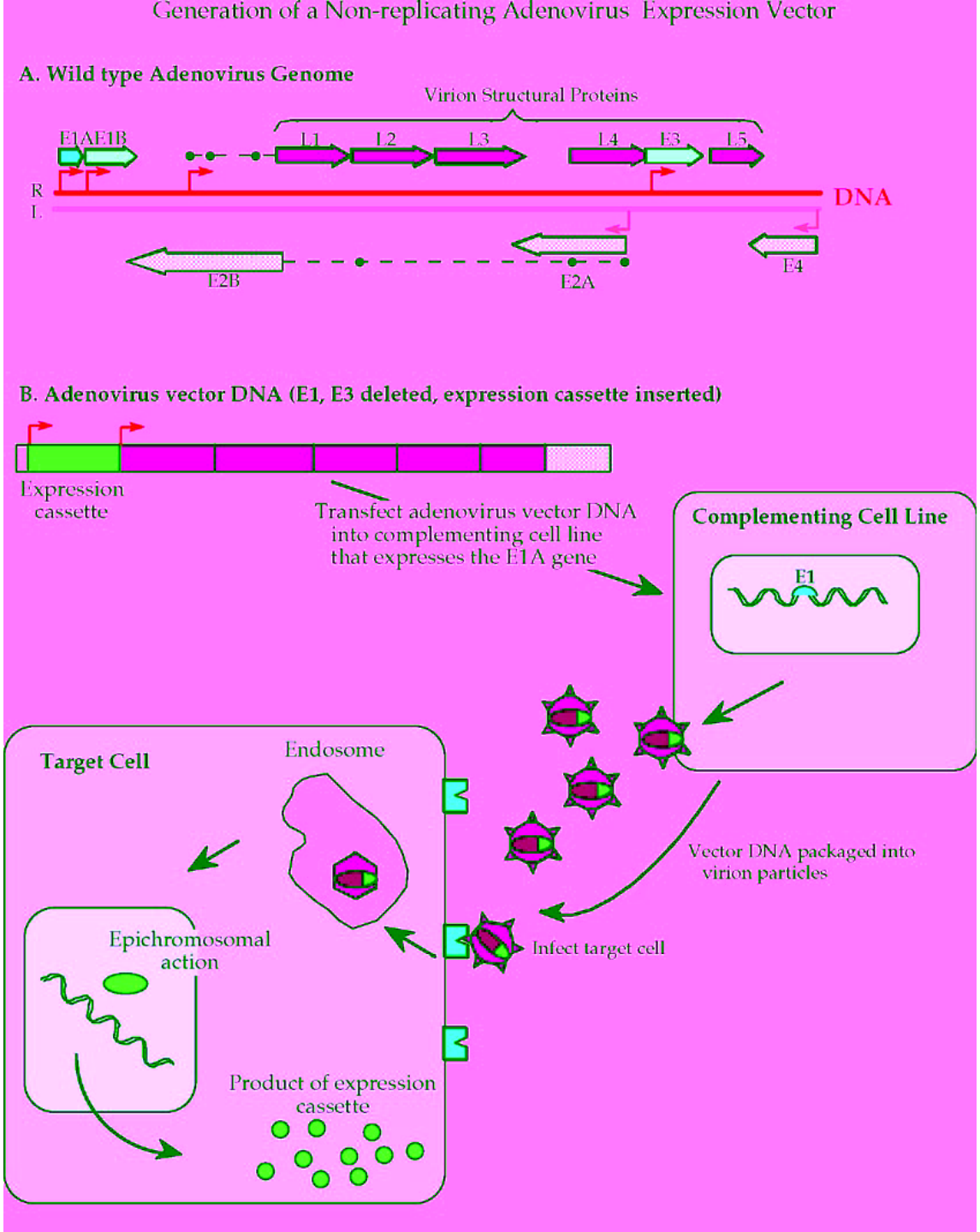
- Adenovirus E1A gene product is needed to initiate replication. Essential E1 genes are deleted, and cDNA plus promoter/enhancer sequences inserted in place of E1, or non-essential E3 genes (as shown below).
- Recombinant DNA genome is transfected into E1-producing 293 cells, to recover recombinant viral particles.



[figure modified from Flint *et al.*, Principles of Virology, ASM Press, 2000]

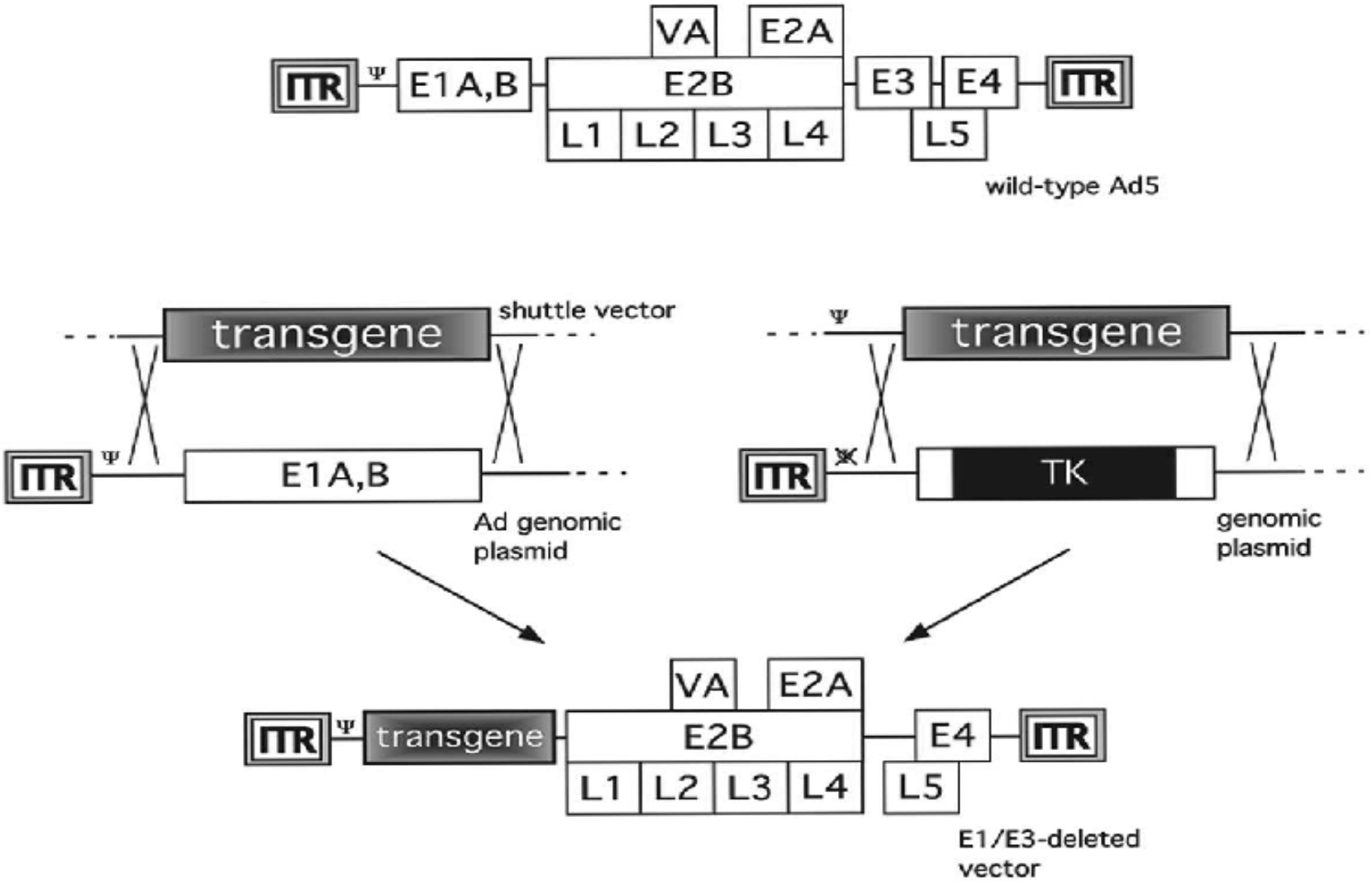
Adenoviral vectors:

- Double-stranded DNA viruses, usually cause benign respiratory disease; serotypes 2 and 5 are used as vectors.
- Can infect dividing and non-dividing cells, can be produced at high titers.
- Replication-deficient adenovirus vectors can be generated by replacing the E1 or E3 gene, which is essential for replication.
- The recombinant vectors are then replicated in cells that express the products of the E1 or E3 gene and can be generated in very high concentrations.
- Cells infected with recombinant adenovirus can express the therapeutic gene, but because essential genes for replication are deleted, the vector can’t replicate.

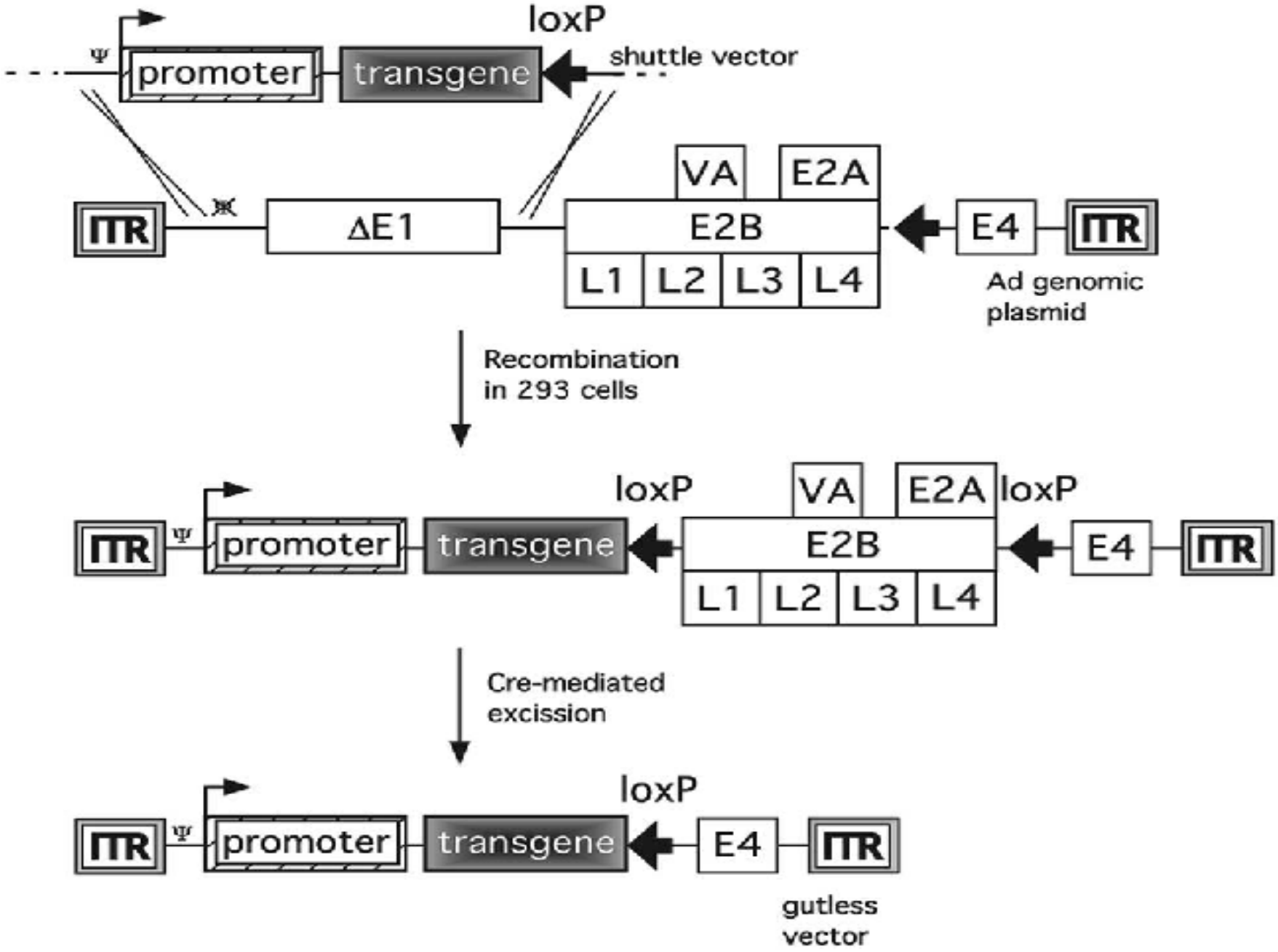

Generation of a Non-replicating Adenovirus Expression Vector
A. Wild type Adenovirus Genome
Virion Structural Proteins
E1AE1B
L1
L2
L3
L4
E3
L5
R
L
DNA
E2B
E2A
E4
B. Adenovirus vector DNA (E1, E3 deleted, expression cassette inserted)
Expression cassette
Transfect adenovirus vector DNA into complementing cell line that expresses the E1A gene
Complementing Cell Line
E1
Target Cell
Endosome
Vector DNA packaged into virion particles
Epichromosomal action
Infect target cell
Product of expression cassette

Early generations of adenoviral vectors

(replication defective)



Gutless Adenoviral vector (Amplicon)



Characteristics of adenoviral vector

- Advantages
  - High titers
  - Both dividing and non-dividing cells
  - Wide tissue tropism
  - Easily modify tissue tropism
- Disadvantages
  - Transient expression (not good for genetic diseases)
  - Highly immunogenic
  - High titers of virus can be toxic
  - More suitable for cancer immunotherapy

Adenoviral vectors- Limitations

- Adenoviral vectors can infect cells in vivo, causing them to express high levels of the transgene. However, expression lasts for only a short time (5-10 days post-infection).

- Immune response is the reason behind the short-term expression.

- Immune reaction is potent, eliciting both the cell-killing “cellular” response and the antibody producing “humoral “ response.

- Humoral response results in generation of antibodies to adenoviral proteins and prevents any subsequent infection if a second injection of the recombinant adenovirus is given.

Modification of the tropism of adenovirus vector

- Adenovirus fiber binds to CAR (coxsakie and adenovirus receptor, CAR), receptor which is ubiquitous

- Modify the fiber protein

Death of 18-year old Jesse Gelsinger

- **Liver disease: OTC deficiency (genetic disease)**
- **University of Pennsylvania**
- **High dose of adenoviral vector (E1 and E4 genes deleted) carrying the normal copy of OTC gene was administered**
- **Suspected cause of death**
  - **Toxicity of high titer adenoviral vector**
  - **High immunogenicity of adenoviral vector (an immune revolt)**

Adeno-associated viral vectors:

- AAV is a simple, non-pathogenic, single stranded DNA virus dependent on the helper virus (usually adenovirus) to replicate.

- It has two genes (cap and rep), sandwiched between inverted terminal repeats that define the beginning and the end of the virus and contain the packaging sequence.

- The cap gene encodes viral capsid proteins and the rep gene product is involved in viral replication and integration.

- It can infect a variety of cell types and in the presence of the rep gene product, the viral DNA can integrate preferentially into human chromosome 19.

- To produce an AAV vector, the rep and cap genes are replaced with a transgene.

- The total length of the insert cannot exceed 4.7 kb, the length of the wild type genome.

- Production of the recombinant vector requires that rep and cap are provided in trans along with the helper virus gene products.

- The current method is to cotransfect two plasmids, one for the vector and another for rep and cap into cells infected with adenovirus.

- This method is cumbersome, low yielding and prone to contamination with adenovirus and wild type AAV.

- Interest in AAV vectors is due to their integration into the host genome allowing prolonged gene expression.

Adeno-associated virus vectors:

Advantages:

All viral genes removed

Safe

Transduction of nondividing cells

Stable expression

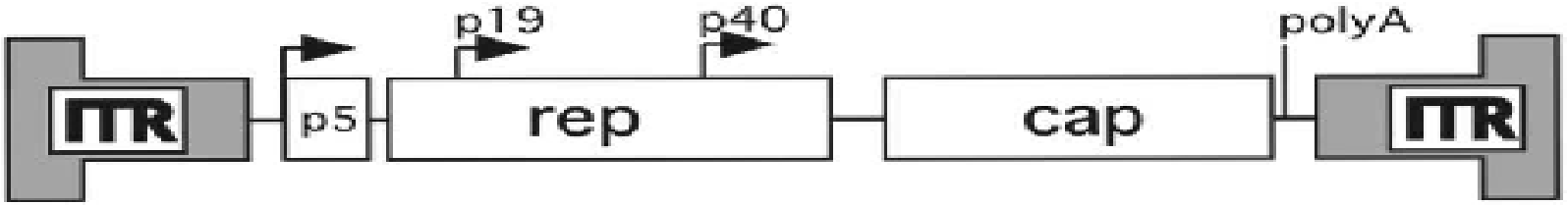
Disadvantages:

Small genome limits size of foreign DNA

Labor intensive production

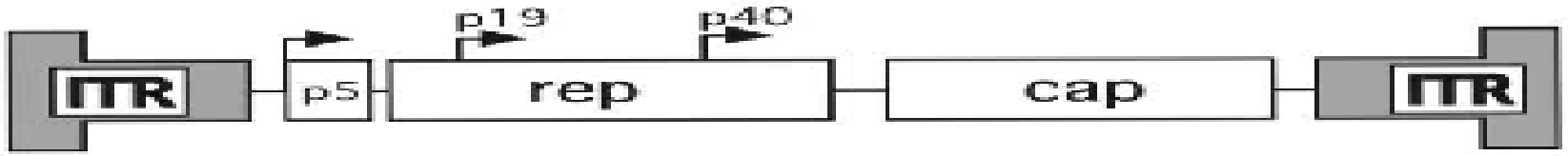
Status of genome not fully elucidated

Generation of adeno-associated virus vector



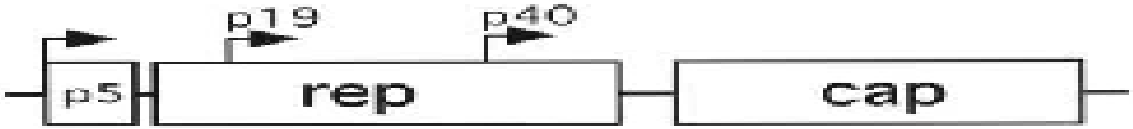
wild-type AAV

AAV vector
(psub201)

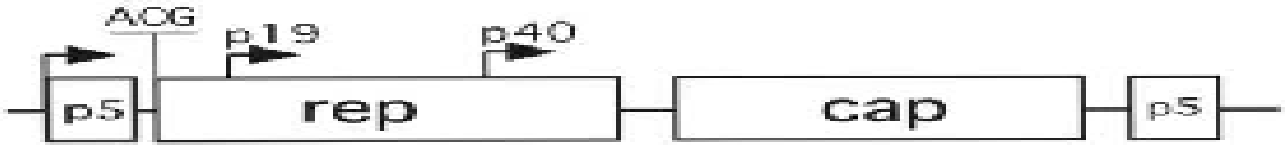


wild-type AAV

AAV packaging plasmids

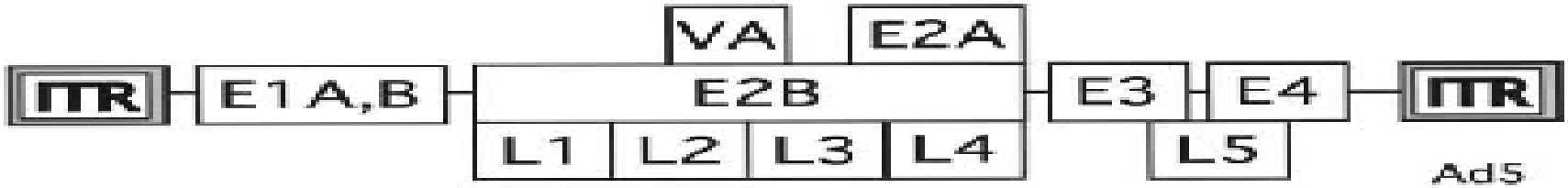


pAAV/Ad

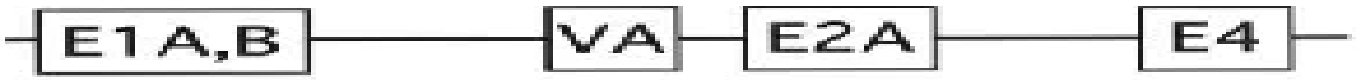


AAV-packaging

AAV helper plasmids



Ad5



Ad-helper

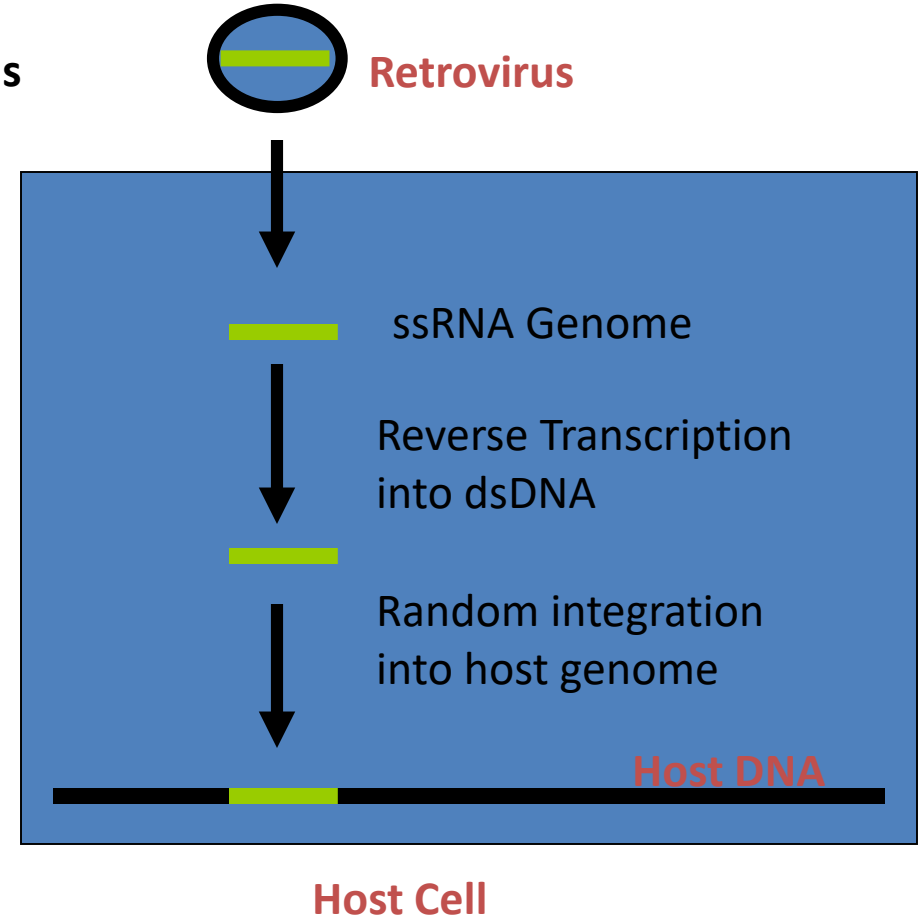
Characteristics of AAV vector

- Advantages
  - Integration and persistent expression
  - No insertional mutagenesis
  - Infecting dividing and nondividing cells
  - Safe
- Disadvantages
  - Size limitation, 4.9 kb
  - Low titer of virus, low level of gene expression

Retroviruses

(including Lentivirus, HIV and MMLV based vectors)

- Single stranded RNA genome
- Lipid membrane enveloped
- Host range determined by envelope proteins



The Retroviral Genome

Long Terminal Repeat (LTR): **Necessary for integration into host genome**

ψ (Psi): **packaging signal**

***gag*:** **Packages viral genome into viral particles**

***pol*:** **viral polymerase necessary for viral replication**

***env*:** **viral envelope proteins, necessary for entry into host cells, dictate host range**

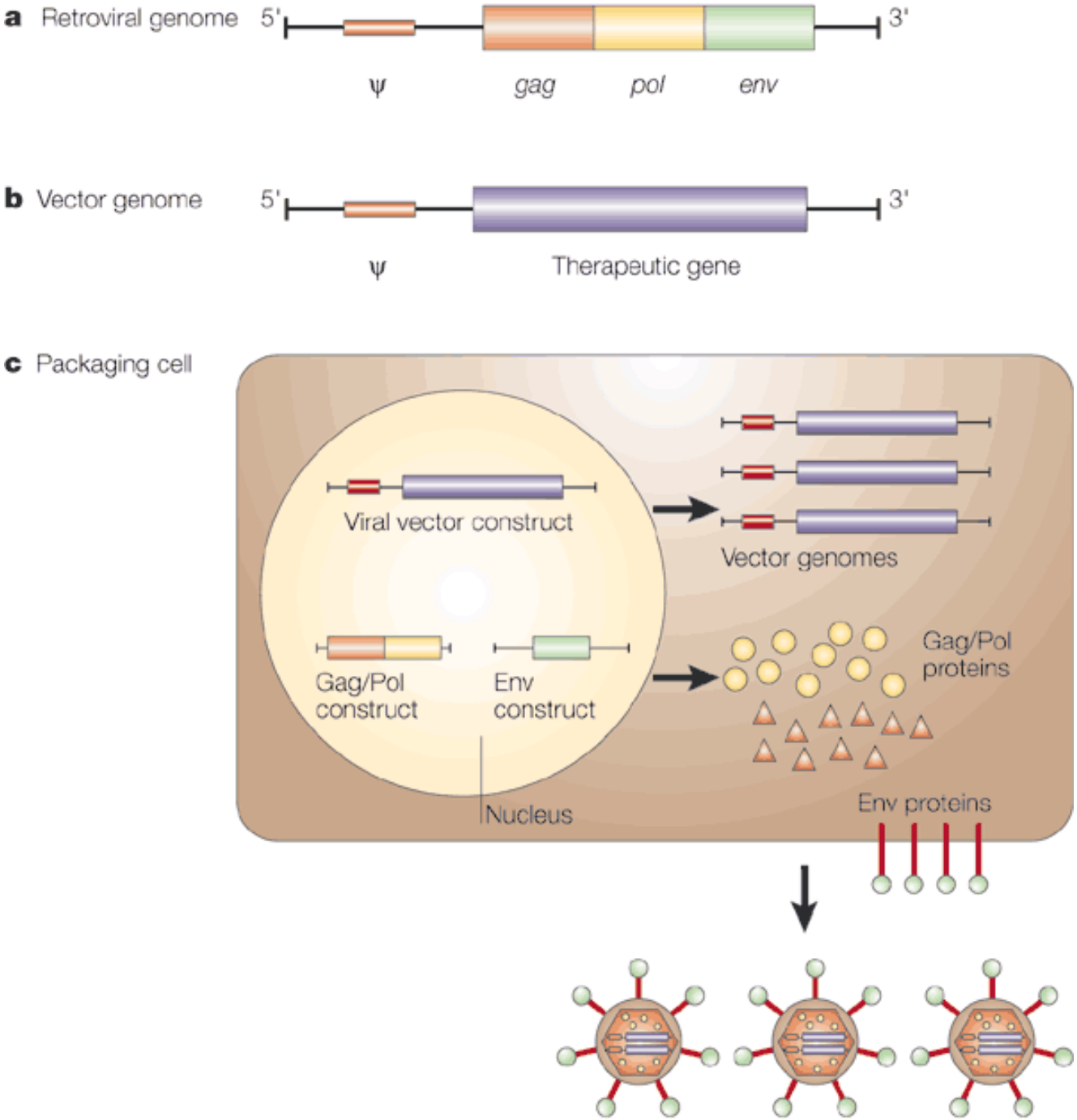
Retroviral vectors:

• Retroviral vectors are based on Moloney murine leukemia virus (Mo-MLV) which is capable of infecting both mouse and human cells.

• The viral genes, gag, pol and env, are replaced with the transgene of interest and expressed on plasmids in the packaging cell line.

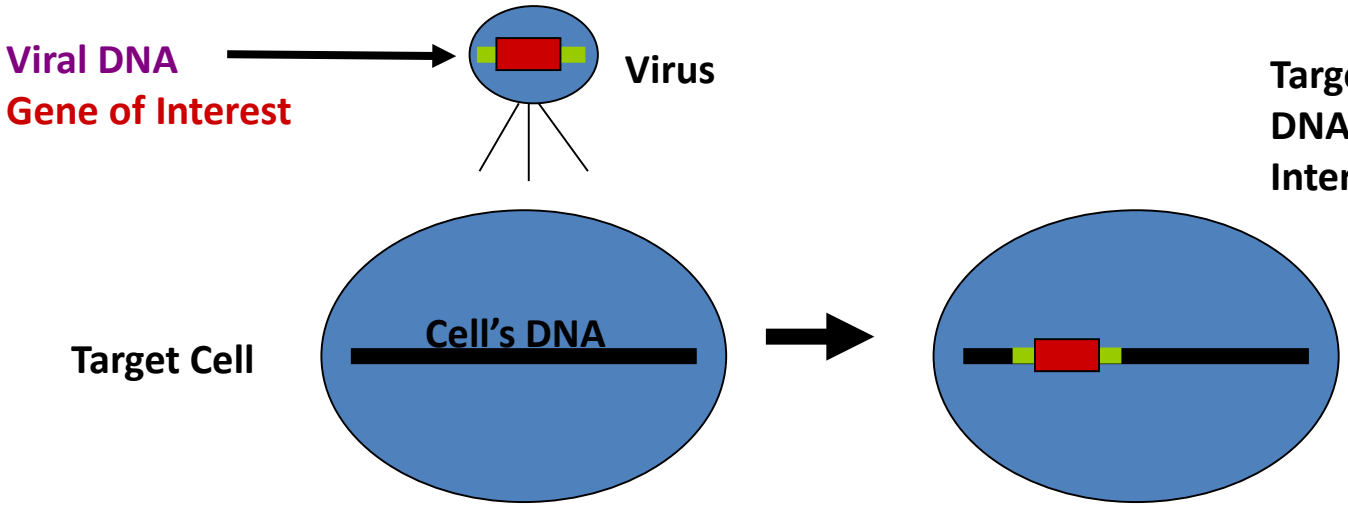
• Because the non-essential genes lack the packaging sequence, they are not included in the virion particle.

• To prevent recombination resulting in replication competent retroviruses, all regions of homology with the vector backbone is removed.


a Retroviral genome
5'
3'
ψ
gag
pol
env
b Vector genome
5'
3'
ψ
Therapeutic gene
c Packaging cell
Viral vector construct
Vector genomes
Gag/Pol
construct
Env
construct
Gag/Pol
proteins
Nucleus
Env proteins

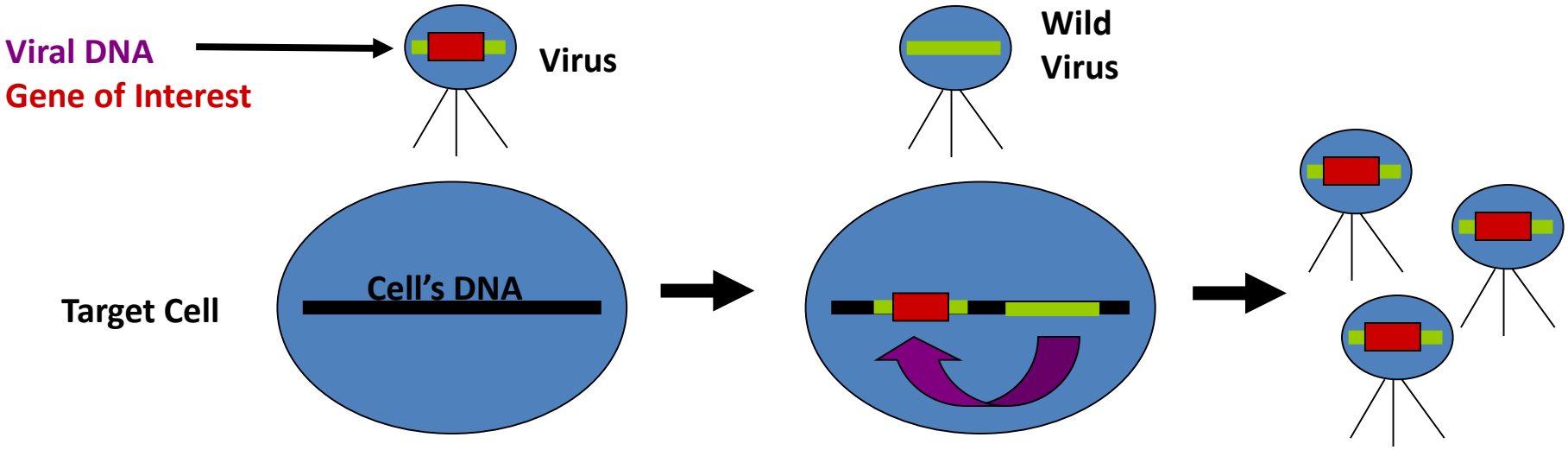
Replication Deficient Viral Vectors: Genetically Engineered So The Viral Infection Cannot Spread

- The viral DNA does not contain the viral genes needed to make more viruses.



No New Viral Particles are Created
Infection dose not spread

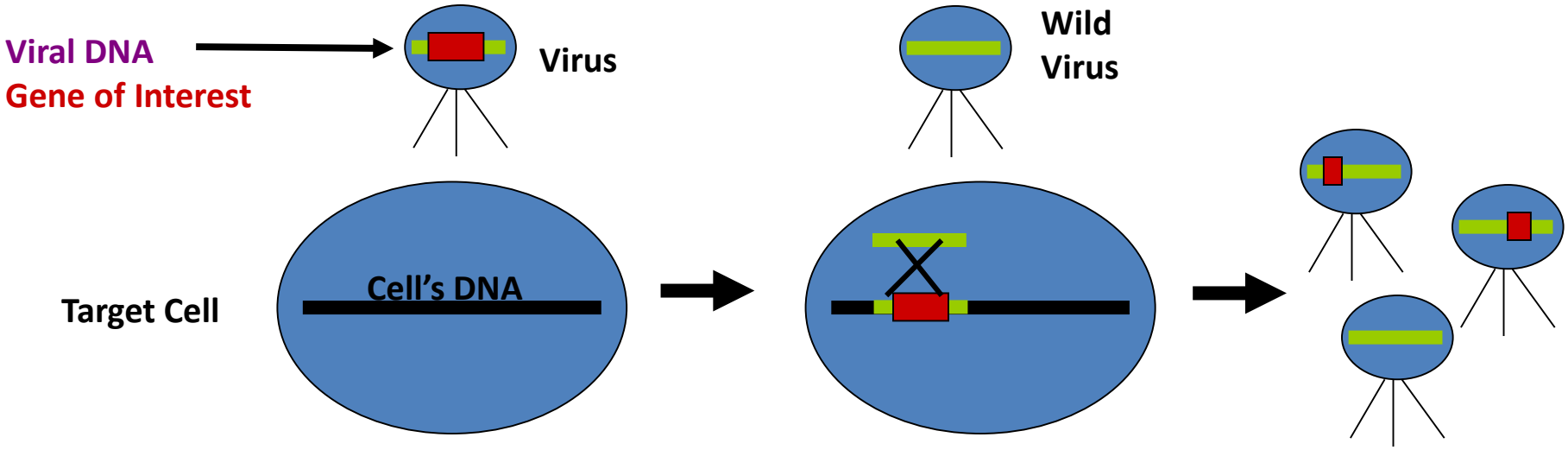
Rescue of Replication Deficient Viruses by superinfection with Wild Viruses



Complementation:

The genome from the wild virus provides the missing proteins needed for the viral vector to replicate. The superinfected cell functions similarly to a packaging line.

Rescue of Replication Deficient Viruses by superinfection with Wild Viruses

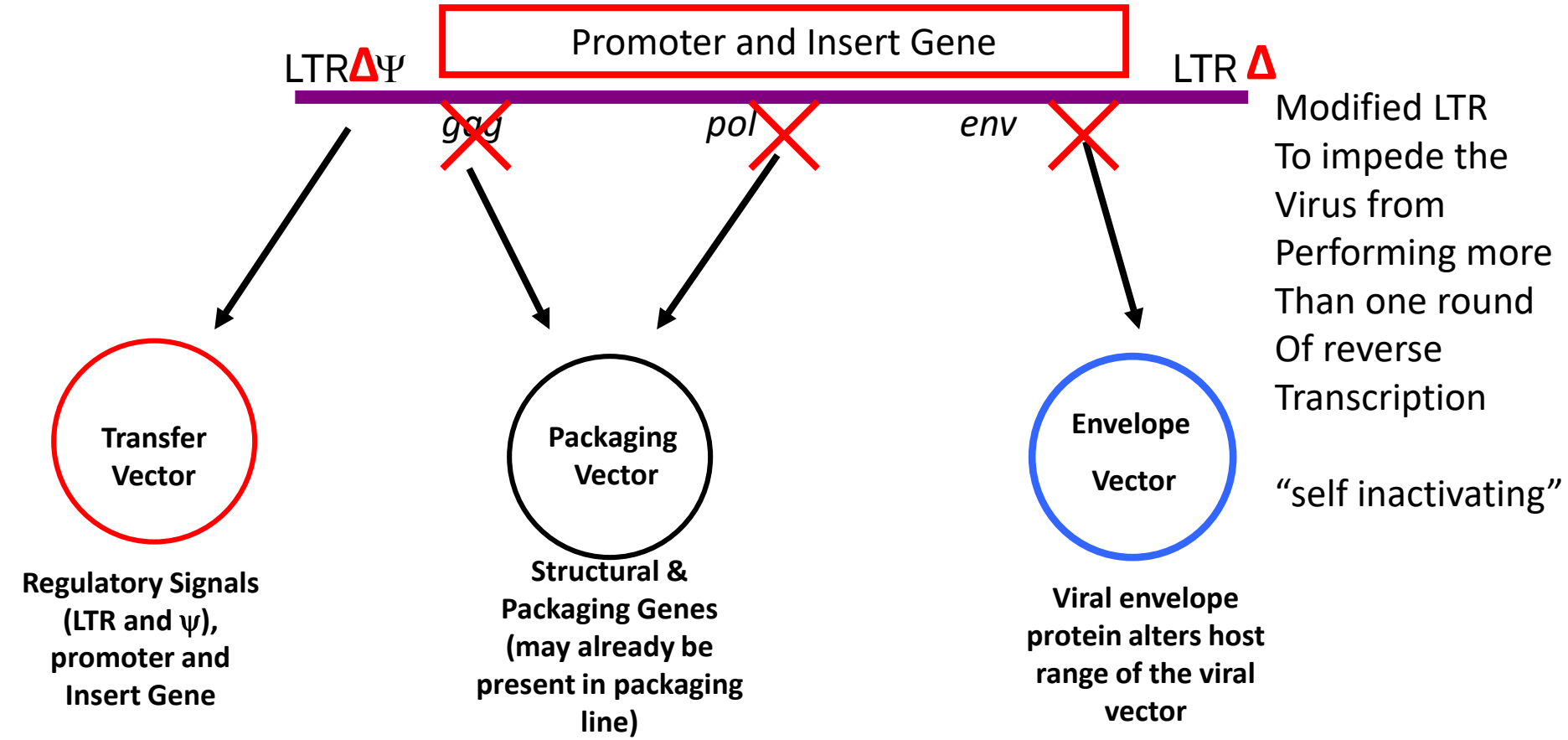


Recombination:

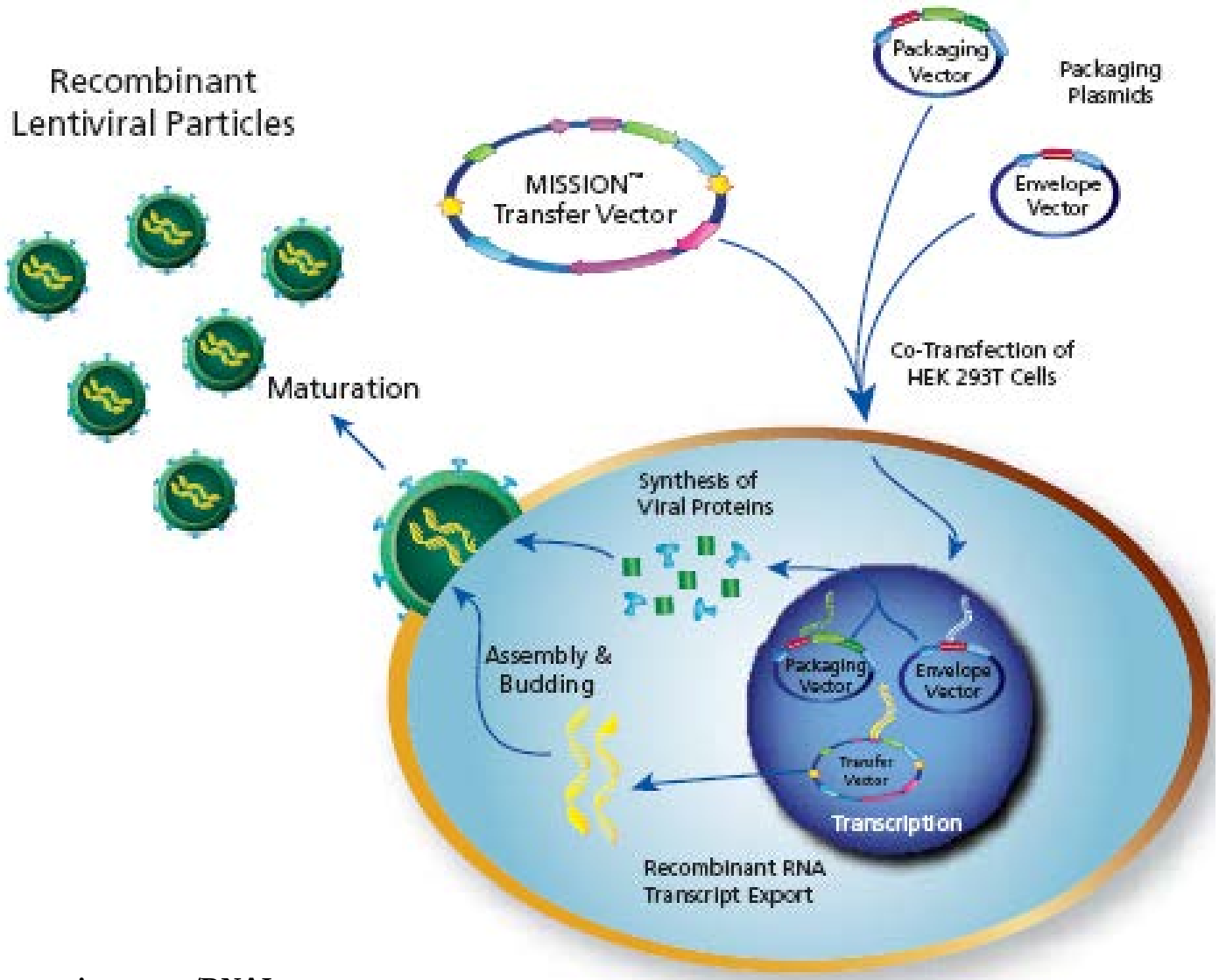
The genome from the wild virus randomly recombines with the viral vector, providing sufficient genetic material for the viral vector to replicate. The resulting rescued virus may possess pieces of the original insert gene. The viral genome is impossible to predict due to random recombination. The virus may exhibit altered virulence.

Design of Replication Incompetent Lentiviral Vectors (3rd Generation)

The viral vector is "gutted" as much as possible to create room for the insert gene and to divide the viral genome into cis- and trans- acting regions



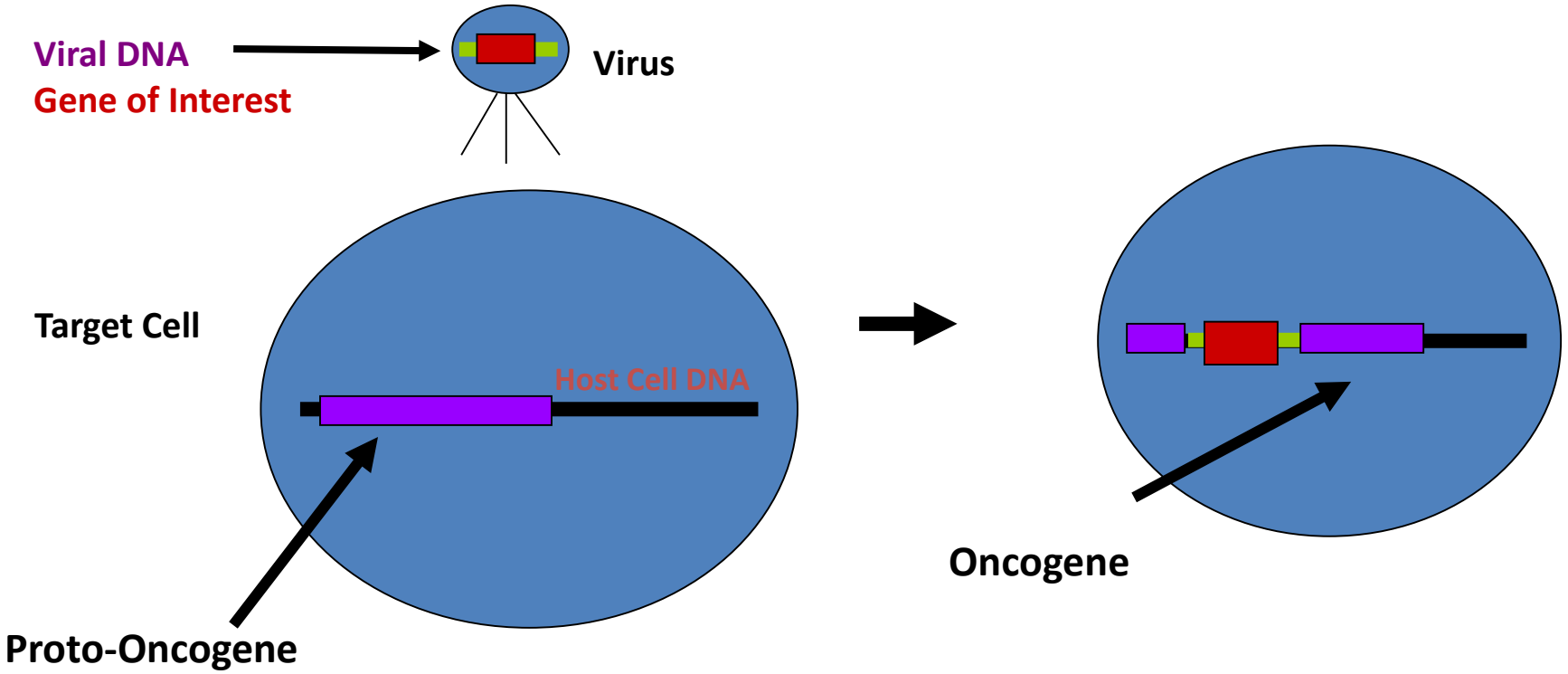
Packaging Recombinant Lentiviral Particles



The three plasmids containing the viral genome components are transfected into the packaging line to create the infectious viral particles.

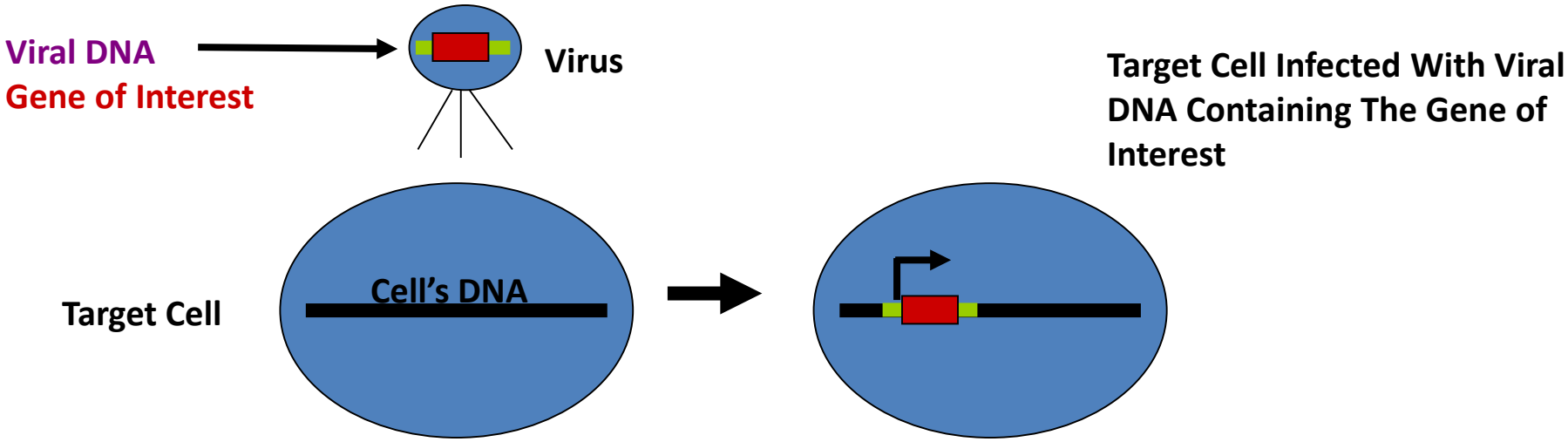
Multiple plasmids are used so multiple recombination events would be required to reconstitute a replication competent virus.

Risks Associated with Retroviruses: Insertional Mutagenesis



Random integration of viral genome may disrupt endogenous host genes. Of special concern Is disruption of proto-oncogenes, which can lead to increased cancer risk.

Risks Associated with Retroviral Vectors: Viral Transduction



Individuals infected with the viral vector may express the insert gene at the site of infection.

Retroviral vectors- Limitations

- A critical limitation of retroviral vectors is their inability to infect nondividing cells, such as those that make up muscle, brain, lung and liver tissue.
- The cells from the target tissue are removed, grown in vitro and infected with the recombinant vector, the target cells are producing the foreign protein are then transplanted back into the animal (ex vivo gene therapy).
- Problems with expression being shut off, prolonged expression is difficult to attain.
- Expression is reduced by inflammatory interferons acting on viral LTRs, as the retroviral DNA integrates, viral LTR promoters are inactivated.
- Possibility of random integration of vector DNA into the host chromosome.

Characteristics of retroviral vector

- Advantages
  - Integration: permanent expression
  - Pseudotyped virus
- Disadvantages
  - Only infecting dividing cells
  - Insertional mutagenesis (tumor formation)
    - Activate oncogenes
    - Inhibit tumor suppressor genes

Lentiviral Vectors:

- Belong to the retrovirus family but can infect both dividing and non-dividing cells.

- They are more complicated than retroviruses, containing an additional six proteins, tat, rev, vpr, vpu, nef and vif.

- Human immunodeficiency virus (HIV) has been disabled and developed as a vector for in vivo gene delivery.

- Low cellular immune response, thus good possibility for in vivo gene delivery with sustained expression over six months.

- No potent antibody response.

Likely Symptoms of Lab Acquired Infections with Retro/Lentiviral Vectors

- **Fever / flu-like symptoms**
- **Possible inflammation of infected tissues**
- **Random integration of viral genome into host genome can result in insertional mutagenesis and oncogenesis (see slide 15)**
- **Expression of insert genes in infected tissues (oncogenes, inflammatory mediators and toxins are of special concern) (see slide 16)**

Herpesvirus vectors

- HSV-1 is a mild disease
- Linear dsDNA
- 50 kb of foreign DNA can be accomodated (150 kb for the new generation of vectors)
- Neurotropic virus
- Highly deleted defective virus (half of the 80 herpes genes can be deleted) + packaging cell lines with mutant strains.

The First Case

- The first gene therapy was performed on September 14th, 1990
  - Ashanti DeSilva was treated for SCID
    - Sever combined immunodeficiency
  - Doctors removed her white blood cells, inserted the missing gene into the WBC, and then put them back into her blood stream.
  - This strengthened her immune system
  - Only worked for a few months ☹

A case of leukemia in a SCID child treated with a retroviral vector

- **SCID disease or 'Bubble boy disease' (T cell deficiency)**
- **Overall quite successful, over 1000 peoples received retroviral gene therapy**
- **A French baby's treated with retroviral vector 3 years ago**
- **A leukemia-like illness developed this summer.**
- **Nine other children treated same time show no sign of leukemia**
- **But the side effect isn't a big enough risk yet that genetic experiments for children with an often fatal immune disease should stop**
- **People receiving retroviral gene therapy should be warned about the risk of developing leukemia**

Successful One Year Gene Therapy Trial For Parkinson's Disease

- Neurologix a biotech company announced that they have successfully completed its landmark Phase I trial of gene therapy for Parkinson's Disease using AAV vectors.

- This was a 12 patient study with four patients in each of three dose escalating cohorts. All procedures were performed under local anesthesia and all 12 patients were discharged from the hospital within 48 hours of the procedure, and followed for 12 months. Primary outcomes of the study design, safety and tolerability, were successfully met. There were no adverse events reported relating to the treatment.

Vectors Based on RNA Viruses

Features	Retroviral	Lentiviral	Alphaviral
Maximum Insert size	7-7.5 kb	7-7.5 kb	5 kb
Concentrations viral particles/ml	$>10^8$	$>10^8$	$>10^9$
Route of gene delivery	Ex vivo	Ex/In vivo	In vivo
Integration	Yes	Yes	No
Duration of expression in vivo	Shorter than theorized	Long	Short
Stability	Good	Not tested	Good
Ease of Preparation scale up	Pilot scale up up to 20-50 liters	Not known	Not known
Immunological problems	Few	Few	Unknown
Pre-existing host immunity	Unlikely	Unlikely, except in AIDS patients	No
Safety problems	Insertional mutagenesis?	Insertional mutagenesis?	Few

Vectors Based on DNA and on DNA Viruses

Features	Adenoviruses	Adeno-associated viruses	Herpesviruses	Vaccinia virus	Naked DNA /Lipid DNA
Maximum Insert size	7.5 kb	4.5kb	~30kb	>25 kb	Unlimited size
Concentrations viral particles/ml	$>10^{10}$	$>10^{12}$	$>10^{8}$	10^{7}-10^{9}	No limitation
Route of gene delivery	Ex/In vivo	Ex/In vivo	Ex vivo	Ex/In vivo	Ex/In vivo
Integration	No	Yes/No	No	No	very poor
Duration of expression in vivo	Short	Long	Short/ Long in CNS?	Short	Short
Stability	Good	Good	Unknown	Good	Very good
Ease of Preparation scale up	Easy to scale up	Difficult to purify, difficult to scale up	Not yet tried	Vaccine production facilities exist	Easy to scale up
Immunological problems	Extensive	Not known	Not known	Extensive	None
Pre-existing host immunity	Yes	Yes	Yes	Diminishing as unvaccinated population grows	No
Safety	Inflammatory response, toxicity	Inflammatory response, toxicity	Neurovirulence? Insertional mutagenesis	Disseminated vaccinia in immunocompromised hosts	None?

Clinical Trials of Gene Therapy for Monogenic Diseases in the United States in 2000

Disease	Gene	Vector	Number of Trials	Number of patients	Results
Gaucher disease	GC[c]	Retrovirus	3	9	One trial shows long term elevation of GC expression, other trials primarily Phase I
OTC deficiency	OTC	Adenovirus	1	14	Trial suspended after one fatality (see text)
ADA-SCID	ADA + NeoR	Retrovirus	1	6	Ongoing since 1990
Cystic Fibrosis	CFTR	Adenovirus	9	83	Some correction of defect in 30% of patients, but inflammation at clinical doses, and reduction in therapeutics with repeated injection.
	CFTR	AAV	4	36	Some correction of defect, Phase II study started
	CFTR	Cationic Lipids	4	25	30% to 50% of patients showed showed improvement
Chronic granulomatous	p47 phox/ gp91 phox	Retrovirus	3	9	Phase I/II, study closed in 1998
Familial hypercholesterolemia	LDLR	Retrovirus	1	5	Phase I, closed in 1994

Other Clinical Trials of Gene Therapy in the United States in 2000

Disease	Gene	Vector	Number of Trials	Number of patients	Results
Chronic Diseases					
Rheumatoid arthritis	IRAP	Retrovirus	1	7	?
Artery disease and restenosis	VEGF	Naked DNA	2	29	?
Infectious Diseases					
AIDS	HIV-IT(V)	Retrovirus	3	298	Most gene trials for HIV are in Phase I, with a few in phase II. Few results reported.
	CD4-Zeta TcR	Retrovirus	3	54	
	Anti-HIV ribozyme	Retrovirus	2	12	
	TK + HyR	Retrovirus	2	14	
	Antisense to TAR	Retrovirus	3	17	

Clinical Trials of Gene Transfers for Cancer Therapy in the United States in 2000

Location	Gene	Vector	Number of Trials	Number of patients	Phase
Brain cancers					
Neuroblastoma	IFNγ	Retrovirus	1	4	I
	IL-2	Retrovirus	1	12	I
	IL-2	Adenovirus	1	6	I
Central nervous system	TK	Adenovirus	2	22	I
Pediatric tumor	TK	Retroviral producing cells	1	2	I
Adult brain tumor	TK	Retroviral producing cells	1	15	I
Ovarian cancer	HSV-TK	Adenovirus	1	10	I
	TK	Retroviral producing cells	3	42	I
	BRCA-1	Retrovirus	1	40	I/II
	p53	Adenovirus	1	16	I
Small cell lung cancer	IL-2 + NeoR	Lipofection	1	8	I
	Anti-sense to k-*ras*	Retrovirus	1	9	I
	p53	Adenovirus	2	59	I/II
Prostate cancer	GM-CSF	Retrovirus	1	8	I/II
	PSA	Poxvirus	1	3	I
	HSV-TK	Adenovirus	1	18	I
Breast cancer	BRCA-1	Retrovirus	1	21	I
	E1A	Lipofection	1	16	I
	MDR-1+ NeoR	Retrovirus	4	39	I
	CD80	Lipofection	1	15	I
	CEA	Poxvirus	4	53	I
	CEA	RNA transfer	1	30	I