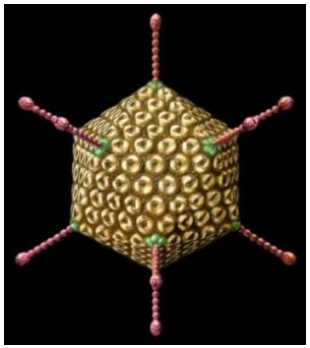
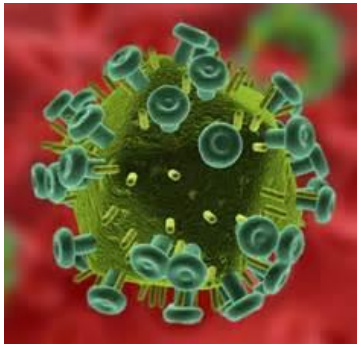
M1 D²HP Development of Drugs and Health Products

TU08 Biotechnology





Introduction to gene therapy: Viral vectors

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November 6th 2024

Content

- The viral vectors
- Retroviral / Lentiviral vectors
- Adenoviral vectors
- rAAV vectors (adeno-associated viruses)

The viral vectors

Viral vector

☞ Definition

Genetically modified virus to transfer and express its own genes or foreign genes

☞ Advantages of viral vectors

Cloning of the genome of different viruses

Possible handling in bacterial plasmids

Production of recombinant viruses by reintroduction into their host cells

☞ 4 main objectives

Production of specific proteins in cell culture

Vaccination

Gene therapy

Anticancer agents

Characteristics sought for a viral vector

Targeting and expression

- ☞ Adaptable cellular tropism
  - * high efficiency
- ☞ Expression of the transgene
  - * expression level control
  - * expression stability: integration or episomal form
- ☞ Large genetic material

Production

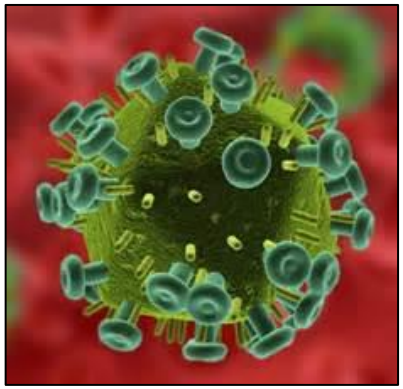
- ☞ Easy to produce vectors, low cost
- ☞ Production of high titers of viruses

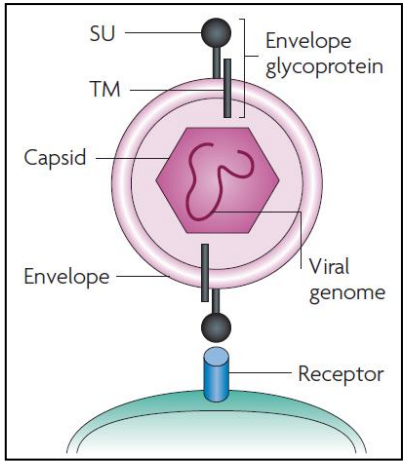
Safety

- ☞ Low or absent recombination
- ☞ Limiting the generation of RCV (Replication Competent Viruses)
- ☞ Minimal toxicity
  - * safe
- ☞ Minimal immune response
  - * directed against the components of the viral vector and / or the transgene
  - * Little / no inflammatory response

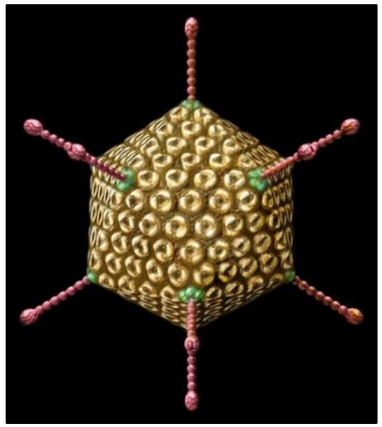
Main viral vectors

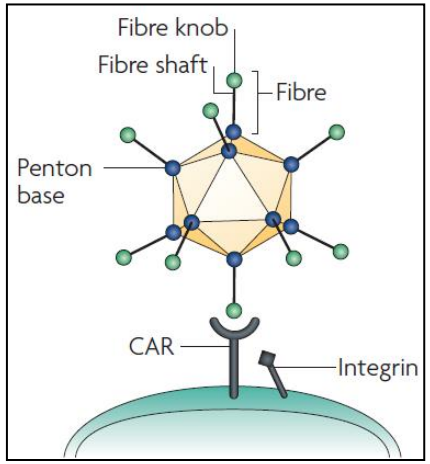
Retroviral / Lentiviral vectors



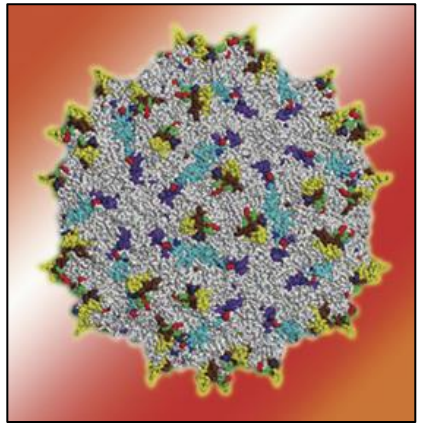


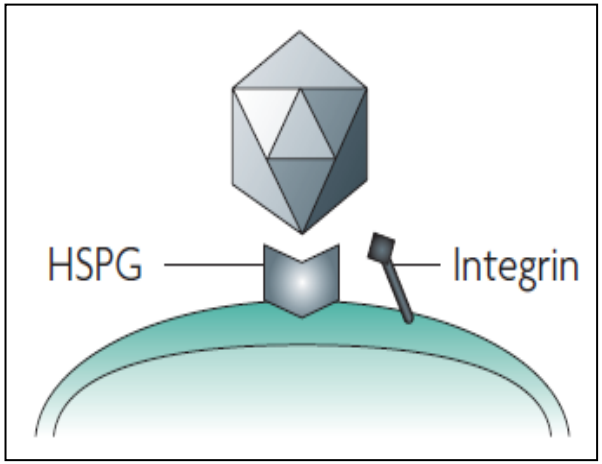
Adenoviral vector





rAdeno-associated virus (rAAV)





2 types of viral vectors

☞ **Replicative vectors**

* independently replicating vectors

  - no use in gene therapy

  - use in molecular biology, cell biology, vaccinology and protein expression

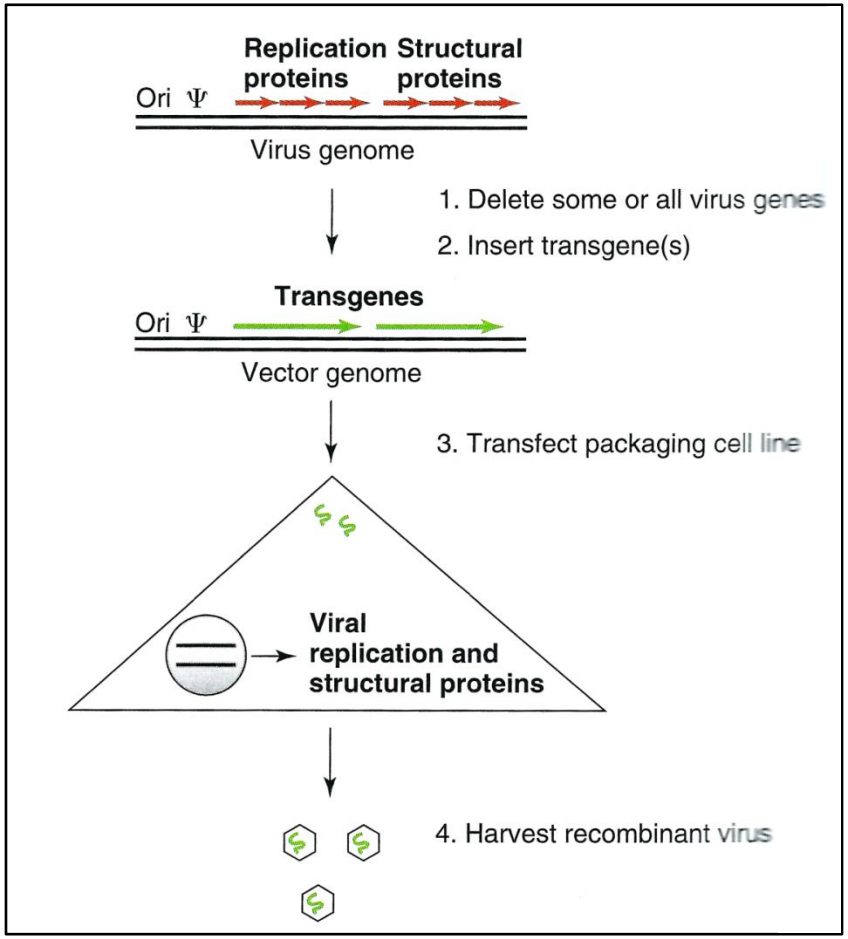
☞ **Non replicative vectors**

* recombinant viruses that replicate only in cells expressing deleted viral genes

* use in molecular biology, cell biology and gene therapy

Construction and production of a typical viral vector

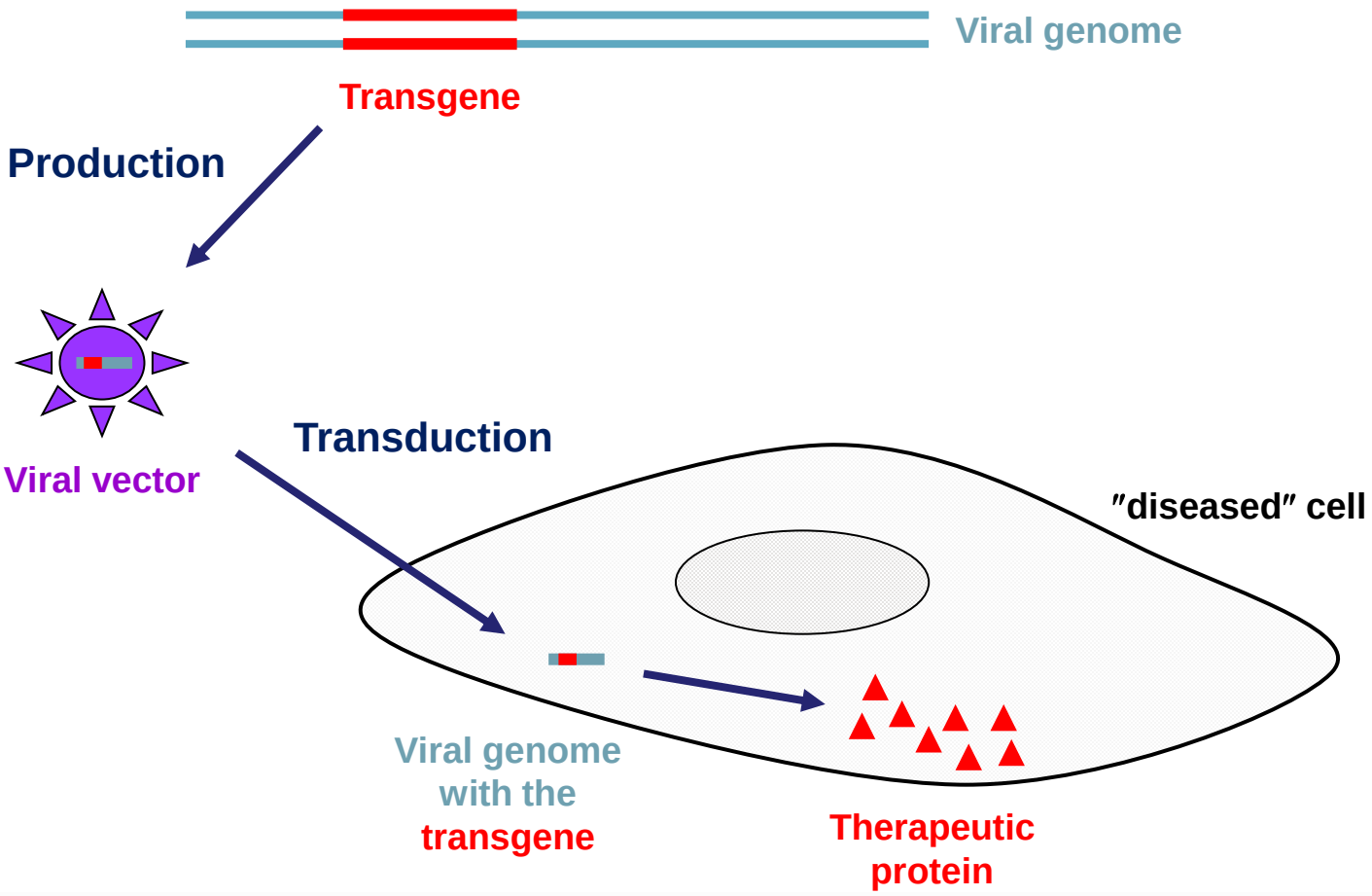
- Identify a gene not essential for replication
  or conditionally necessary
- Replace this (these) gene(s) with the transgene



Viral vector and transgene

Limiting factor = size of the transgene

- Adenovirus 8 - 35 kb
- Retrovirus 6 to 8 kb
- Lentivirus 8 kb
- AAV 4.5 kb



Large-scale production

Example with figures on AAV

⇨ **Gene therapy Hemophilia B Factor IX**

Amount of AAV injected per patient = 2.10^{11} viral particles per kg

so $1.5.10^{13}$ (15,000 billion) for a 70 kg patient

In theory, Y = 100% so 1 liter of culture

In pratice, Y = 1% so 100 liters of culture

Bioreactors of 100 - 200 L

Purification and quality control of vectors

Control of viral vectors

Identity and purity	DNA identity	Sequencing Restriction map
	Physical titration of the viral suspension	Photometry
	Biological titration of the viral suspension	Lysis plaques / Cytopathic effect (RT-)PCR
	Viral vector purity (empty viral capsids)	Photometry
Activity	*In vitro* transgene transfer efficiency	PCR
	Expression of the protein of interest *in vitro*	SDS-PAGE Western-Blot ELISA Immunocytochemistry
	Functionality of the protein of interest	Cell / animal models
Safety / Sterility	Testing for bacterial and fungal contaminants	Sterility test
	Search for endotoxins	Endotoxin test (LAL)
	Search for mycoplasmas	Culture in specific media Cell culture Epifluorescence, PCR
	Search for adventitious viruses *in vitro*	Tests on indicator cells PCR
	Search for replicative particles	Tests on permissive cells PCR

Biosecurity and risk levels (High / Low)

Biosafety	High risk	Low risk
Replication of the vector	Replicative vector	Non replicative vector
Design of the vector	Use of 2 plasmids for production Viral genes present and expressed	Use of 3 plasmids for production Viral genes deleted
Transgene	Oncogene, gene encoding a toxin, tumor suppressor gene	Non oncogene, structural gene
Production scale	Large	Laboratory
Animal host	Permissive host Humanized animals	Non permissive host
Handling of animals	Vector administration (use of needles)	-

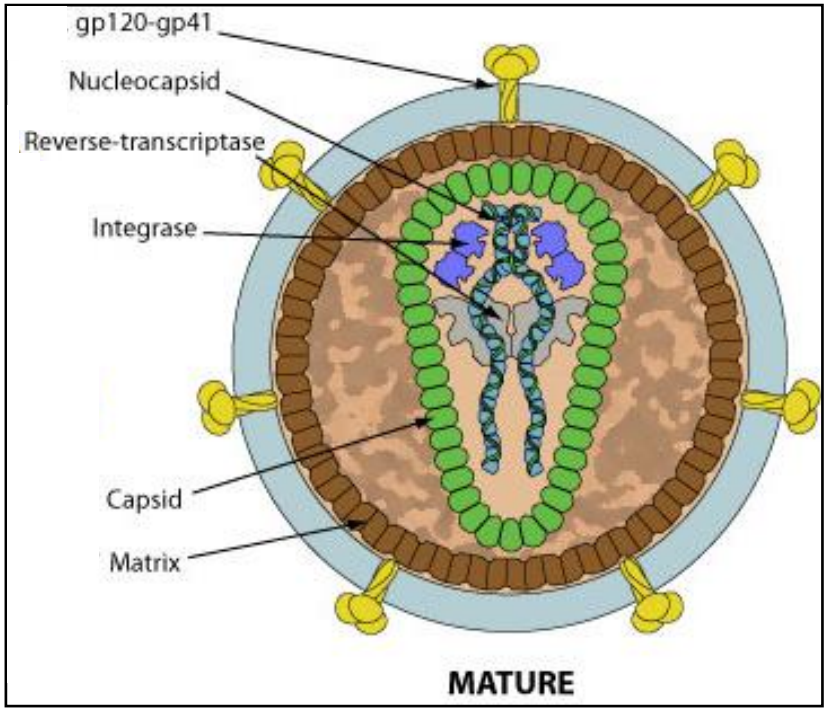
Difficulties in using viral vectors

- Size of the transgene
- Transient stability of the transgene
- Random insertion of the transgene into the genome
  - risks of endogenous gene activation
    - cancer occurrence
- Difficulty regulating therapeutic gene expression
  - completely inappropriate expression
- Infection of non-target cells
- Potential immunogenicity
- Risk of recombination with a wild-type virus
- Large-scale production
  - difficult and expensive

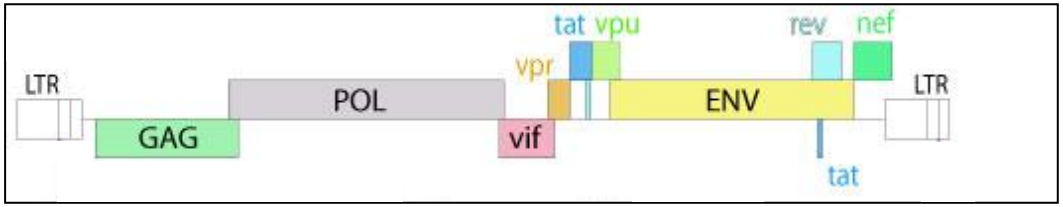
Retroviral and lentiviral vectors

Lentiviral particle

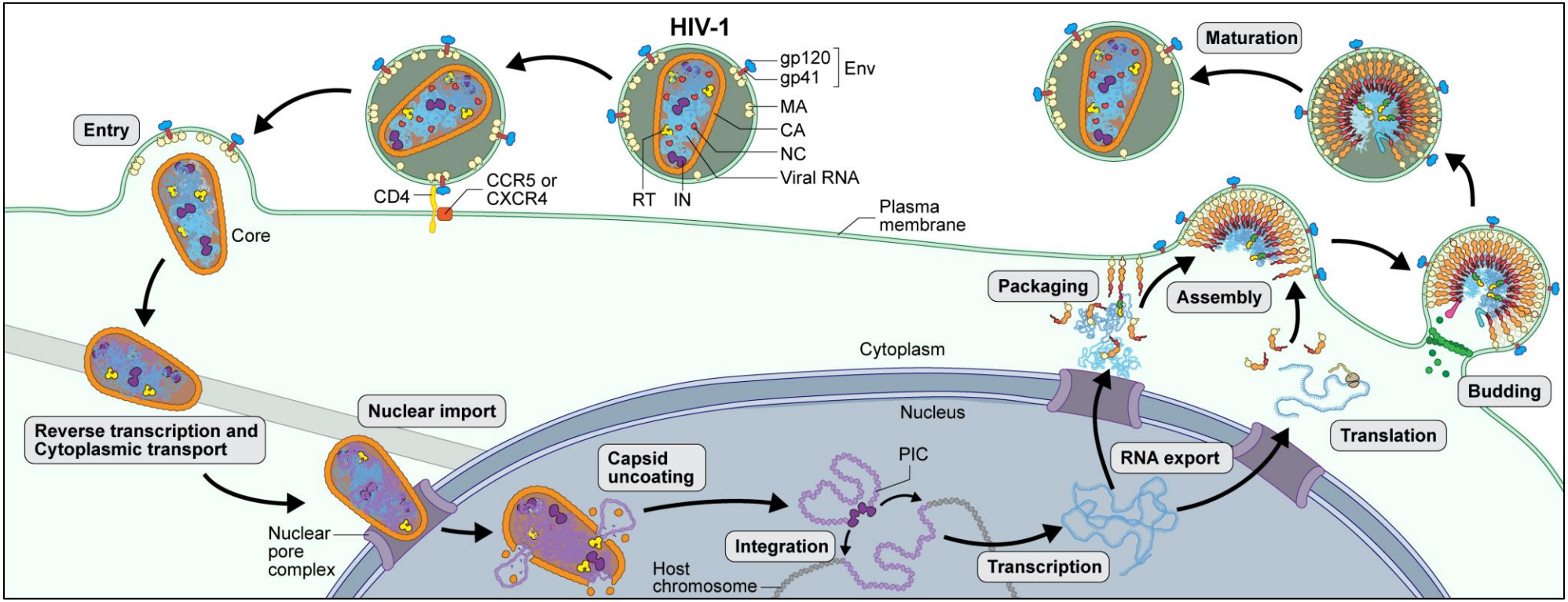
Virion



Genome



Multiplication cycle of the HIV (Genus *Lentivirus*)



[scienceofhiv.org]

Production of lentiviral vectors (1)

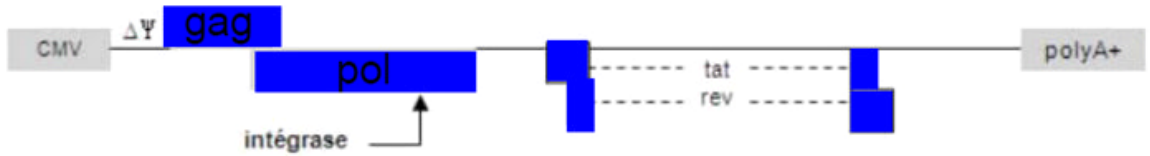
⇨ **3 plasmids to be transfected to make recombinant lentivirus**

1

Plasmid vector (pTrip CMV GFP) that contains the therapeutic gene

2

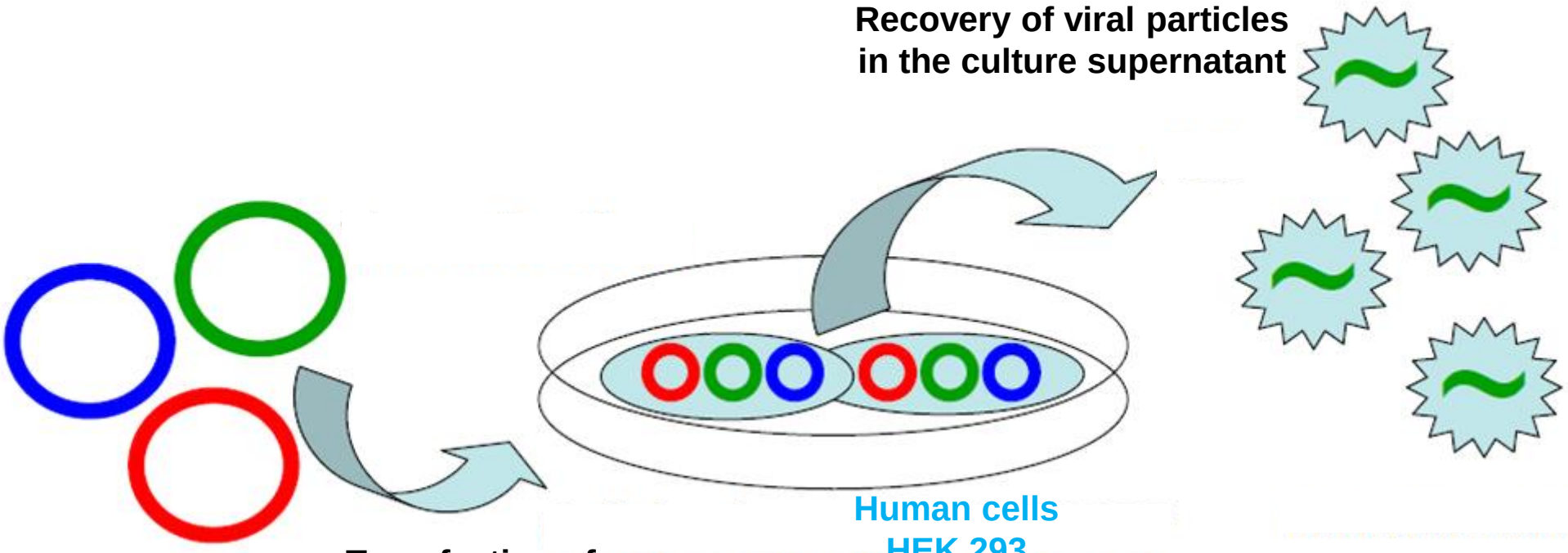
Encapsidation plasmid (p8.91) that expresses the proteins necessary for the formation of the capsid (*gag*, *pol*) and the regulating elements (*tat*, *rev*) and is deleted of the sequence ψ



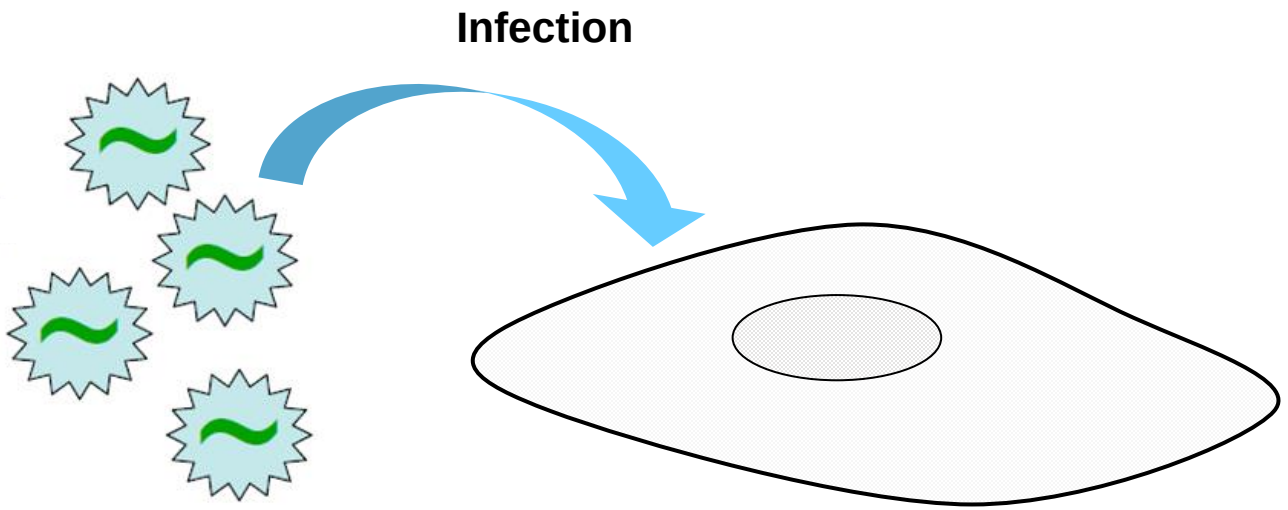
3

Envelope plasmid (pVSV-G) that expresses the envelope glycoproteins of the vesicular stomatitis virus (VSV-G)

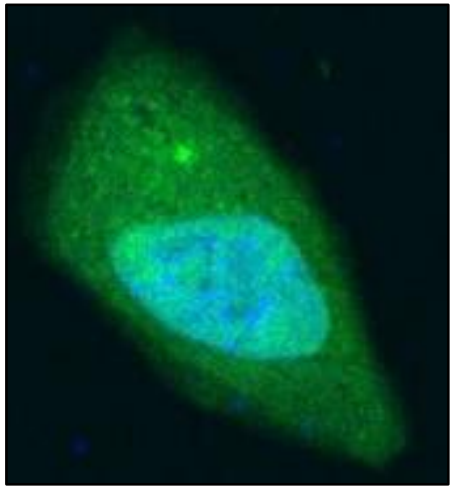
Production of lentiviral vectors (2)



Infection of target cells



Cells expressing GFP



Advantages of lentiviral / retroviral vectors

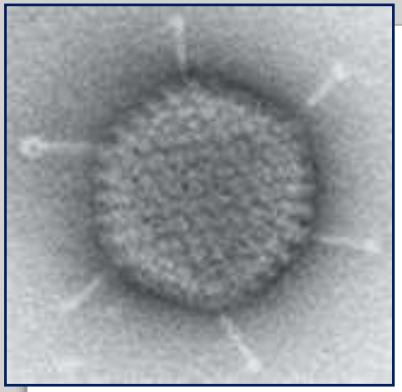
- Envelope glycoprotein that can be substituted by another protein allowing infection ⇨ modification of the tropism
  - G protein of VSV
- Ideal vectors for gene replacement
  - transgene stably integrated into the cell genome
- Possible integration of the transgene into mitotic and non mitotic cells
  - true only for lentiviral vectors
- Long-term expression of the transgene
- Very limited immune response
- Low risk of recombination
  - construction fractionation

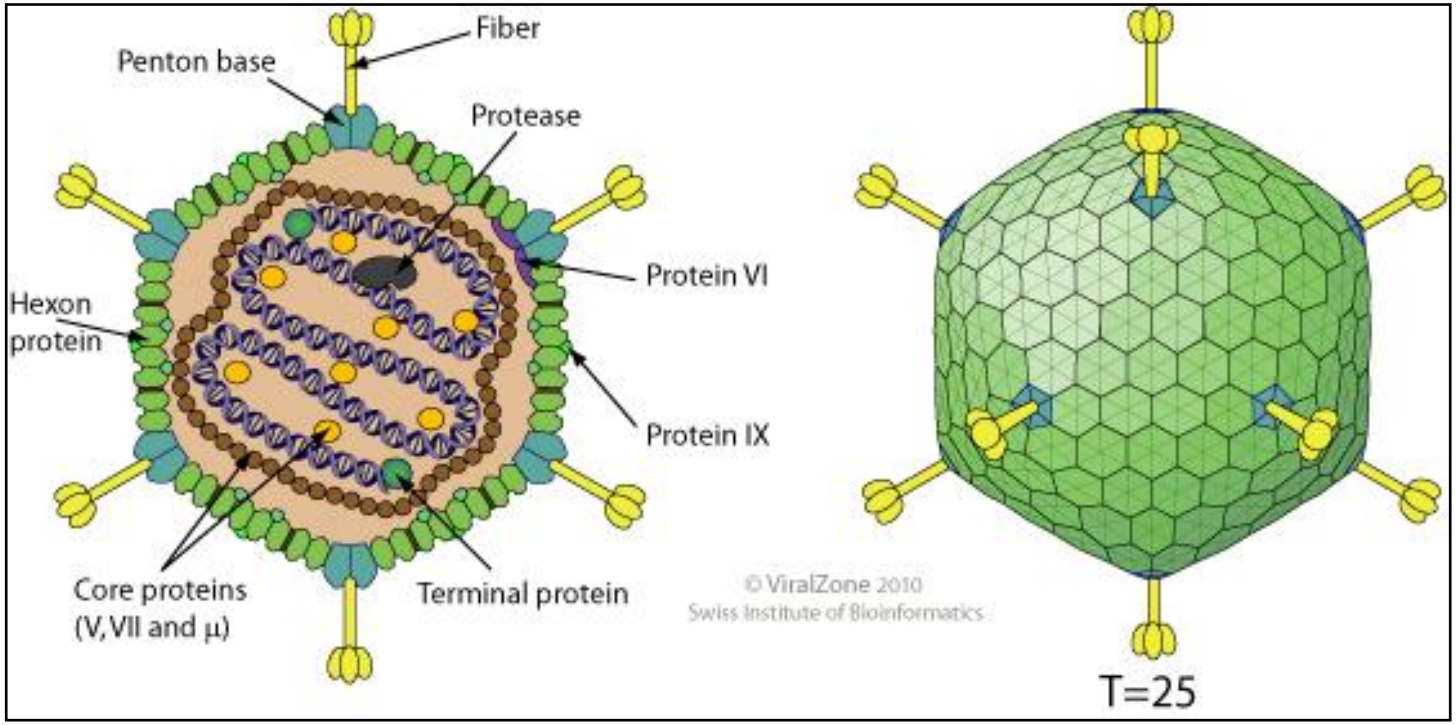
Limitations of lentiviral / retroviral vectors

- Small insert
  - limited to 7-8 kb
- Problem to produce high titers of infectious viruses
- Lack of effective cell targeting
- Some cell types difficult to infect
- Random integration
  - risk of insertional mutagenesis
- Safety problems

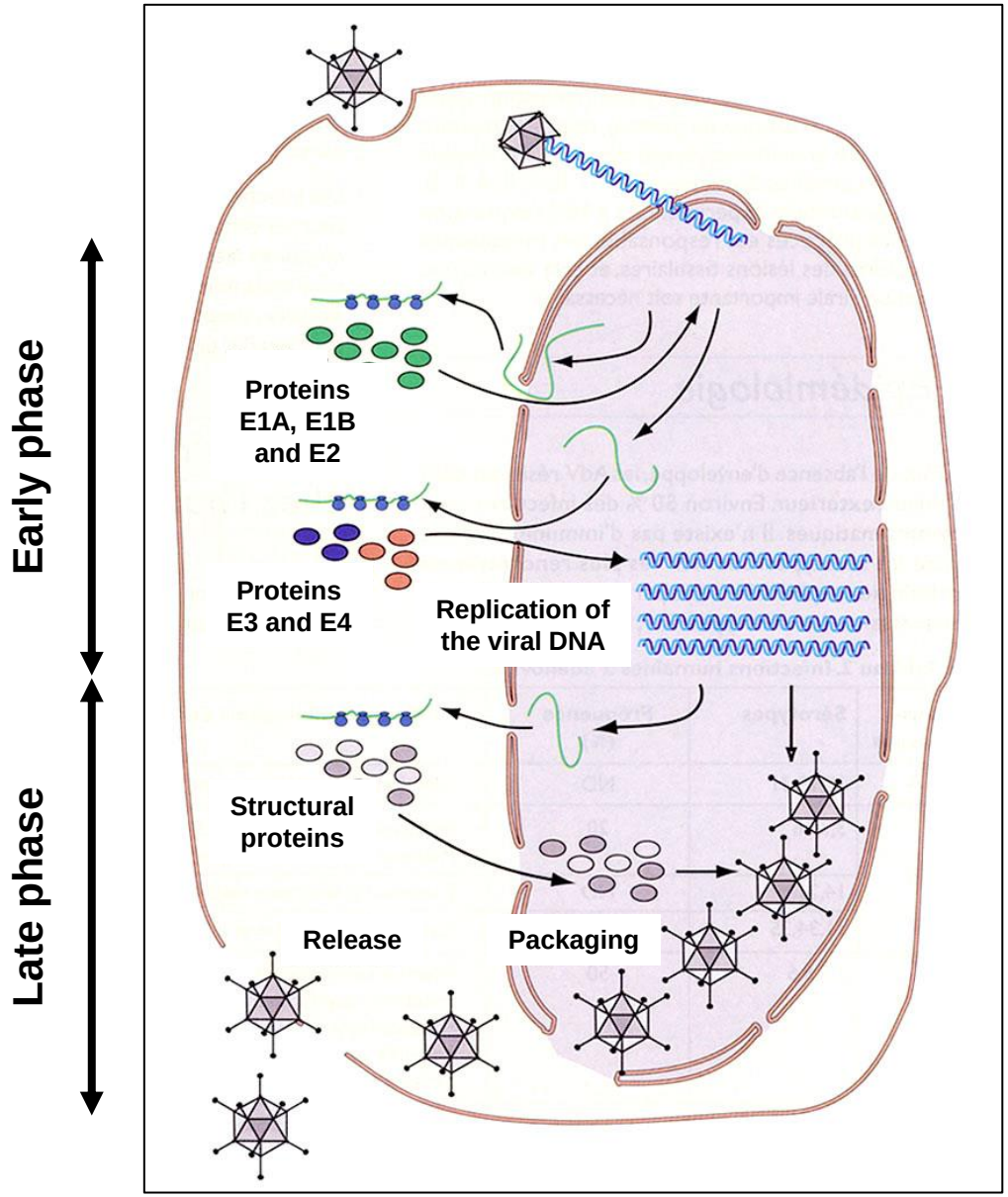
Adenoviral vectors

Adenoviral particle



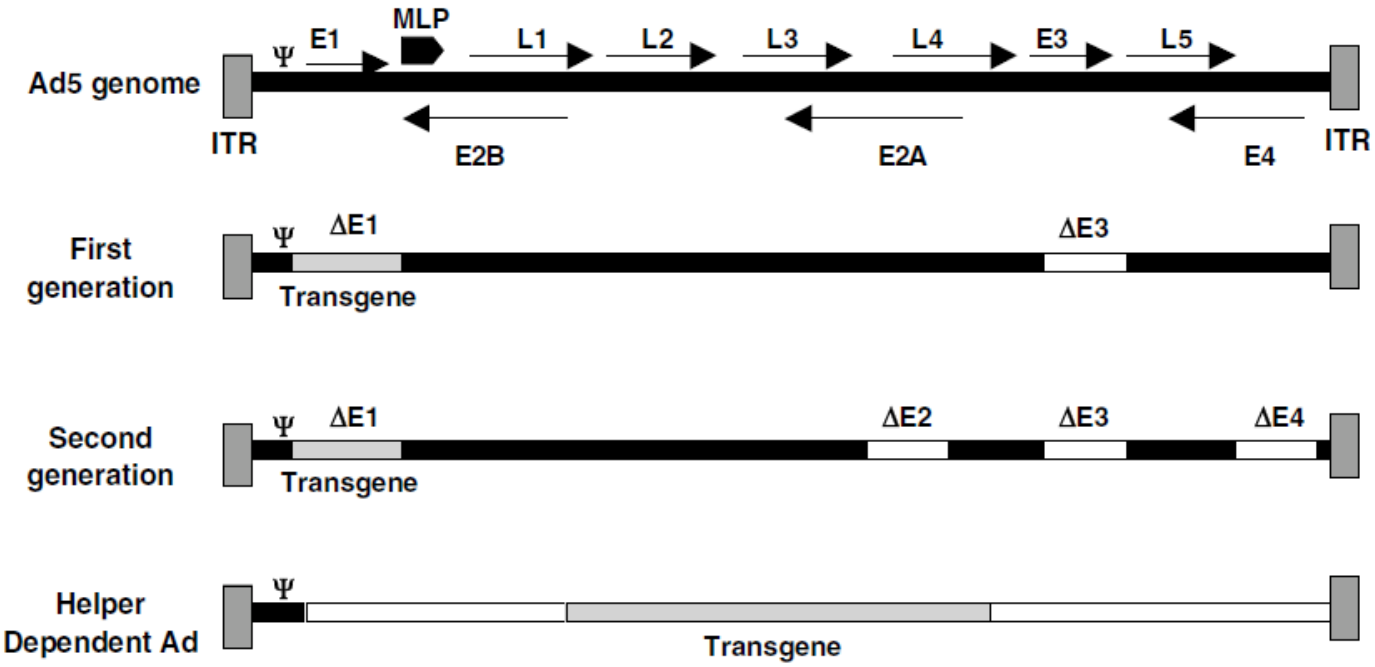


Multiplication cycle of the adenovirus



[Huraux, 2003,Traité de Virologie Médicale]

Different generations of adenoviral vectors



ITR: Inverted Terminal Region of 100 bp

ψ: encapsidation sequence at 5' extremity

MLP: *Major Late Promotor*

Presence of early regions (E) and one late region (L)

Production in HEK 293 cells expressing the necessary viral proteins = transcomplementation

[Figure adaptée Alba et al., 2005, Gene Therapy]

Advantages of adenoviral vectors

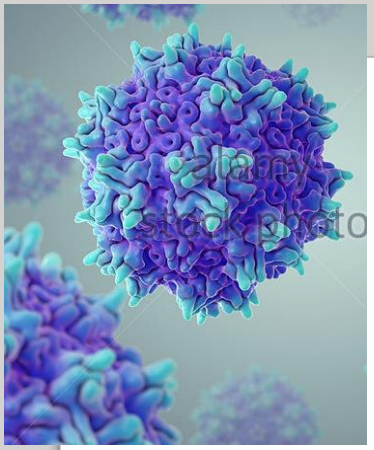
- Low pathogenicity in an immunocompetent person
- Well characterized and easily manipulated genome
- Production of high titers of infectious viruses
- Tropism for widely expressed receptors
- Vectors transducing quiescent cells
- No integration of the viral genome
  - persistence of the viral genome in episomal form
- High expression of the transgene
- Induction of strong cellular and humoral immune, systemic and mucous responses
  - adapted to vaccination strategies (oral route for example)

Limitations of the adenoviral vectors

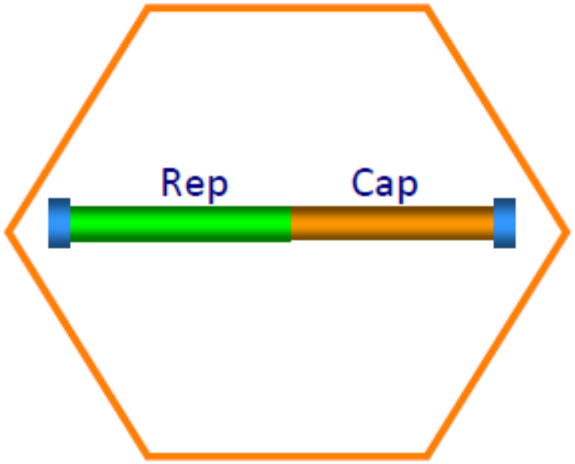
- Persistence of transgene expression limited in time
- Immune response = major obstacle
  - prevalence = 80% in the population
- Inflammatory response
- Difficult re-administration
- Risk of theoretical recombination with endogenous viruses

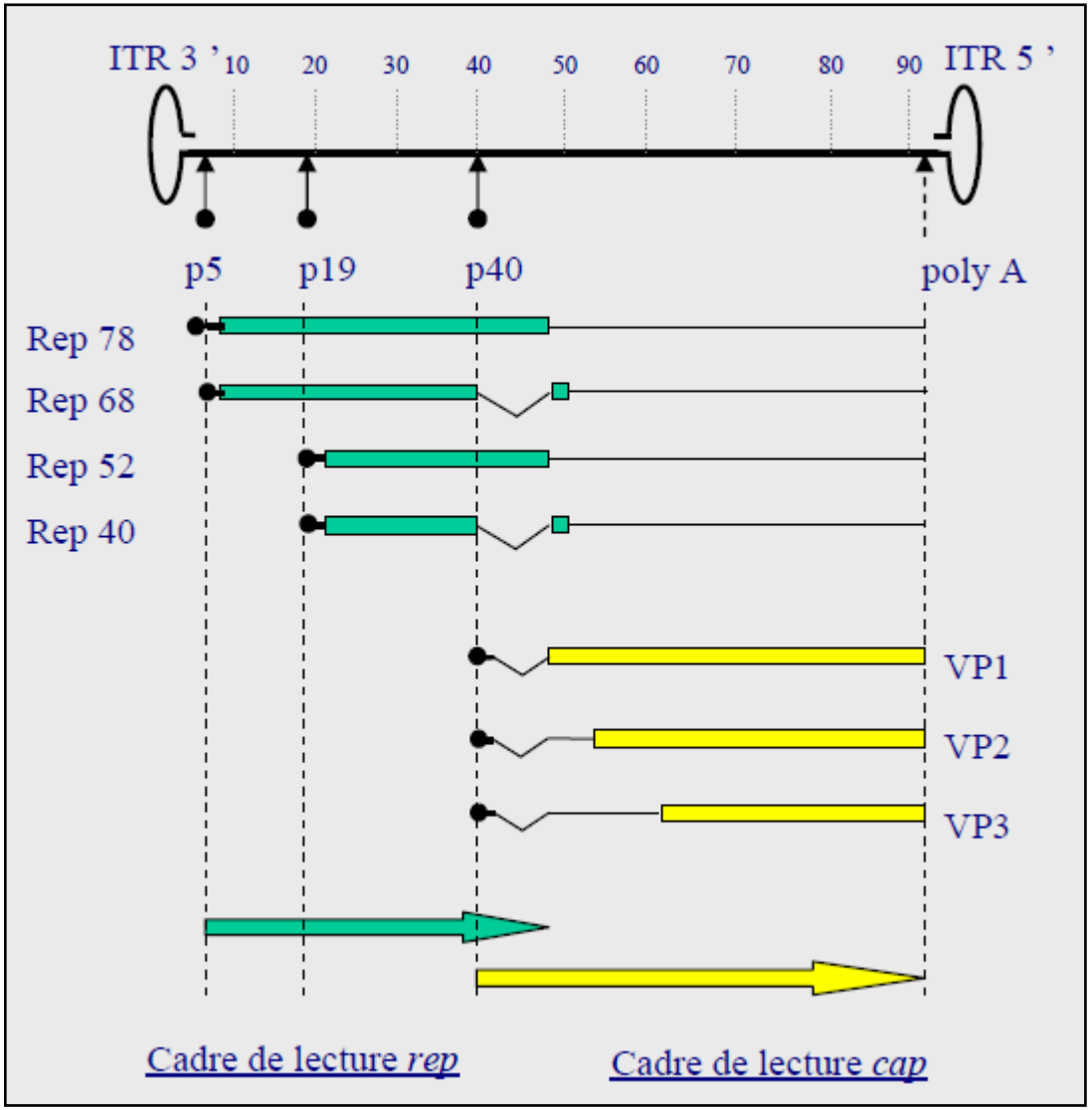
rAAV (Adeno-Associated Virus) vectors

Genome of serotype 2 AAV

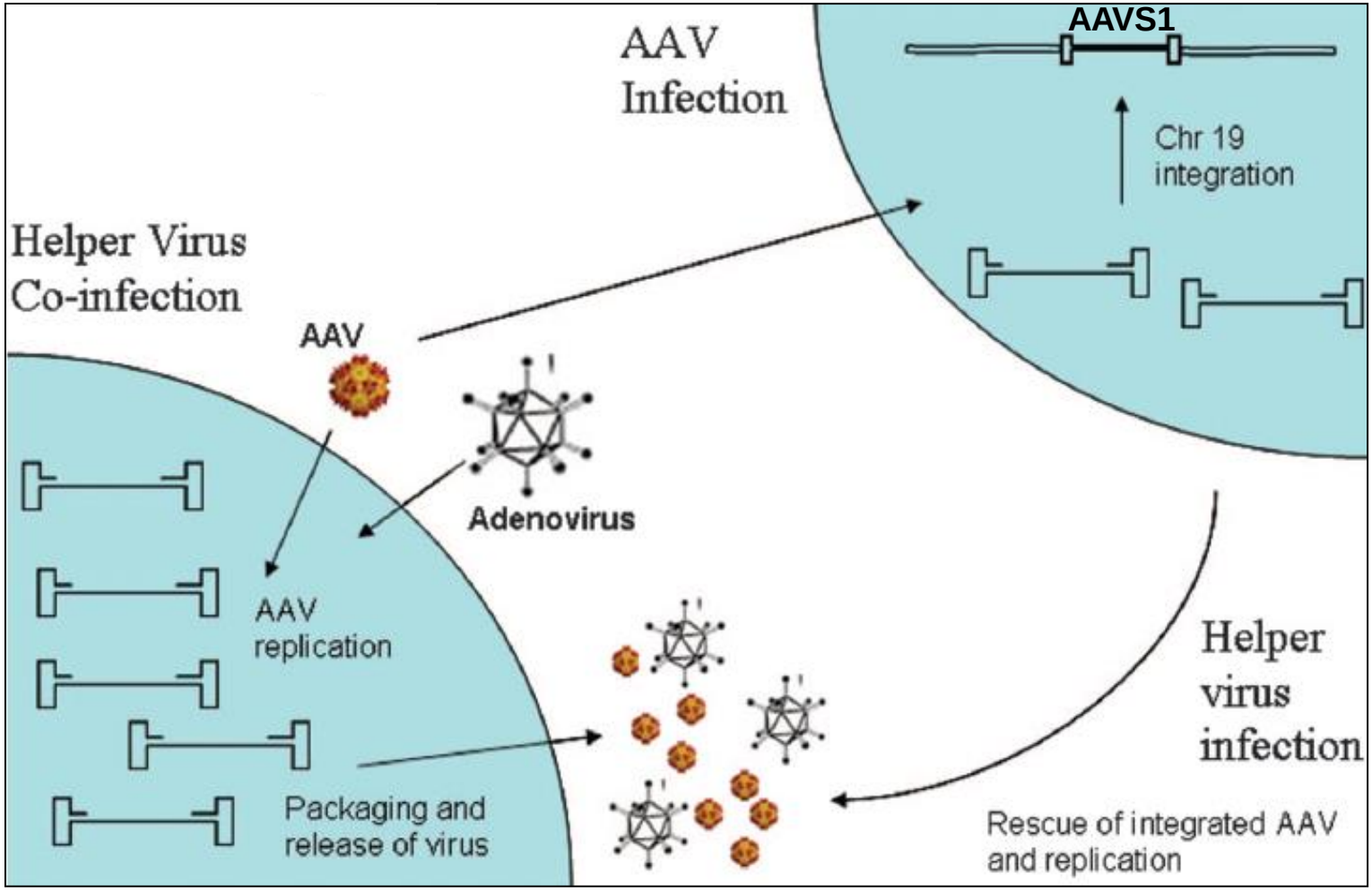


AAV-WT





Multiplication cycle of the AAV

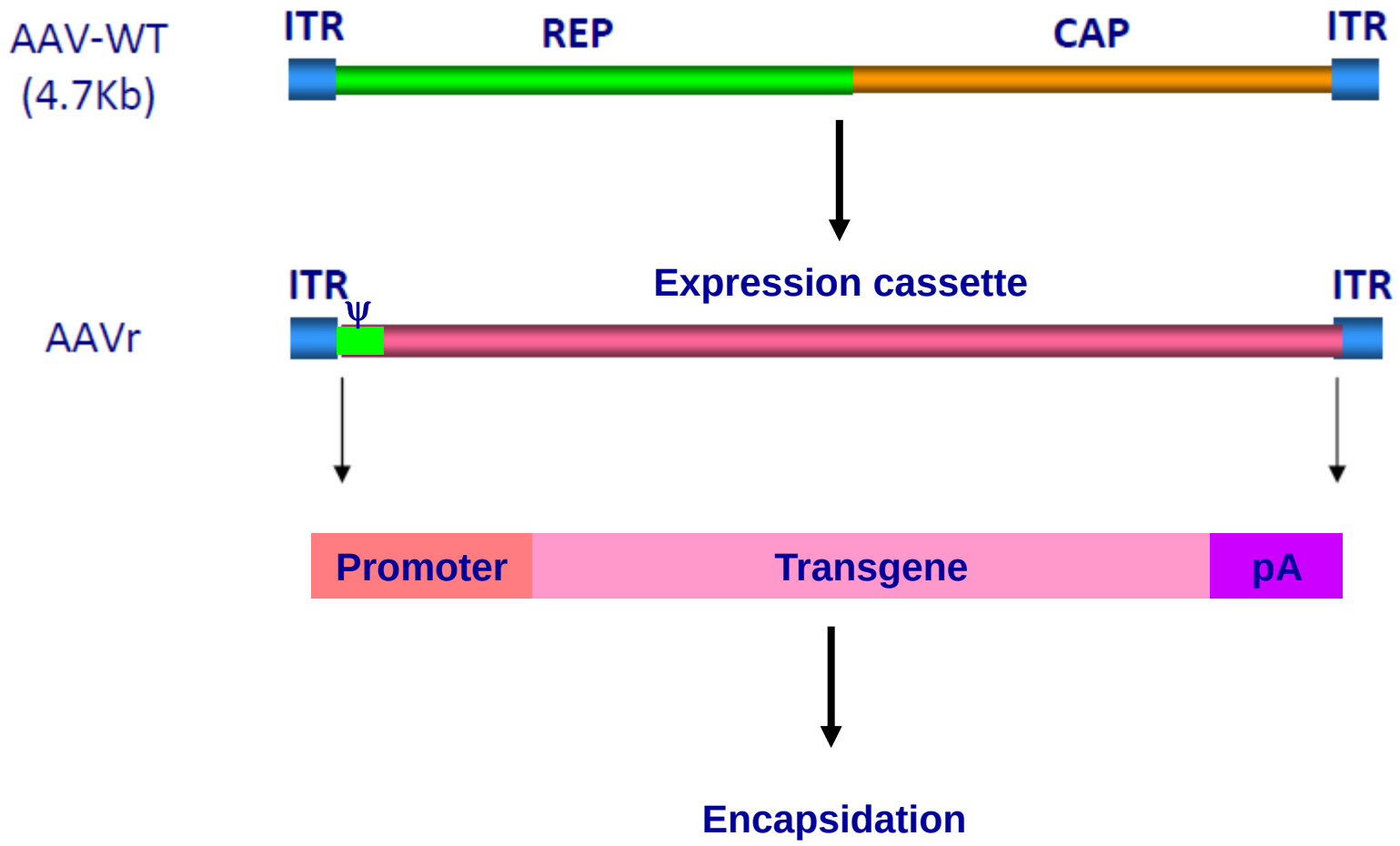


Lysogenic cycle

Lytic cycle

[Daya and Berns, 2008, Clinical Microbiology Reviews]

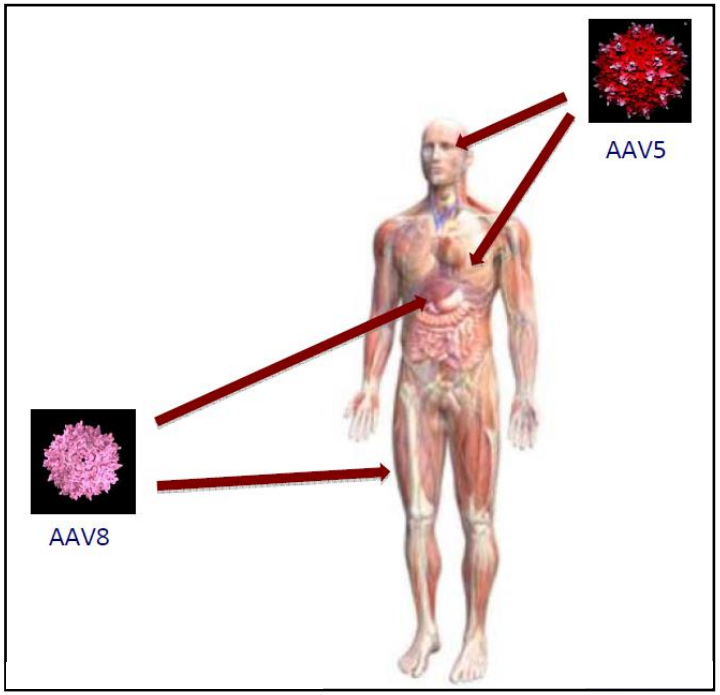
Recombinant AAV



⇨ **Obtaining a non replicative vector carrying the transgene of interest**

Tissue targeting possible with the AAV

- **Serotypes of AAV**
  - **different tropism depending on serotype**



Another example = AAV-2 (widely used) can transduce muscle, brain, retina, liver, and lungs

⇨ Select the specific serotype of the target tissue

AAV receptors/co-receptors and tissue preferences

Serotypes	Main receptors	Co-receptors	Tissue tropism
AAV1	Sialic acid (N-bound)	Unknown	SM,CNS, retina, pancreas
AAV2	HSPG	FGFR1, HGFR, LamR, CD9 tetraspanine	SM, CNS, liver, kidneys
AAV3	HSPG	FGFR1, HGFR, LamR	Hepatocarcinoma, SM
AAV4	Sialic acid (O-bound)	Unknown	CNS, retina
AAV5	Sialic acid (N-bound)	PDGFR	SM, CNS, lungs, retina
AAV6	Sialic acid (N-bound), HSPG	EGFR	SM, SM (IV), heart, lungs
AAV7	Unknown	Unknown	SM, retina, CNS
AAV8	Unknown	LamR	Liver, SM, CNS, retina, pancreas, heart
AAV9	Galactose (N-bound)	LamR	Liver, heart (IV), brain (IV), SM (IV), lungs, pancreas, kidneys (IV)

CNS: Central Nervous System ; **EGFR**: Epidermal Growth Factor Receptor ; **FGFR1**: Fibroblast Growth Factor Receptor 1 ; **HGFR**: Hepatocyte Growth Factor Receptor ; **HSPG**: Heparan Sulfate Proteoglycan ; **IV**: Intravenous ; **PDGFR**: Platelet-Derived Growth Factor Receptor ; **SM**: skeletal muscle

[Millet et al., 2013, Virologie]

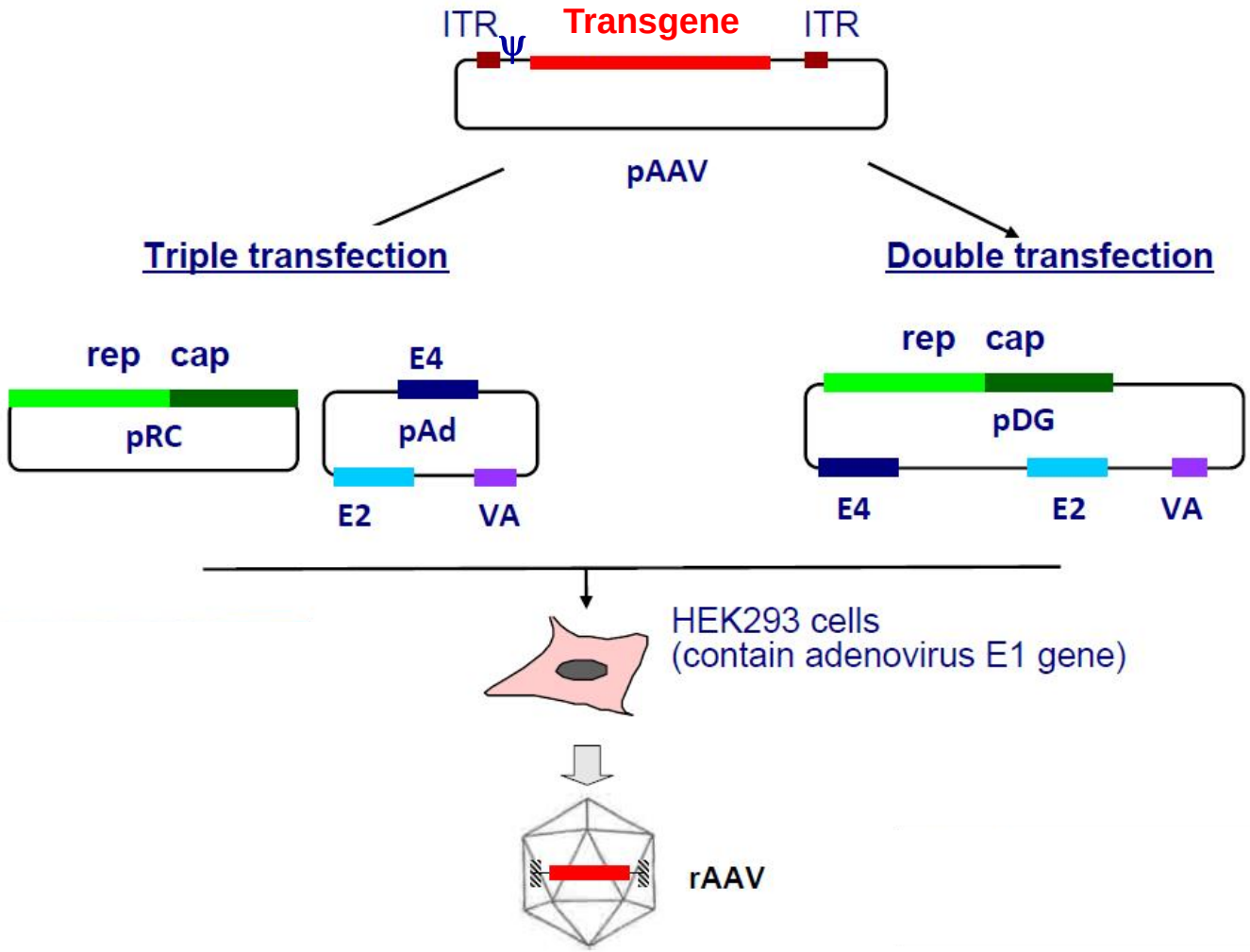
Production of recombinant AAV (1)

☞ **Different methods of production**

- **Classical method of transfection** ✓
- **Stable encapsidation and production cell lines**
- **Herpes Simplex Virus RepCap system** ✓
- **Baculovirus / insect cells system**

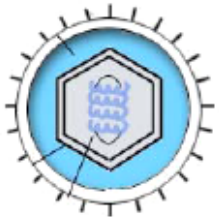
Production of recombinant AAV (2)

☞ Transfection



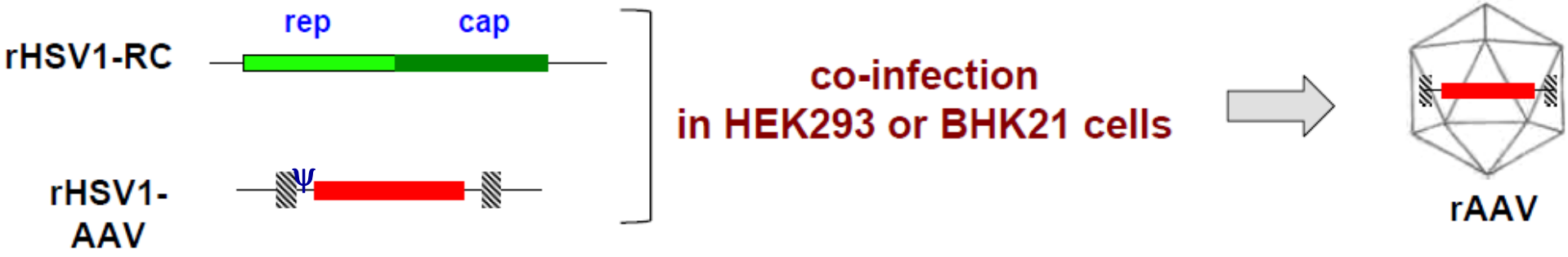
Production of recombinant AAV (3)

☞ **Use of a helper Herpes Simplex virus**



Herpes virus used for rAAV production:

Replication deficient HSV-1 (e.g. ΔICP27), produced on trans-complementing cells (expressing ICP27)



Advantages of AAV vectors

- Non-pathogenic AAV in an immunocompetent person
- Replication dependent on a helper virus
  - adenovirus or herpesvirus
- Potential infection of mitotic and non mitotic cells
- Persistence of the viral genome
  - * possible integration specifically into the AAVS1 site of chromosome 19
    - only if the *REP* gene is maintained
  - * no risk of insertional mutagenesis

Limitations of AAV vectors

- Small insert
  - maximum 4.5 kb
- Low production of viruses
- Low level of transgene expression
- Risk of contamination of the production
  - if using a helper virus

Summary of the different viral vectors (1)

Vector	Maximum size of the transgene	Transduction of quiescent cells	Transgene stability (term of expression)	Immune response	Risks	Cell targeting
Retrovirus	8 kb	No	Long	No	Integration Mutagenesis	Large
Lentivirus	8 kb	Yes	Long	No	Integration Mutagenesis	Large
Adenovirus	7.5-35 kb	Yes	Transient	Yes	Inflammatory response	Large
AAV	4.5 kb	Yes	Long / Transient	No	-	Specific according to serotype

Summary of the different viral vectors (2)

Feature	Adenoviral vector	Helper-dependent adenoviral vector	AAV vector	Retroviral vector	Lentiviral vector
Particle size (nm)	70–100	70–100	20–25	100	100
Cloning capacity (kb)	8–10	~30	4.9 (10 after heterodimerization of two AAV virions)	8	9
Chromosomal integration	No	No	No (yes if *rep* gene is included)	Yes	Yes
Vector yield (transducing units/ml)	High (10^{12})	High (10^{12})	High (10^{12})	Moderate (10^{10})	Moderate (10^{10})
Entry mechanism	Receptor (CAR)-mediated endocytosis, endosomal escape and microtubule transport to the nucleus		Receptor-mediated endocytosis, endosomal escape and transport to the nucleus	Receptor binding, conformational change of Env, membrane fusion, internalization, uncoating, nuclear entry of reverse-transcribed DNA	
Transgene expression and practical application	Weeks to months; highly efficient short-term expression (e.g. for cancer or in acute cardiovascular diseases)	>1 year; highly efficient medium- to long-term expression	>1 year; medium- to long-term gene expression for non-acute diseases (onset of transgene expression after ~3 weeks)	Long-term correction of genetic defects	
Oncolytic potential?	Yes	No	No	No (but has potential to spread through the tumour without lysis, thereby spreading a suicide gene that encodes a pro-drug-converting enzyme)	
Emergence of replication-competent vector *in vivo*?	Possible but not a major concern	Negligible, low risk	Possible but not a major concern	Risk is a concern	Risk is a concern
Infects quiescent cells?	Yes	Yes	Yes	No	Yes
Transcriptional targeting affected by chromosomal integration site?	No	No	No	Yes	Yes
Risk of oncogene activation by the vector?	No	No	No	Yes	Yes

AAV, adeno-associated virus; CAR, coxsackie and adenovirus receptor; Env, viral envelope protein.

[Waehler et al., 2007, Nature Reviews Genetics]