

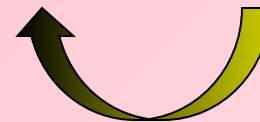
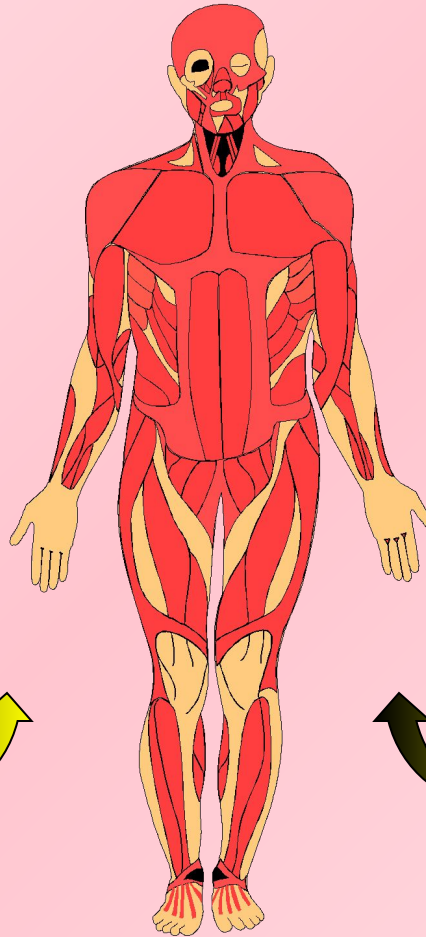
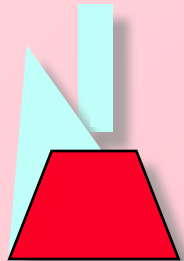


**BIOL 670 /**  
**775**  
***Viral VECTORS***

# Gene Delivery

- *Ex vivo*

Transplantation  
of recombinant  
cells with virus



- *In vivo*

Direct  
administration  
of virus

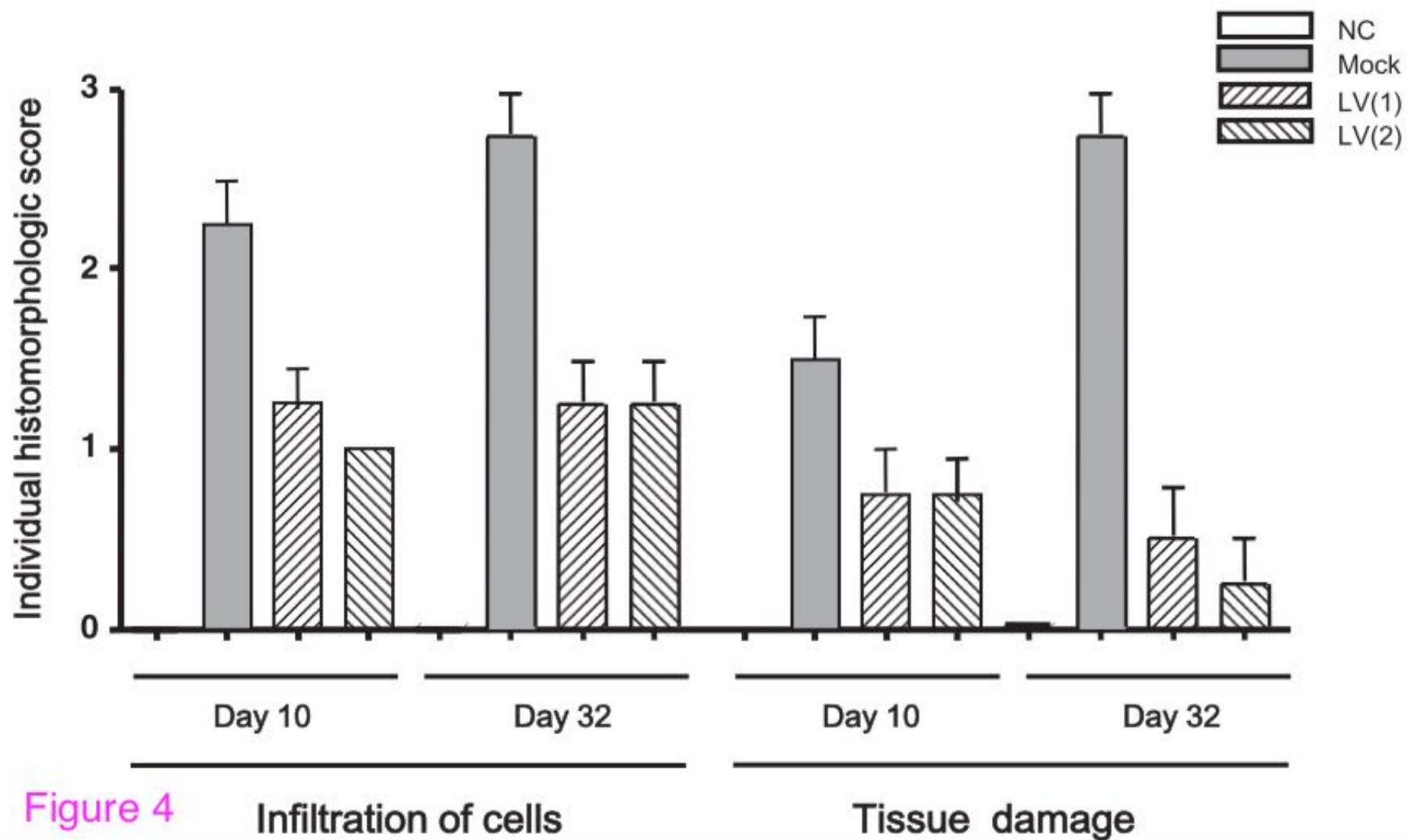


# “*New*” Gene Therapy

**Mucosal gene therapy using a pseudotyped lentivirus vector encoding murine interleukin-10 (mIL-10) suppresses the development and relapse of experimental murine colitis**

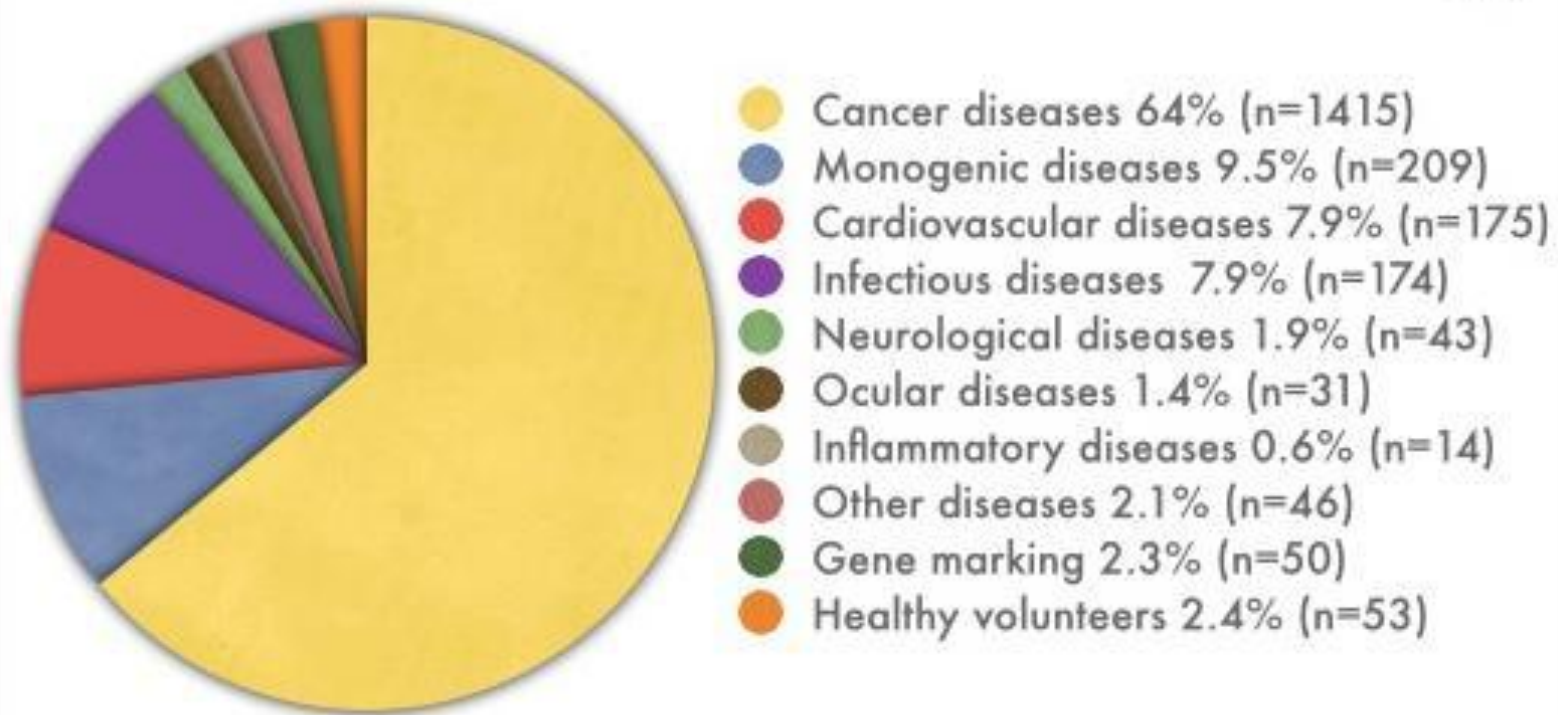
H. Matsumoto et al. (2014)

*BMC Gastroenterology* 14:68

**c****Figure 4****Infiltration of cells****Tissue damage**

# Gene Therapy Trials

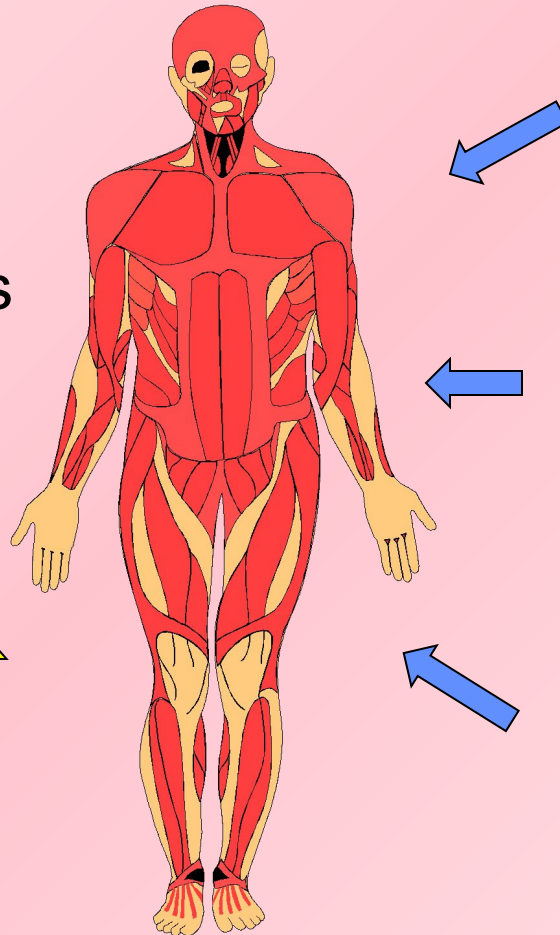
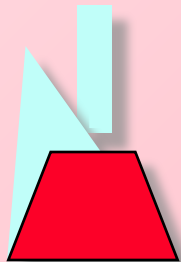
Indications Addressed by Gene Therapy Clinical Trials



# Gene Therapy

- ***Ex vivo***

Transplantation  
of transduced cells



*myoblasts*

*hepatocytes*

*blood*

*bone marrow*

*fibroblasts*

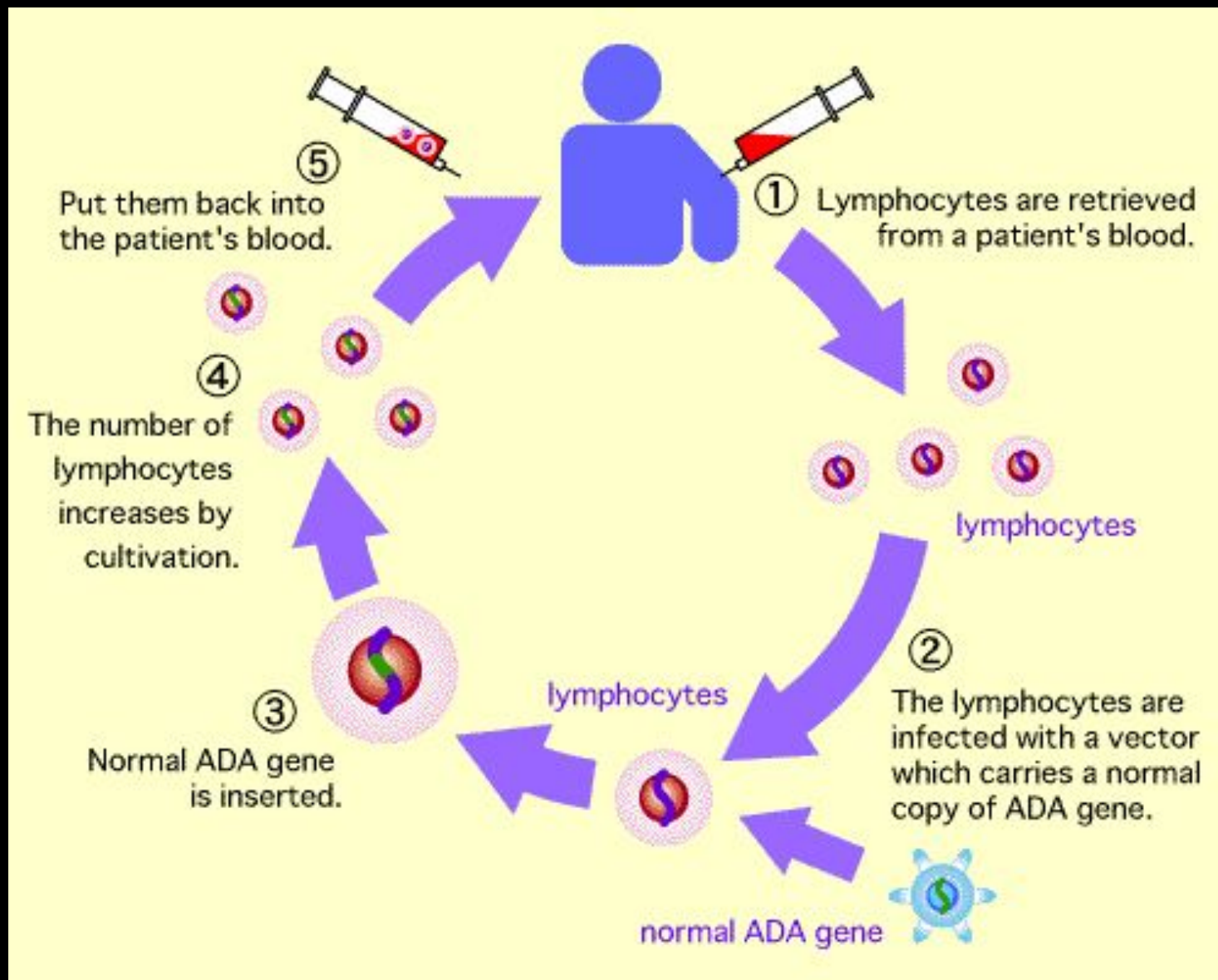
*skin*

*stem cells*



1990

# Ex vivo



# Cells? Which cells?



- Focus on the patient!
- Then focus on the disease (cells, tissues...)



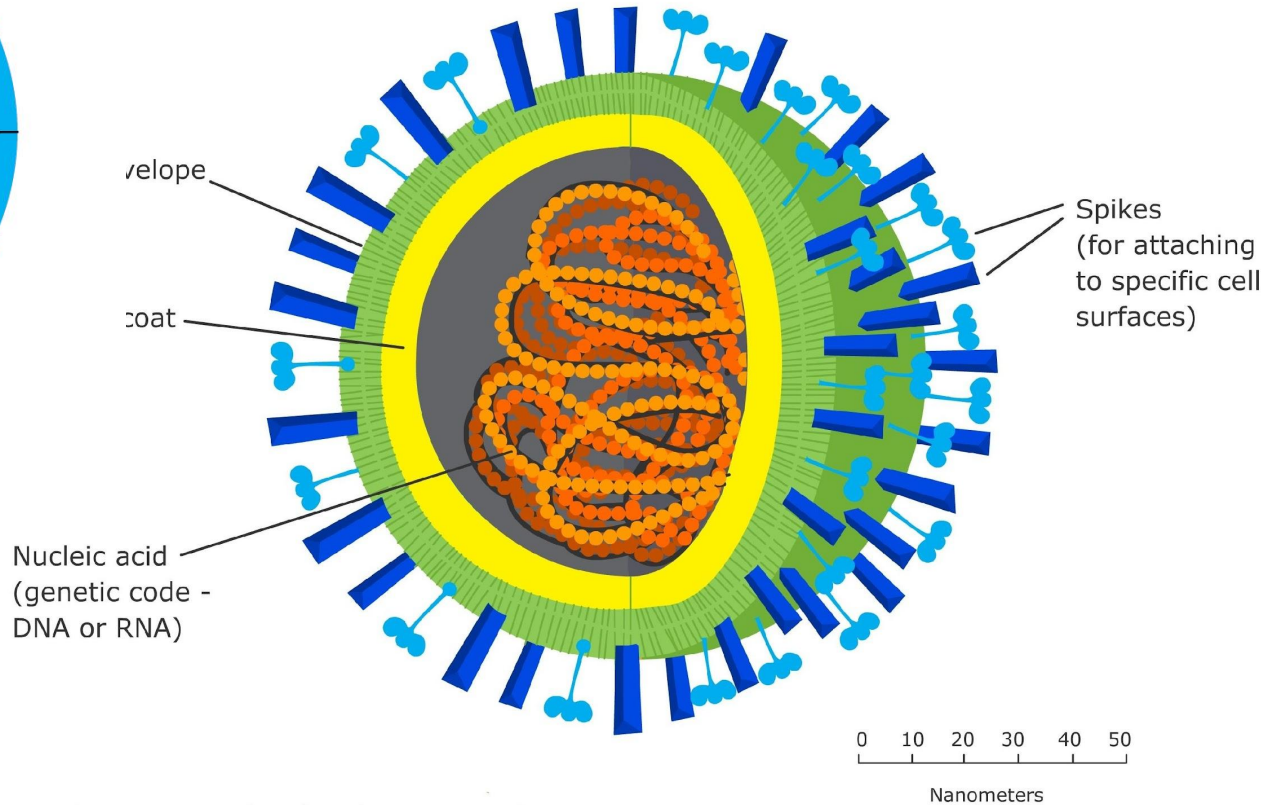
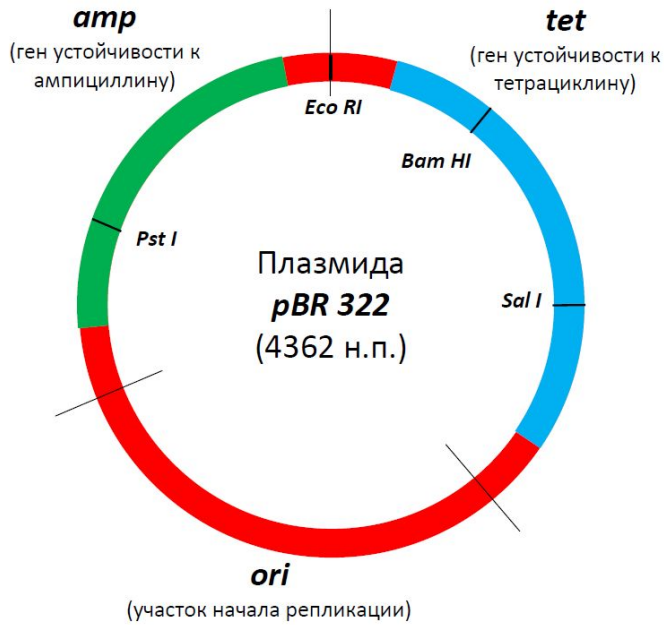
# Ex vivo Gene Therapy

## Lentiviral Hematopoietic Stem Cell Gene Therapy Benefits Metachromatic Leukodystrophy

*Alessandra Biffi et al. Luigi Naldini's laboratory (Italy); Science 2013*

Metachromatic leukodystrophy (MLD) is a neurodegenerative lysosomal storage disease caused by arylsulfatase A (ARSA) deficiency. The disease primarily affects children and invariably leads to premature death. In previous work with a mouse model of MLD, we used a lentiviral vector (LV) to introduce a functional ARSA gene into hematopoietic stem cells (HSCs) ex vivo and showed that reinfusion of the engineered HSCs prevented and corrected disease manifestations in the animals. To determine whether this gene therapy strategy is safe and can offer therapeutic benefit to patients with early-onset MLD, we designed a phase I/II trial. There was high-level stable engraftment of the transduced HSCs in the bone marrow and peripheral blood of all patients. Findings were associated with a clear therapeutic benefit.

# Which vector to use? (rocket)



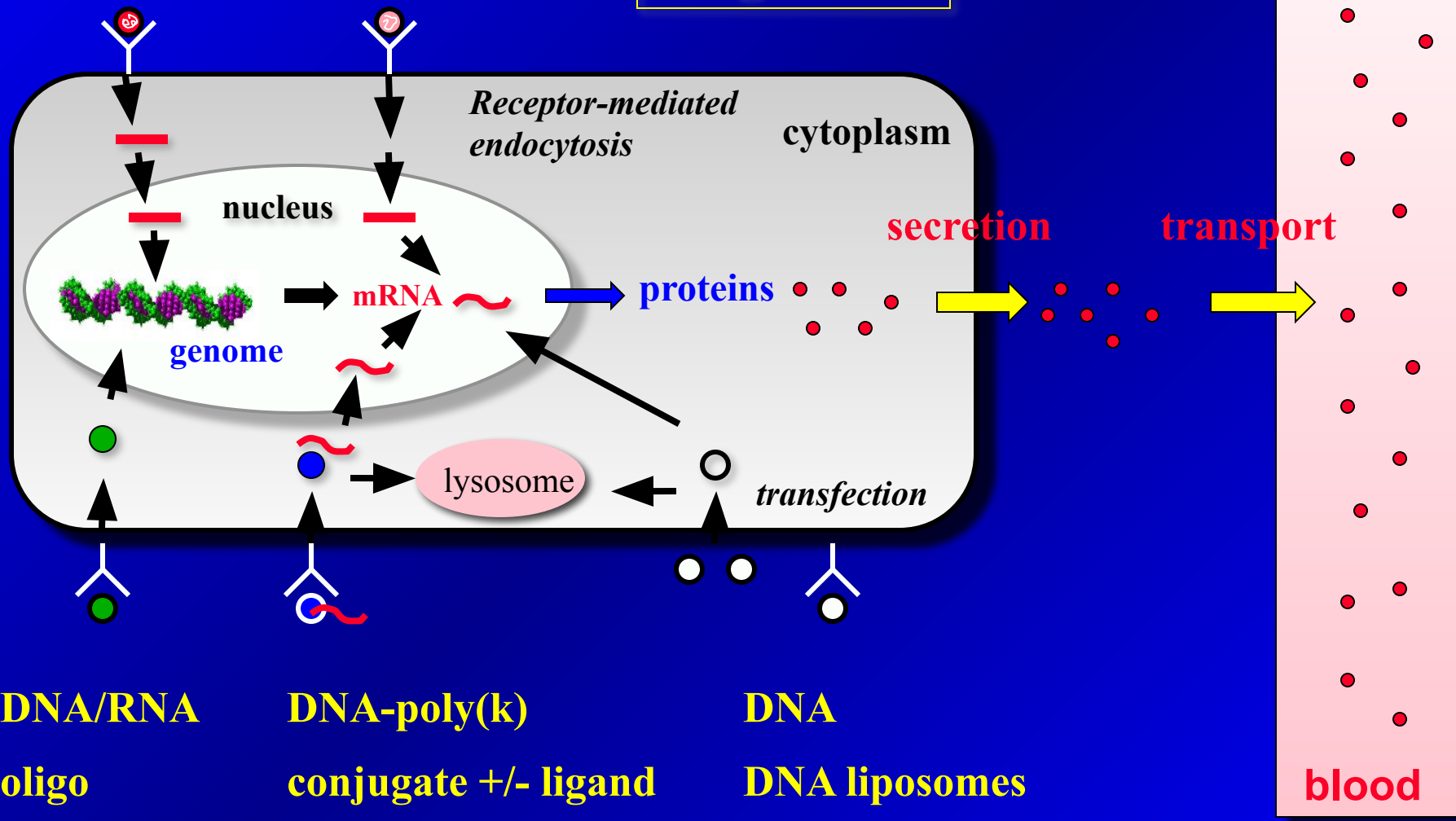
**Retrovirus**

**Adenovirus**

**AAV**

**Herpes**

**Target cell**



**DNA/RNA**

**DNA-poly(k)**

**DNA**

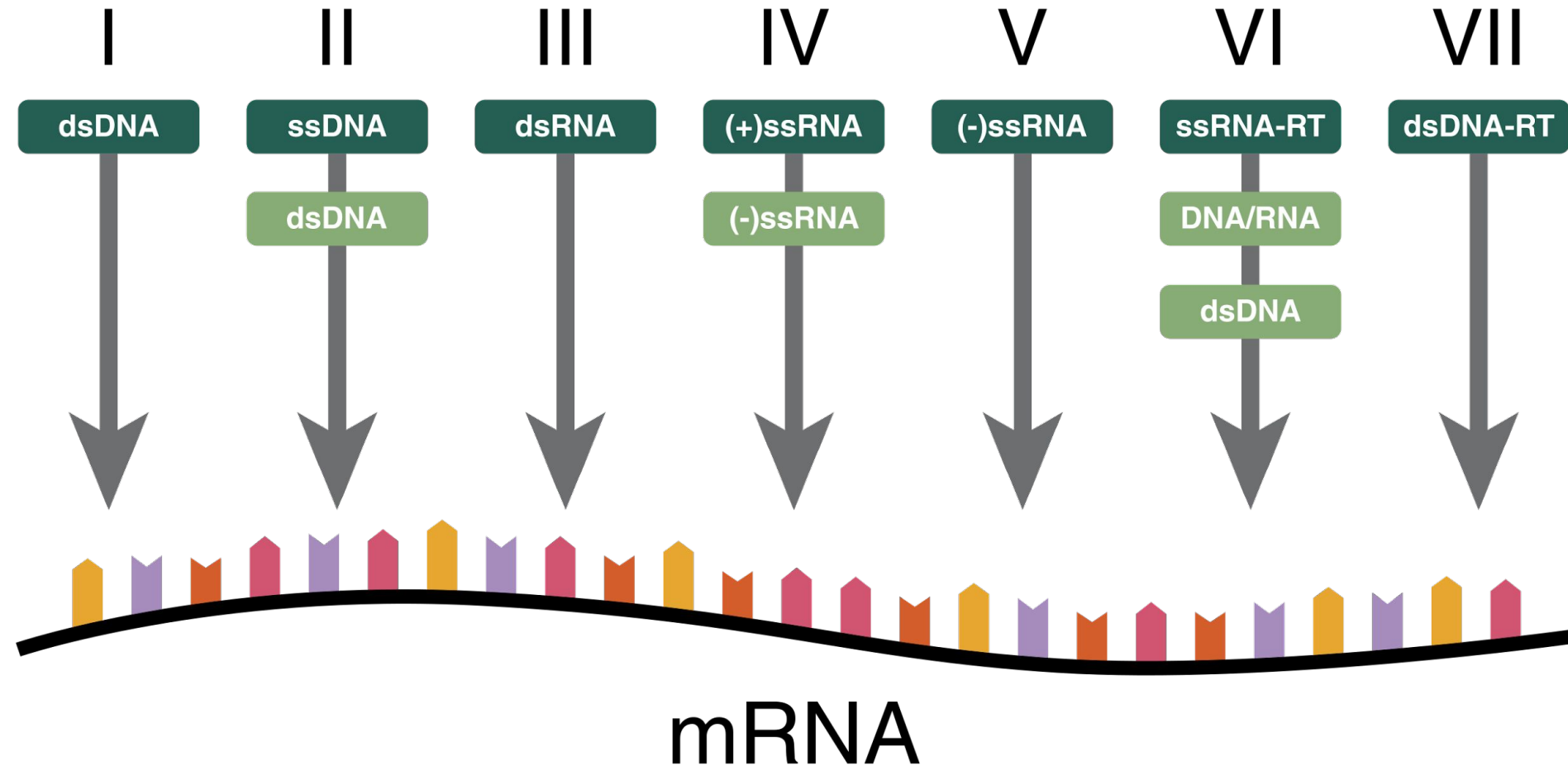
**oligo**

**conjugate +/- ligand**

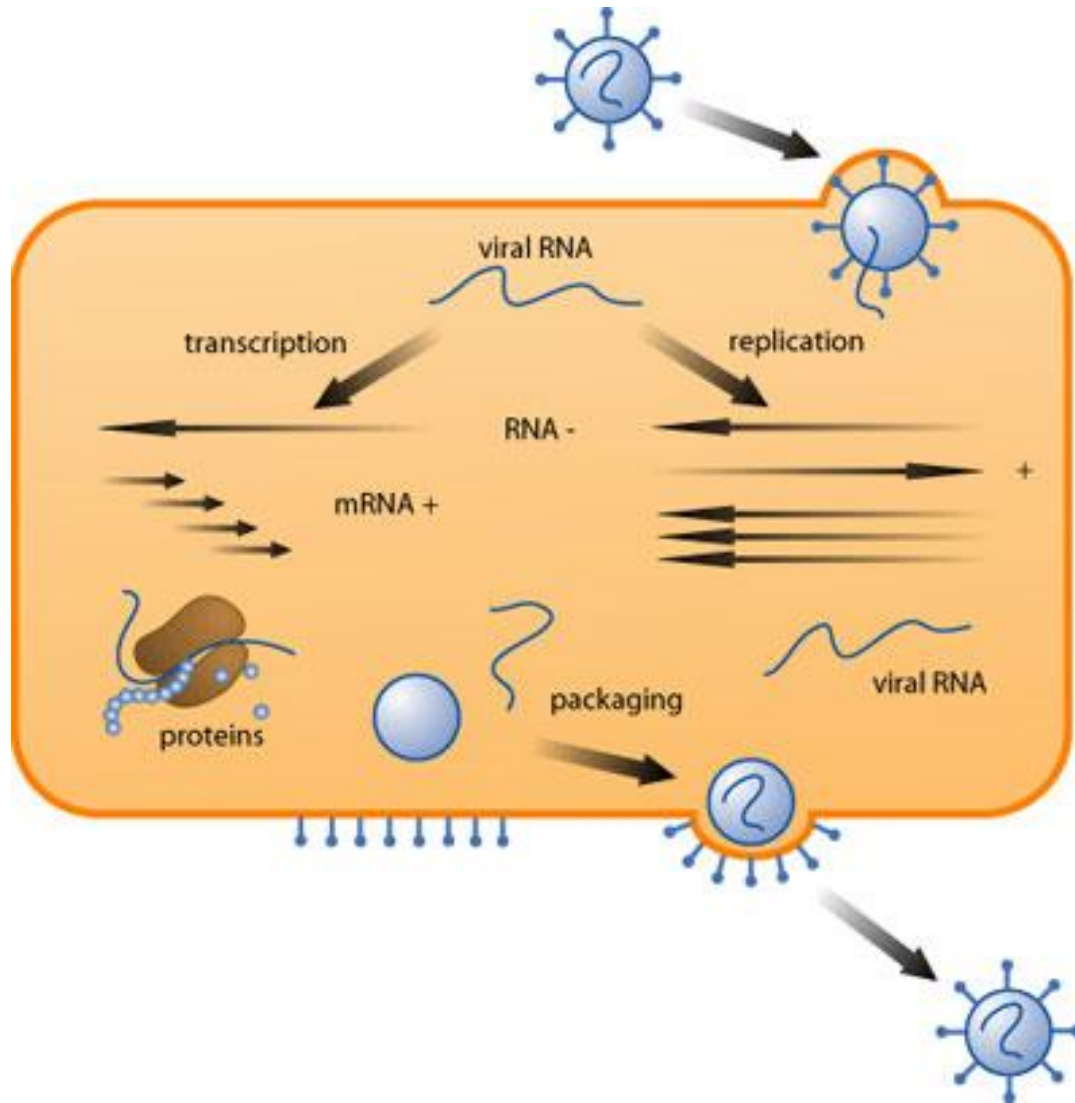
**DNA liposomes**

**blood**

# Virus Classification (classes)

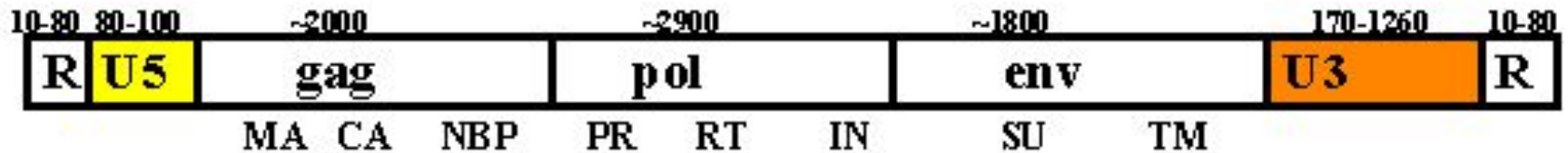


# Viral Replication



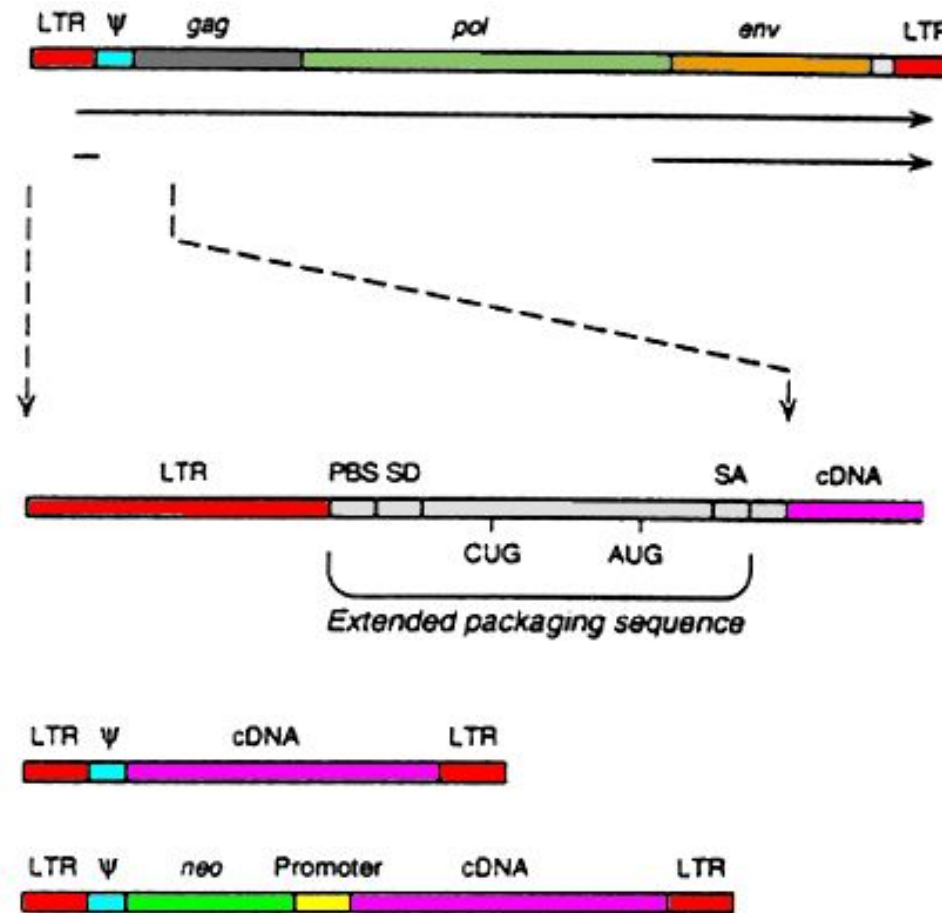


# Viral Proteins

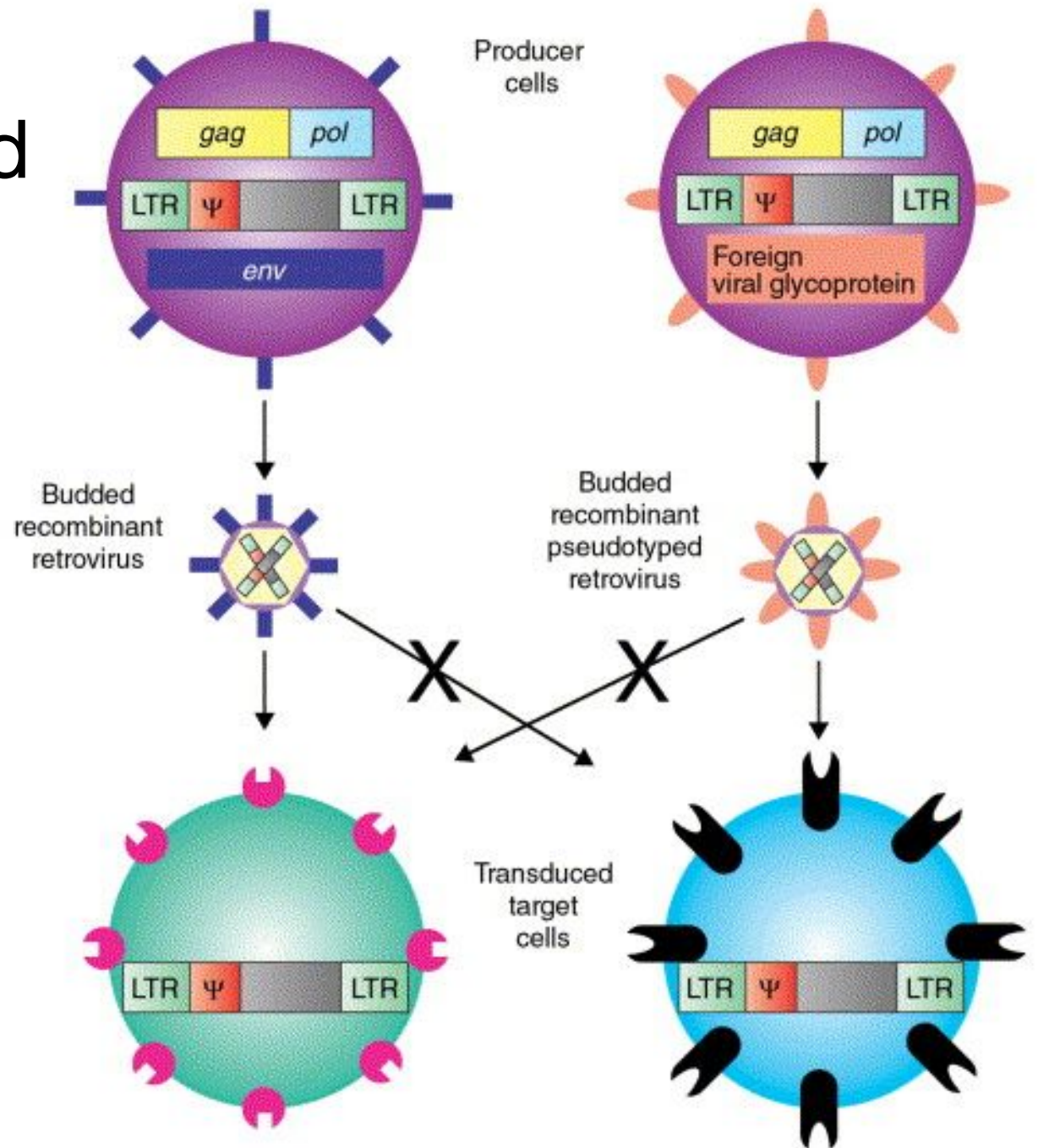


- MA Matrix
- CA Capsid
- NBP Nuclear Binding Protein
- PR Protease
- RT Reverse Transcriptase
- IN Integrase
- SU Surface protein
- TM Transmembrane Protein

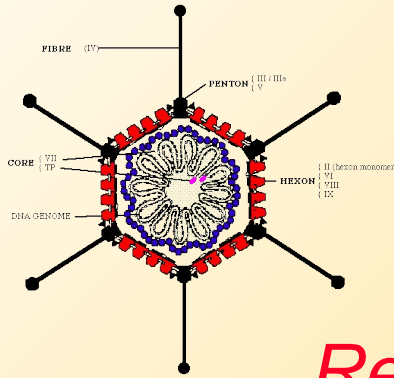
# $\Psi$ is essential for viral replication



# Pseudotyped virus



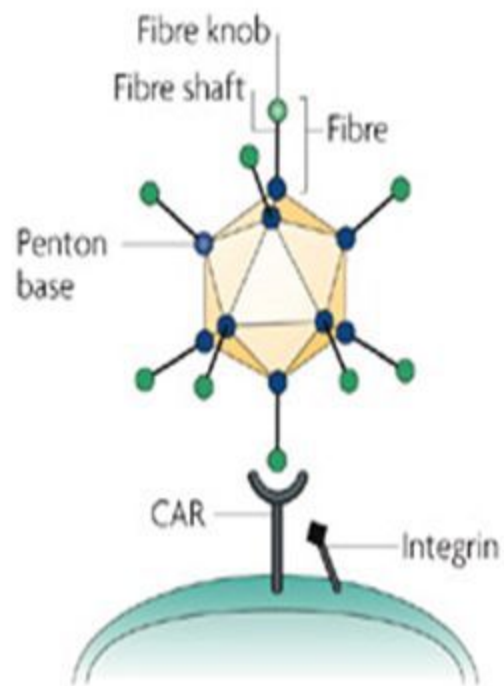
# Viral vectors



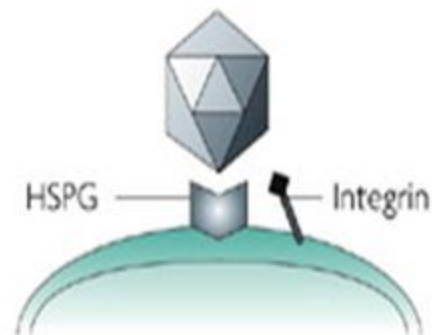
*Retrov. / Adenov. / AAV / Lentiv. / Herpes*

<i>Genome</i>	<i>RNAs</i>	<i>DNA</i>	<i>dDNAs</i>	<i>RNAs</i>	<i>DNA</i>	<i>dAd</i>
<i>Size</i>	9	30	4.7	9	150	
<i>Capacity</i>	>7	8-30	4	>7	130	
<i>Target cells</i>	<i>Div</i>	<i>Div/no</i>	<i>Div/no</i>	<i>Div/no</i>	<i>Div/no</i>	<i>Div/no</i>
<i>Integration</i>	<i>yes</i>	<i>no</i>	<i>yes/no</i>	<i>yes</i>	<i>no</i>	<i>no</i>

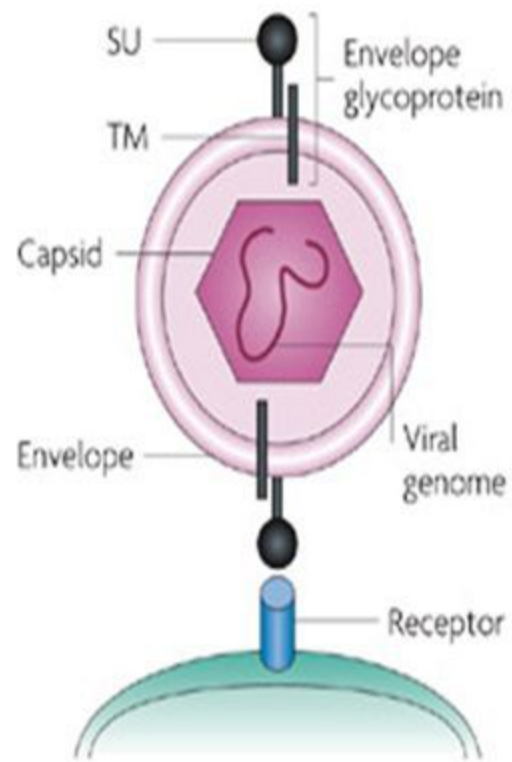
**a Adenovirus 5**



**b AAV2**



**c Retrovirus (lentivirus)**

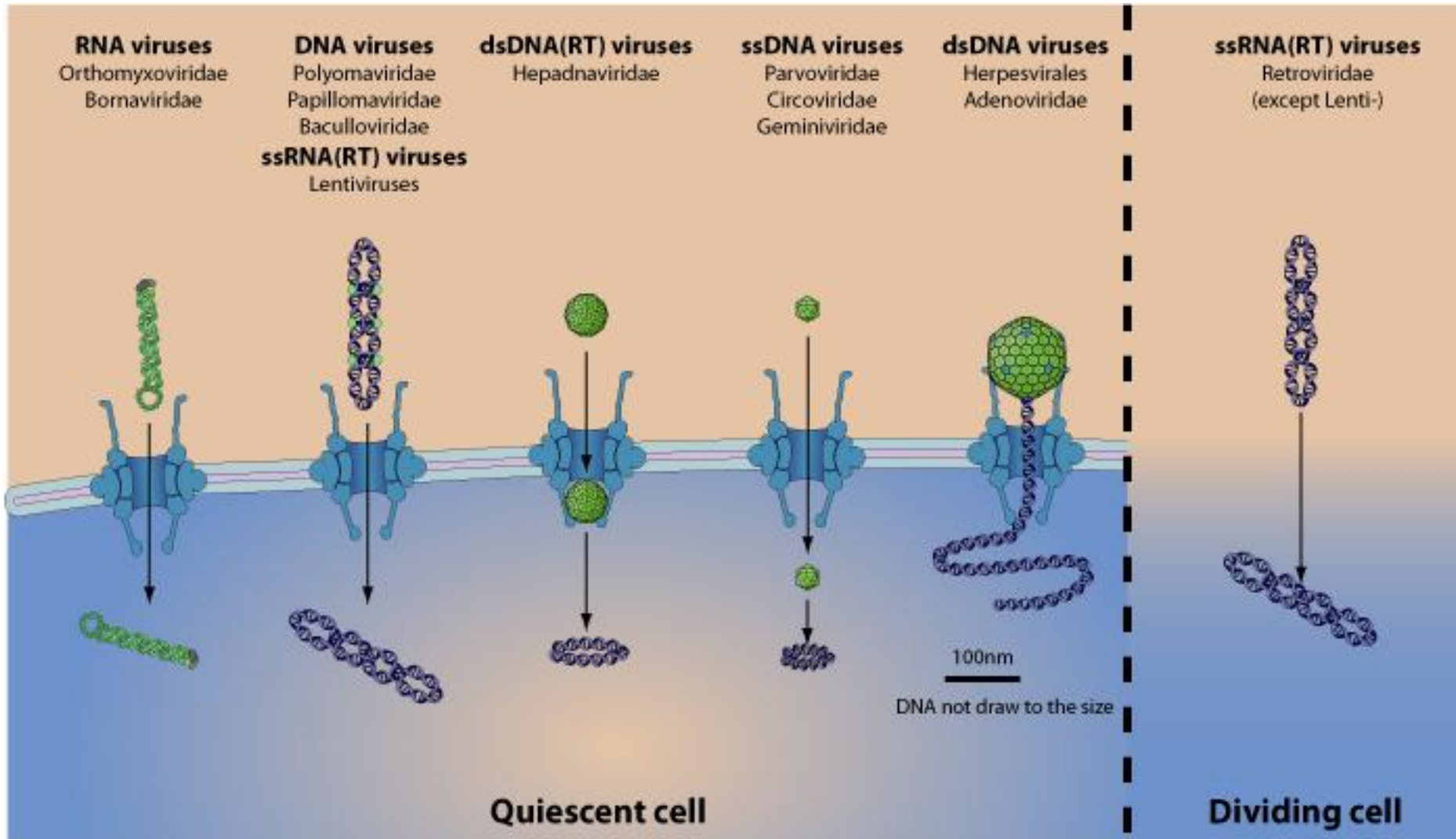


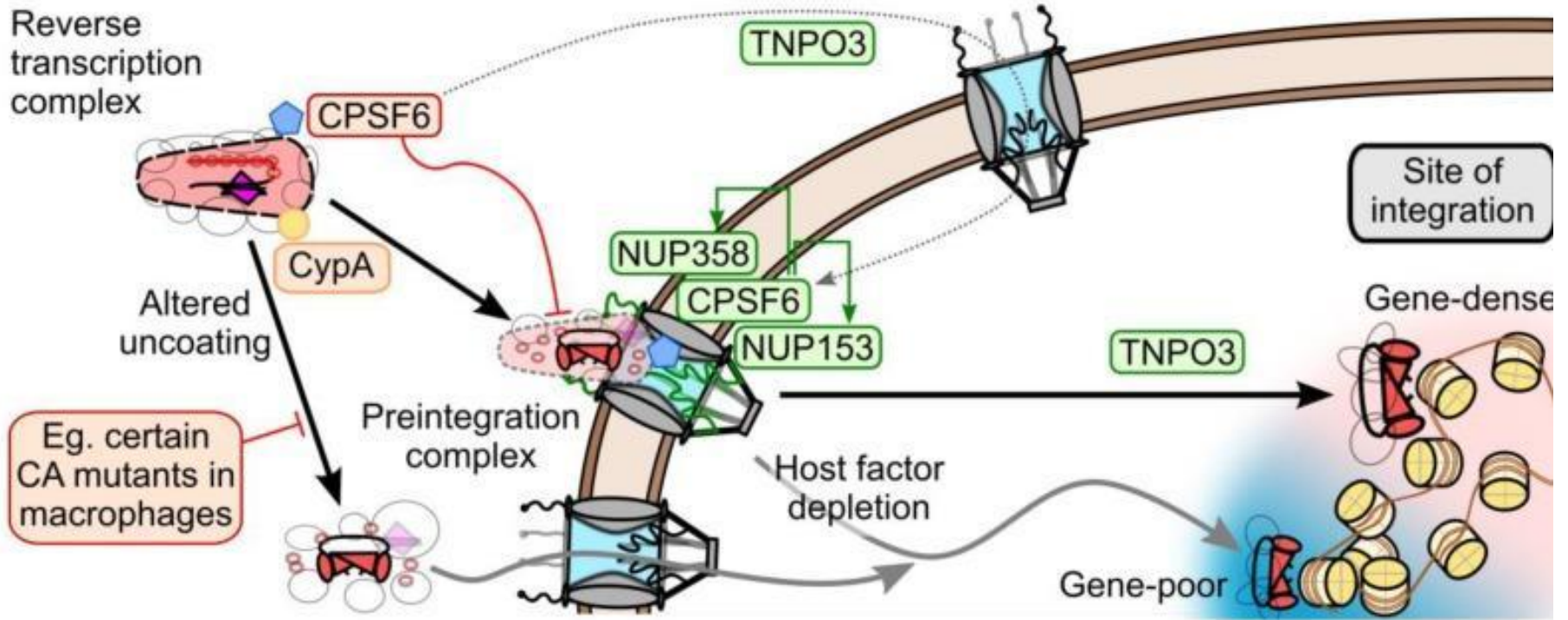
Nature Reviews | **Genetics**

# VIRAL VECTORS



# Viral Entry into Nucleus





# RELEVANT QUESTIONS WHEN CHOSING A VECTOR

- **What disease am I going to target?**
- **How long do I need to express the transgene for?  
Is it likely that re-administrations are required?**
- **Which cells do I want to target?**
- **What medical conditionings do patients have?**
- **Choice of promoter? Viral? Mammalian?**
- **Is regulation of expression required?**
- **Vector tropism?**

# **IDEAL VECTOR CANDIDATE**

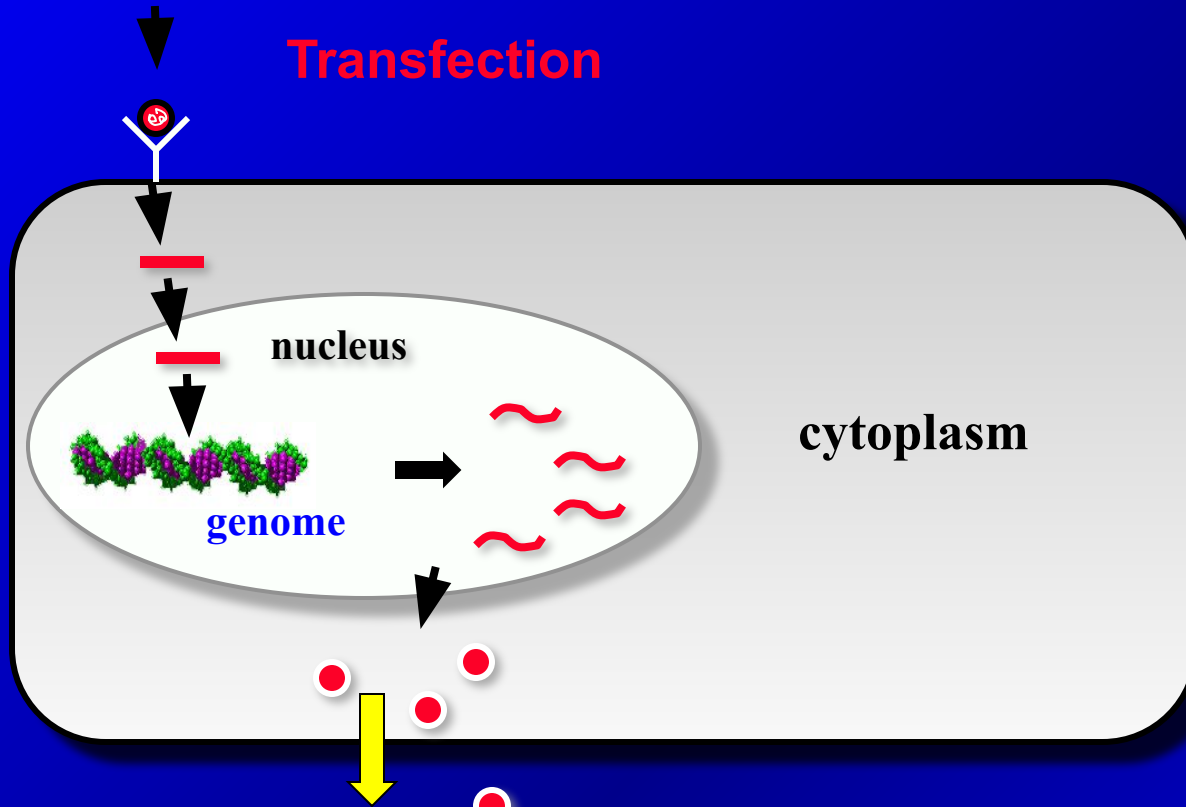
## **(does not yet exist)**

- **High titer or concentrations ( $>10^8$  particles/ml)**
- **Method of production is convenient and reproducible**
- **Precise introduction of the transgene**
- **The transgene is responsive to its regulatory elements**
- **Ability to target specific cells (pseudotyped)**
- **Does not elicit host immune response**
- **Persistence as required**

**DNA of interest**

# **Production of Viral particles**

**Transfection**



***Packaging cell***

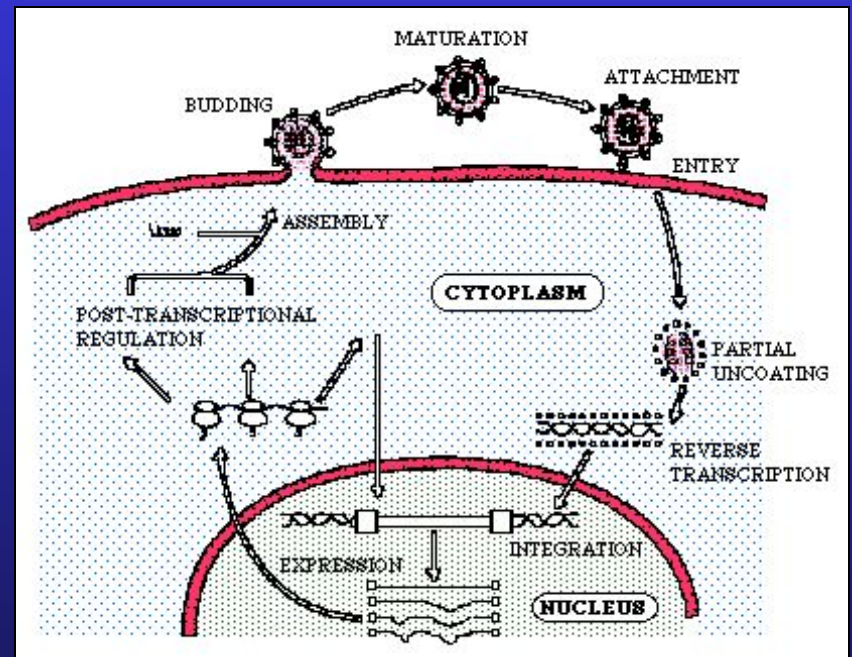
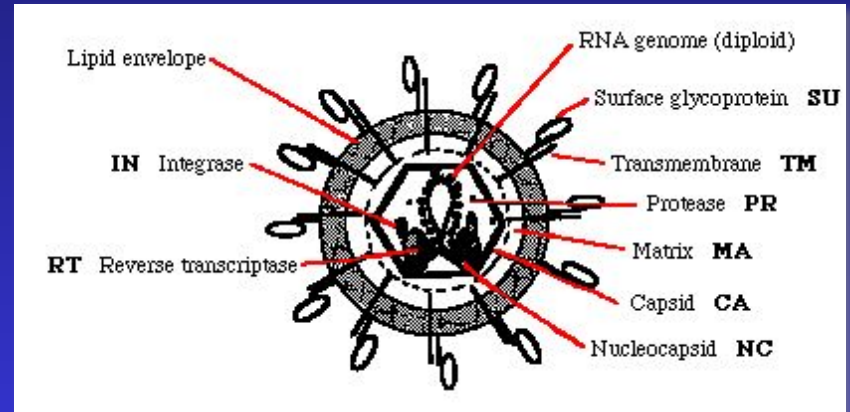
**Viral particles**

- Size limitation
- Viral titer
- Replication ability

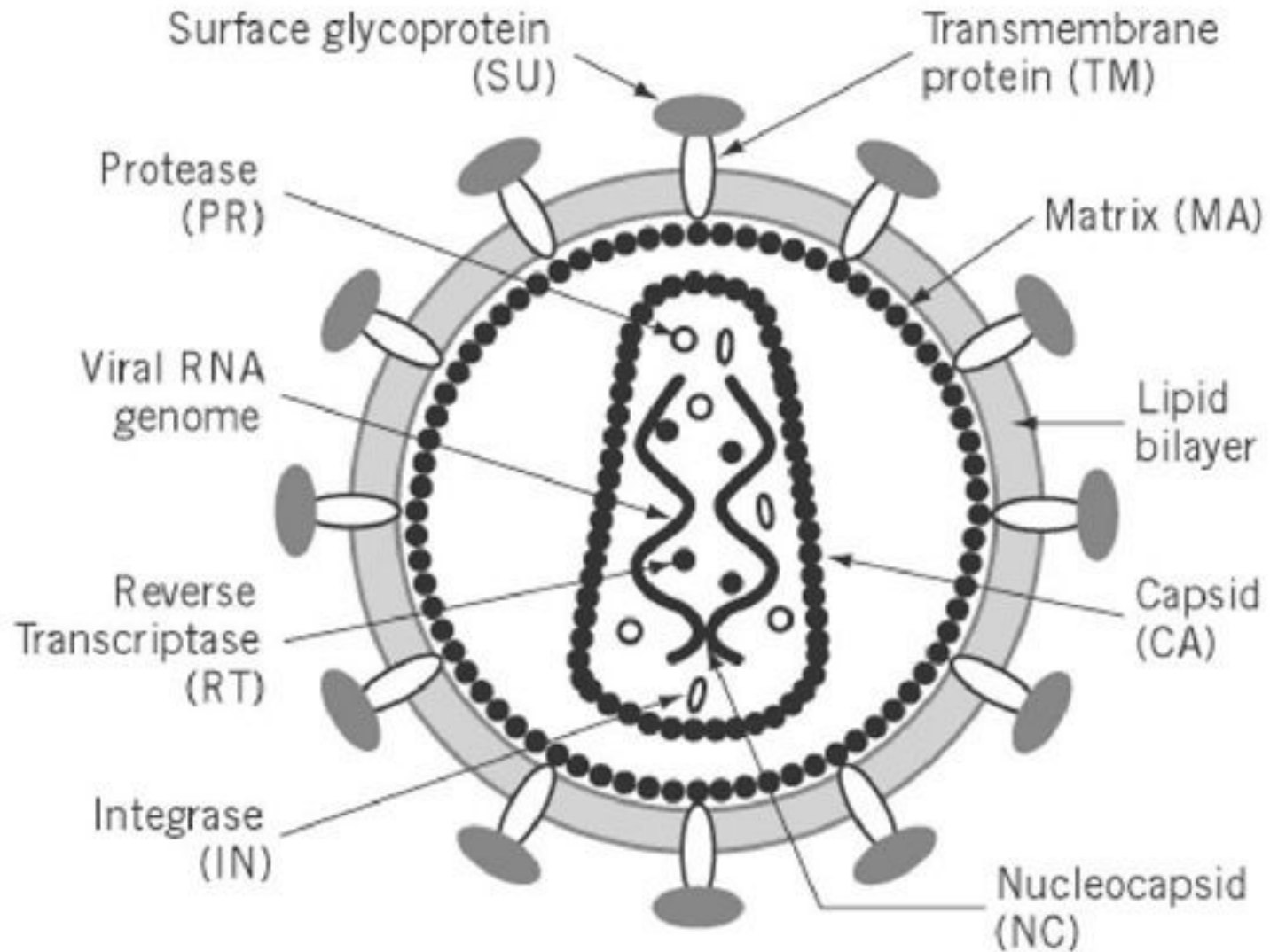


# RETROVIRUS

- Single stranded RNA molecule
- Only infects dividing cells
- eco, amphotrophic
- Mouse: cationic amino acid transporter
- Integrates into host genome
- Pseudotyped

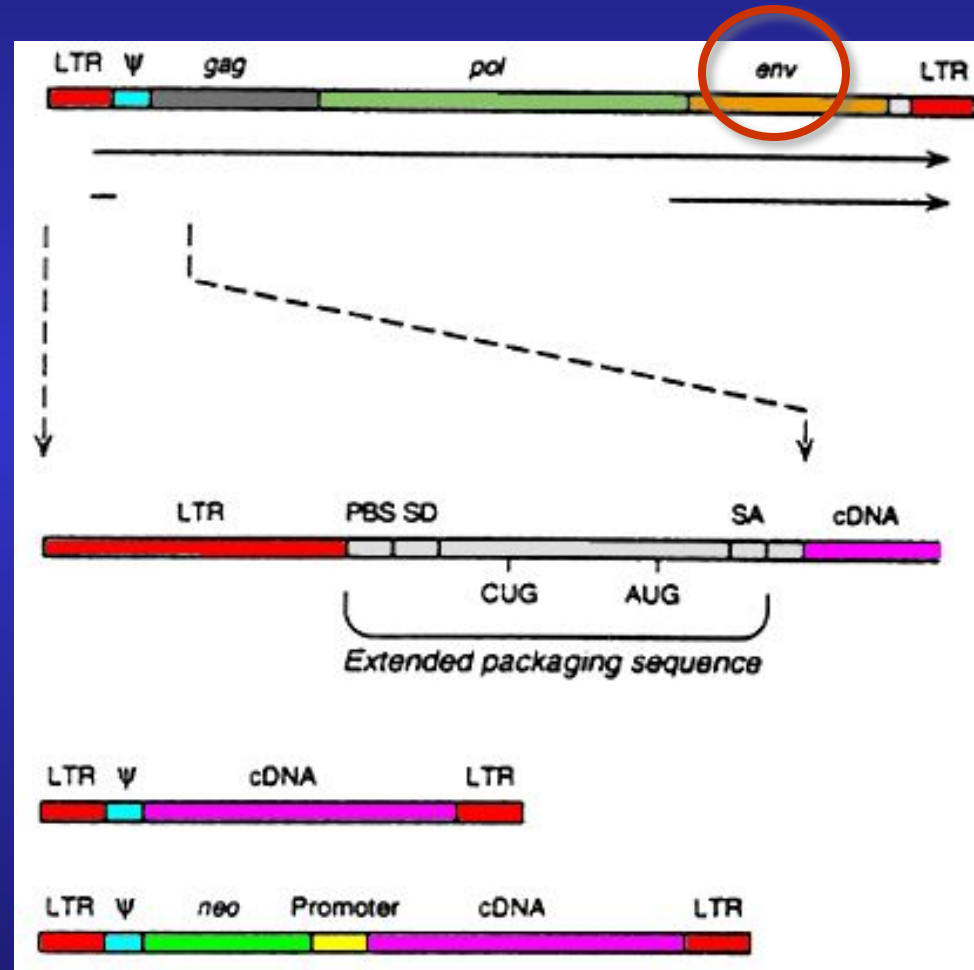


# RETROVIRUS



# RETROVIRUS

- Single stranded RNA molecule
- Long terminal repeats LTR with promoter/enhancer sequences
- Long-term persistence of DNA

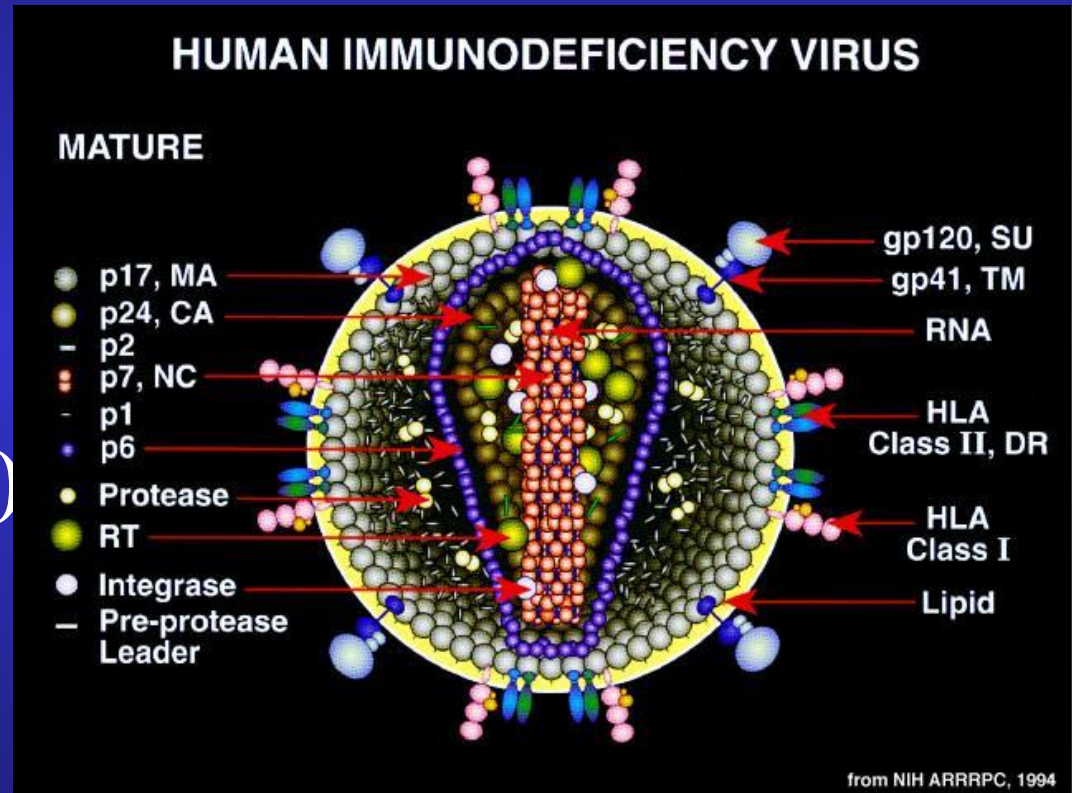


# LIMITATIONS OF RETROVIRUS

- **Retroviruses are inactivated by human sera**
- **Transgene expression from LTR is often inactivated**
- **Potential insertional mutagenesis**
- **Oncogene activation**

# LENTIVIRUS

- Based on HIV genome
- Infect dividing / non-dividing cells
- CD4/CCR5 receptor (co-receptor)
- Integrates into host genome
- Sustained transgene expression



# ADVANTAGES OF LENTIVIRUS

- Targeting of stem cells
- Gene expression is sustained, and often sustained through cellular differentiation
- Promising in preclinical studies:
  - Hematopoietic cells
  - inhibition of genes (interference)



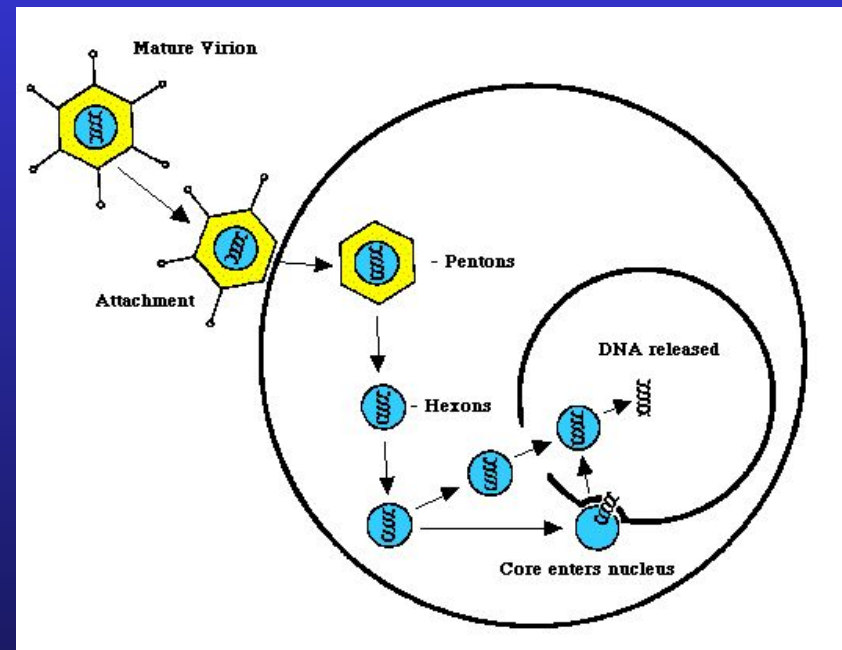
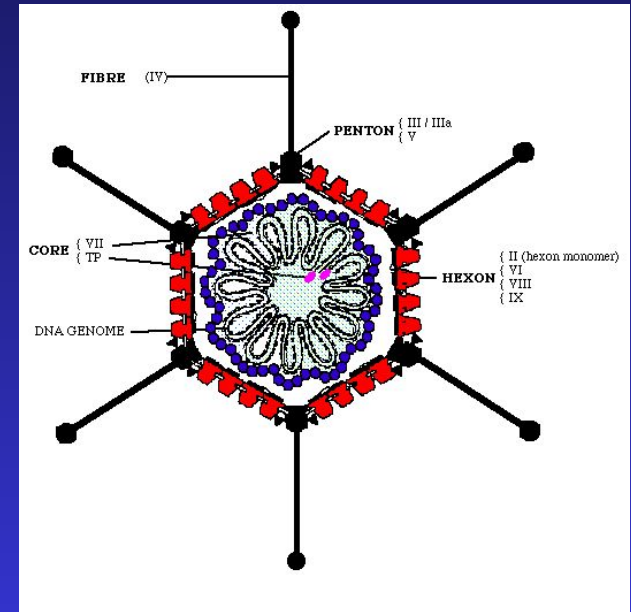
# LIMITATIONS OF LENTIVIRUS

- Gene expression is often not as high as with adenovirus
- Same as retrovirus (except it can target non-dividing cells)
- Potential use in gene therapy provided safety is proven

# ADENOVIRUS

There are at least 10 proteins in the Adenovirus capsid

- Double stranded DNA molecule
- Infects dividing and non-dividing cells
- Human CD46 receptor
- Does not integrate into host genome (episomal)
- Very high titer



- Large capacity as a vector
- Very broad cell tropism
- Infects dividing / non-dividing cells
- Very high expression

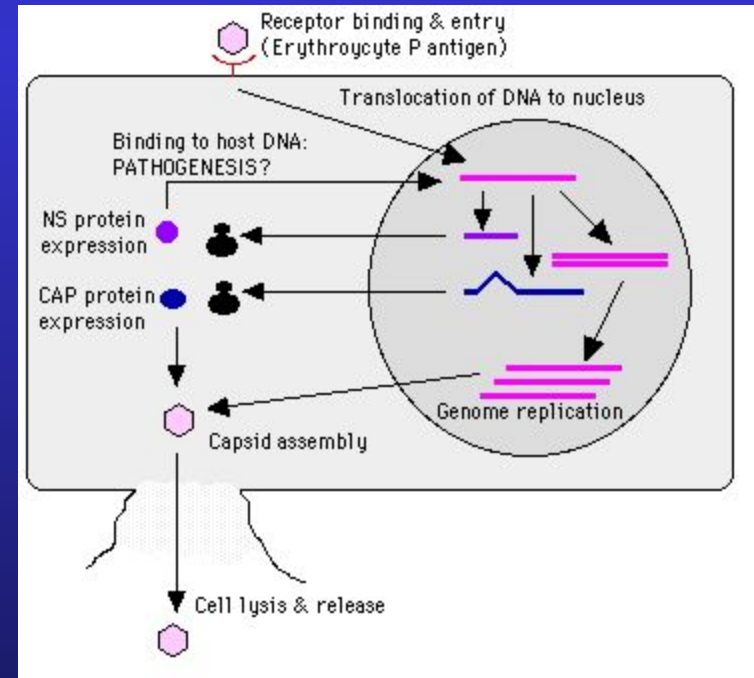
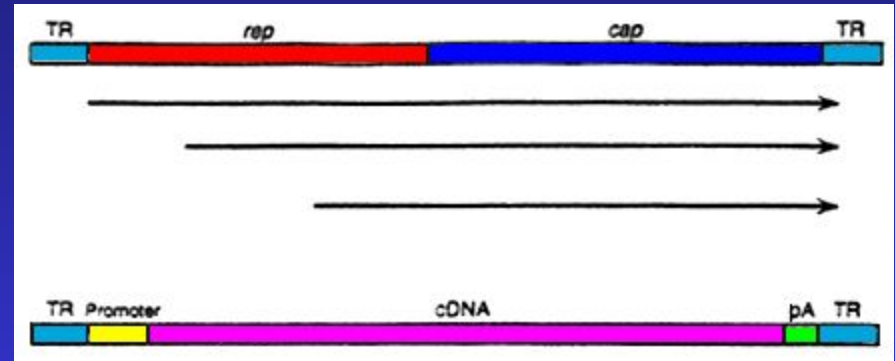
- Very antigenic
- Expression is typically transient
- Gutless
- oncolytic
- replication selective
- Serotypes

# *Adenovirus*

Bar = 100nm

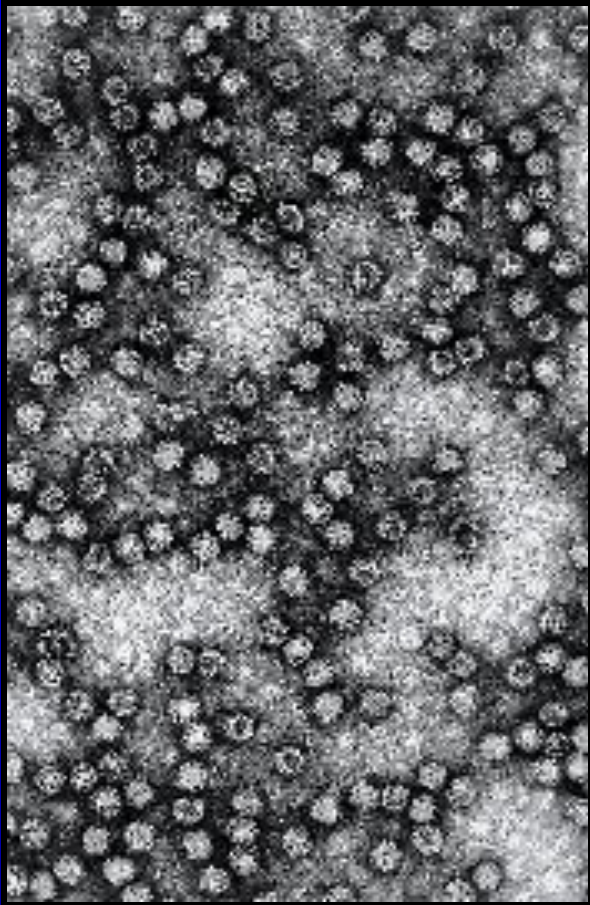
# AAV

- Small size virus (< 5kbp)
- S/s DNA genome
- Adenovirus-dependent for efficient replication
- Infects dividing / non-dividing cells
- Heparin sulfate receptor
- Integrates into host genome ??
- Episomal vs integrated



# *Adeno-Associated (AAV)*

---



- **not very antigenic**
- **high expression**
- **long term (>1 year)**
- **AAV vectors are virtually empty of viral genes**
- **most promising viral vector**

# AAV

- **Lag phase (6 weeks) for max delivery**
- **Neutralizing Abs to capsid do not prevent long-term delivery of therapeutic product**
- **Small size of load (unsuitable for large genes)**
- **Difficult to produce**
- **Multiple administrations ?**
- **Serotypes**



# HERPES

- **Large size DNA genome (150 kbp)**
- **Human neurotropic virus**
- **Suitable for targeting the CNS**
- **Infects dividing / non-dividing cells**
- **Very large payload**
- **Does not integrate into host genome, but replicates as episome**
- **Cytotoxic / inflammation**

# HYBRID VECTORS

- **AAV / adenovirus**
- **Retrovirus / adenovirus**
- **Retrovirus / Herpes**

# ALTERNATIVE VIRUS

- **Simbis**
- **Poxvirus**
- **Vaccinia**
- **Baculovirus**
- **Sendai**
- **Foamy virus**
- **SV40.....**

# ***KEY ISSUES***

- **Delivery**
- **Immune response**
- **Logistics**
- **Tropism**
- **Persistence**

# *IMMUNITY OF VIRAL VECTORS*

- **Delivery**
- **Immune response**
- **Logistics**
- **Tropism**
- **Persistence**

# Viral Vectors

“Yea”

“Nay”

- Excellent expression
  - Off-the-shelf drug
  - Industrial production
  - Superior delivery
  - Available vectors
  - Most viral vectors are benign to humans
- Immunogenicity
  - Insertional mutagenesis
  - Germ-line transmission
  - Narrow efficacy range + huge human variability
  - Human variability