BIOL 670 / What vectors

Gene Delivery

• Ex vivo

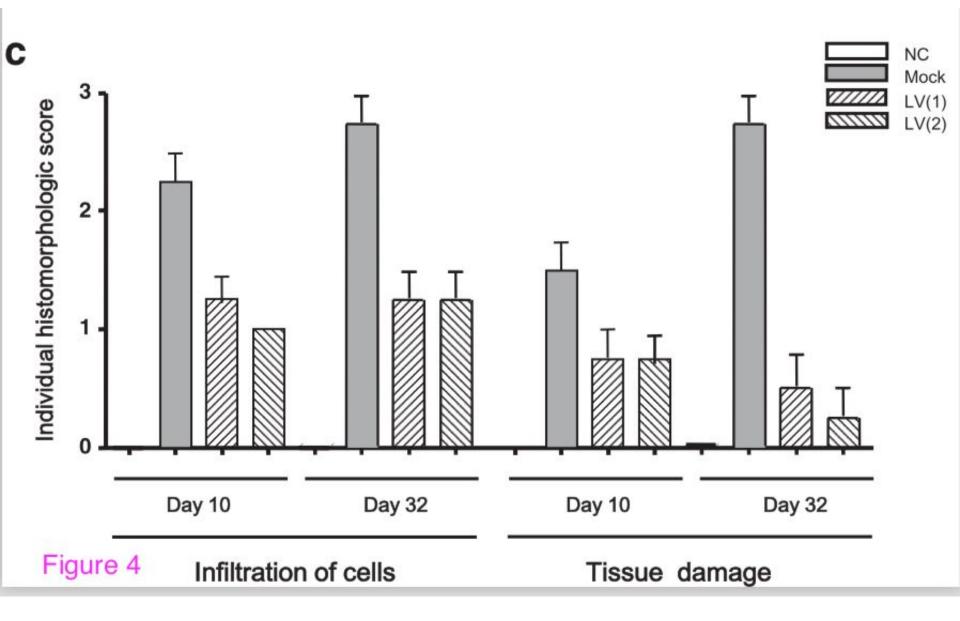
Transplantation of recombinant cells with virus In vivo

Direct administration of virus

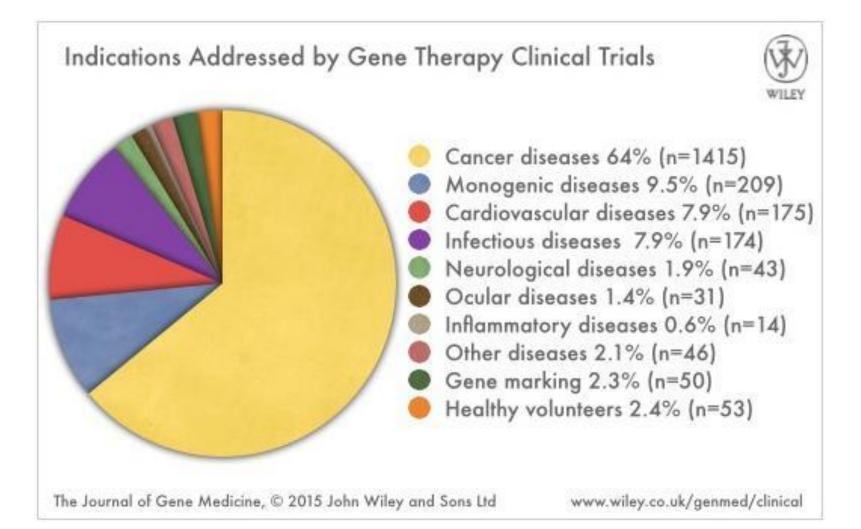
"New" Gene Therapy

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

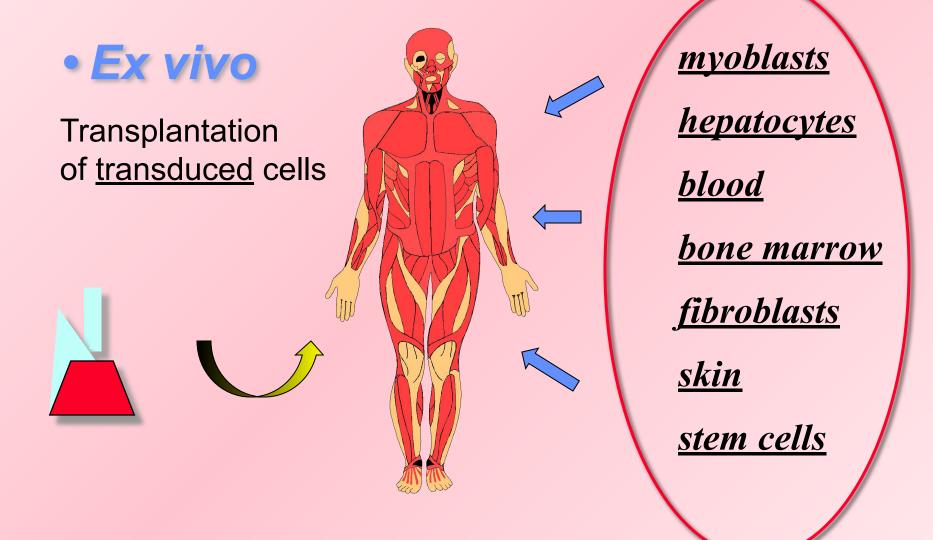
H. Matsumoto et al. (2014) *BMC Gastroenterology* 14:68



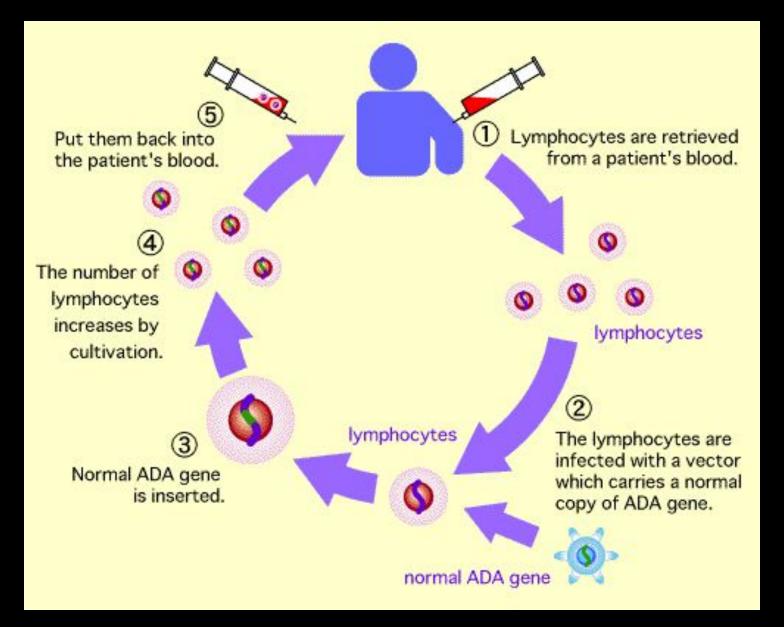
Gene Therapy Trials



Gene Therapy







Cells? Which cells?



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

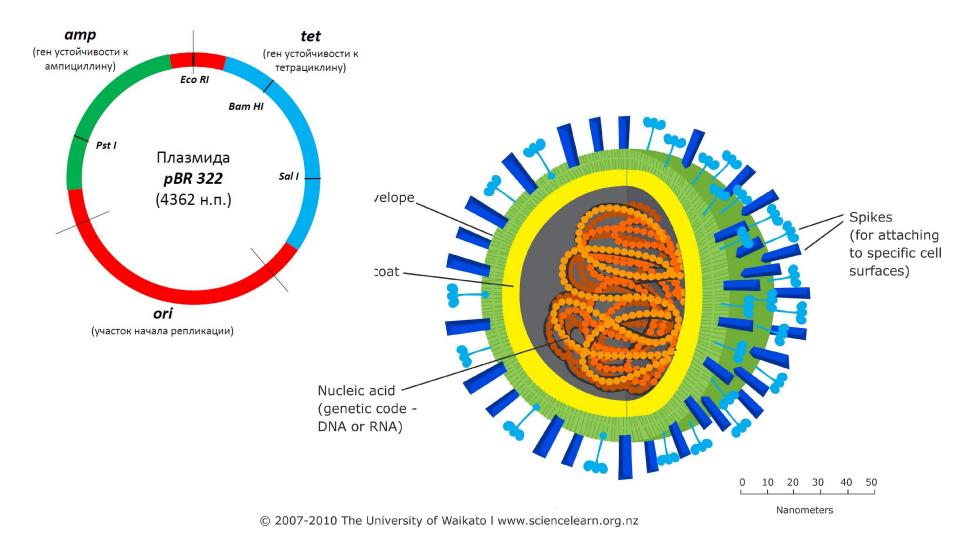
Ex vivo Gene Therapy

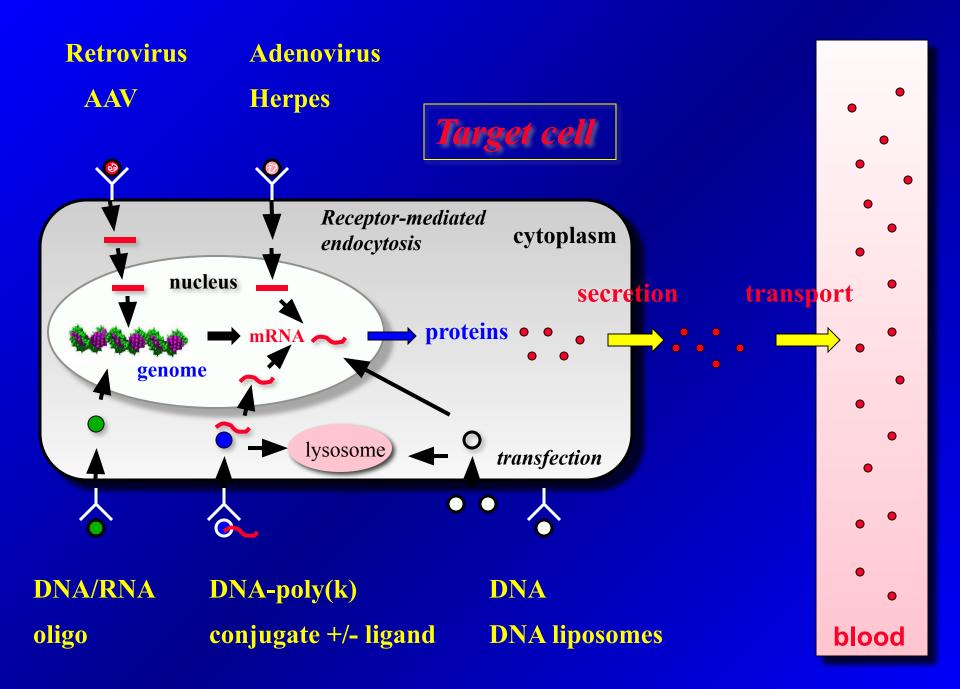
Lentiviral Hematopoietic Stem Cell Gene Therapy Benefits Metachromatic Leukodystrophy

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

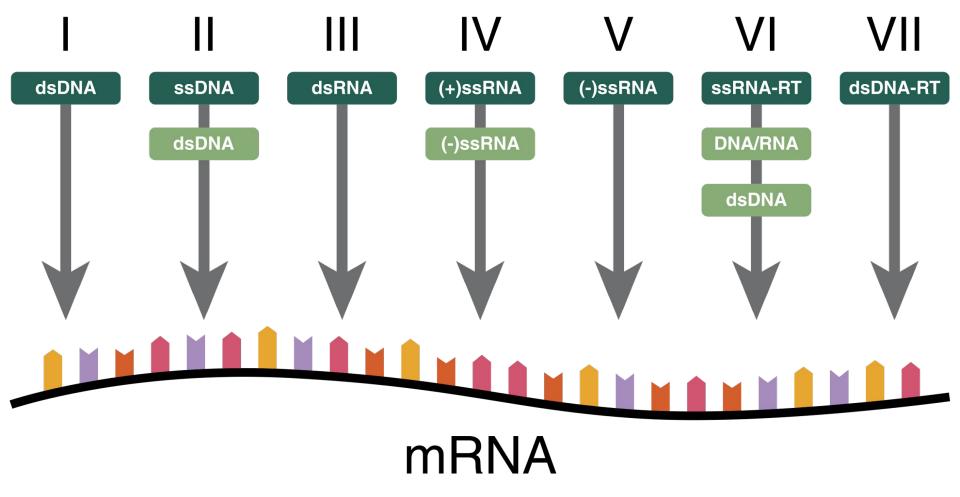
Metachromatic leukodystrophy (MLD) is a neurodegenerative lysosomal storage disease caused by arylsulfatase A (ARSA) deficiency. The disease primarily affects children and invariably leads to premature death. In previous work with a mouse model of MLD, we used a lentiviral vector (LV) to introduce a 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 <u>clear therapeutic benefit</u>.

Which vector to use? (rocket)

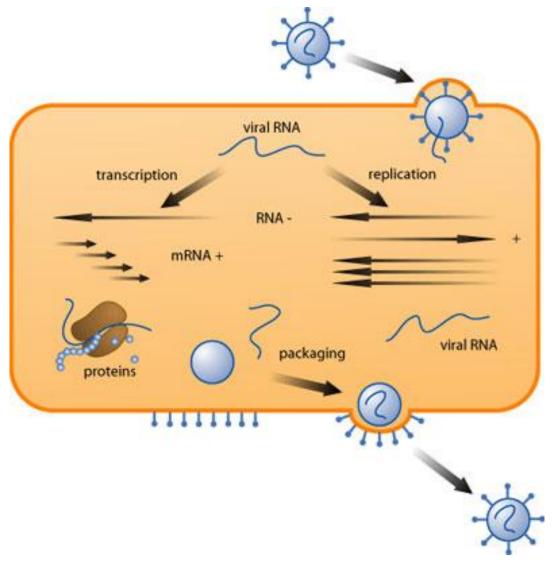




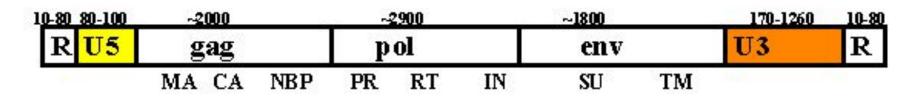
Virus Classification (classes)



Viral Replication

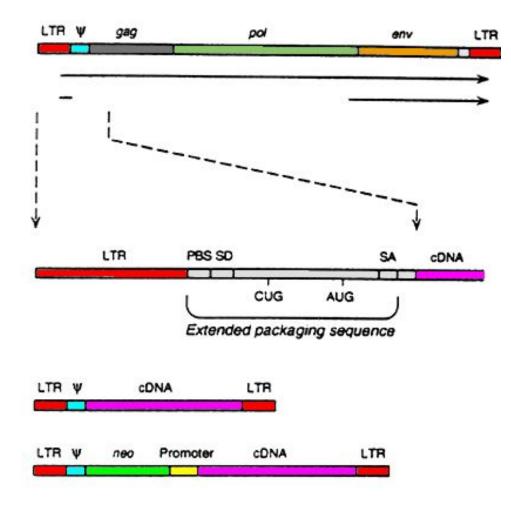


Viral Proteins

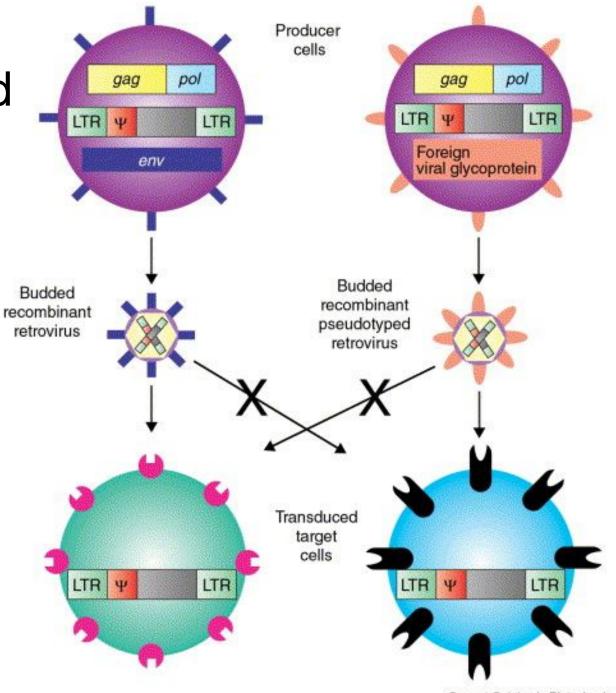


- MA Matrix
- CA Capsid
- NBP Nuclear Binding Protein
- PR Protease
- RT Reverse Transcriptase
- IN Integrase
- SU Surface protein
- TM Transmembrane Protein

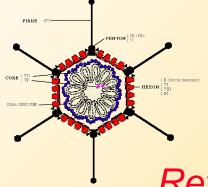
Ψ is essential for viral replication



Pseudotyped virus



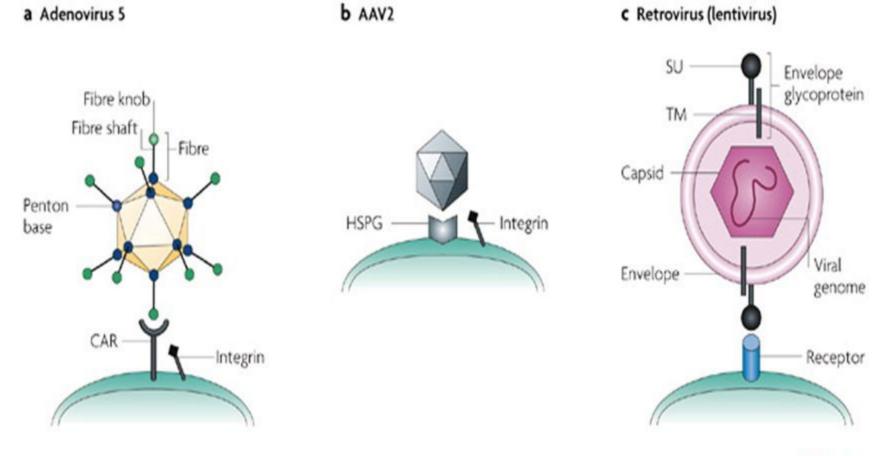
Current Opinion in Biotechnology





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

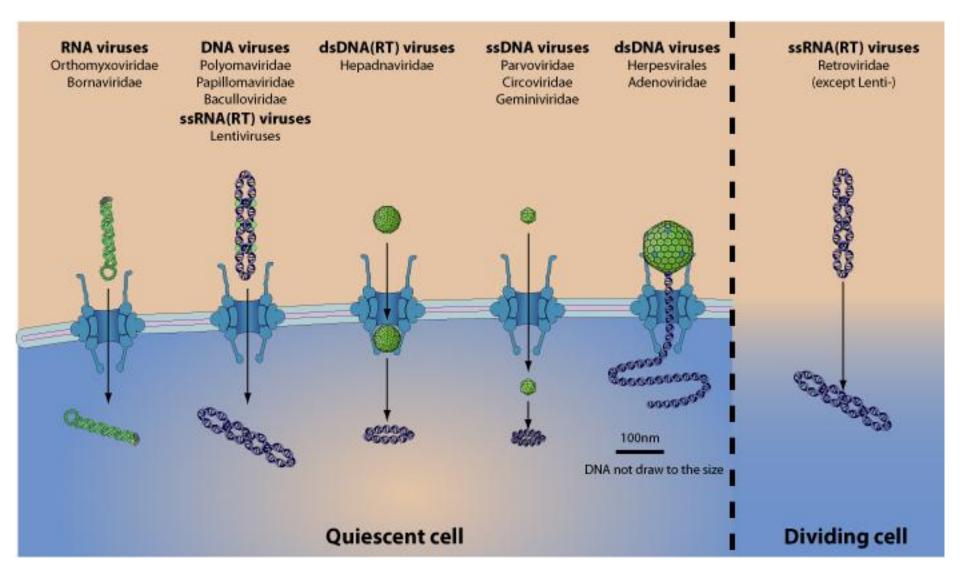
Genome F	NAs	DNAd	DN	As RNA	As DNA	d
Size	9	30	4.7	9	150	
Capacity	>7	8-30	4	>7	130	
Target cells	Div	Div/	'no	Div/no	Div/no	Div/no
Integration	yes	no		yes/no	yes	no

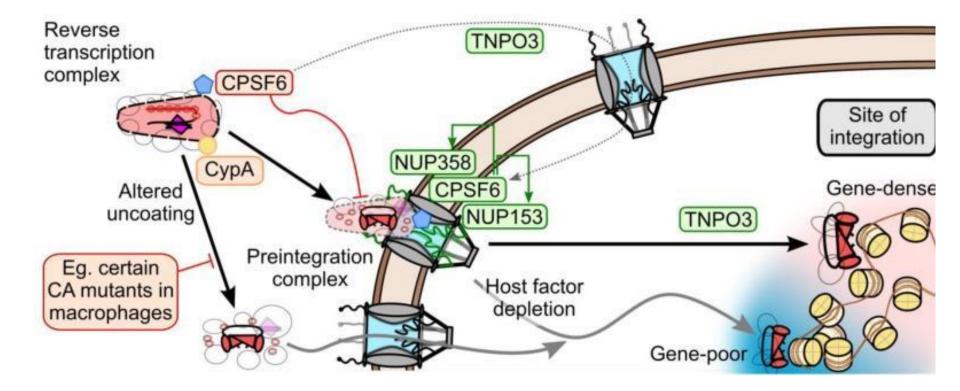


Nature Reviews | Genetics

VIRAL VECTORS

Viral Entry into Nucleus



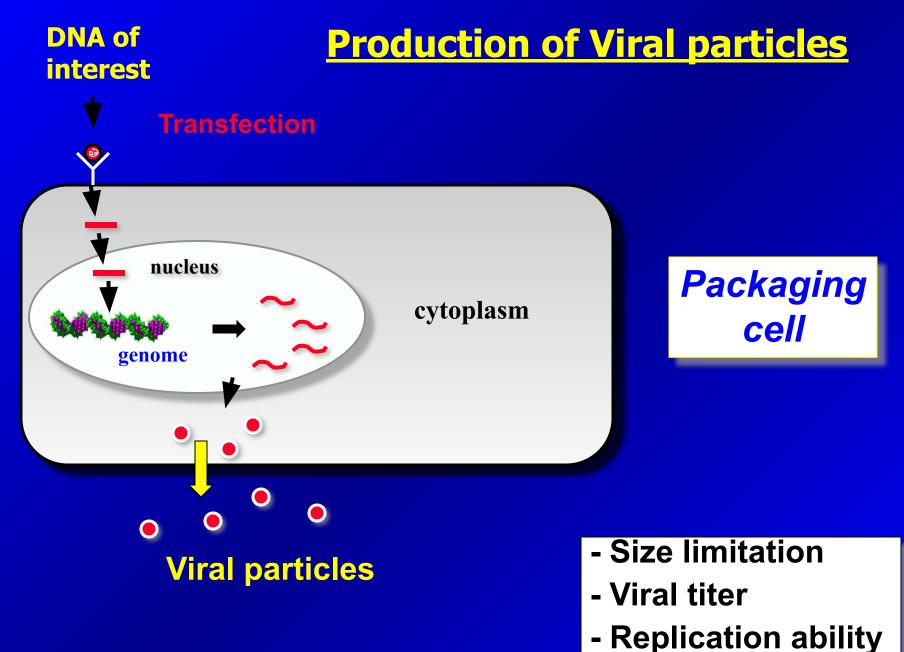


RELEVANT QUESTIONS WHEN CHOSING A VECTOR

- 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?

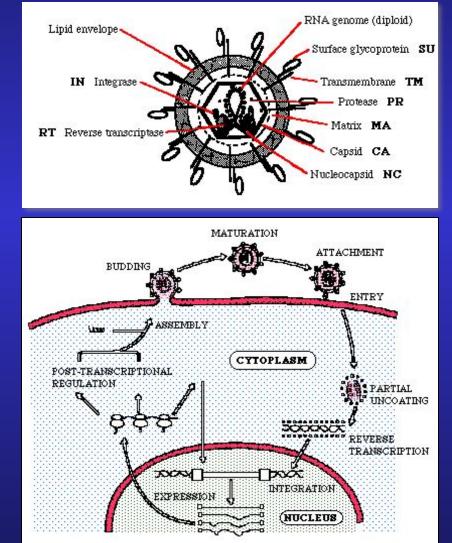
IDEAL VECTOR CANDIDATE (does not yet exist)

- High titer or concentrations (>10⁸ 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 (<u>pseudotyped</u>)
- Does not elicit host immune response
- Persistence as required

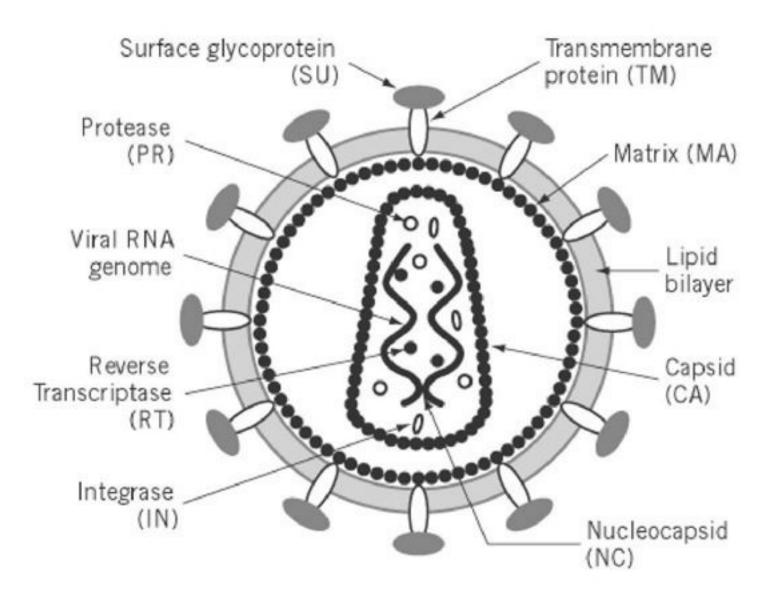


RETROVIRUS

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

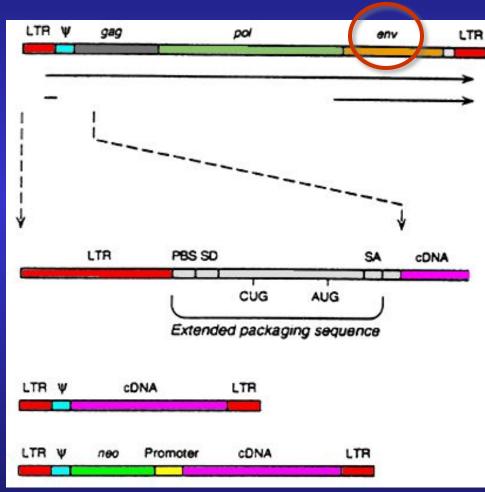


RETROVIRUS



RETROVIRUS

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

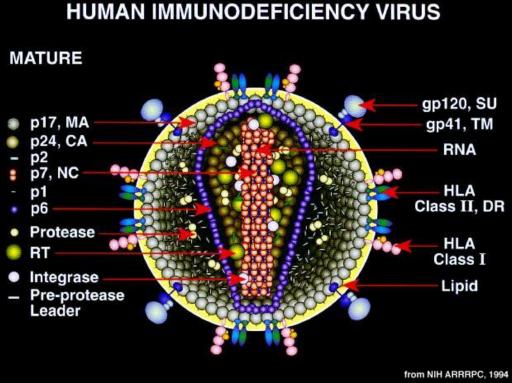


LIMITATIONS OF RETROVIRUS

- 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-recep)
- 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)

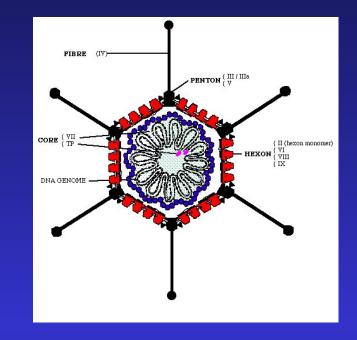
LIMITATIONS OF LENTIVIRUS

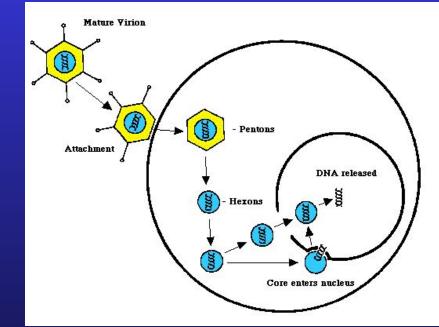
- Gene expression is often not as high as with adenovirus
- Same as retrovirus (except it can target non-dividing cells)
- Potential use in gene therapy provided safety is proven

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

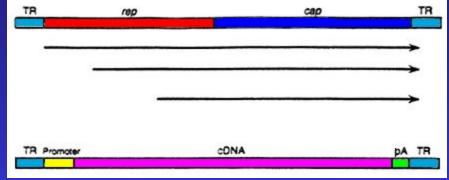
 Very antigenic
 Expression is typically transient

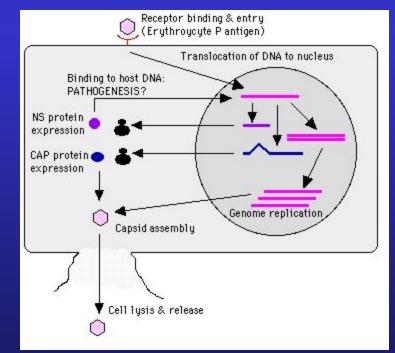
Gutless
oncolytic
replication selective
Serotypes

Adenovirus

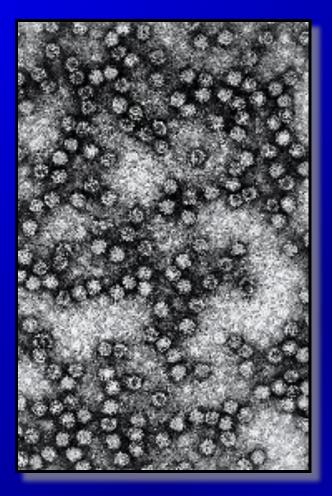
AAV

- 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 Iong 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.....

KEYISSUES

- 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