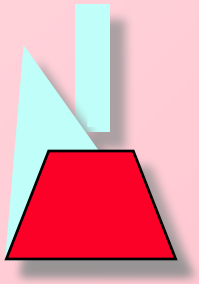
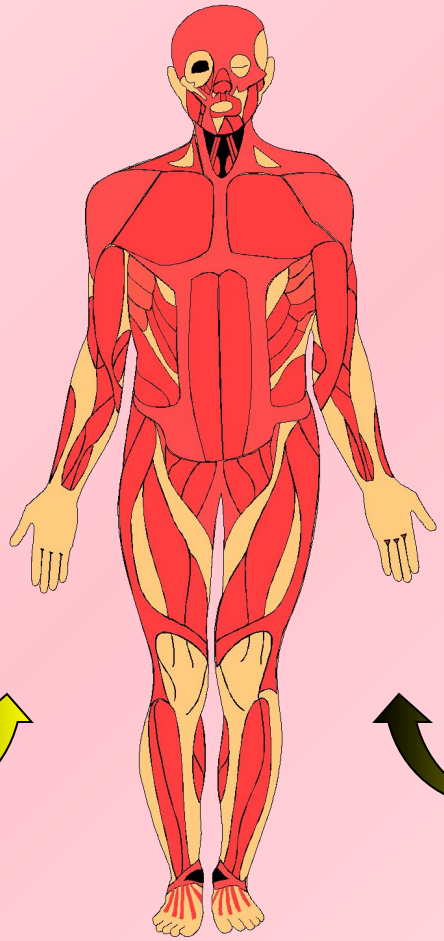

BIOL 670 /
775
Viral VECTORS

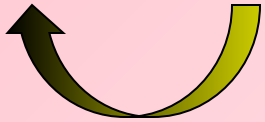
Gene Delivery

- ***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

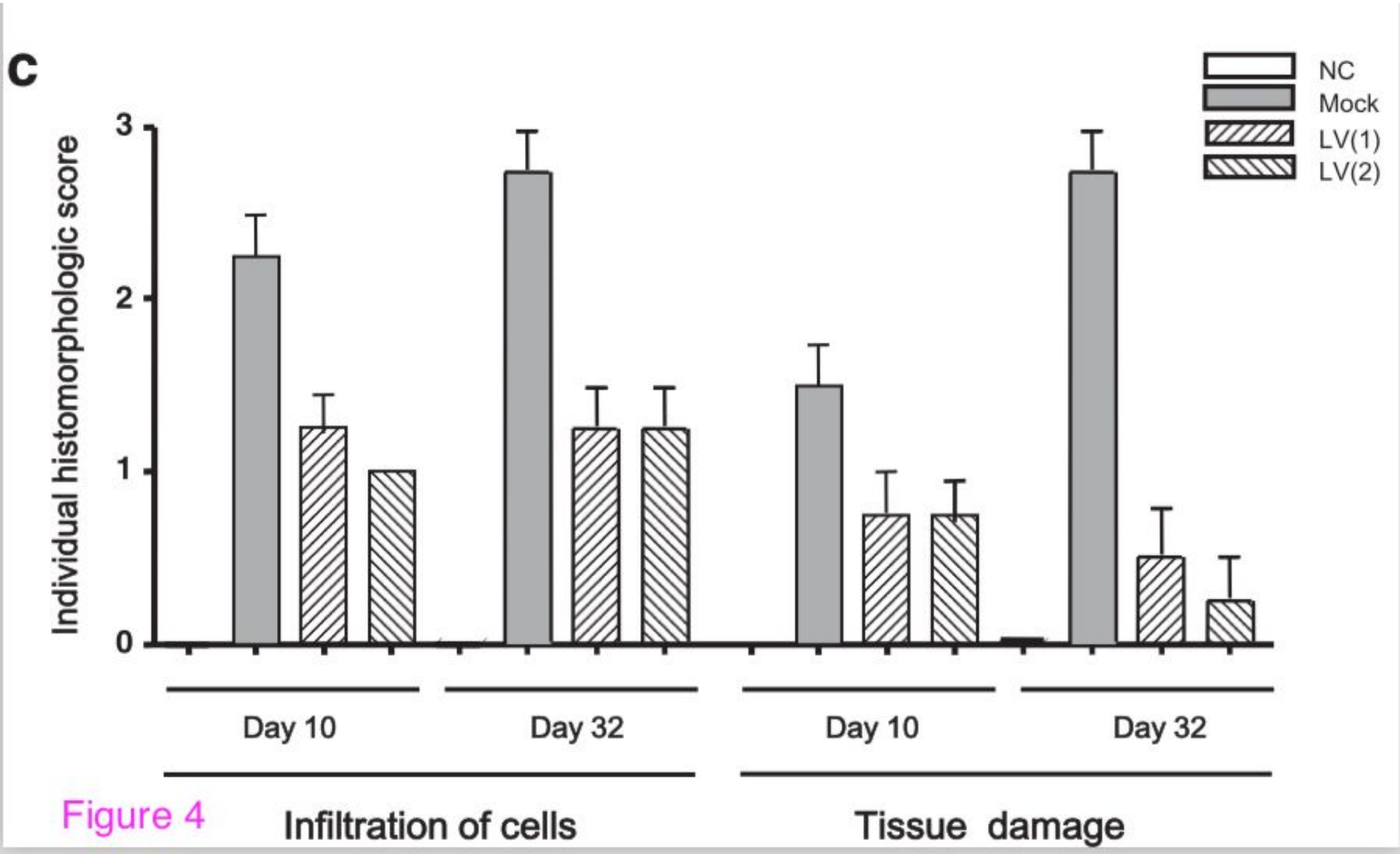
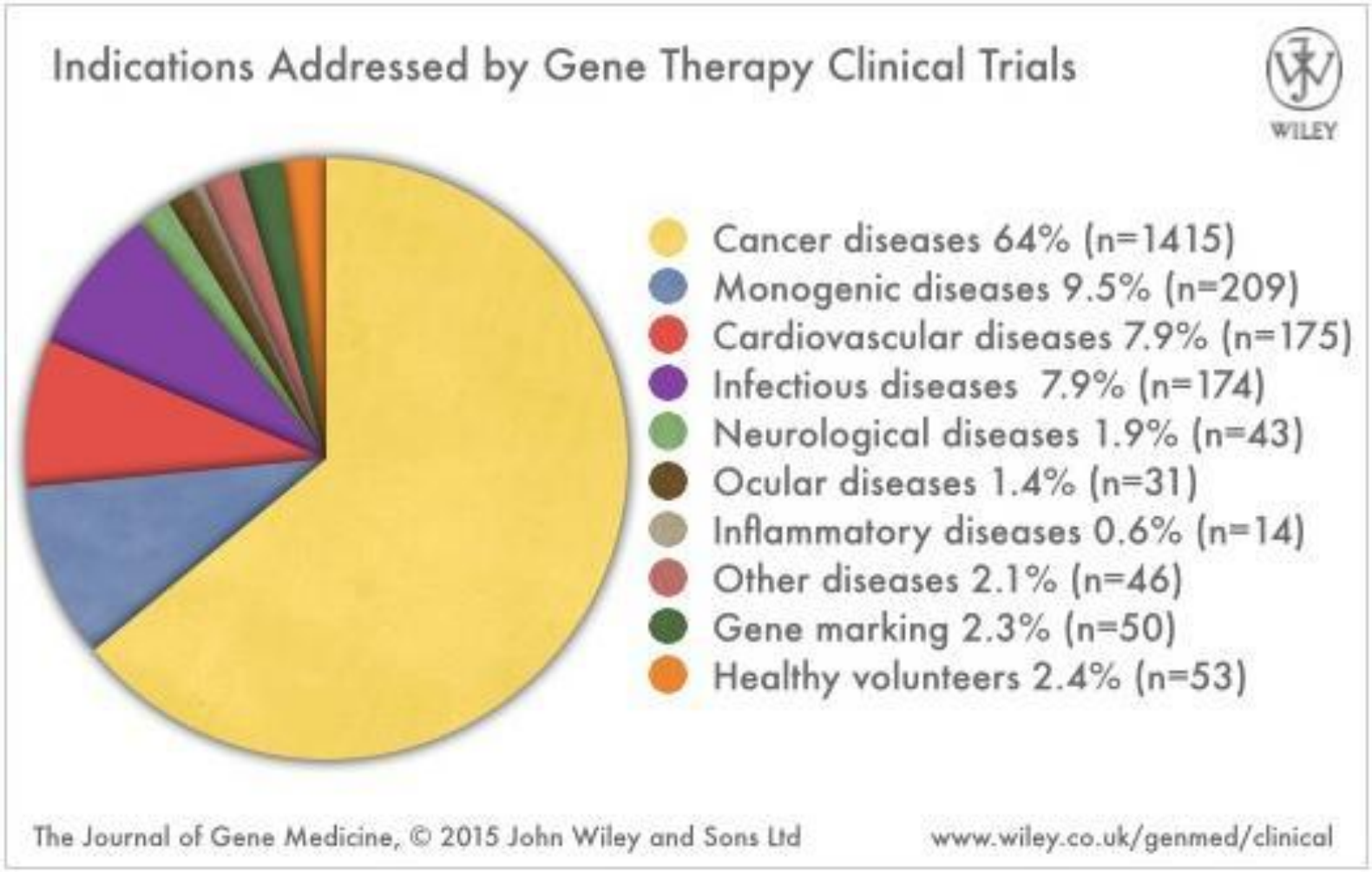

c
NC
Mock
LV(1)
LV(2)
Individual histomorphologic score
3
2
1
0
Day 10
Day 32
Day 10
Day 32
Infiltration of cells
Tissue damage

Figure 4

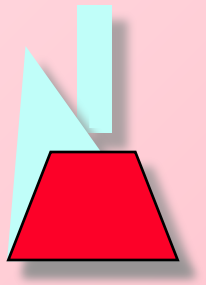
Gene Therapy Trials

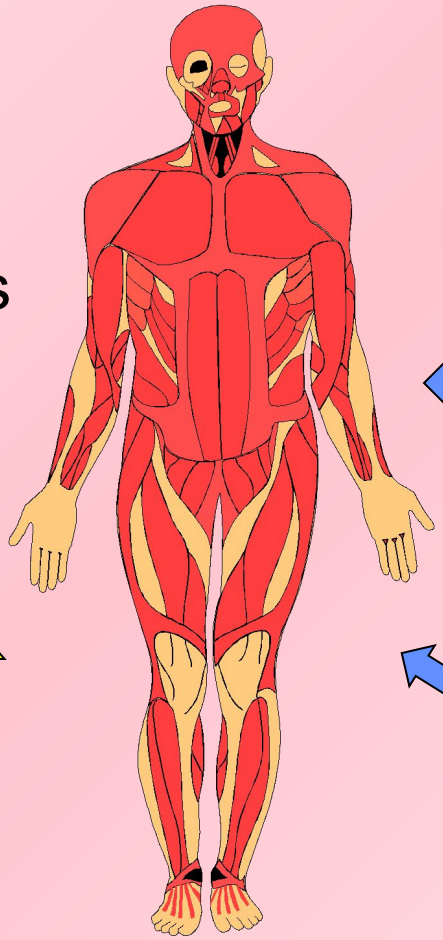


Gene Therapy

- ***Ex vivo***

Transplantation
of transduced cells





myoblasts

hepatocytes

blood

bone marrow

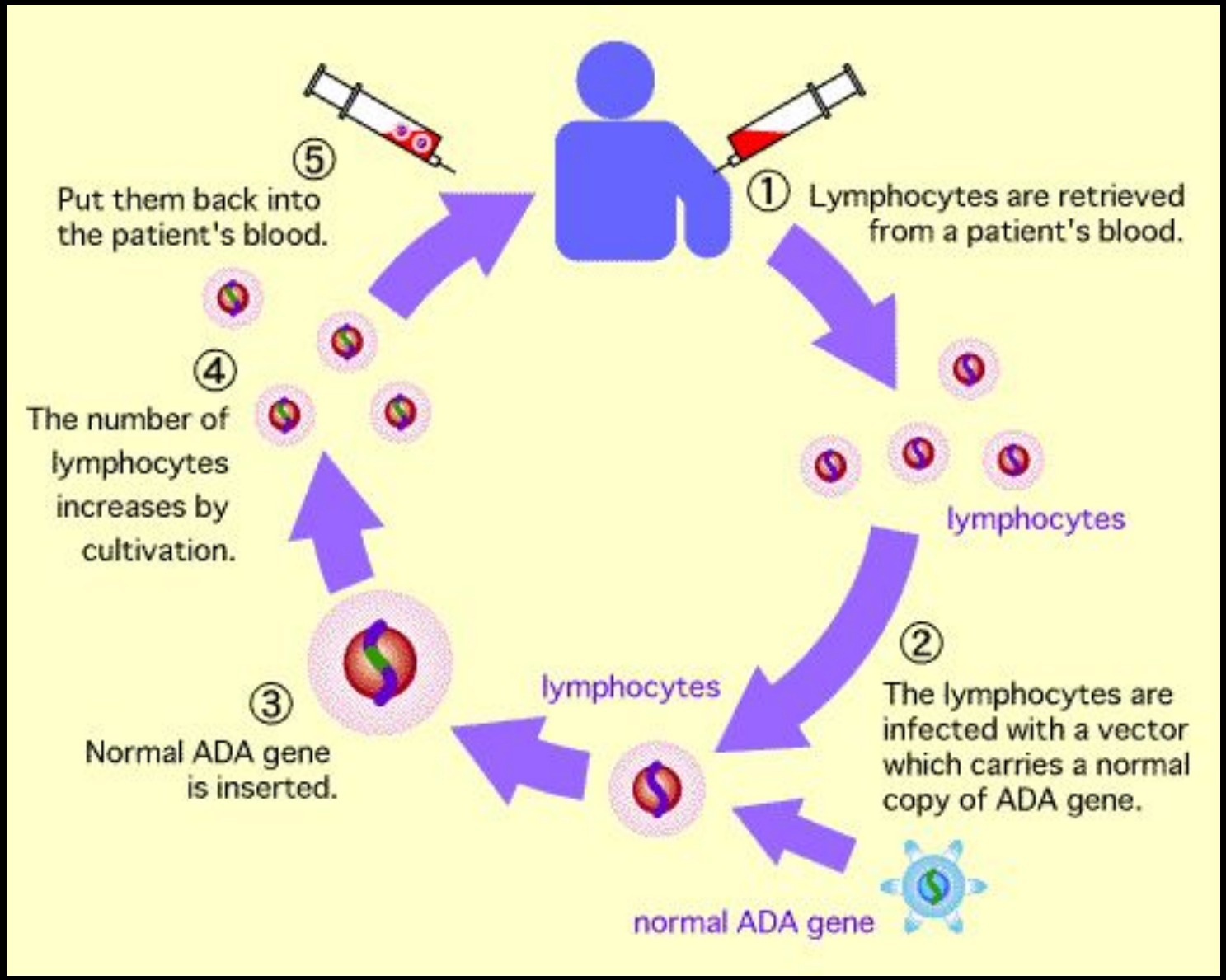
fibroblasts

skin

stem cells

1990

Ex vivo


⑤
Put them back into the patient's blood.
① Lymphocytes are retrieved from a patient's blood.
④
The number of lymphocytes increases by cultivation.
lymphocytes
②
The lymphocytes are infected with a vector which carries a normal copy of ADA gene.
③
Normal ADA gene is inserted.
lymphocytes
normal ADA gene

Cells? Which cells?

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

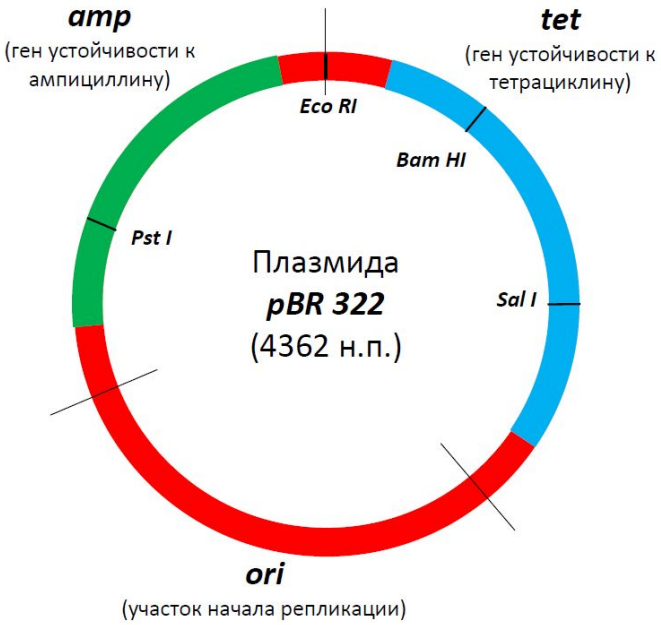
Ex vivo Gene Therapy

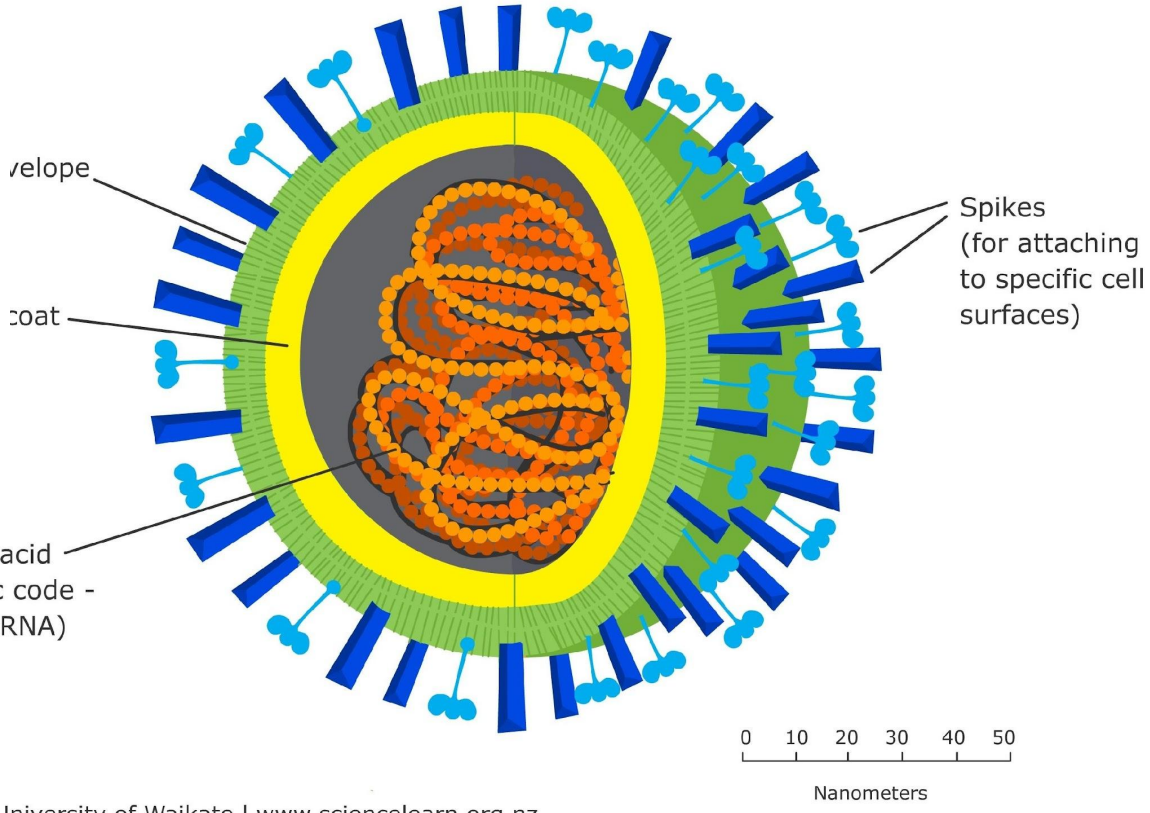
Lentiviral Hematopoietic Stem Cell Gene Therapy Benefits Metachromatic Leukodystrophy

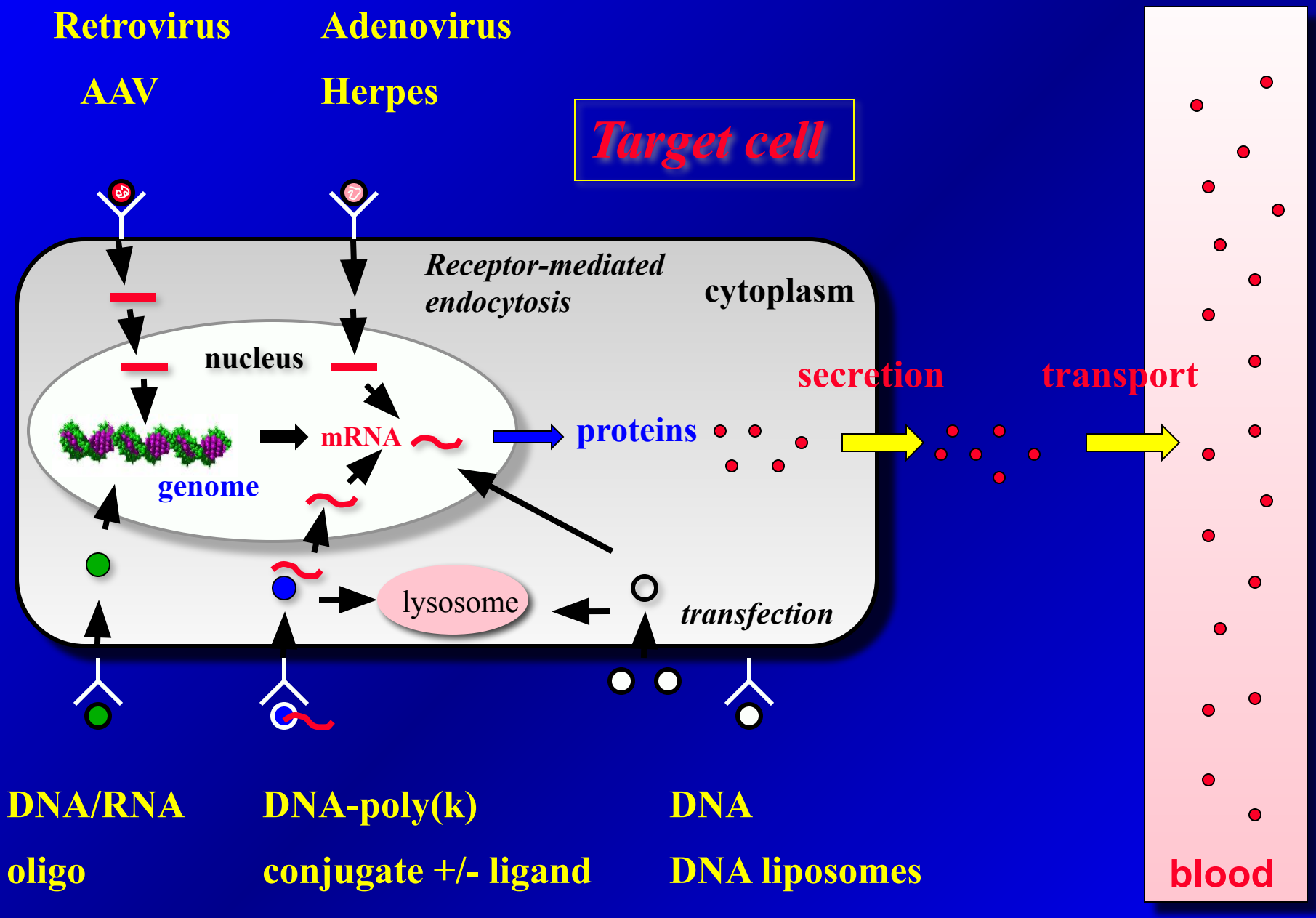
Alessandra Biffi et al. Luigi Naldini's laboratory (Italy); Science 2013

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.

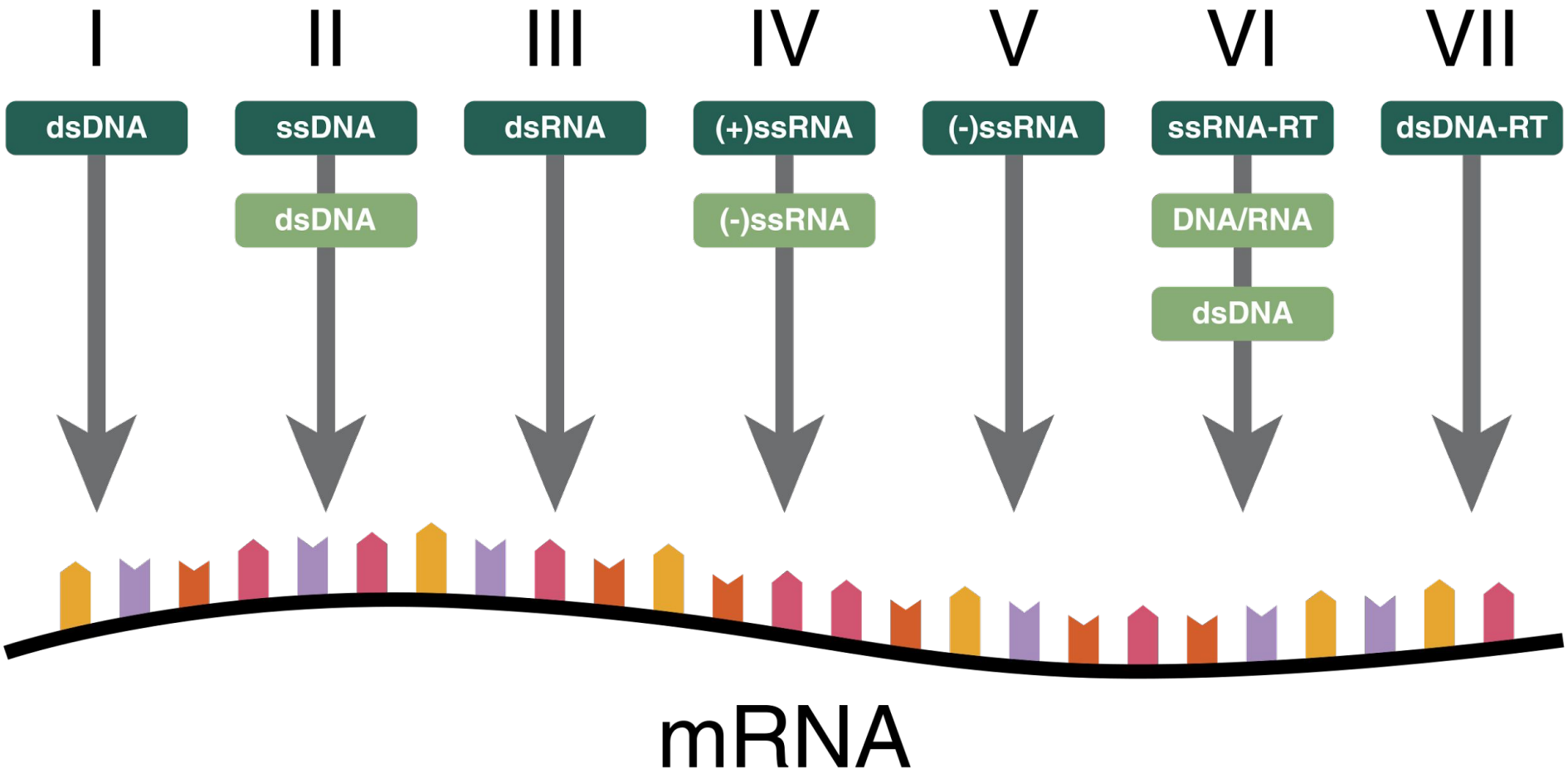
Which vector to use? (rocket)



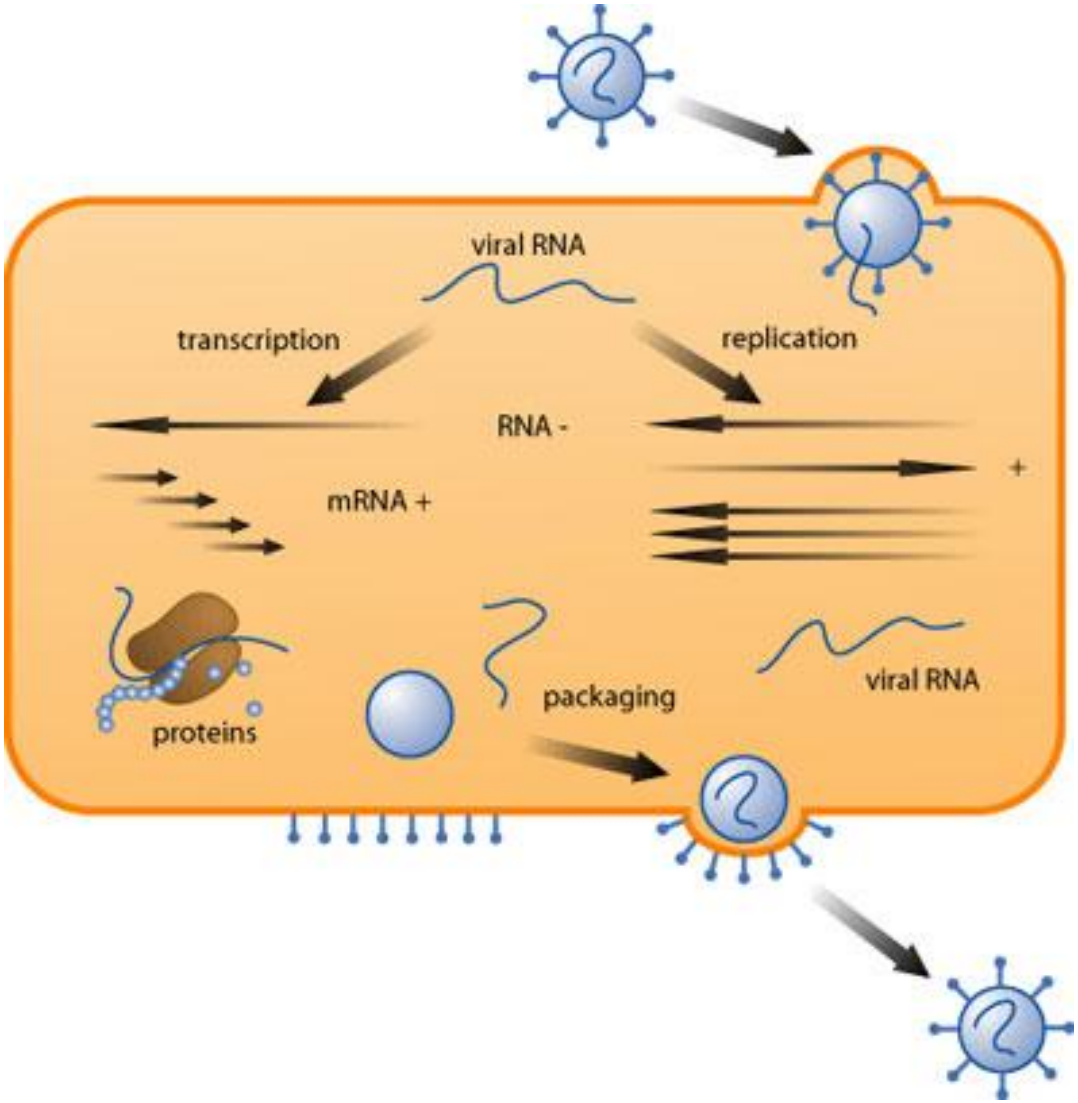



Retrovirus
AAV
Adenovirus
Herpes
Target cell
Receptor-mediated endocytosis
cytoplasm
nucleus
mRNA
genome
proteins
secretion
transport
lysosome
transfection
blood
DNA/RNA
oligo
DNA-poly(k)
conjugate +/- ligand
DNA
DNA liposomes

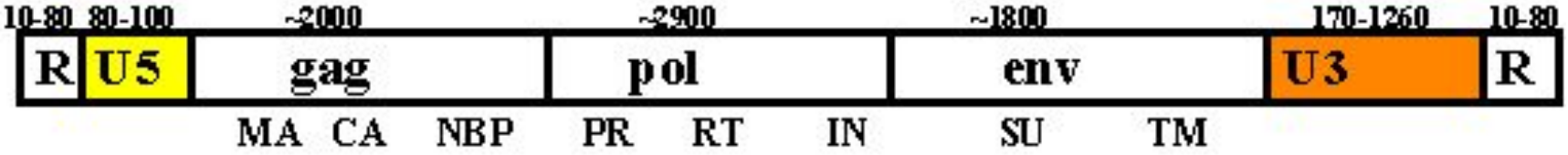
Virus Classification (classes)



Viral Replication

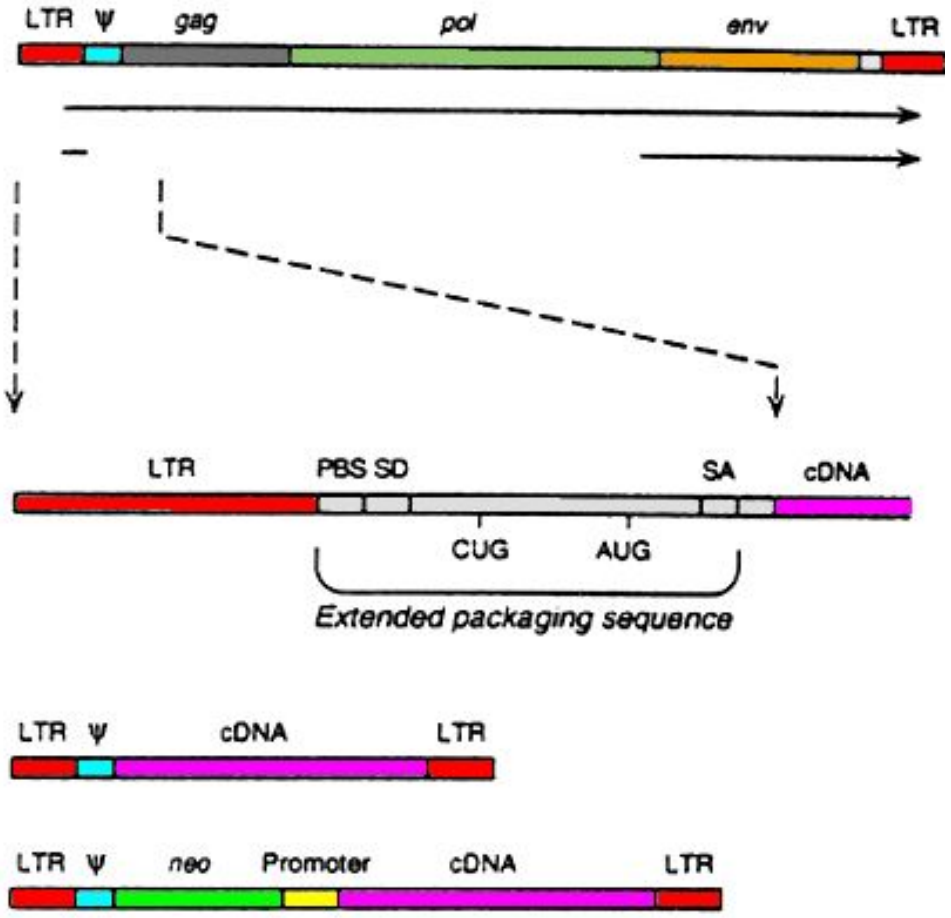


Viral Proteins

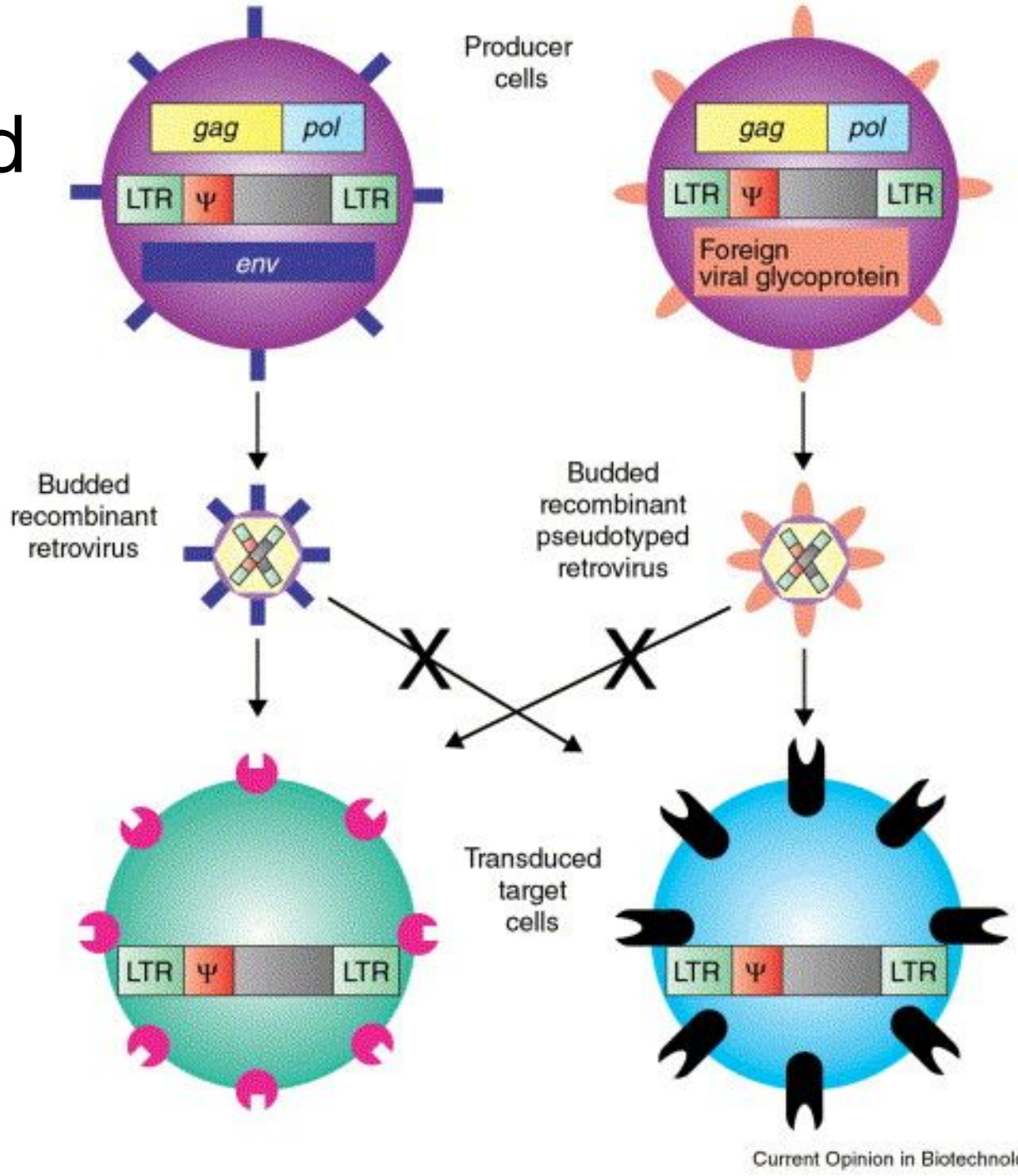


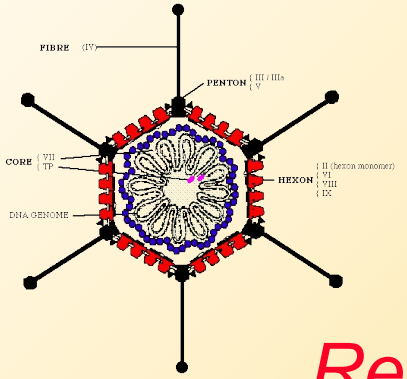
MA	Matrix
CA	Capsid
NBP	Nuclear Binding Protein
PR	Protease
RT	Reverse Transcriptase
IN	Integrase
SU	Surface protein
TM	Transmembrane Protein

Ψ is essential for viral replication



Pseudotyped virus

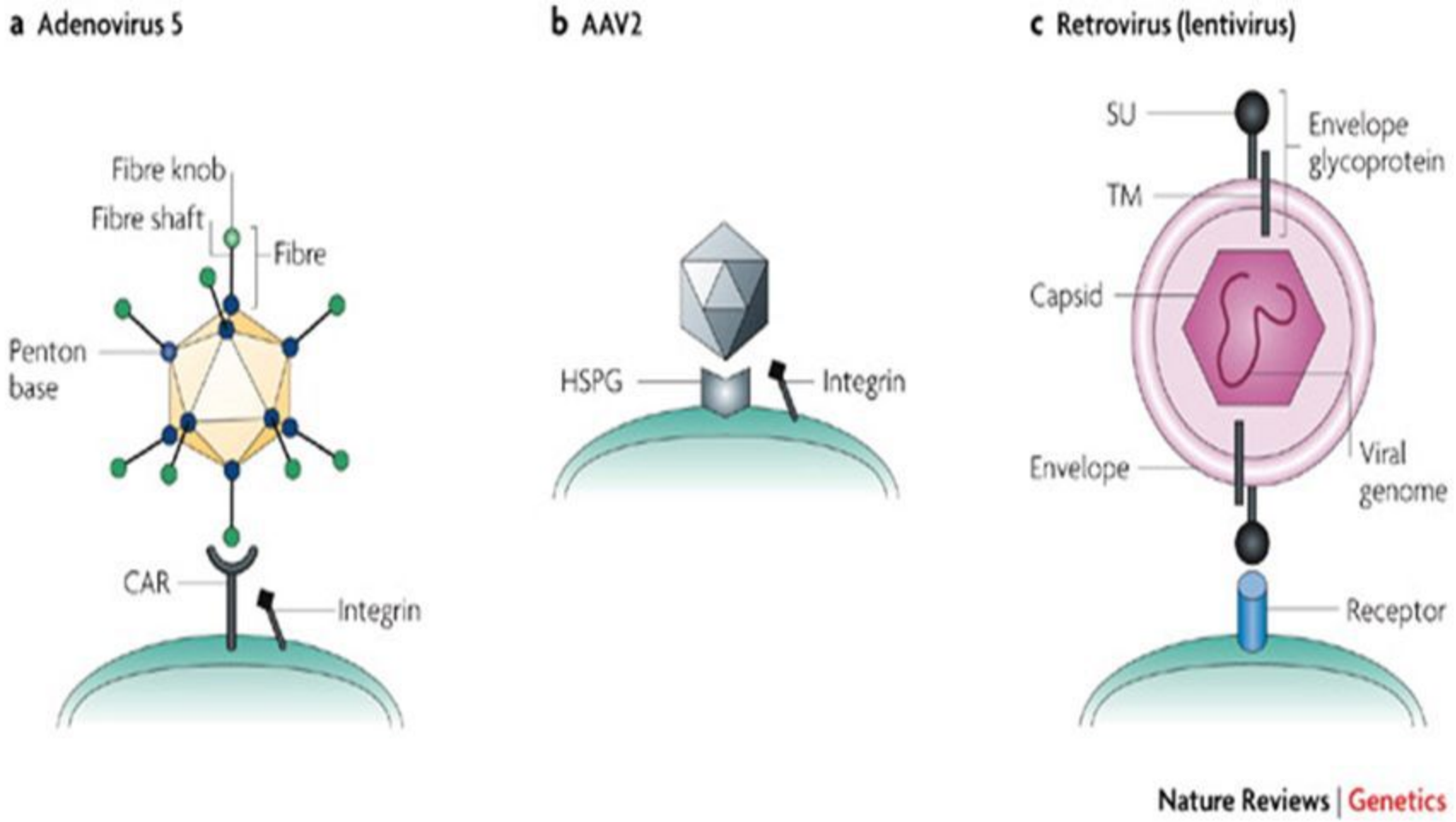




Viral vectors

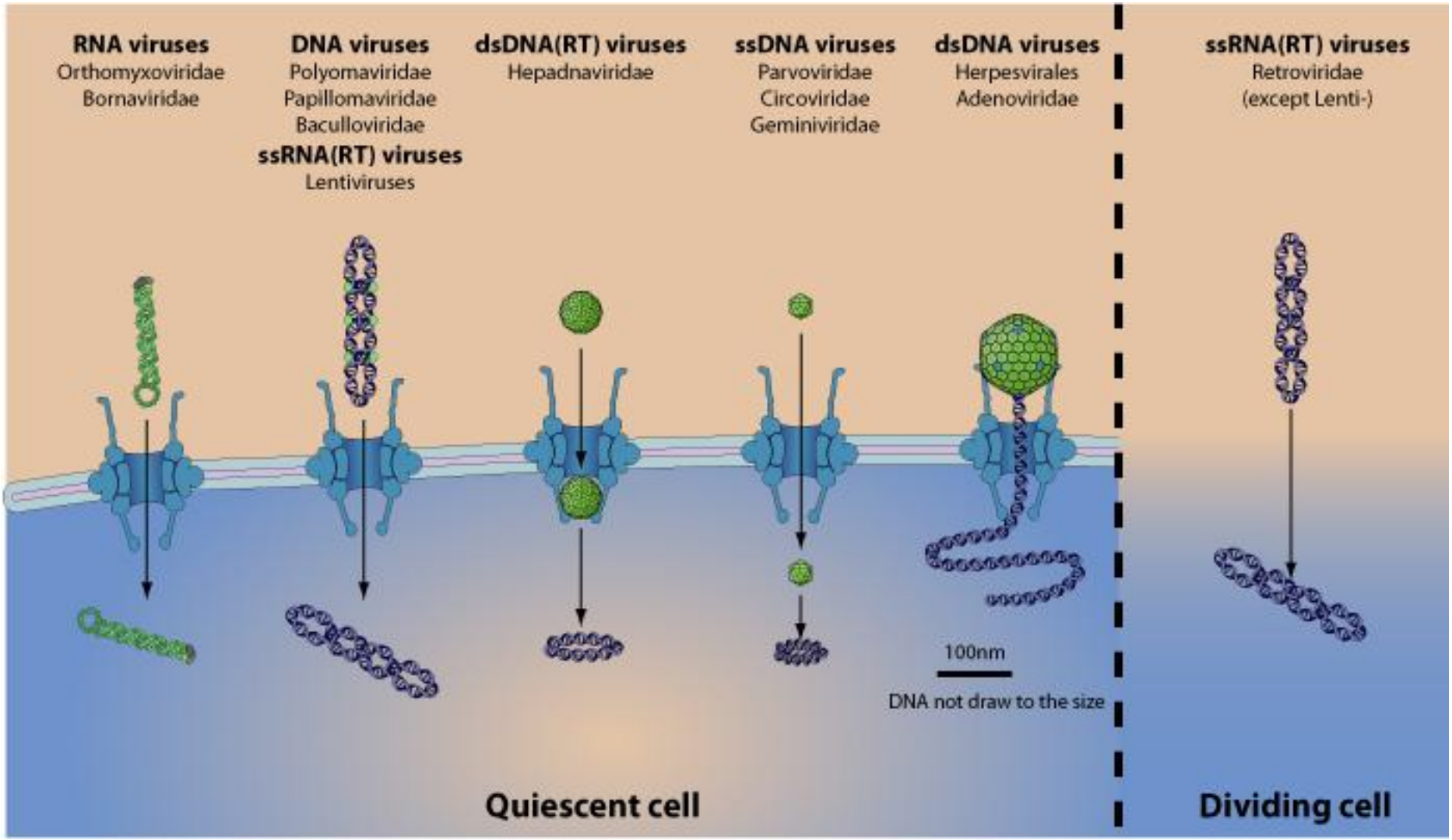
Retrov. / Adenov. / AAV / Lentiv. / Herpes

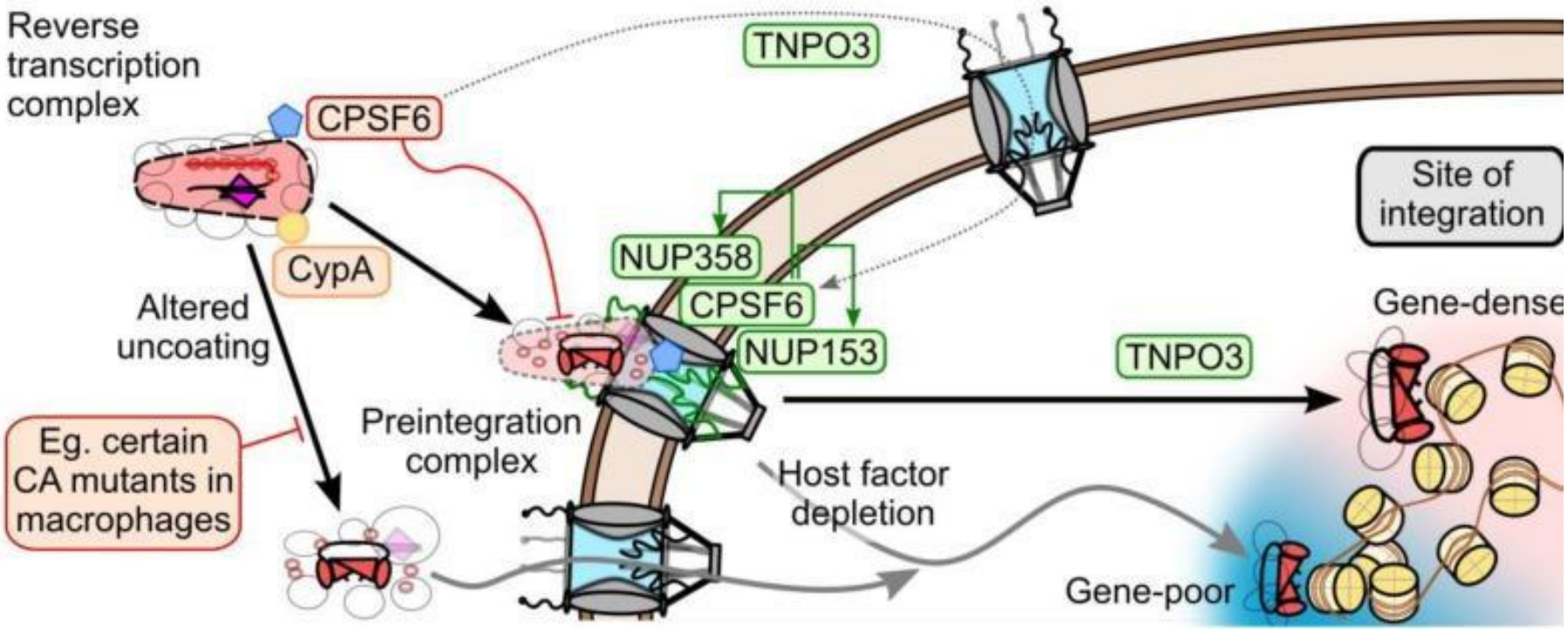
Genome	*RNAs*	*DNAd*	*DNAs*	*RNAs*	*DNAd*
Size	*9*	*30*	*4.7*	*9*	*150*
Capacity	*>7*	*8-30*	*4*	*>7*	*130*
Target cells	*Div*	*Div/no*	*Div/no*	*Div/no*	*Div/no*
Integration	*yes*	*no*	*yes/no*	*yes*	*no*



VIRAL VECTORS

Viral Entry into Nucleus




Reverse transcription complex
CPSF6
CypA
TNPO3
Site of integration
NUP358
CPSF6
NUP153
Altered uncoating
Eg. certain CA mutants in macrophages
Preintegration complex
Host factor depletion
TNPO3
Gene-dense
Gene-poor

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?

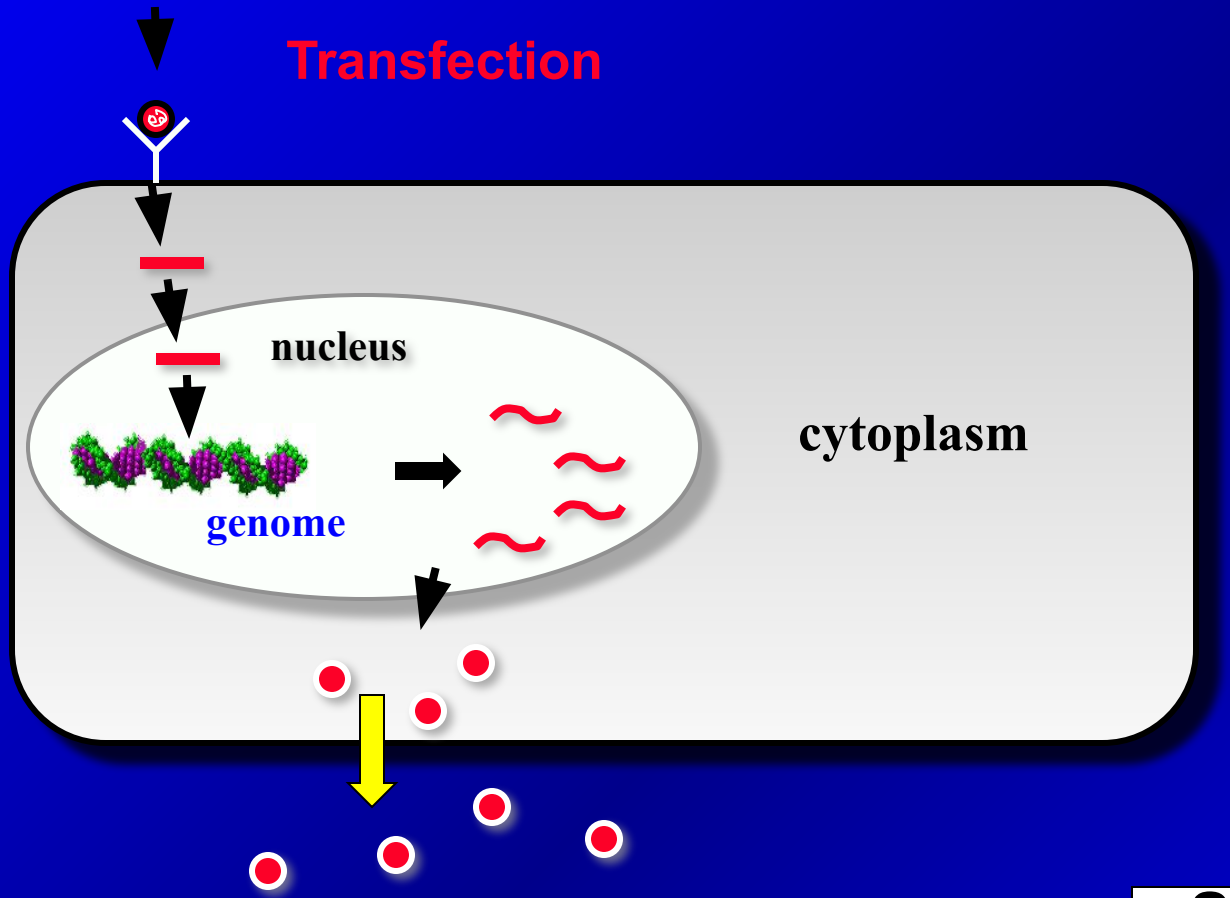
IDEAL VECTOR CANDIDATE (does not yet exist)

- 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

Production of Viral particles

DNA of interest

Transfection

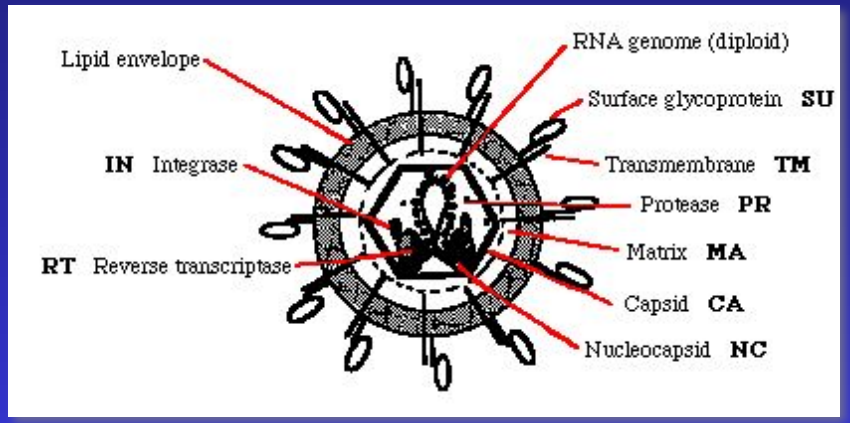


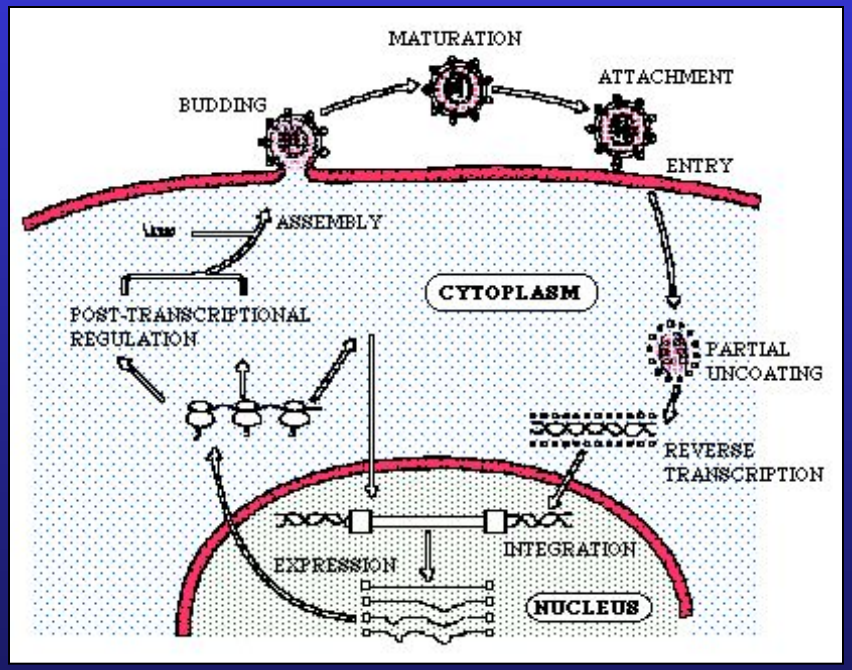
Packaging cell

- Size limitation
- Viral titer
- Replication ability

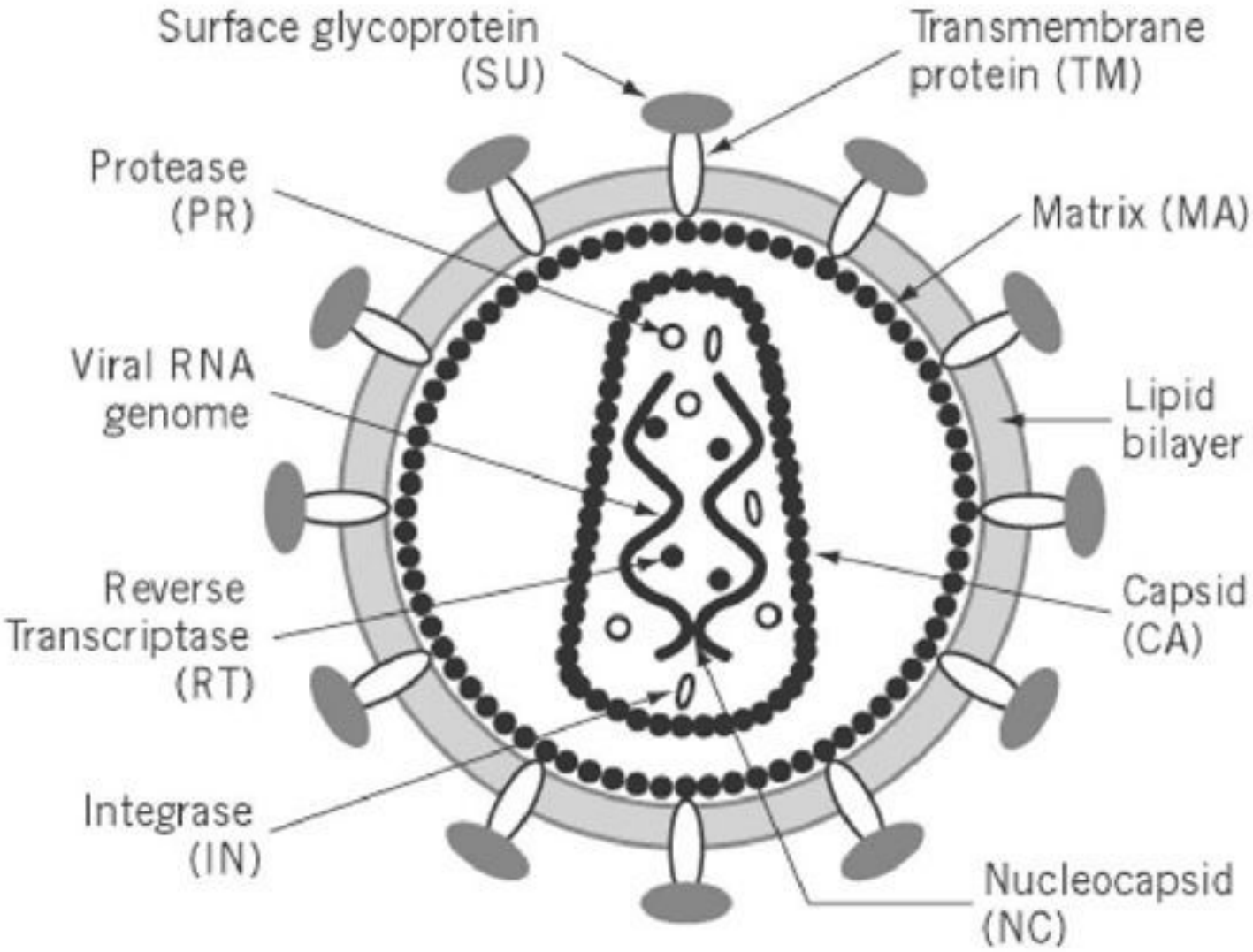
RETROVIRUS

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



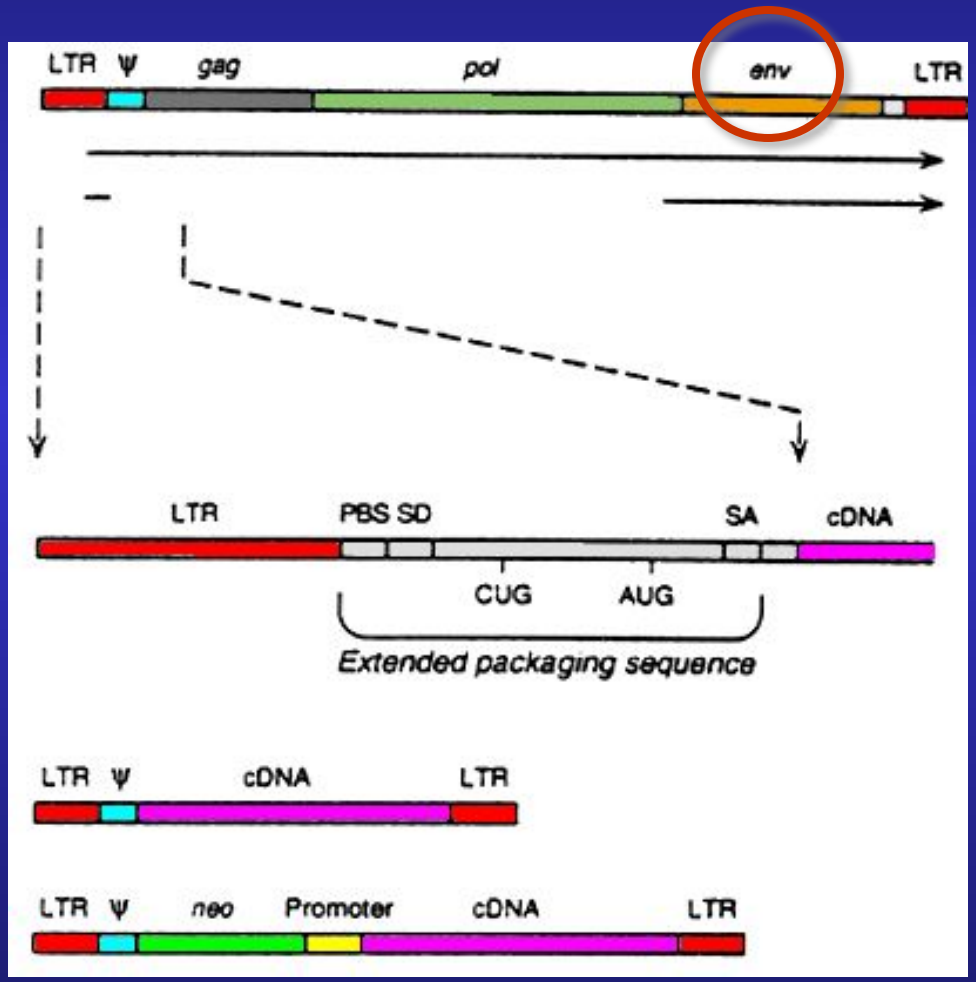


RETROVIRUS


Surface glycoprotein (SU)
Transmembrane protein (TM)
Protease (PR)
Matrix (MA)
Viral RNA genome
Lipid bilayer
Reverse Transcriptase (RT)
Capsid (CA)
Integrase (IN)
Nucleocapsid (NC)

RETROVIRUS

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

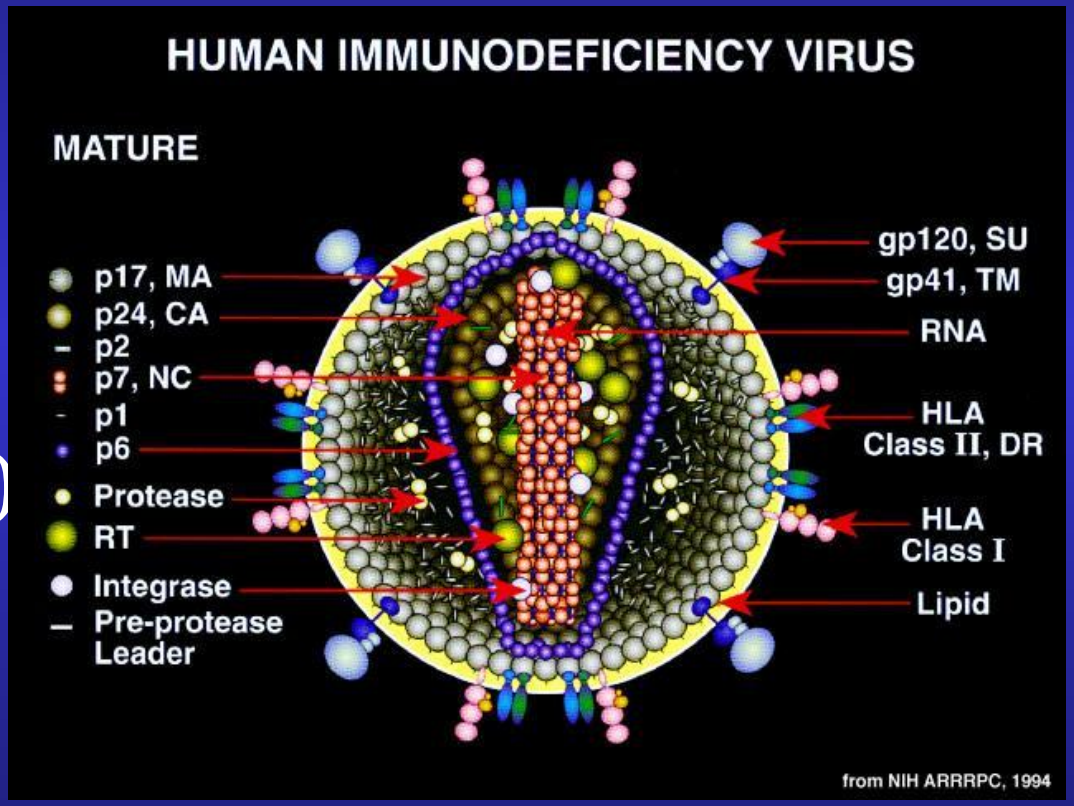


LIMITATIONS OF RETROVIRUS

- 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

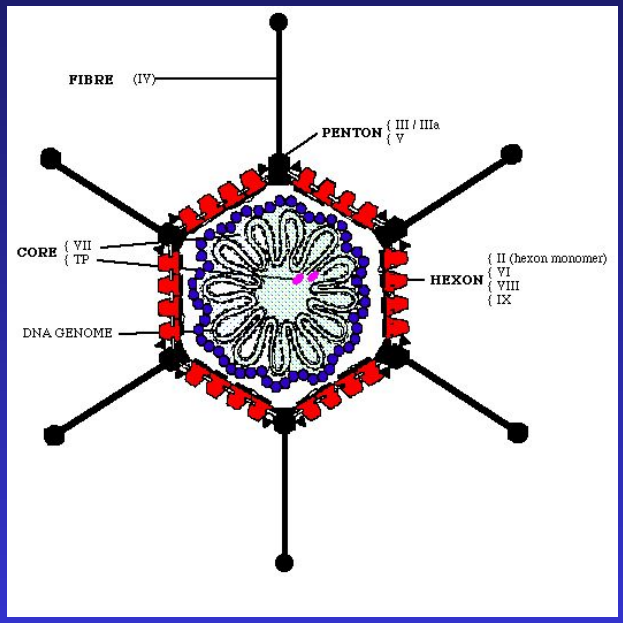
- Targeting of stem cells
- Gene expression is sustained, and often sustained through cellular differentiation
- Promising in preclinical studies:
  - Hematopoietic cells
  - inhibition of genes (interference)

LIMITATIONS OF LENTIVIRUS

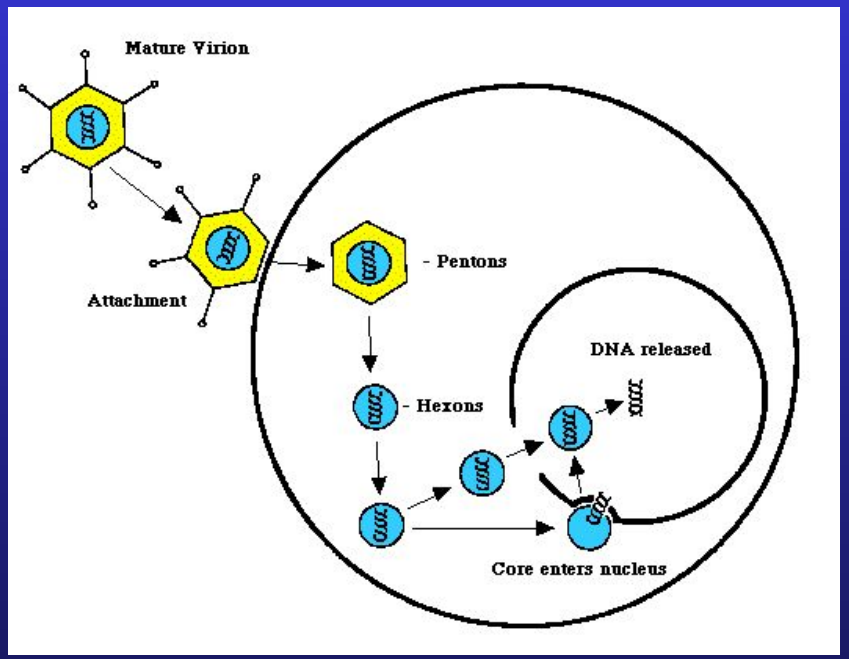
- Gene expression is often not as high as with adenovirus
- Same as retrovirus (except it can target non-dividing cells)
- Potential use in gene therapy provided safety is proven

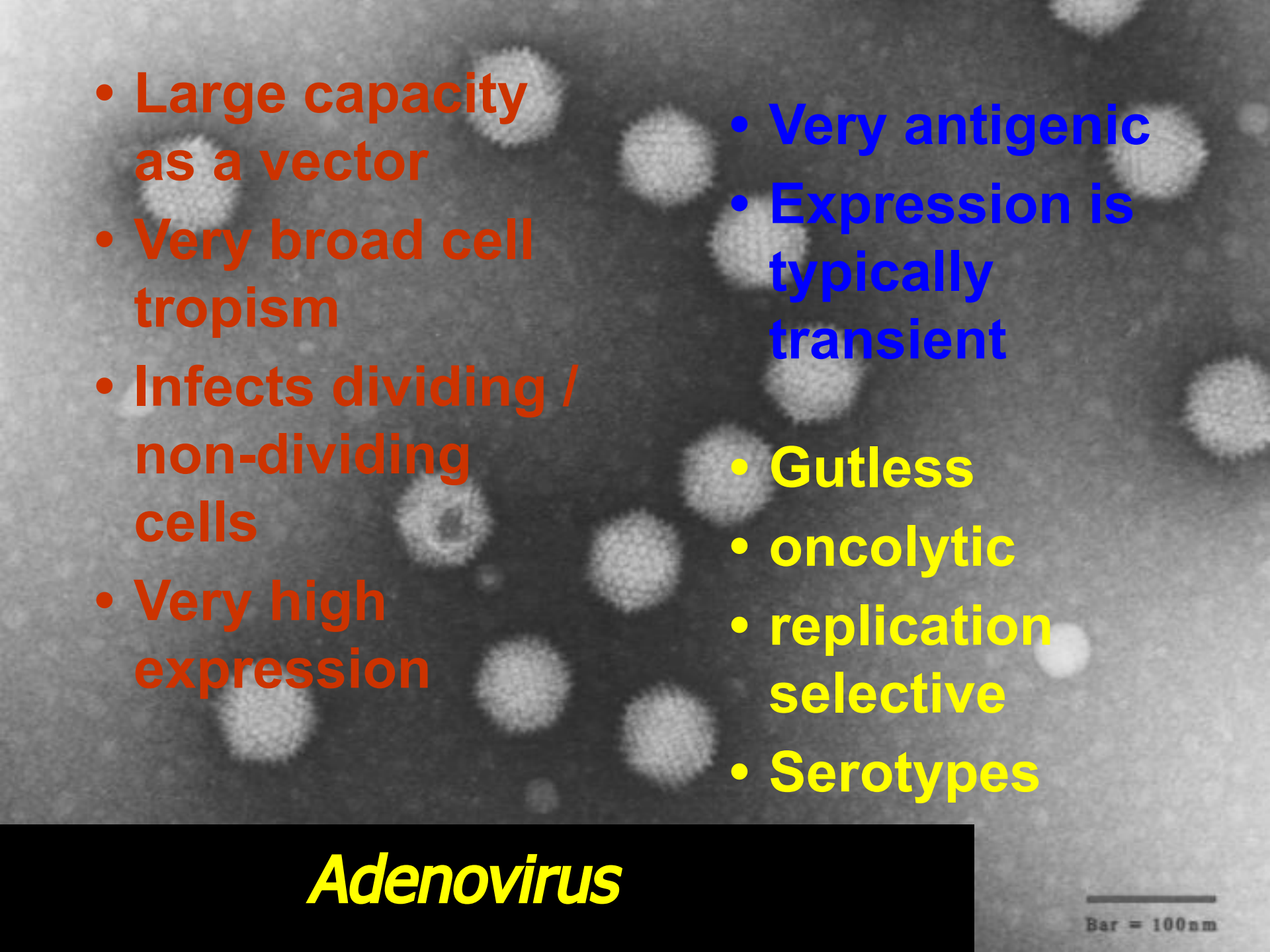
ADENOVIRUS

There are at least 10 proteins in the Adenovirus capsid



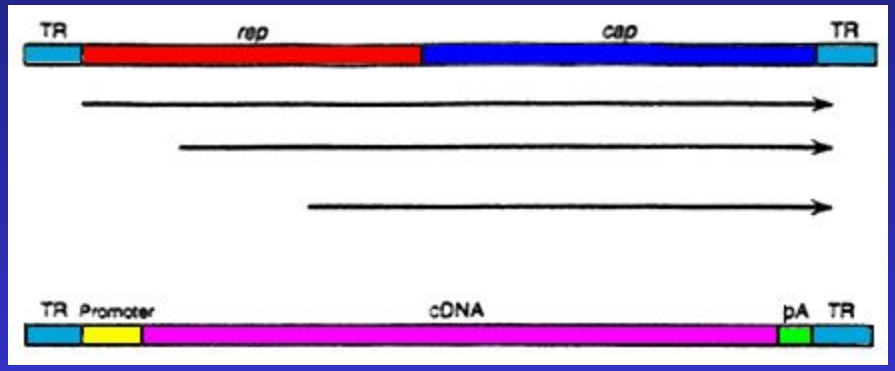
- **Double stranded DNA molecule**
- **Infects dividing and non-dividing cells**
- **Human CD46 receptor**
- **Does not integrate into host genome (episomal)**
- **Very high titer**

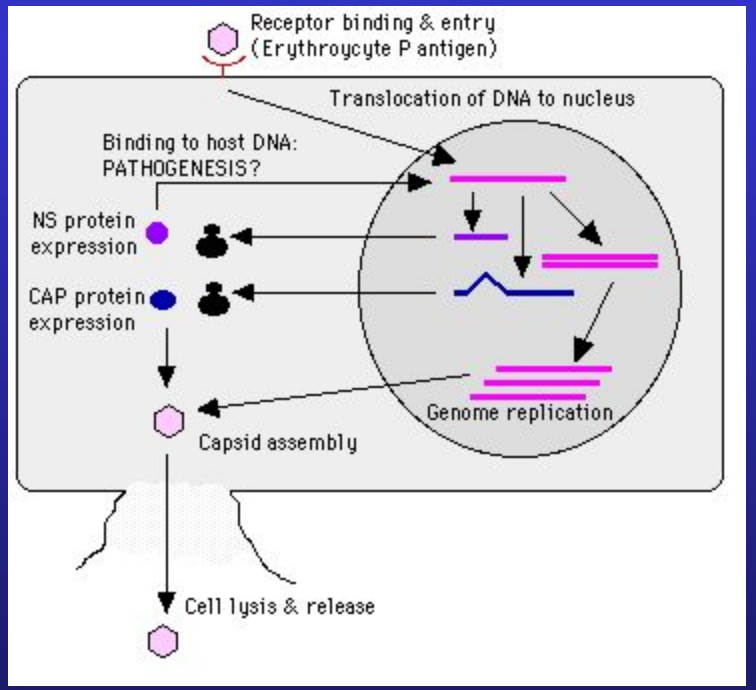



• Large capacity as a vector
• Very broad cell tropism
• Infects dividing / non-dividing cells
• Very high expression
• Very antigenic
• Expression is typically transient
• Gutless
• oncolytic
• replication selective
• Serotypes
Adenovirus
Bar = 100nm

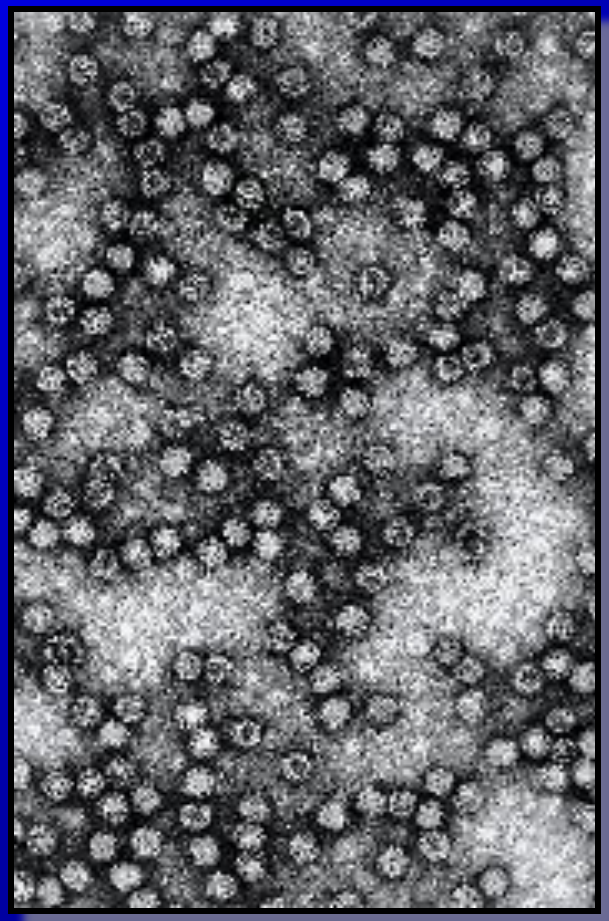
AAV

- 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"

- Excellent expression
- Off-the-shelf drug
- Industrial production
- Superior delivery
- Available vectors
- Most viral vectors are benign to humans

"Nay"

- Immunogenicity
- Insertional mutagenesis
- Germ-line transmission
- Narrow efficacy range + huge human variability
- Human variability