Methods of Gene Transfer (I)

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Objectives of this lecture

By the end of this lecture you will be able to:

1. Identify the two main methods for gene transfer
2. Compare between different viral vectors
3. Select a specific vector according to the therapeutic need

Gene Transfer

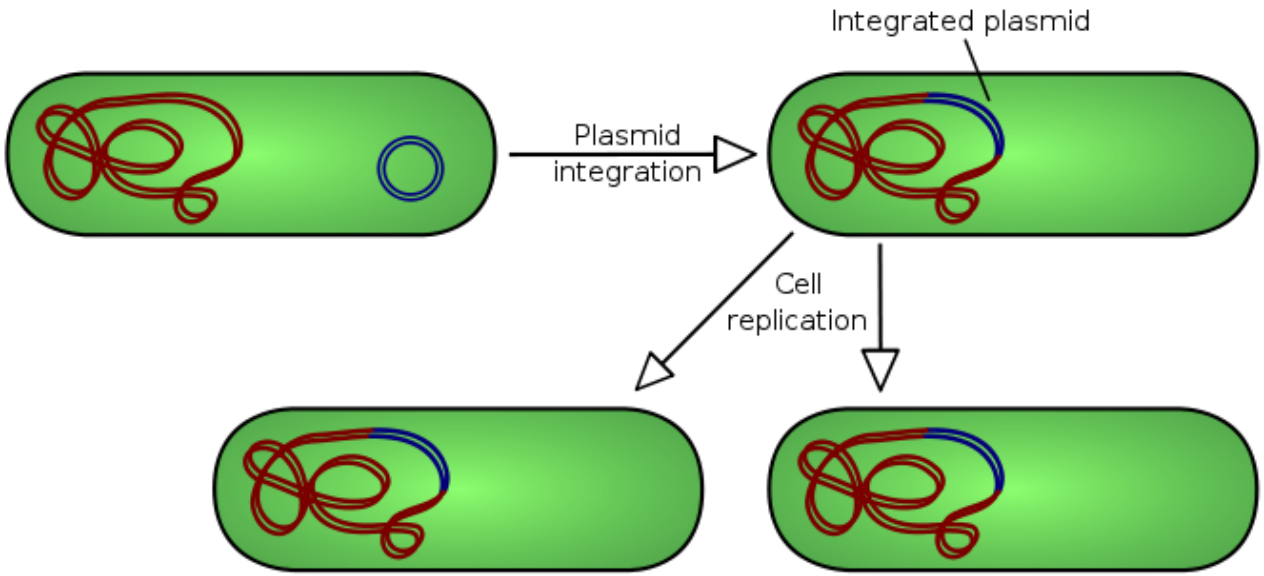
- **Transformation:** introduction of genetic materials into bacteria
- **Transfection:** introduction of genetic materials into eukaryotic cells (e.g. fungi, plant, or animal cells)
- **Transduction:** introduction of genetic materials ***using*** viruses
- **Lipofection:** introduction of genetic materials ***using*** liposomes

Stable vs. Transient Gene Transfer

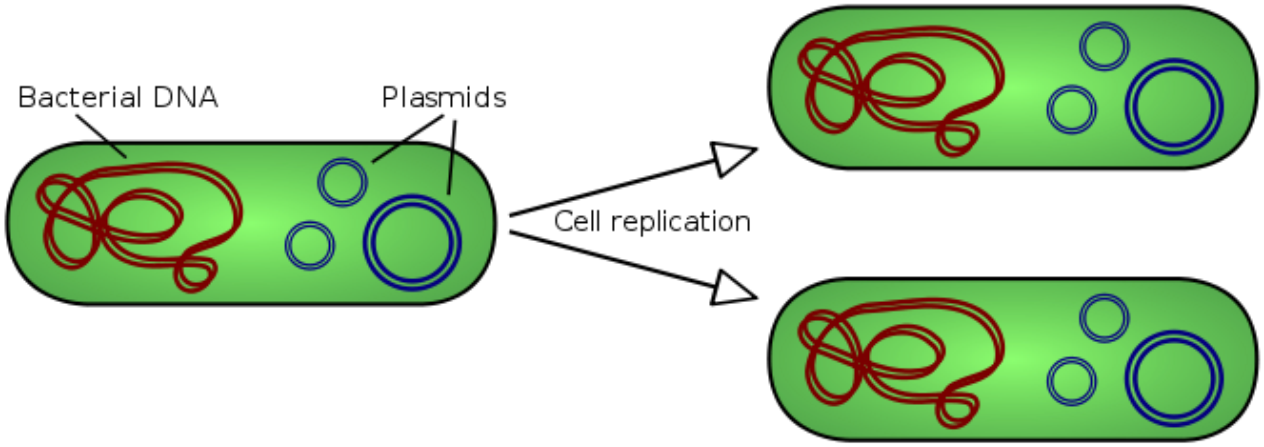
- **Stable Gene Transfer:** achieved by plasmid integration in the host genome or episomal replication of the transferred plasmid.

- **Transient Gene Transfer:** the foreign DNA is usually not integrated into the nuclear genome and will be degraded or diluted through mitosis

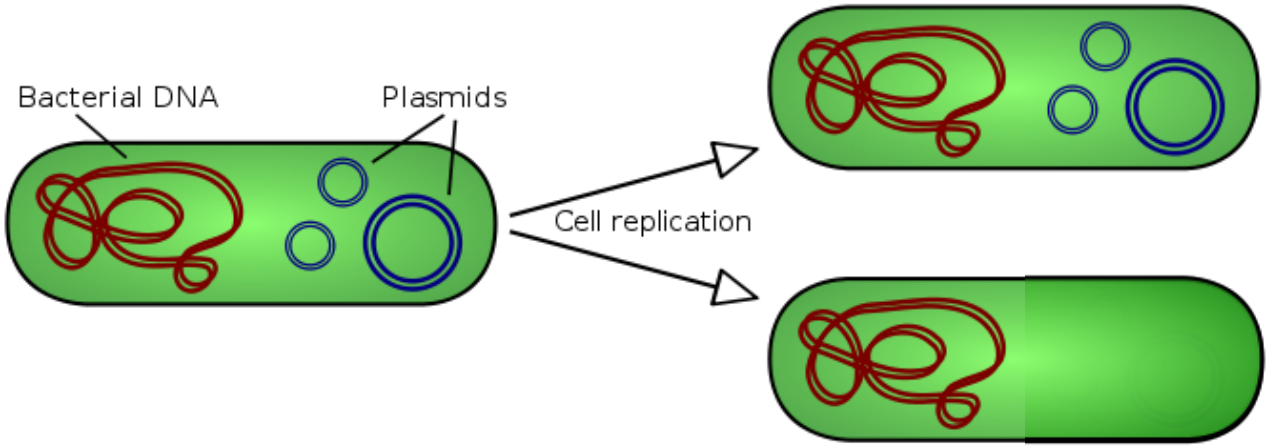
Plasmid Integration

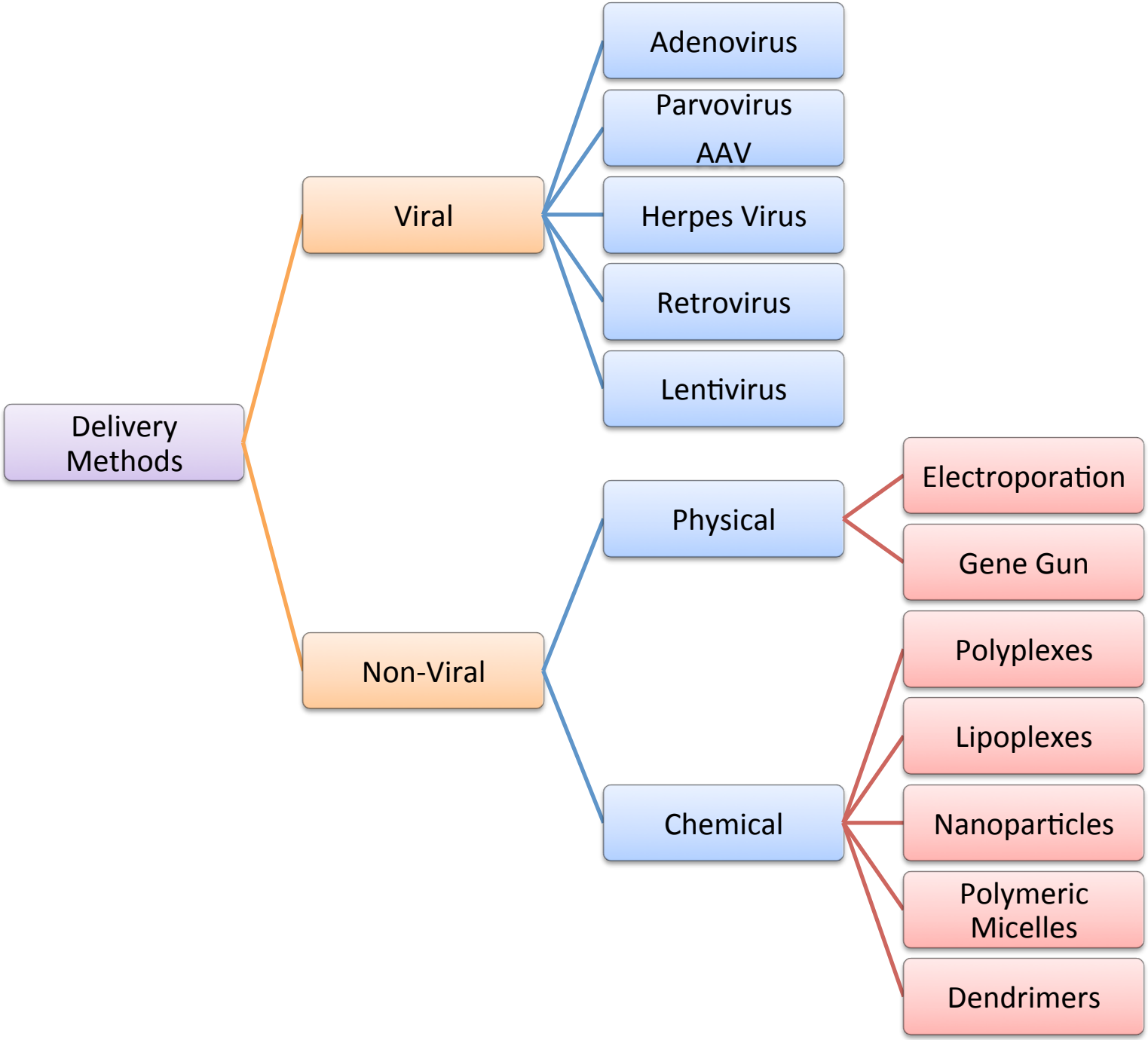


Episomal Vector with Ori

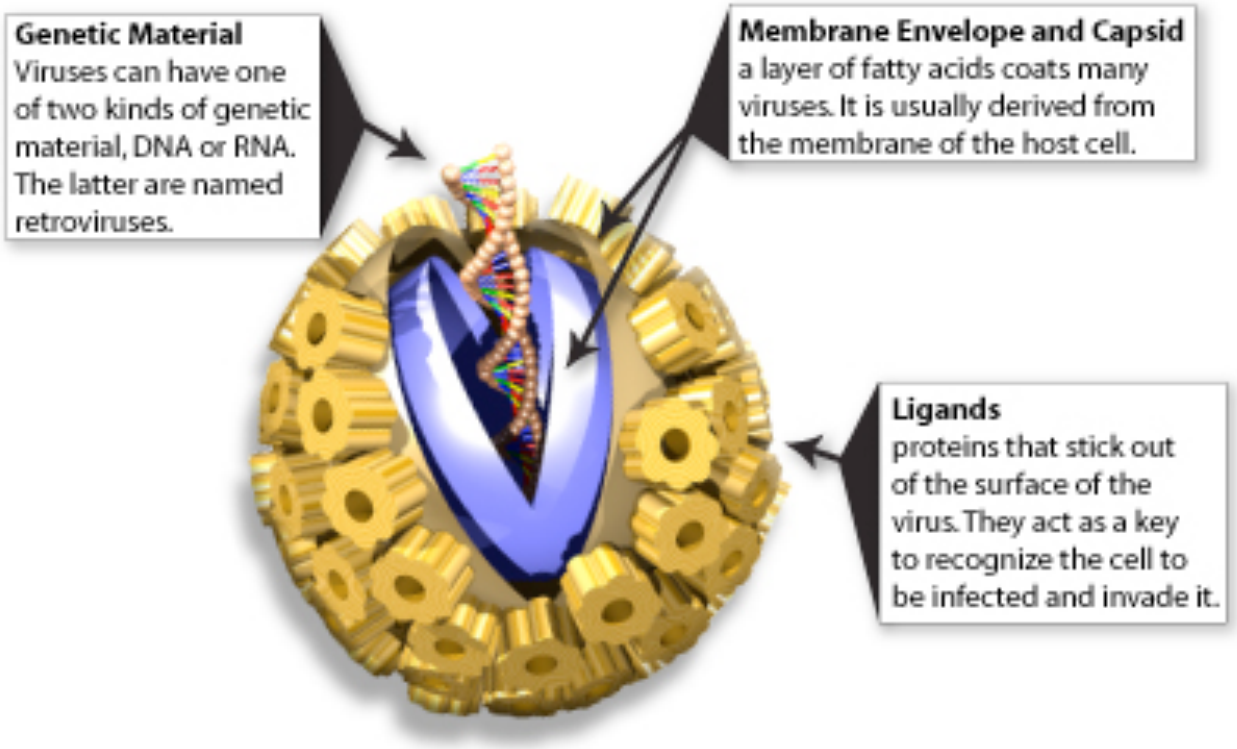


Transient Gene Transfer

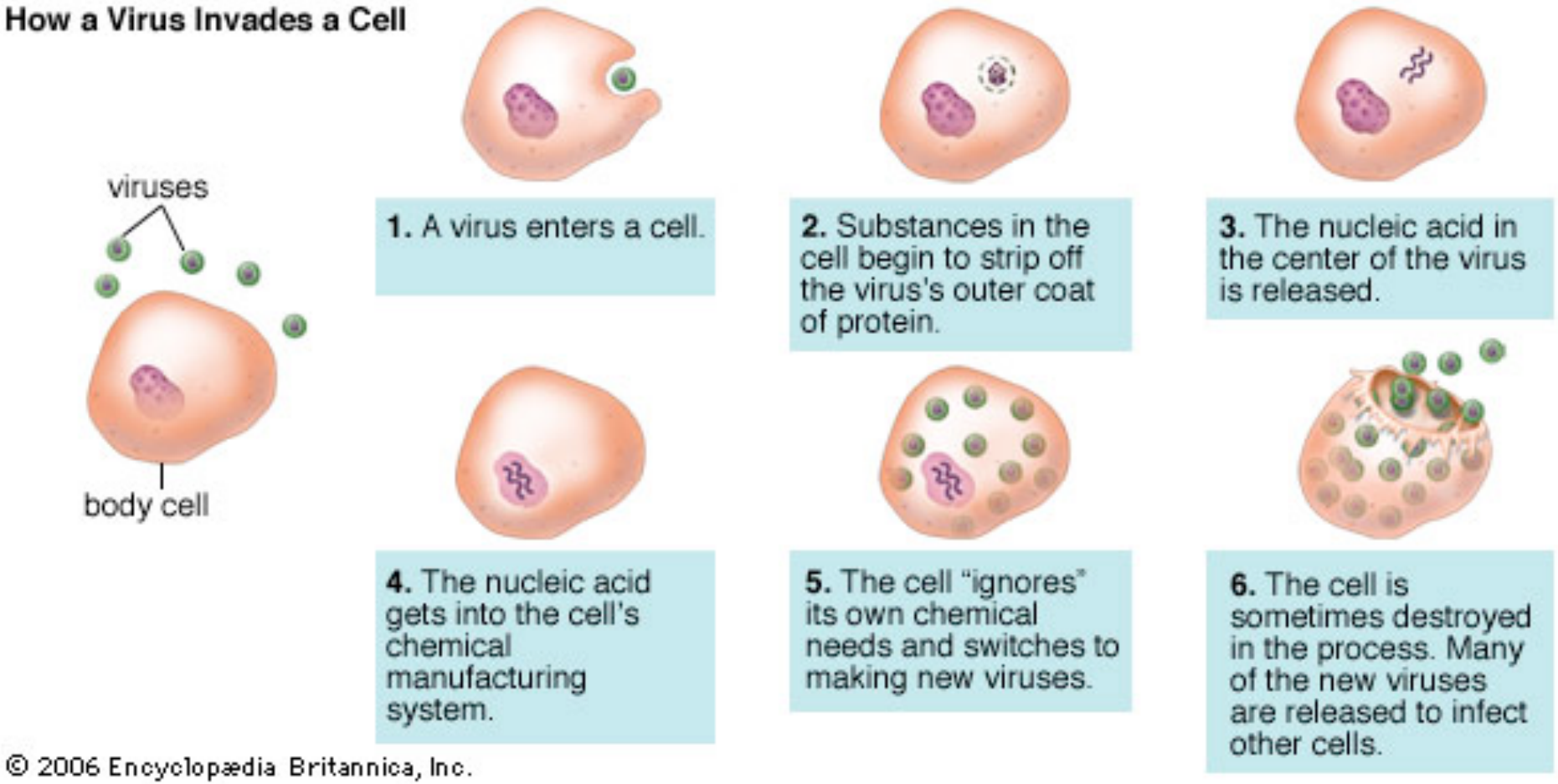



Delivery Methods
Viral
Adenovirus
Parvovirus AAV
Herpes Virus
Retrovirus
Lentivirus
Non-Viral
Physical
Electroporation
Gene Gun
Chemical
Polyplexes
Lipoplexes
Nanoparticles
Polymeric Micelles
Dendrimers

Why Viruses?

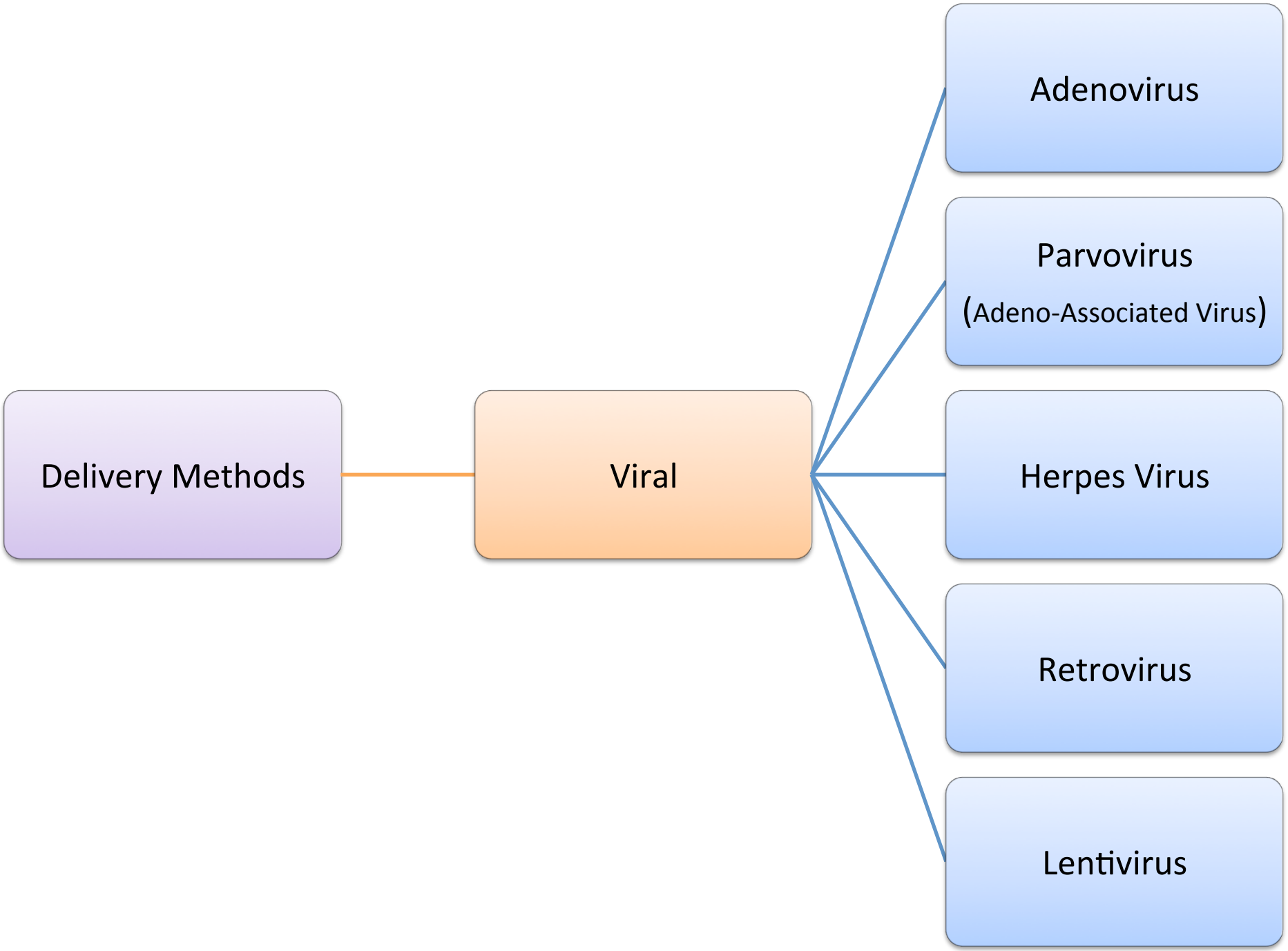

Genetic Material
Viruses can have one of two kinds of genetic material, DNA or RNA. The latter are named retroviruses.
Membrane Envelope and Capsid
a layer of fatty acids coats many viruses. It is usually derived from the membrane of the host cell.
Ligands
proteins that stick out of the surface of the virus. They act as a key to recognize the cell to be infected and invade it.

How viruses work

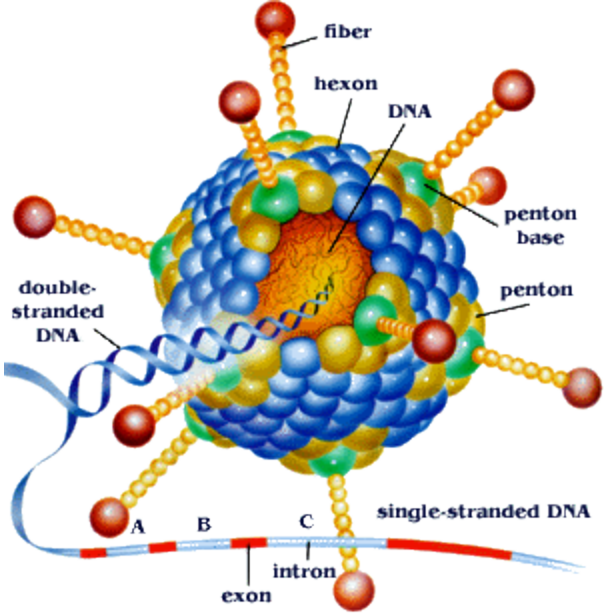


General Requirements for Viral Vectors

- Must be replication defective to prevent uncontrolled spreading *in vivo*
- Should not process undesirable properties
- The viral genome should be able to accommodate the therapeutic gene


Delivery Methods
Viral
Adenovirus
Parvovirus
(Adeno-Associated Virus)
Herpes Virus
Retrovirus
Lentivirus

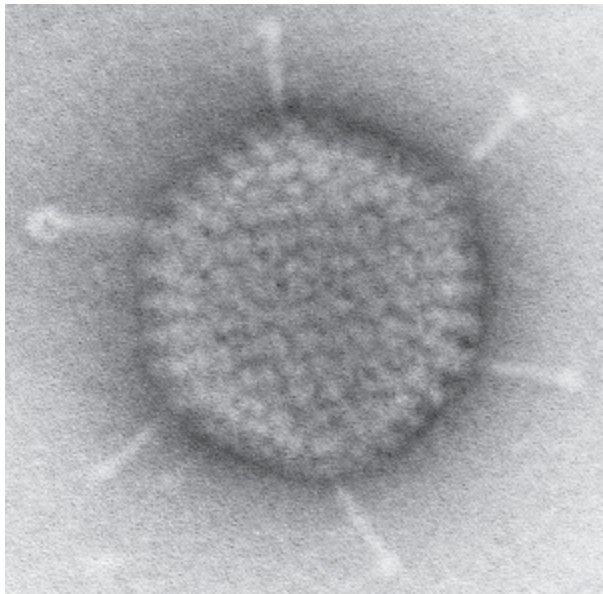
Adenovirus



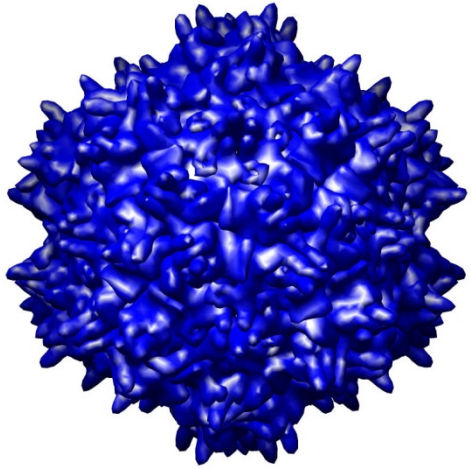
- dsDNA virus
- Recombinant vector
- Replication deficient

- Advantages:
  - High titer viral stocks
  - Broad host species spectrum
  - Wide range of target cells
  - Extremely high transduction efficiency
  - Transduction of primary or non-dividing cells

- Disadvantages:
  - Transient gene expression
  - Immunogenicity



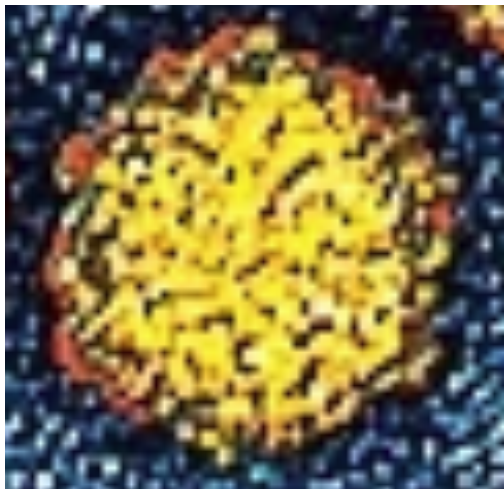
Adeno-Associated Virus (Parvovirus)



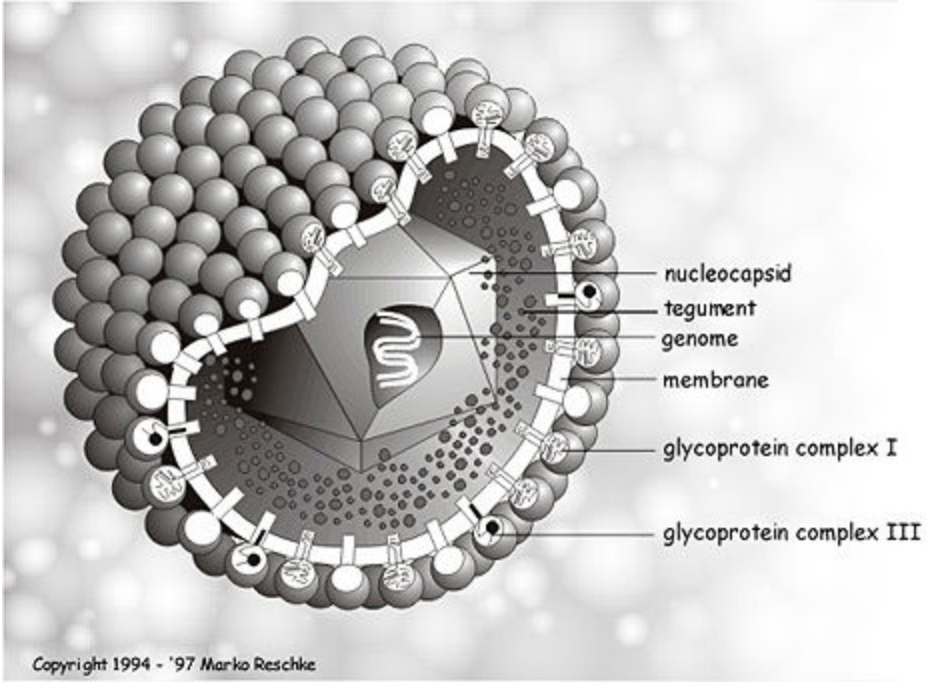
- ssDNA virus
- Wild type and Recombinant

- Advantages:
  - Transduction of non-proliferating cells
  - Possible chromosomal integration
  - Specific site for gene insertion in host cells (only with wild-type virus)

- Disadvantages:
  - Possible mutagenesis
  - Immunogenicity



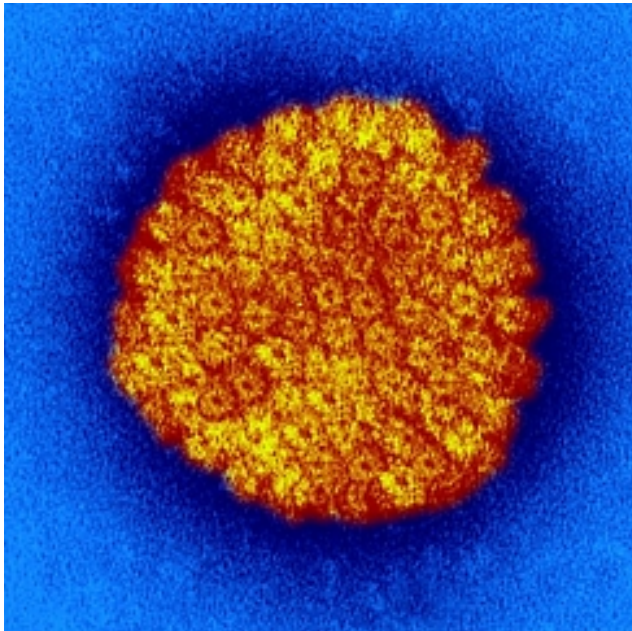
Herpes Virus



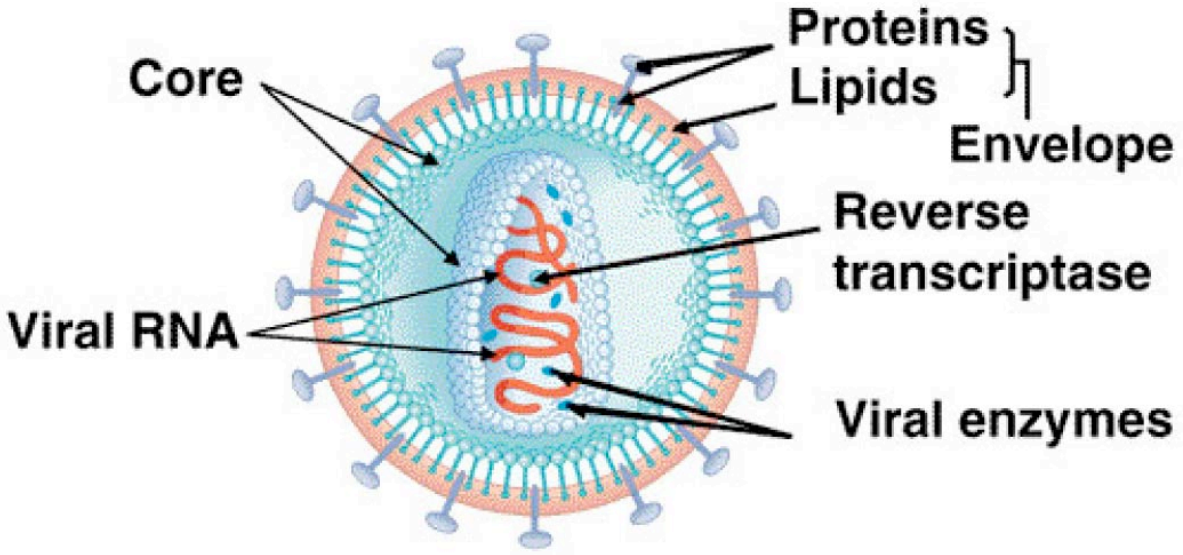
- dsDNA virus
- Recombinant vector
- Replication deficient

- Advantages:
  - Transduction of non-dividing cells
  - Affinity to neuronal tissues
  - Accommodates large fragment of foreign DNA

- Disadvantages:
  - Transient gene expression



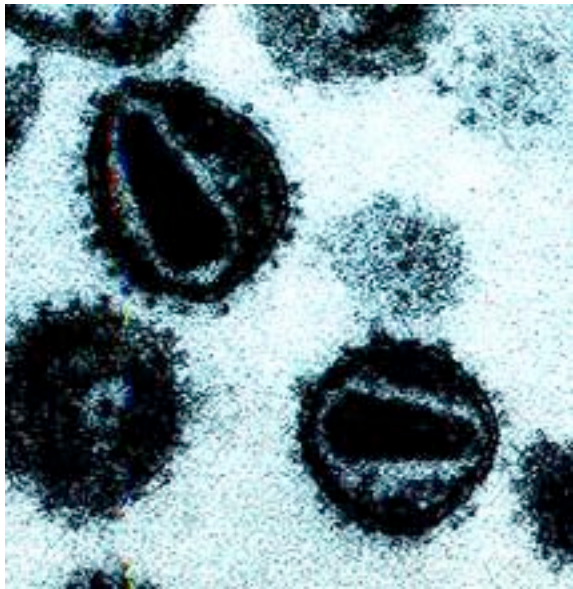
Retrovirus

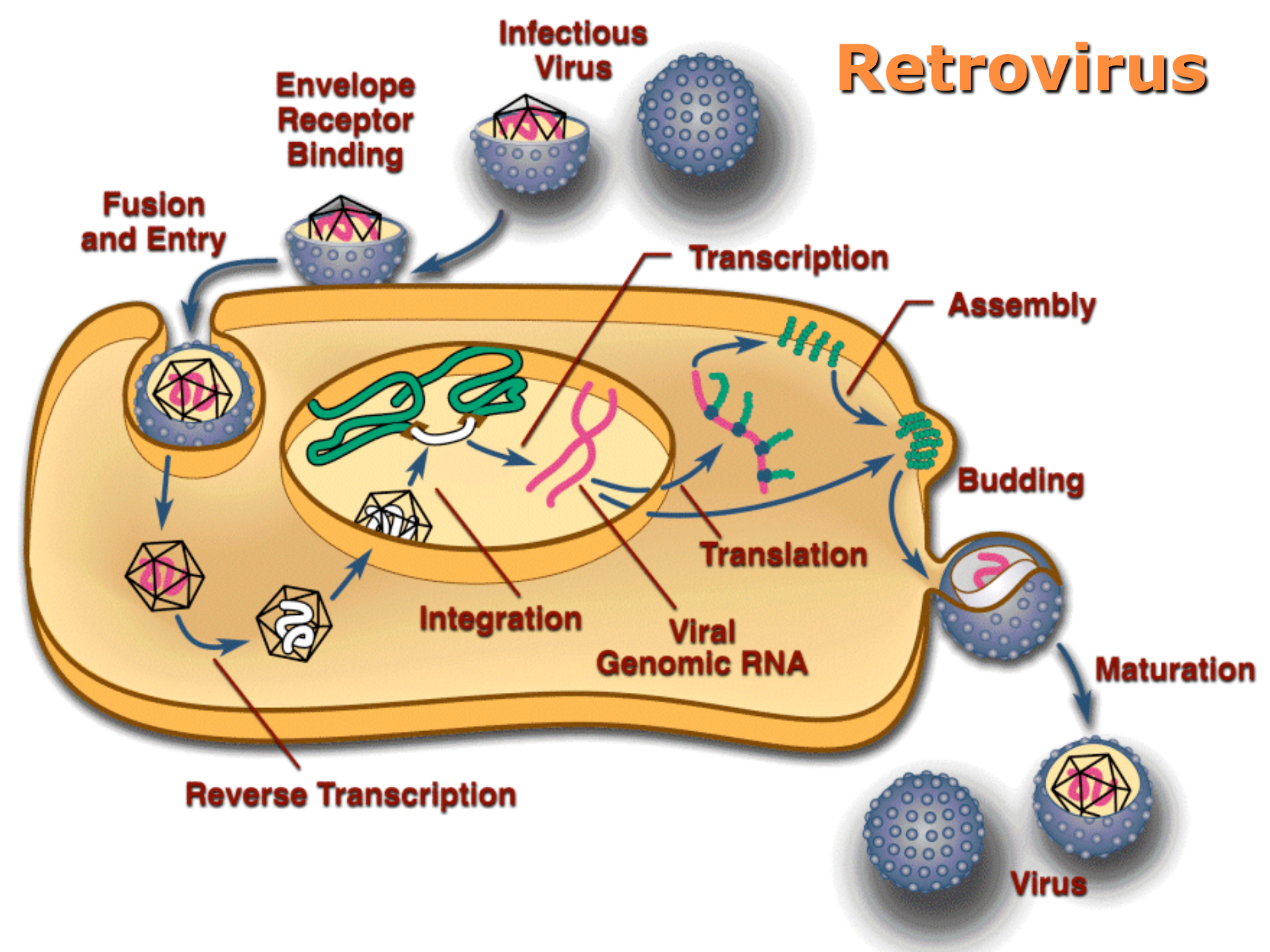


- ssRNA virus

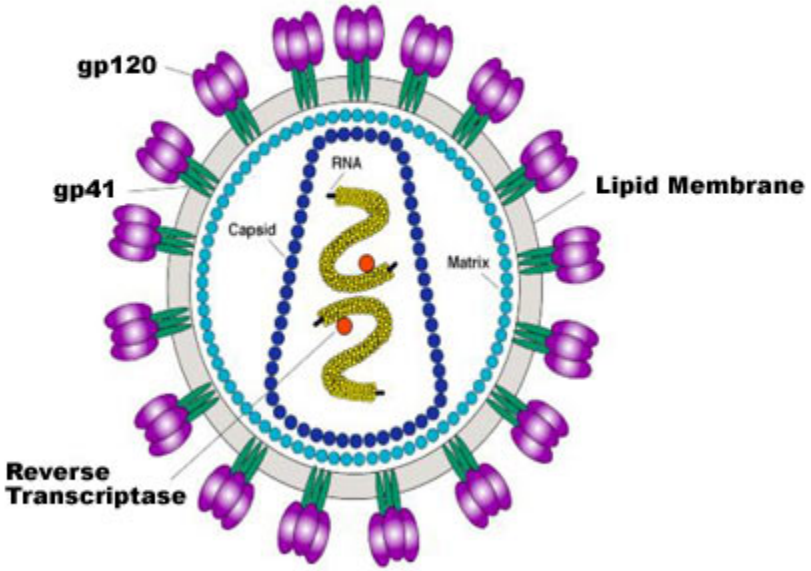
- Advantages:
  - Wide host range
  - Stable integration in host genome

- Disadvantages:
  - Difficult to get high titer stocks
  - Limited to proliferating cells
  - No evidence of permanent gene expression
  - Possibility of tumor formation




Retrovirus
Infectious Virus
Envelope Receptor Binding
Fusion and Entry
Transcription
Assembly
Budding
Translation
Integration
Viral Genomic RNA
Reverse Transcription
Maturation
Virus

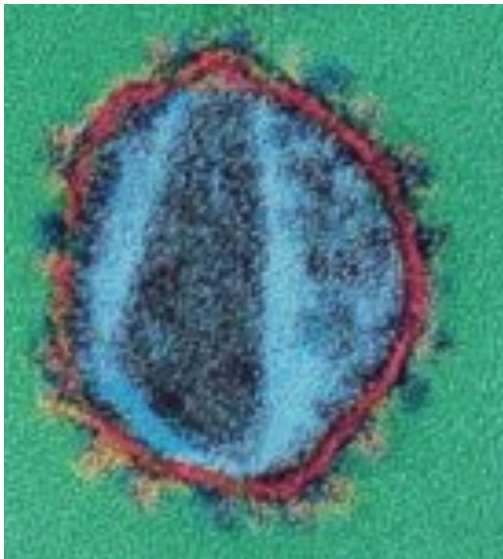
Lentivirus



- Subclass of Retroviruses
- ssRNA virus

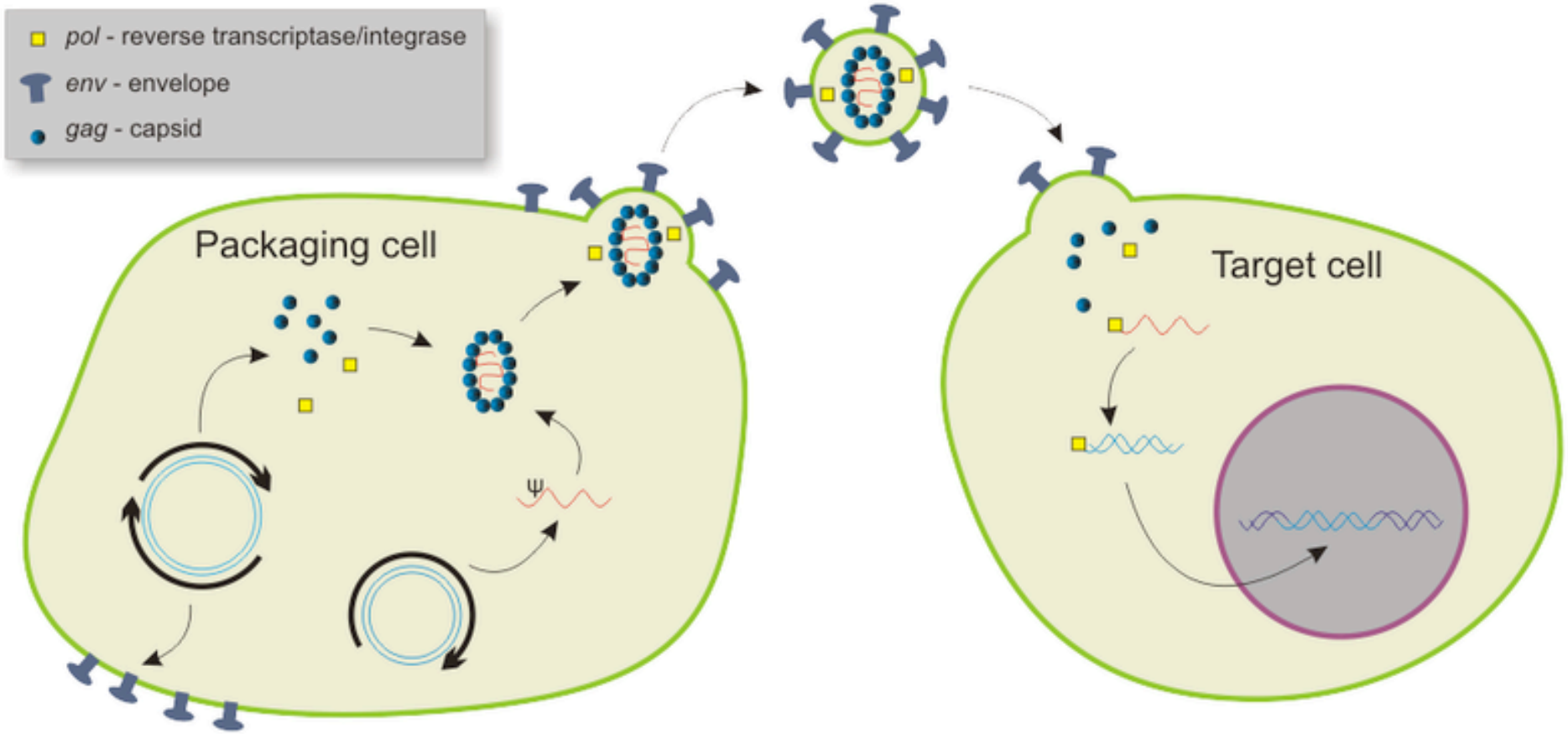
- Advantages:
  - Stable integration in host genome
  - Ability to integrate in the genome of non-dividing cells
  - Less tendency for oncogenesis

- Disadvantages:
  - Possibility of tumor formation

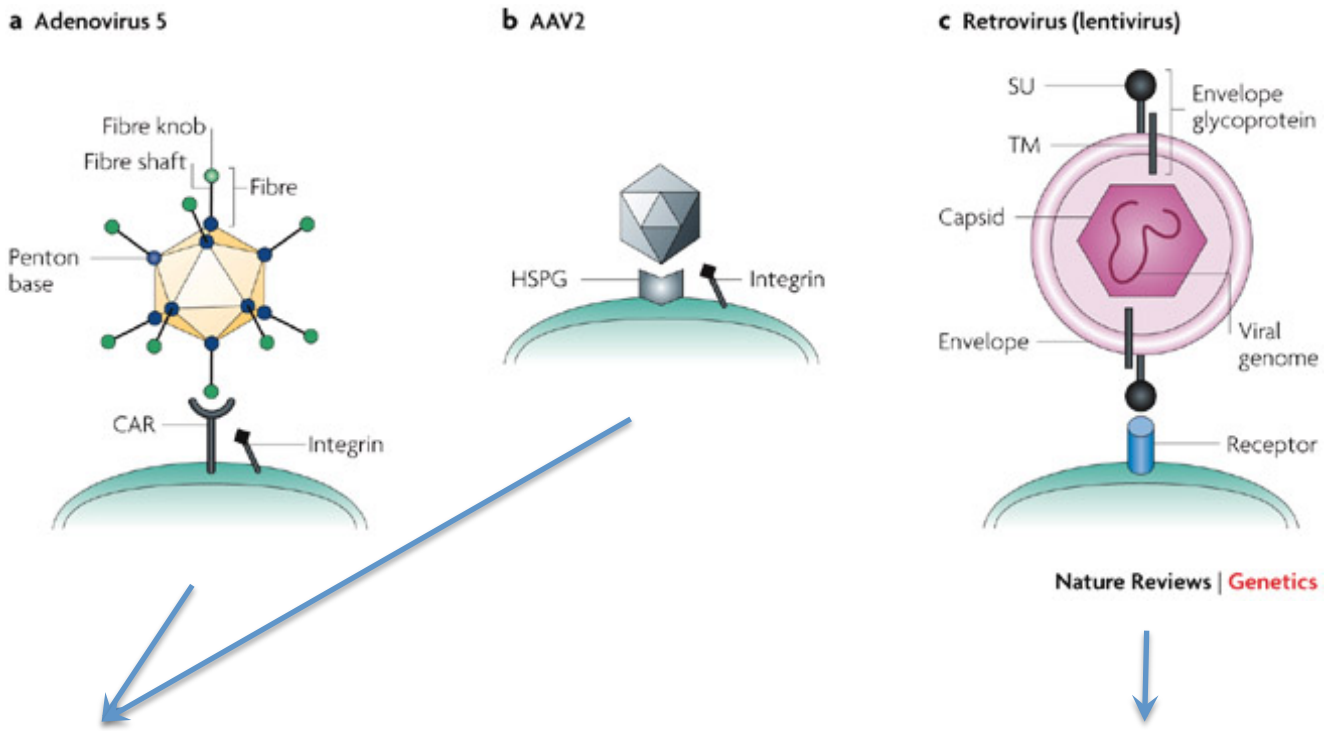


Lentivirus Packaging & Replication

- For safety reasons, Lentivirus used for gene therapy never carries gene required for its replication. It is rather packaged in a packaging cell (a mammalian cell line e.g. HEK 293)



Mechanism of Cell Entry



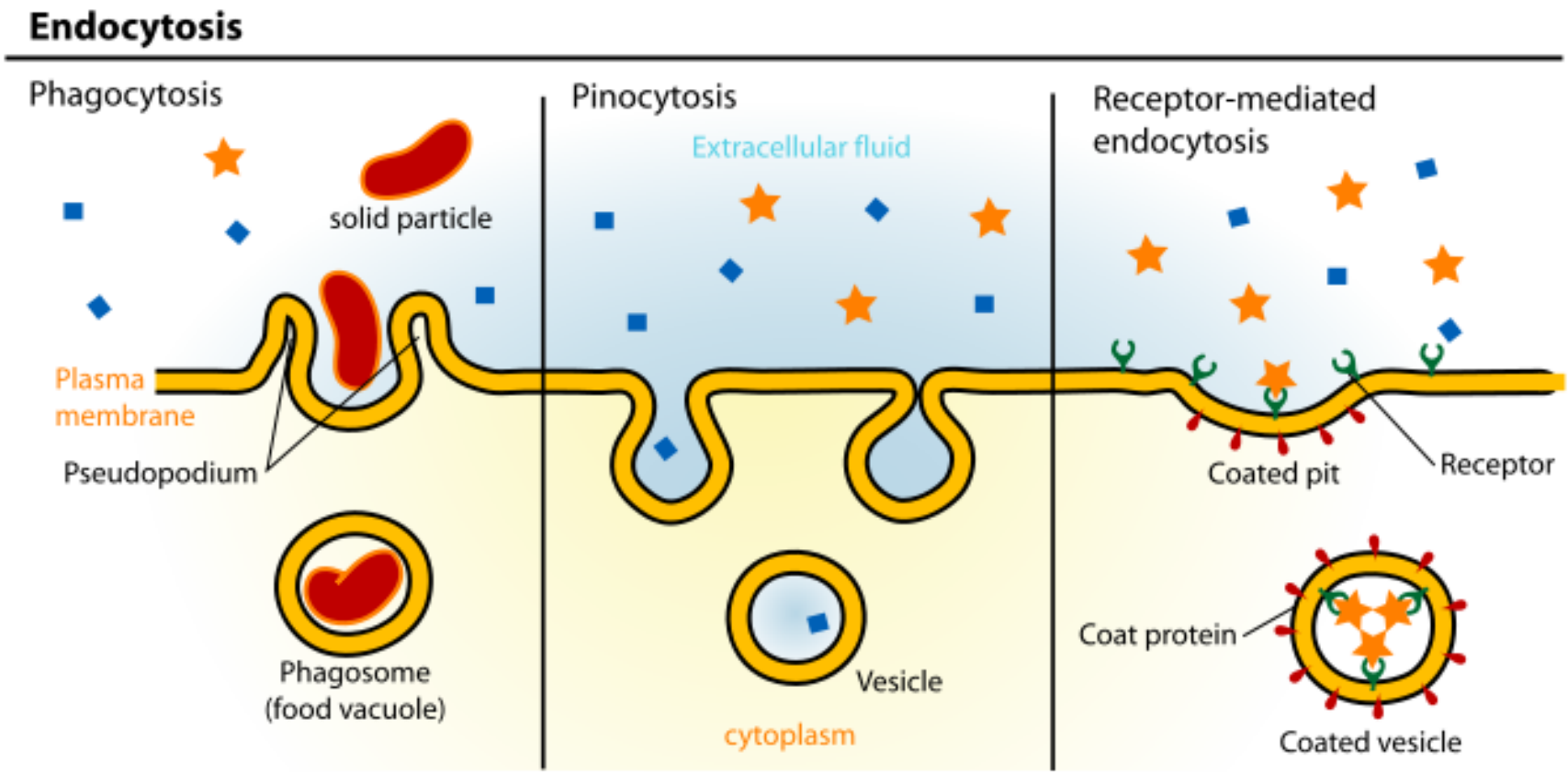
receptor-mediated endocytosis

non-enveloped viruses

membrane fusion

enveloped viruses

Endocytosis



Feature	Adenovirus	AAV	HSV	Retrovirus	Lentivirus
Particle size (nm)	70-100	20-25	150-200	100	100
Cloning capacity (kb)	8-10	4.9	40-50	8	9
Chromosomal integration	No	Possible	No	Yes	Yes
Vector yield (transducing unit/ mL)	High (10^{12})	High (10^{12})		Moderate (10^{10})	Moderate (10^{10})
Entry mechanism	RME[a]	RME[a]	MF[b]	MF[b]	MF[b]
Transgene expression	Short (weeks)	Moderate (months)	Moderate (months)	Long (years)	Long (years)
Oncolytic potential	Yes	No	Yes	No[c]	No[c]
Oncogenic potential	No	No	No	Yes	Yes
Risk of replication *in vivo*	Not concern	Not concern	Concern	Concern	Concern

[a] RME: receptor-mediated endocytosis
[b] MF: membrane fusion
[c] Retrovirus and lentivirus do not cause cancer cell lysis but they are able to mediate bystander effect

You are now able to:

- ✓ Identify the two main methods for gene transfer
- ✓ Compare between different viral gene transfer vectors
- ✓ Select a specific vector according to the therapeutic need