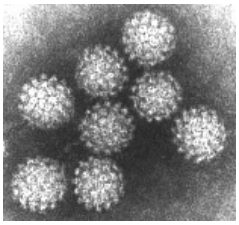
Structure, Function and Use of Viruses

Suggested reading: Molecular Cell Biology, Chapter 6.3

1

Virus

- Simplest organism on earth
- Made of nucleotides (DNA or RNA) & proteins
- Can not reproduce by itself
- Requires cellular machinery to complete its lifecycle
- Most abundant organism
  - ~1 billion virus particles in 1 mL of sea water
- Mostly harmless, but can cause severe disease
  - Influenza; HIV; HCV; etc.
- Genetically modified viruses are used to carry foreign DNA into a cell
  - Gene therapy
  - Recombinant protein expression



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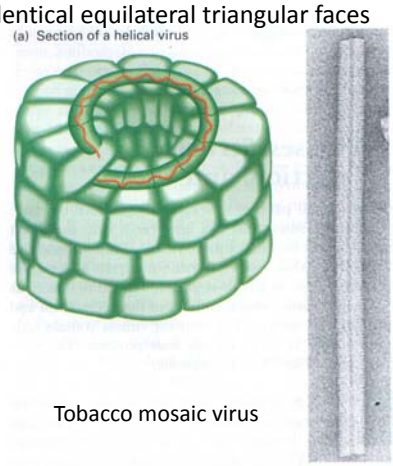
An Egyptian stone tablet from the 18th dynasty (1580-1350 BC)

Earliest depict of viral infection (polio)

3

Virus Structure

- Virus capsid is composed of multiple copies of one protein or a few different proteins encoded by the viral gene.
- Virion Structure
  - Helical: protein subunits form helical arrays around an RNA or DNA molecule
  - Icosahedral: 20 identical equilateral triangular faces



Tobacco mosaic virus

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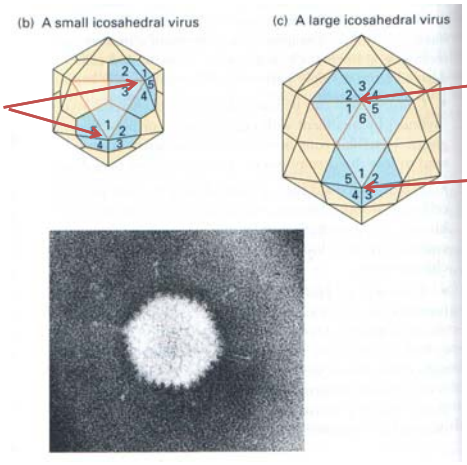
Icosahedral Structure



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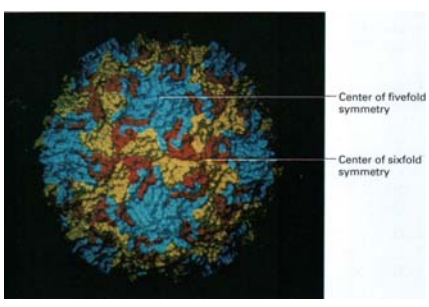

5 VP1

3 VP2 + 3 VP3

The piconavirus capsid

Common cold
foot-and-mouse virus

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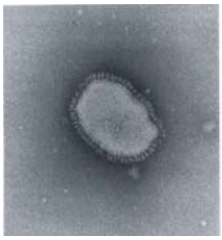
Non-enveloped vs. enveloped virus



- Enveloped virus: capsid is covered by a phospholipid bilayer membrane and has one or two virus-encoded envelop proteins (glycoproteins)
  - e.g. HIV, influenza, hepatitis B & C, smallpox
- Non-enveloped virus: without membrane
  - e.g. hepatitis A, Papilloma virus, polio virus

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Viral Host Range

- Most viruses can only infect a few species
  - Bacteria virus: bacteriophage
  - Animal virus
    - e.g. HIV infects human and monkey;
  - Plant virus
- Many animal viruses can only infect limited cell types
    - e.g. HIV can only infect immune cells (T cells)

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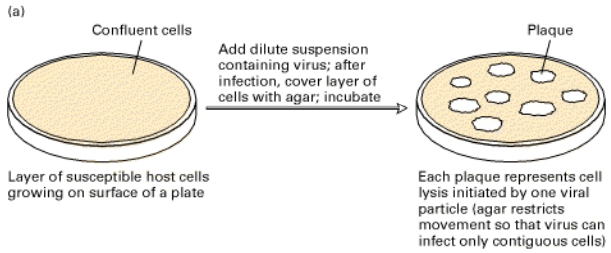
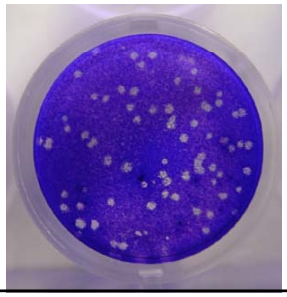
Plaque Assay

- Some viruses kill host cells and can be quantified using plaque assay



All progeny virions in a plaque are derived from a single parental virus.



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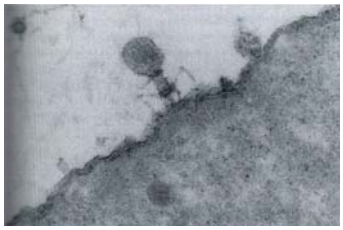
Virus Entry

- First step: association of a viral surface protein with a specific receptor on the host cell surface
- Virus receptor have other cellular functions
- A single type of receptor can be used by many viruses, and some viruses use several different receptors



T4 bacteriophage adsorbed onto an *E. coli* cell.

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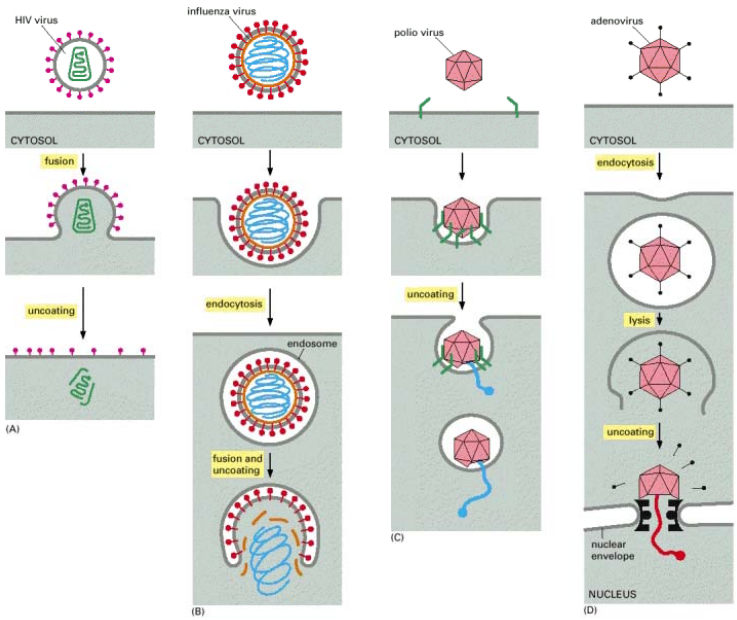
Four Virus Uncoating Strategies



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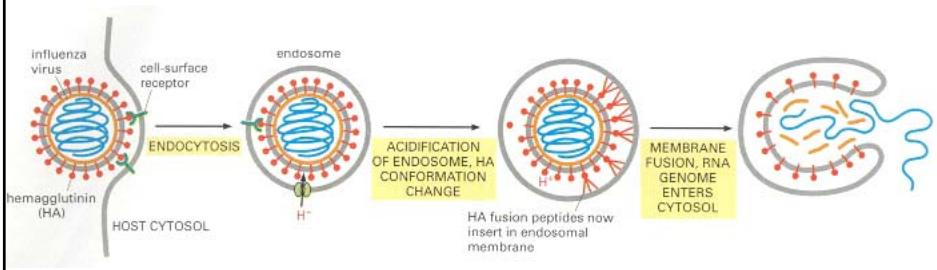
Entry Strategy of the Influenza Virus



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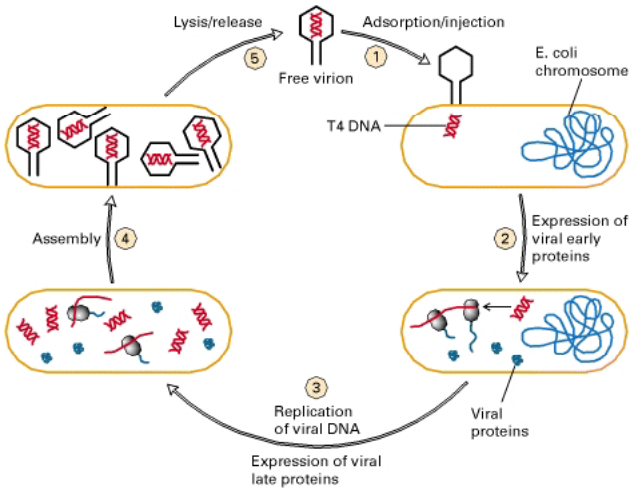
Bacteriophage Life Cycle



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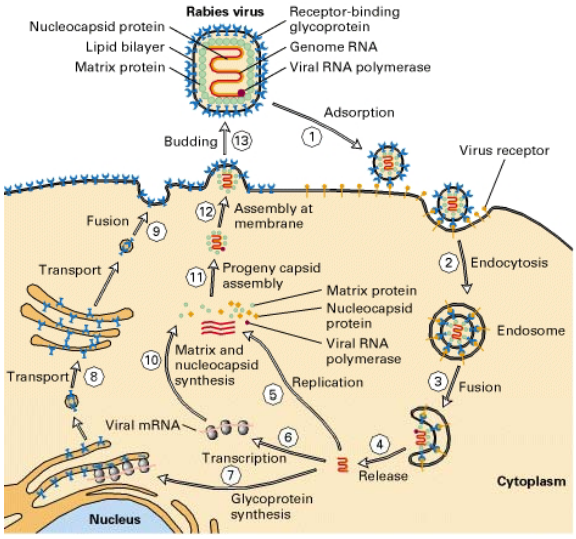
Rabies Virus Life Cycle



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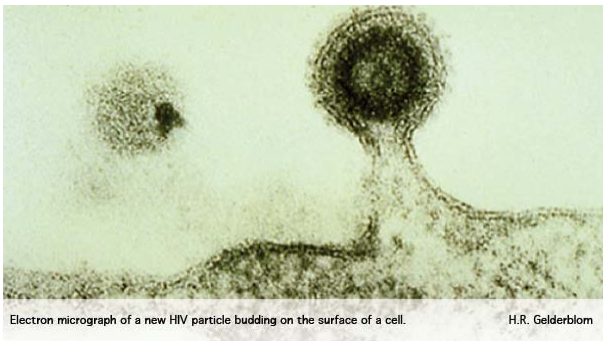
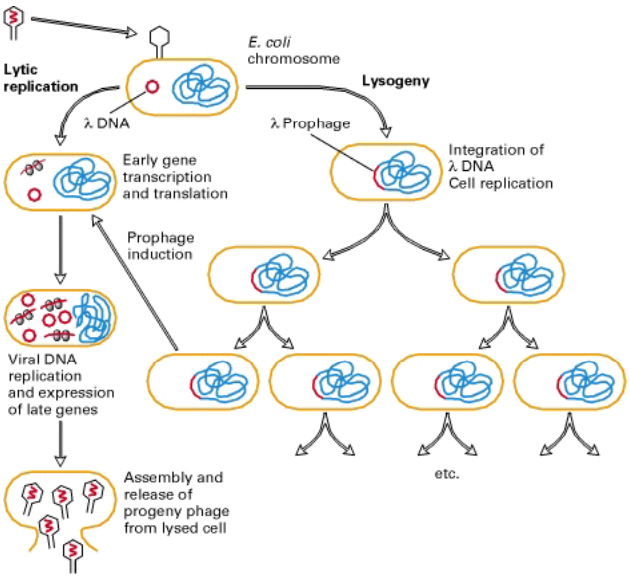


Electron micrograph of a new HIV particle budding on the surface of a cell. H.R. Gelderblom

http://www.soton.ac.uk/chemistry/research/tavassoli/Media/hiv.jpg

λ bacteriophage



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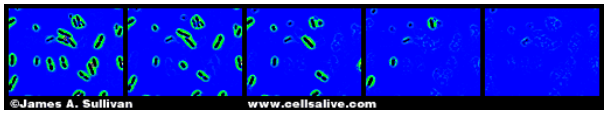
Bacterial Viruses

- T series DNA phage
  - Large lytic phage (T4 bacteria phage, ~170 kbp)
- Temperate phages
  - Bacteriophage λ, extensively used in DNA cloning
- Small DNA phages
  - ΦX174, M13 filamentous phage (6407 nucleotides)
- RNA phage
  - Encode only 4 proteins: RNA polymerase, 2 capsid proteins and an enzyme



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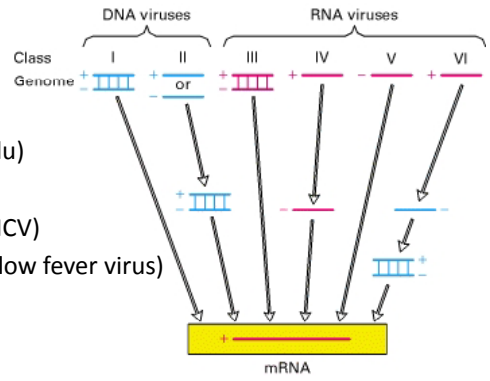
Animal Viruses

- Class I (double-stranded DNA virus)
  - e.g. **Adenovirus** (common cold), Herpesvirus (cold sore), Human papillomavirus (warts)
- Class II (single-stranded DNA virus)
  - e.g. Poxvirus (smallpox)
- Class III (double-stranded RNA virus)
  - e.g. Rotavirus (diarrhea, stomach flu)
- Class IV (positive-strand RNA virus)
  - IVa, single mRNA (e.g. poliovirus, HCV)
  - IVb, 2 mRNA (e.g. sindbis virus, yellow fever virus)
- Class V (negative-strand RNA virus)
  - Va, single RNA (e.g. measles virus)
  - Vb, multiple RNA (e.g. influenza)
- Class VI (retrovirus)
  - e.g. HIV



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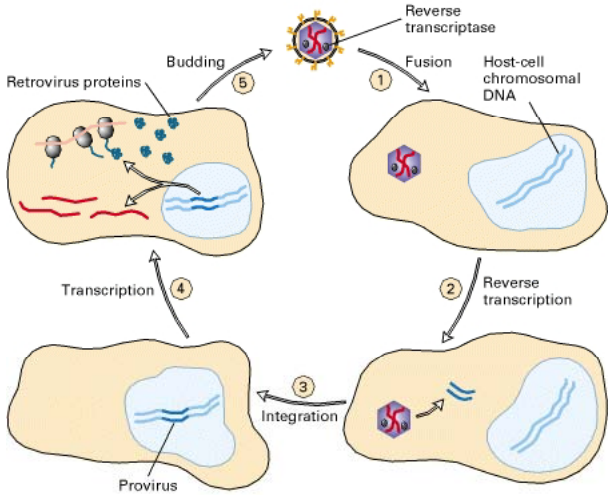
Retroviral life cycle



Show animation

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Gene Therapy

- A technique for correcting defective genes responsible for disease development
  - A normal gene may be inserted to replace a nonfunctional gene (most common)
  - An abnormal gene could be swapped for a normal gene (homologous recombination)
  - An abnormal gene could be repaired (selective reverse mutation)
  - The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered
- Viruses used as gene therapy vectors (most common)
  - Retroviruses
  - Adenoviruses (causes common cold)
    - Double-stranded DNA virus
  - Adeno-associated viruses
    - Single-stranded DNA virus that can insert their DNA to a specific site
  - Herpes simplex viruses (HSP1 causes cold sores)
    - Double-stranded DNA viruses
- Non-viral gene delivery

http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml

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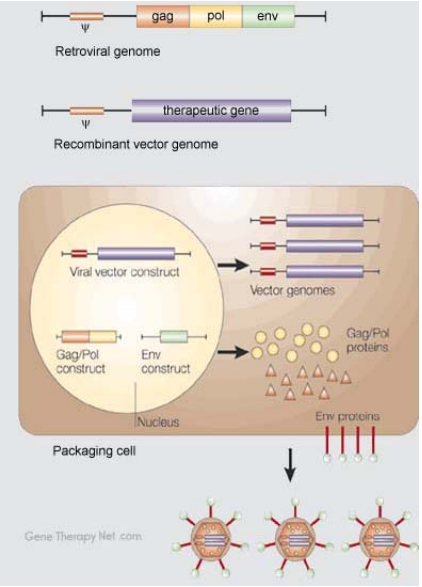
Retrovirus for human gene therapy

Advantage:

gene integration → stable expression
readily infect quiescent and dividing cells
relatively easy manipulation

Disadvantage:

can integrate gene into undesirable loci
→ cancer development



Producing recombinant retrovirus

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Adenovirus

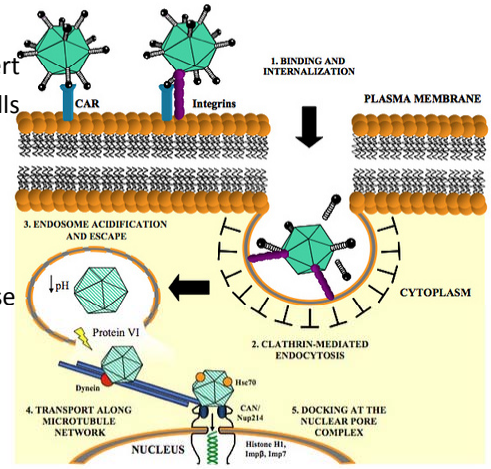
- Non-enveloped double-stranded DNA virus, 51 different serotypes in human and responsible for 5-10% of upper respiratory infection in children, and many infection in adults

Advantage:

Can accommodate large transgene insert
readily infect quiescent and dividing cells
relatively easy manipulation

Disadvantage:

Transient transgene expression
May elicit undesirable immune response



http://www.microbiologybytes.com/blog/tag/adenovirus/

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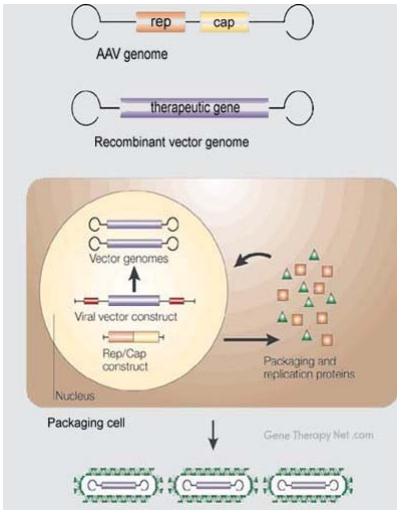
Adeno-associated virus

- Enveloped virus with single-stranded DNA
- Insert genetic material at a specific site on chromosome 19



Producing recombinant AAV

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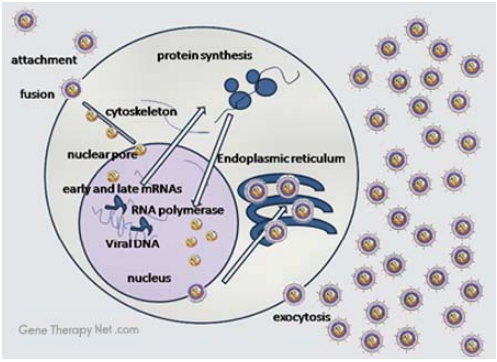
Herpes Simplex Virus

- Enveloped virus with a large linear double-stranded DNA of 150 kb.
- High transgenic capacity



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Types of Gene Therapy

- Somatic cells (most cells of the body)
  - Effects are often short-lived due to cell death, require repeated treatment
  - Difficult to transport gene to the target cells or tissue
  - All gene therapy to date has been directed at somatic cells
  - Diseases target: cystic fibrosis, muscular dystrophy, cancer, etc.
  - *ex vivo* vs. *in vivo*
- Germ cells (eggs or sperm)
  - Result in permanent changes that are passed down to subsequent generations
  - Done during embryologic development
  - So far prohibited

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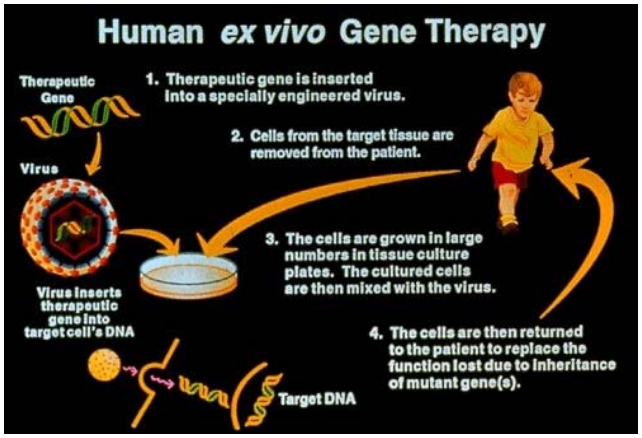
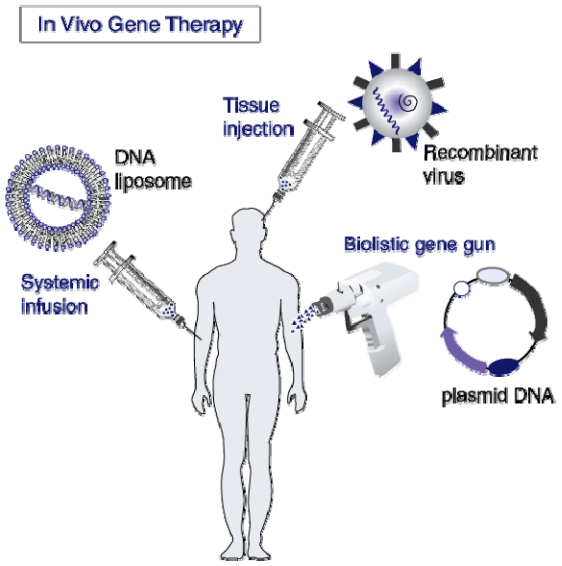
Somatic Gene Therapy- *ex vivo*



First human gene therapy trial
A four-year old girl became the first gene therapy patient on September 14, 1990 at the NIH Clinical Center. She has adenosine deaminase (ADA) deficiency, a genetic disease which leaves her defenseless against infections. White blood cells were taken from her, and the normal genes for making adenosine deaminase were inserted into them. The corrected cells were reinjected into her.

http://history.nih.gov/exhibits/genetics/sect4.htm 26

Somatic Gene Therapy- *in vivo*



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What factors have kept gene therapy from becoming an effective treatment for genetic disease?

- Short-lived nature of gene therapy
  - Therapeutic DNA must remain functional and the cells must be long-lived
  - Patients need to undergo multiple rounds of gene therapy
- Immune response
- Problems with viral vectors
  - Viral vectors may recover its ability to cause disease
- Multigene disorders
  - Heart disease, high blood pressure, Alzheimer's disease, arthritis, diabetes

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Which of the following statements are true of viruses?

A. All contain nucleic acid.
B. All contain lipid.
C. All can reproduce outside of living cells.
D. They always lyse the cells that they infect

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All of the following statements about DNA viruses are correct EXCEPT

A. They can have single-stranded or double-stranded genomes.
B. They can have circular or linear genomes.
C. They must replicate in the cytoplasm
D. They can transform cells.

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Certain chemicals inhibit the step just after the penetration of viruses in eukaryotic cells. If you used this type of chemical in studies of the viral infection process, which step(s) in the replication cycle would still occur?

A. Attachment
B. Uncoating
C. Replication
D. Encapsidation

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