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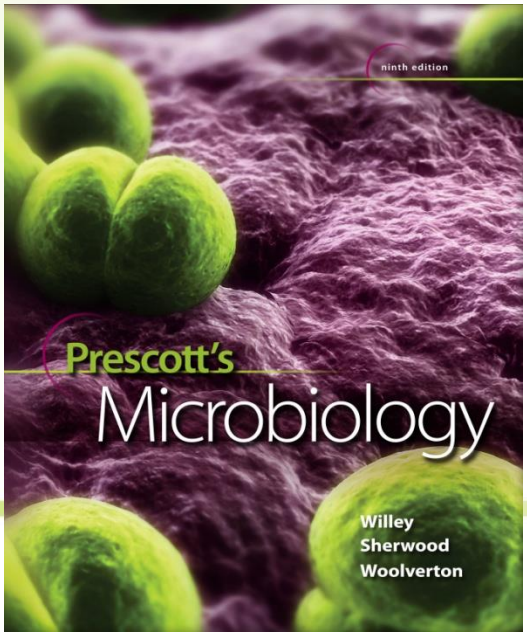


Viruses and Other Acellular Infectious Agents

6.1 Viruses

1. Defines the terms virology, bacteriophages, and phages.
2. List organisms that are hosts to viruses.

Acellular Agents

- Viruses – protein and nucleic acid
- Viroids – only RNA
- Satellites – only nucleic acids
- Prions – proteins only

Viruses

- Major cause of disease
  - also importance as a new source of therapy
  - new viruses are emerging
- Important members of aquatic world
  - move organic matter from particulate to dissolved
- Important in evolution
  - transfer genes between bacteria, others
- Important model systems in molecular biology

General Properties of Viruses

- Virion
  - complete virus particle
  - consists of ≥1 molecule of DNA or RNA enclosed in coat of protein
  - may have additional layers
  - cannot reproduce independent of living cells nor carry out cell division
    - but can exist extracellularly

Virions Infect All Cell Types

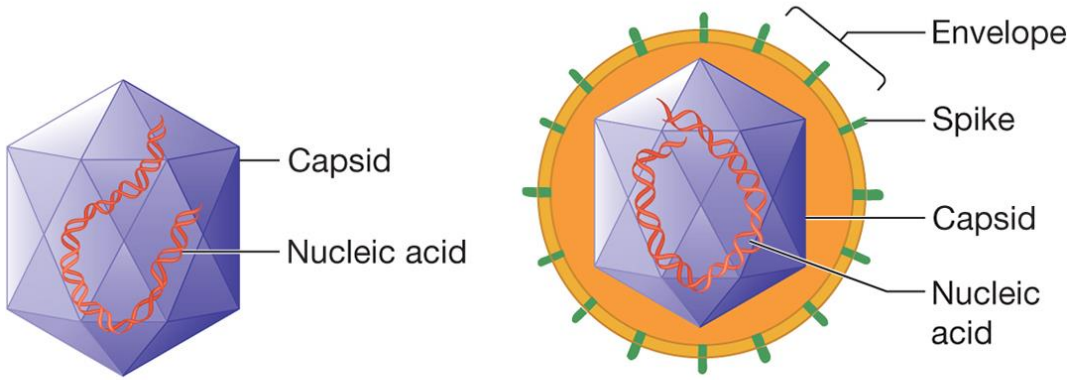
- Bacterial viruses called bacteriophages (phages)
- Few archaeal viruses
- Most are eukaryotic viruses
  - plants, animals, protists, and fungi
- Classified into families based on
  - genome structure, life cycle, morphology, genetic relatedness

6.2 Virion structure

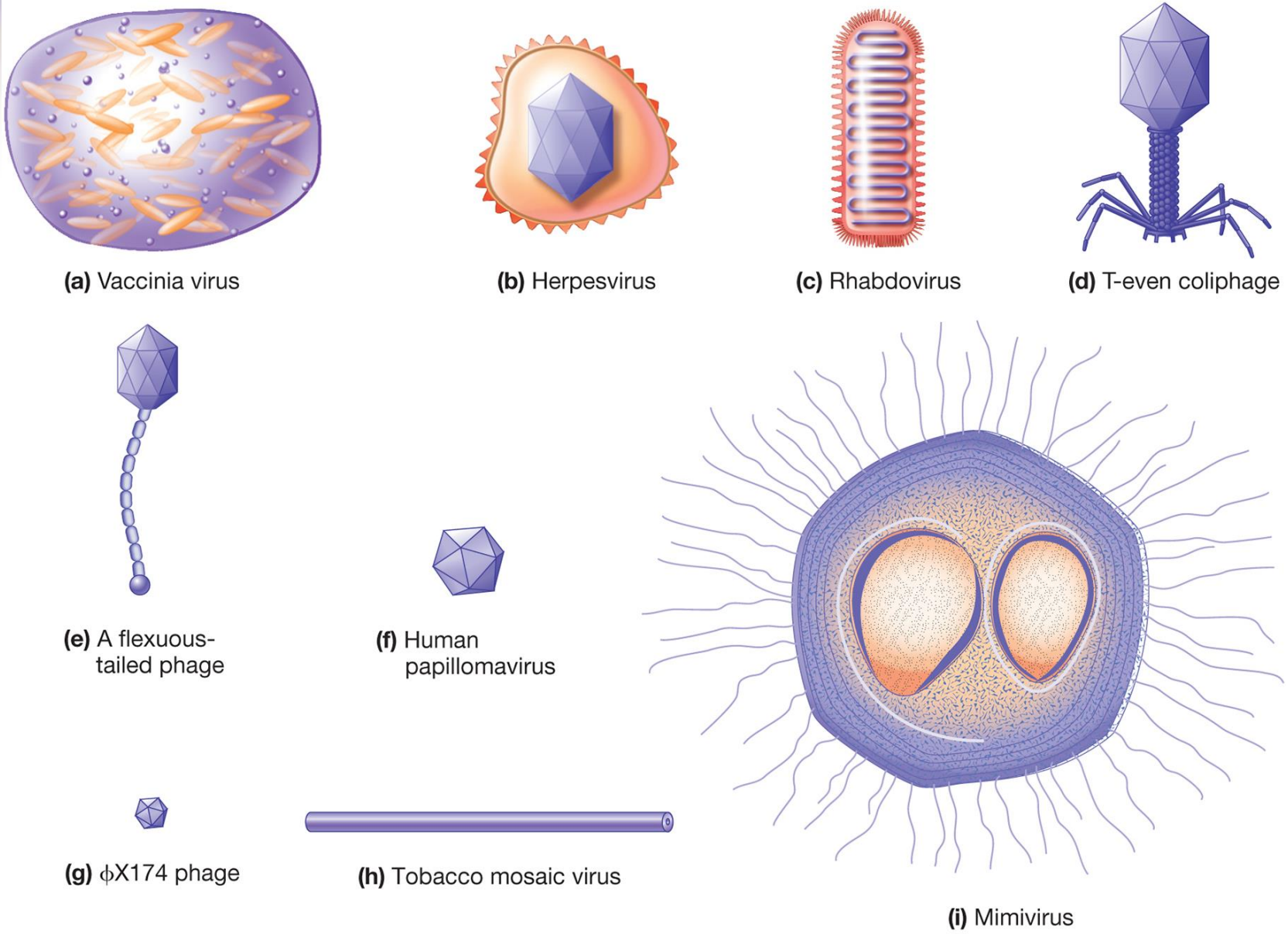
1. State the size range of virions.
2. Identify the parts of a virion and describe their function.
3. Distinguish enveloped viruses from nonenveloped viruses.
4. Describe the types of capsid symmetry.

The Structure of Viruses

- Virion size range is ~10–400 nm in diameter and most viruses must be viewed with an electron microscope
- All virions contain a nucleocapsid which is composed of nucleic acid (DNA or RNA) and a protein coat (capsid)
  - some viruses consist only of a nucleocapsid, others have additional components
- Envelopes



(a) Nonenveloped virus **(b)** Enveloped virus

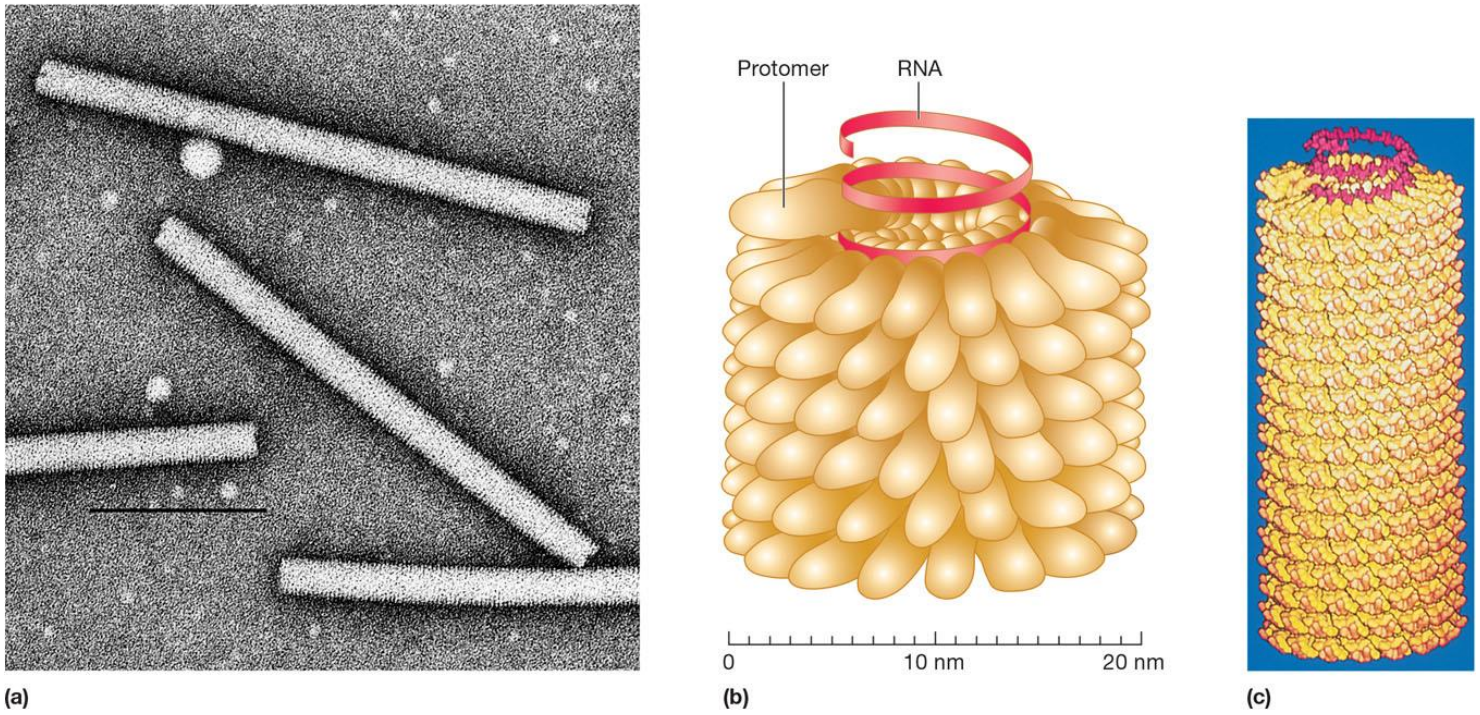


Capsids

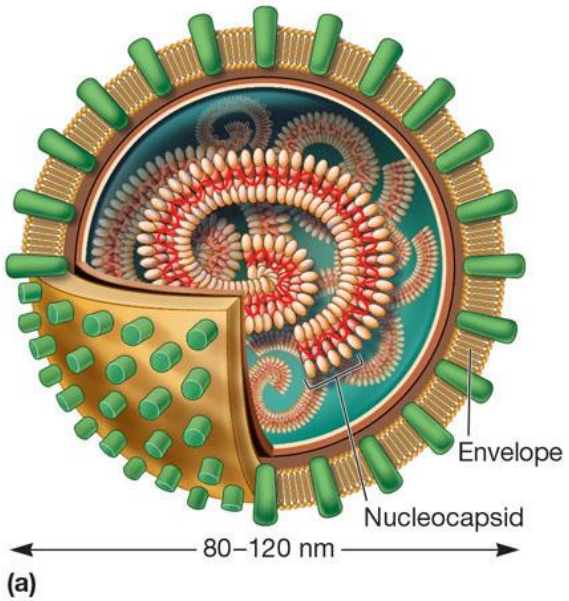
- Large macromolecular structures which serve as protein coat of virus
- Protect viral genetic material and aids in its transfer between host cells
- Made of protein subunits called protomers
- Capsids are helical, icosahedral, or complex

Helical Capsids

- Shaped like hollow tubes with protein walls
- Protomers self assemble
- Size of capsid is a function of nucleic acid



a: Robert G. Milne, Plant Virus Institute National Research Council, Italy; c: Courtesy of Gerald Stubbs and Keiichi Namba, Vanderbilt University; and Donald Caspar, Brandeis University



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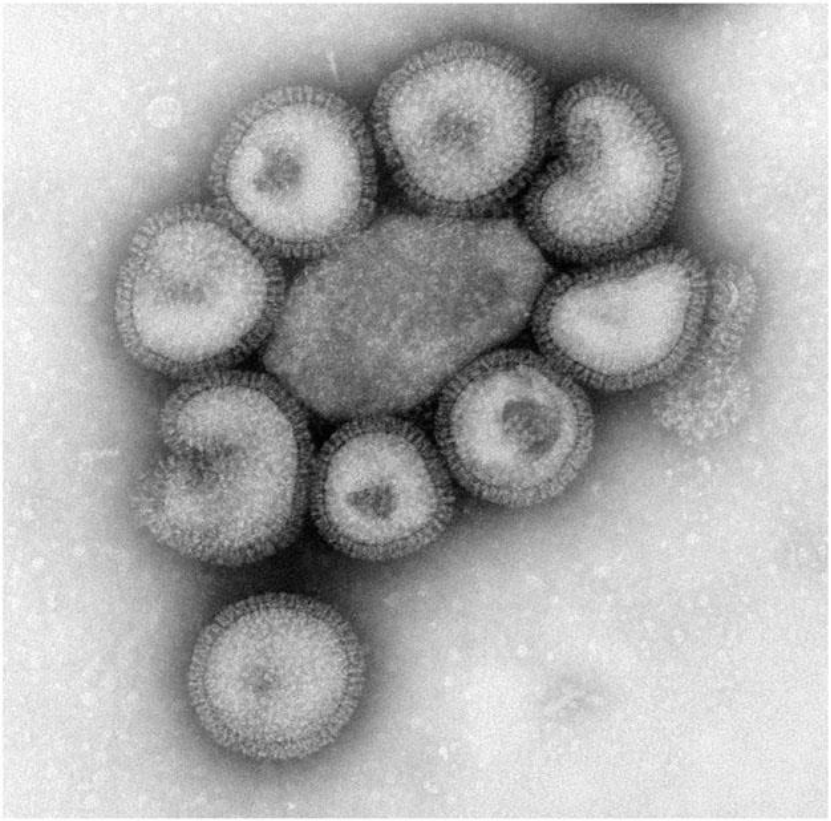
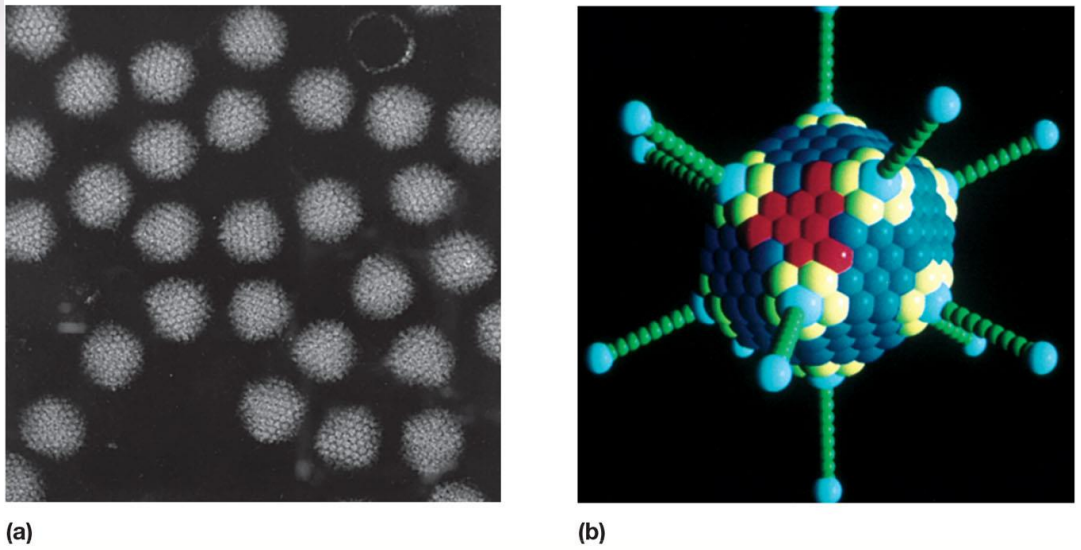


(b)

CDC/Dr. F.A. Murphy

Icosahedral Capsids



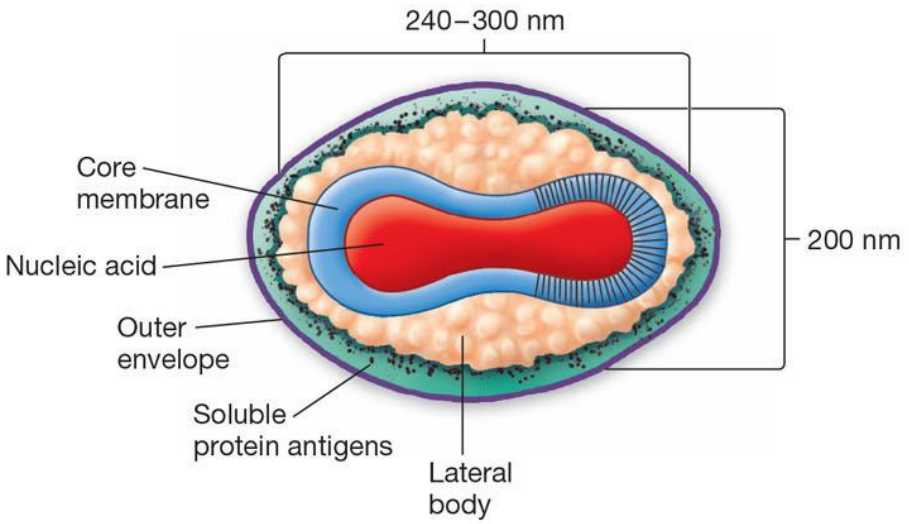
(a) (b)

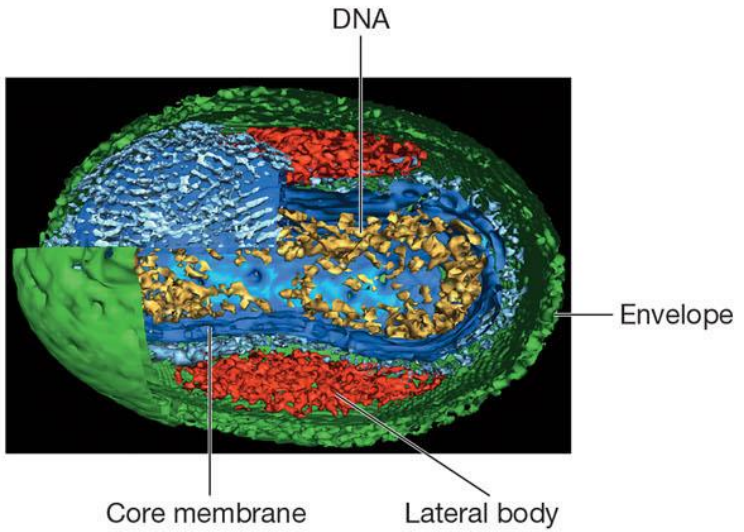
a: Courtesy of Harold Fisher, University of Rhode Island and Robley Williams, University of California at Berkeley; b: © Science VU-NIH, R. Feldman/Visuals Unlimited

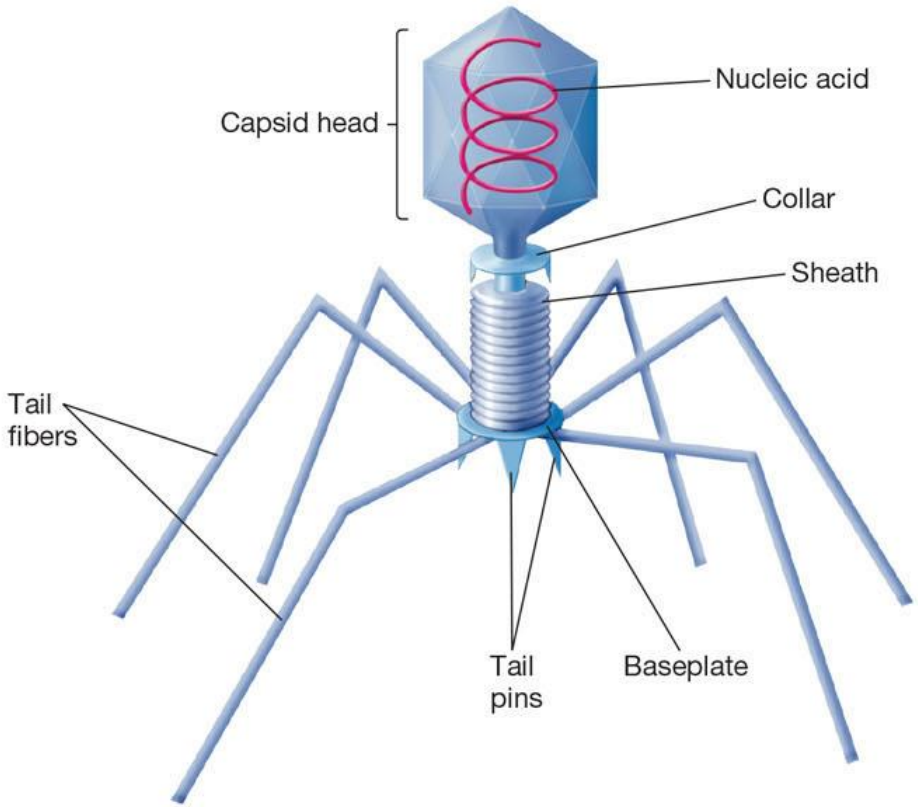
- An icosahedron is a regular polyhedron with 20 equilateral faces and 12 vertices
- Capsomers
  - ring or knob-shaped units made of 5 or 6 protomers
  - pentamers (pentons) – 5 subunit capsomers
  - hexamers (hexons) – 6 subunit capsomers

Capsids of Complex Symmetry

- Some viruses do not fit into the category of having helical or icosahedral capsids
  - poxviruses – largest animal virus
  - large bacteriophages – binal symmetry
    - head resembles icosahedral, tail is helical







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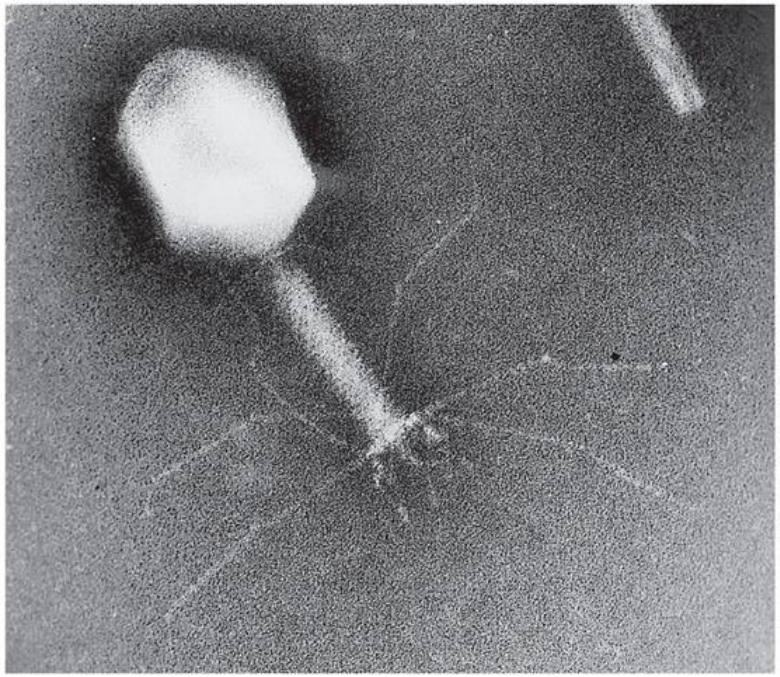
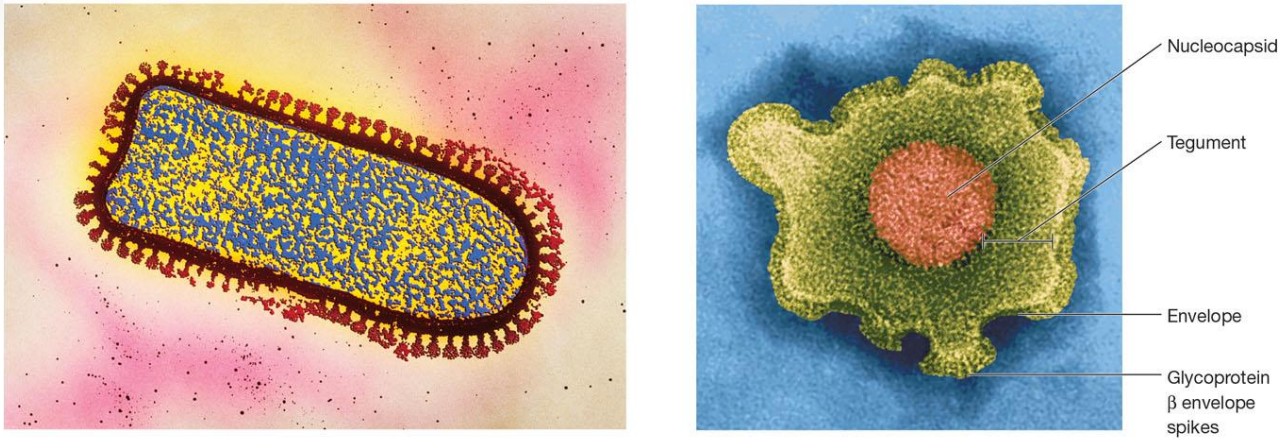


(b)

Viral Envelopes and Enzymes



(a) Rabies virus **(b)** Herpesvirus

a: © Chris Bjornberg/ Photo Researchers, Inc.; b: © Dr. Linda Stannard, UCT/Photo Researchers, Inc.

- Many viruses are bound by an outer, flexible, membranous layer called the envelope
- Animal virus envelopes (lipids and carbohydrates) usually arise from host cell plasma or nuclear membranes

Viral Envelope Proteins

- Envelope proteins, which are viral encoded, may project from the envelope surface as spikes or peplomers
  - involved in viral attachment to host cell
    - e.g., hemagglutinin of influenza virus
  - used for identification of virus
  - may have enzymatic or other activity
    - e.g., neuraminidase of influenza virus
  - may play a role in nucleic acid replication

Virion Enzymes

- It was first erroneously thought that all virions lacked enzymes
- Now accepted that a variety of virions have enzymes
  - some are associated with the envelope or capsid but most are within the capsid

Viral Genome

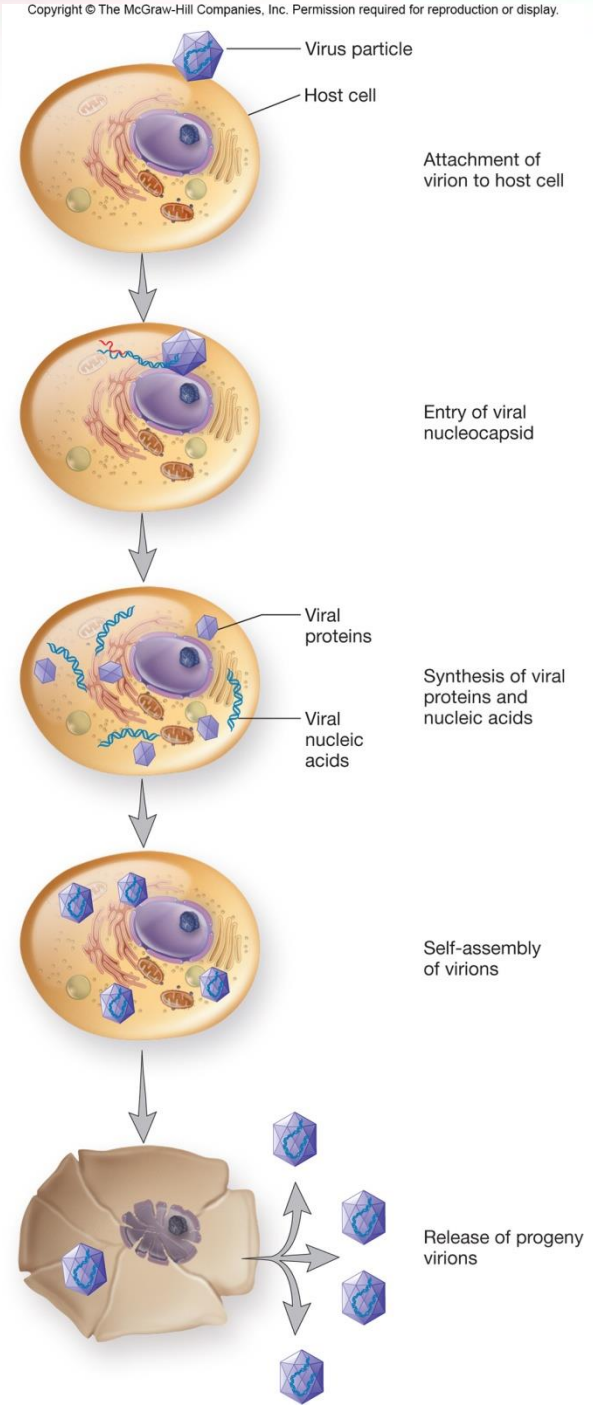
- Diverse nature of genomes
- A virus may have single or double stranded DNA or RNA
- The length of the nucleic acid also varies from virus to virus
- Genomes can be segmented or circular

6.3 Viral multiplication

1. Describe the five steps common to the life cycles of all viruses.
2. Discuss the role of receptors, capsid proteins, and envelope proteins in the life cycles of viruses.
3. Describe the two most common methods for virion release from a host cell.

Viral Multiplication

- Mechanism used depends on viral structure and genome
- Steps are similar
  - attachment to host cell
  - entry
  - uncoating of genome
  - synthesis
  - assembly
  - release

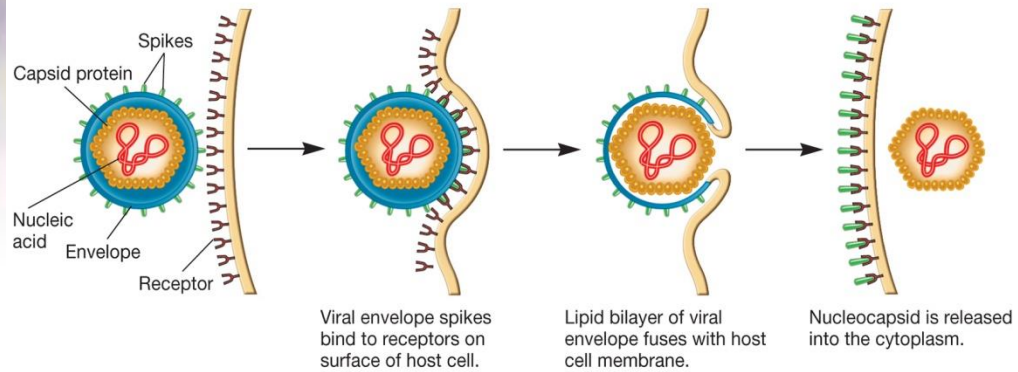


Attachment (Adsorption)

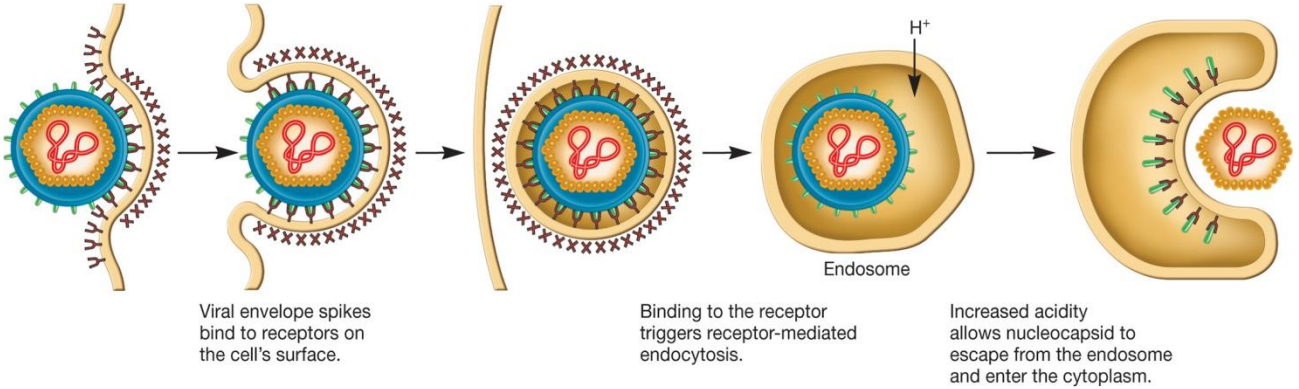
- Specific receptor attachment
- Receptor determines host preference
  - may be specific tissue (tropism)
  - may be more than one host
  - may be more than one receptor
  - may be in lipid rafts providing entry of virus

Viral Entry and Uncoating

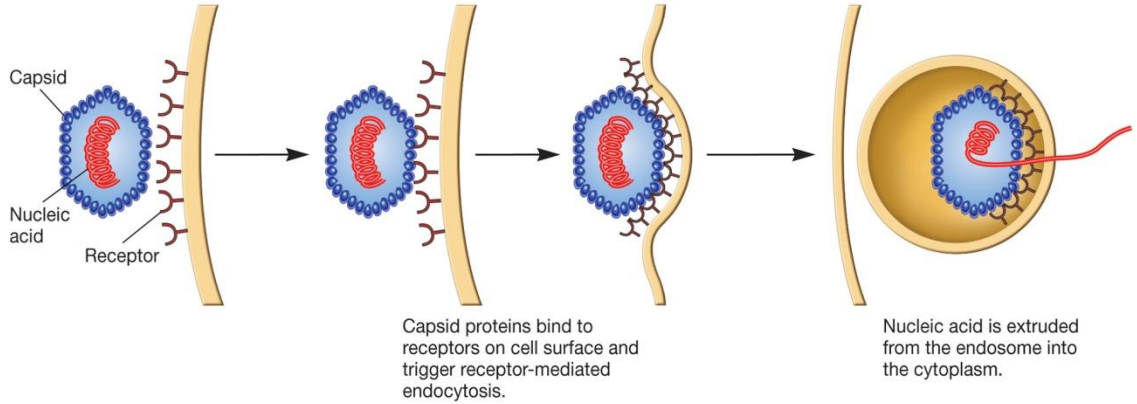
- Entire genome or nucleocapsid
- Varies between naked or enveloped virus
- Three methods used
  - fusion of the viral envelope with host membrane; nucleocapsid enters
  - endocytosis in vesicle; endosome aids in viral uncoating
  - injection of nucleic acid



(a) Entry of enveloped virus by fusing with plasma membrane



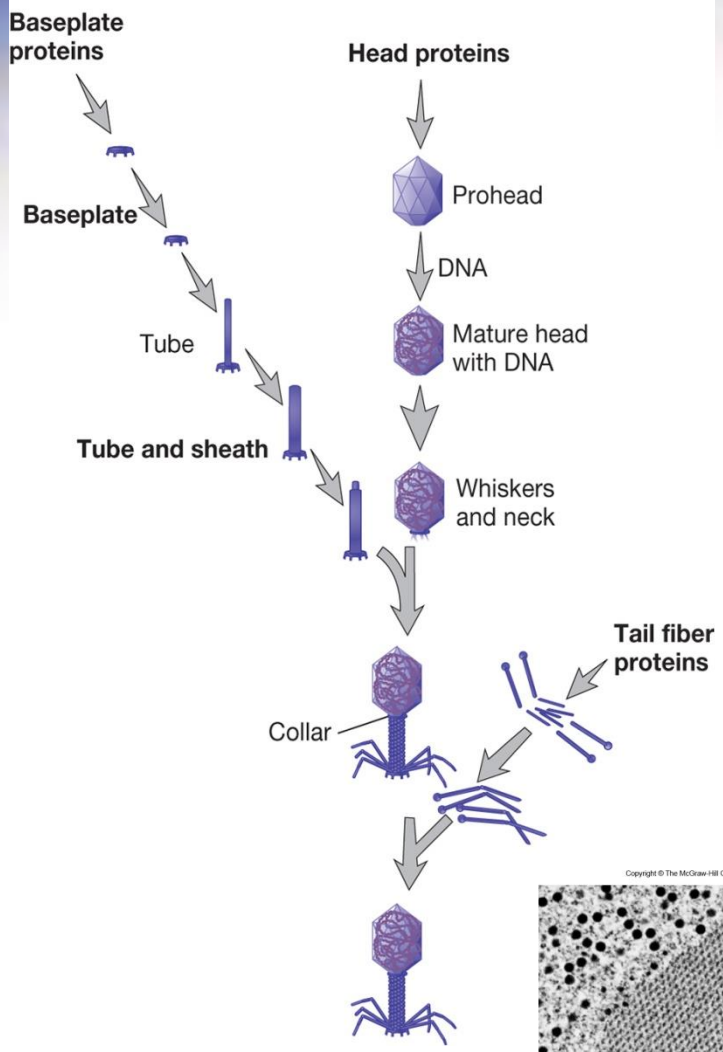
(b) Entry of enveloped virus by endocytosis

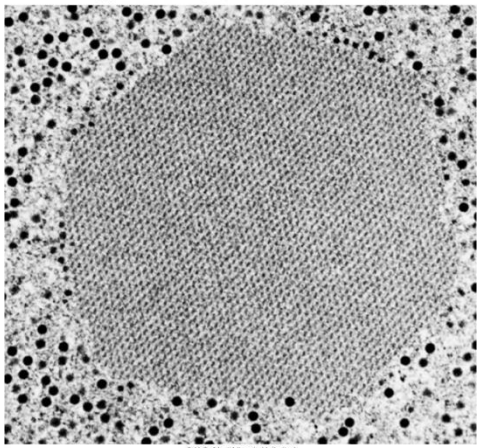


(c) Entry of nonenveloped virus by endocytosis

Synthesis Stage

- Genome dictates the events
- ds DNA typical flow
- RNA viruses
  - virus must carry in or synthesize the proteins necessary to complete synthesis
- Stages may occur, e.g., early and late



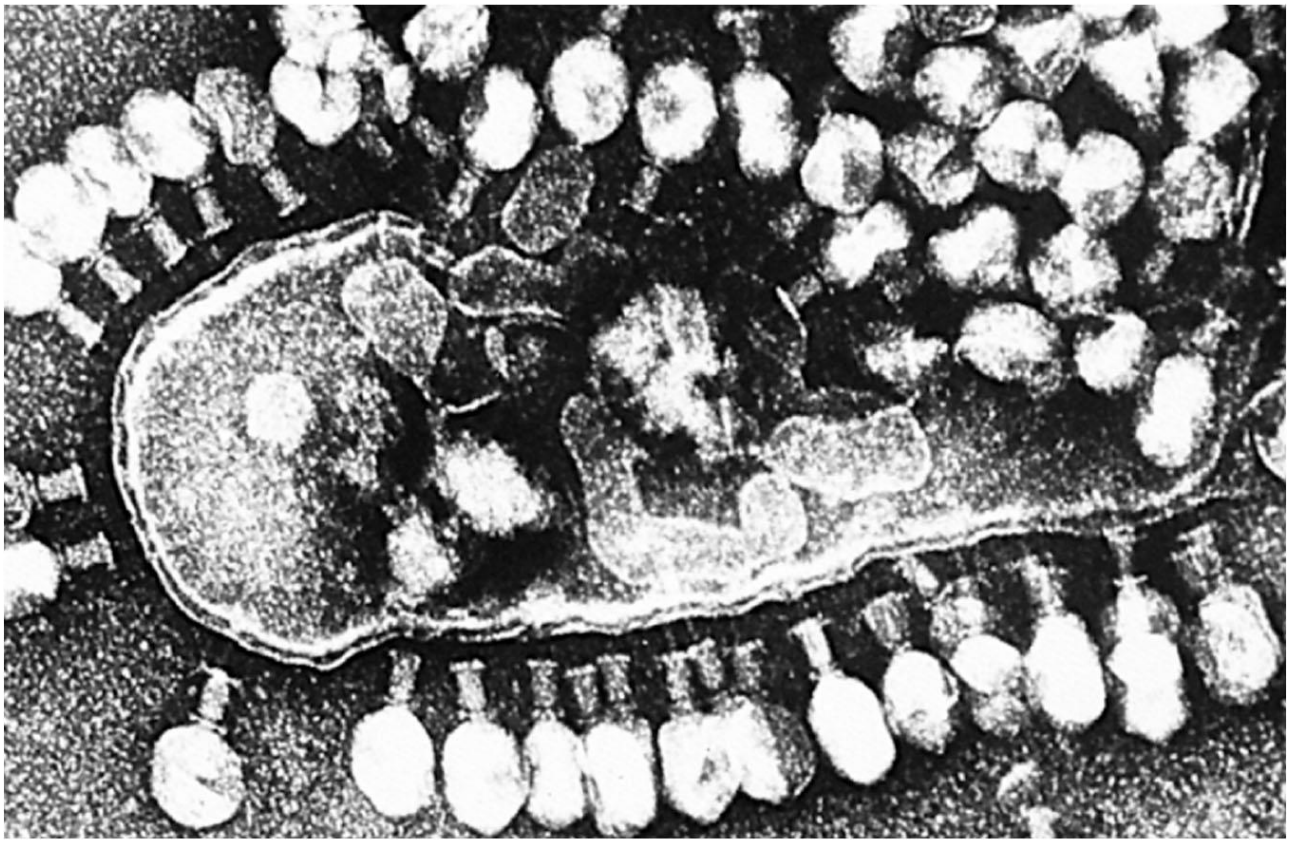


Assembly

- Late proteins are important in assembly
- Assembly is complicated but varies
  - bacteriophages – stages
  - some are assembled in nucleus
  - some are assembled in cytoplasm
  - may be seen as paracrystalline structures in cell

Virion Release

- Nonenveloped viruses lyse the host cell
  - viral proteins may attack peptidoglycan or membrane



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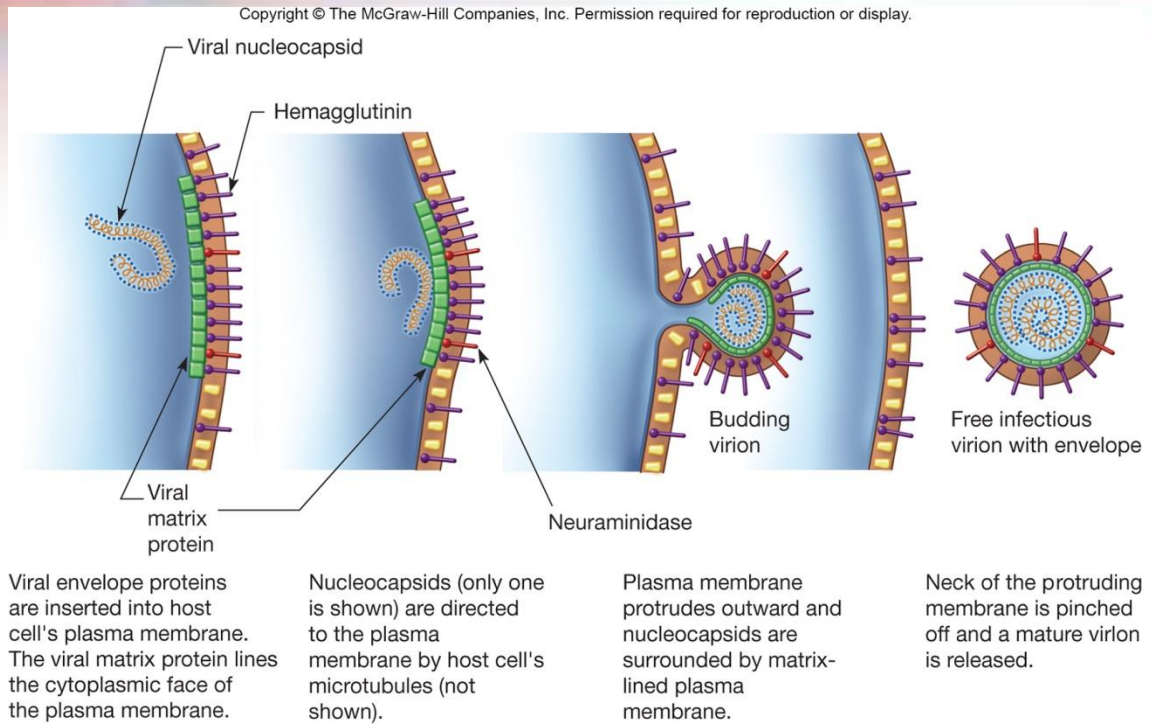
Virion Release



- Enveloped viruses use budding
  - viral proteins are placed into host membrane
  - nucleocapsid may bind to viral proteins
  - envelope derived from host cell membrane, but may be Golgi, ER, or other
  - virus may use host actin tails to propel through host membrane

6.4 Types of viral infections

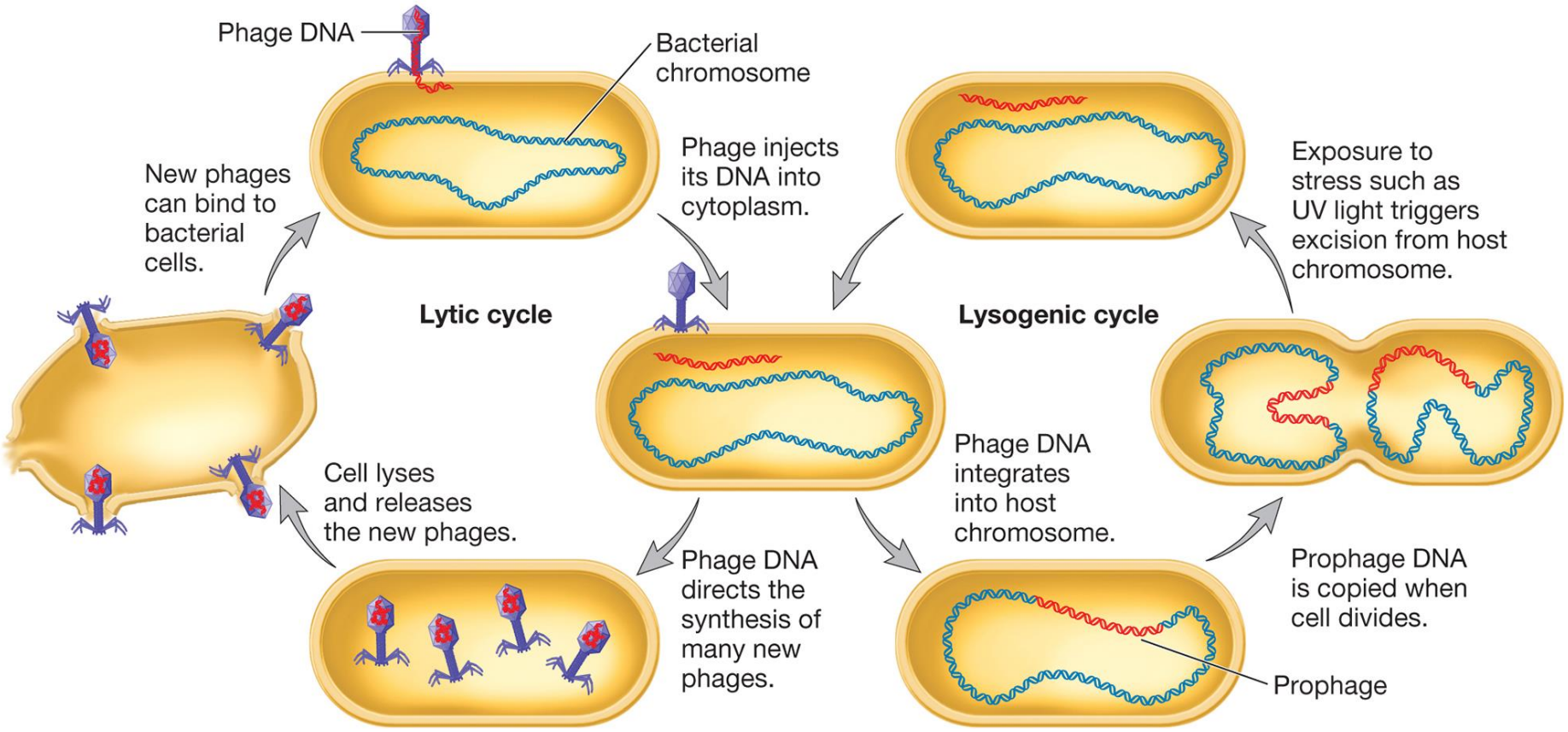
1. Compare and contrast the major steps of the life cycles of virulent phages and temperate phages.
2. List examples of lysogenic conversion.
3. Differentiate among the types of viral infections of eukaryotic cells.
4. Summarize the current understanding of how oncoviruses cause cancer.

Types of Viral Infections

- Infections in *Bacteria* and *Archaea*
- Infections in eukaryotic cells
- Viruses and cancer

Bacterial and Archaeal Viral Infections

- Virulent phage – one reproductive choice
  - multiplies immediately upon entry
  - lyses bacterial host cell
- Temperate phages have two reproductive options
  - reproduce lytically as virulent phages do
  - remain within host cell without destroying it
    - many temperate phages integrate their genome into host genome (becoming a ‘prophage’ in a ‘lysogenic bacterium’) in a relationship called lysogeny



Lysogenic Conversion

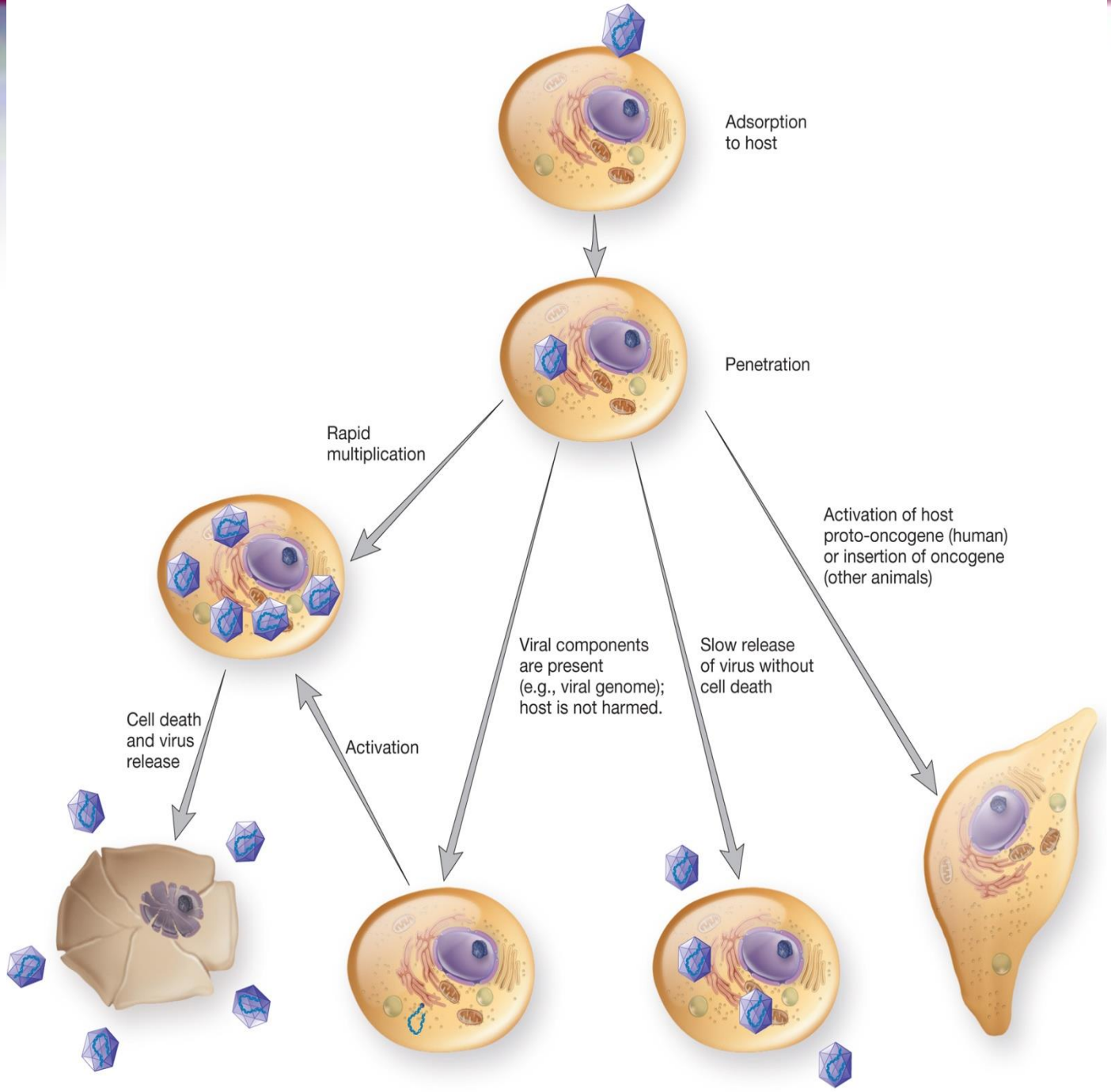
- Temperate phage changes phenotype of its host
  - bacteria become immune to superinfection
  - phage may express pathogenic toxin or enzyme
- Two advantages to lysogeny for virus
  - phage remains viable but may not replicate
  - multiplicity of infection ensures survival of host cell
- Under appropriate conditions infected bacteria will lyse and release phage particles
  - occurs when conditions in the cell cause the prophage to initiate synthesis of new phage particles, a process called induction

Archaeal Viruses

- May be lytic or temperate
- Most discovered so far are temperate by unknown mechanisms

Infection in Eukaryotic Cells

- Cytocidal infection results in cell death through lysis
- Persistent infections may last years
- Cytopathic effects (CPEs)
  - degenerative changes
  - abnormalities
- Transformation to malignant cell


Adsorption to host
Penetration
Rapid multiplication
Activation of host proto-oncogene (human) or insertion of oncogene (other animals)
Viral components are present (e.g., viral genome); host is not harmed.
Slow release of virus without cell death
Cell death and virus release
Activation
(a) Acute infection
(b) Latent infection
(c) Chronic infection
(d) Transformation into malignant

Viruses and Cancer

- Tumor
  - growth or lump of tissue;
  - benign tumors remain in place
- Neoplasia
  - abnormal new cell growth and reproduction due to loss of regulation
- Anaplasia
  - reversion to a more primitive or less differentiated state
- Metastasis
  - spread of cancerous cells throughout body

Carcinogenesis

- Complex, multistep process
- Often involves oncogenes
  - cancer causing genes
  - may come from the virus OR may be transformed host proto-oncogenes (involved in normal regulation of cell growth/differentiation)

Table 6.1 Some Viruses Associated with Human Cancers

Virus	Genome Type	Cancer
Human herpesvirus 8 (HHV8)	Double-stranded (ds) DNA	Several, including Kaposi's sarcoma
Epstein-Barr virus (EBV)	dsDNA	Several, including Burkitt's lymphoma and nasopharyngeal carcinoma
Hepatitis B virus	dsDNA	Hepatocellular carcinoma
Hepatitis C virus	Single-stranded (ss) RNA	Liver cancer
Human papillomaviruses (HPV) strains 6, 11, 16, and 18	dsDNA	Cervical cancer
Human T-cell lymphotropic virus1 (HTLV-1)	ssRNA (retrovirus)	T-cell leukemia

Possible Mechanisms by Which Viruses Cause Cancer

- Viral proteins bind host cell tumor suppressor proteins
- Carry oncogene into cell and insert it into host genome
- Altered cell regulation
- Insertion of promoter or enhancer next to cellular oncogene

6.5 Cultivation and enumeration of viruses

1. List the types of approaches used to cultivate viruses, noting which types of viruses are cultivated by each method.
2. Describe three direct counting methods and two indirect counting methods used to enumerate viruses.
3. Outline the events that lead to the formation of a plaque in a lawn of bacterial cells.
4. Distinguish lethal dose from infectious dose.

The Cultivation of Viruses

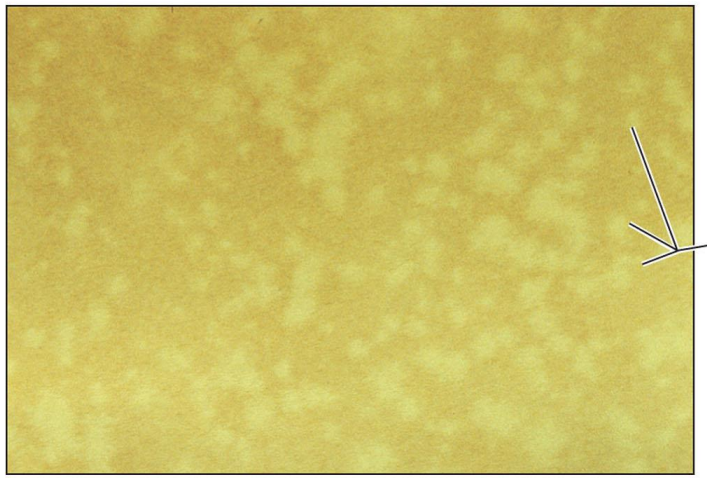
- Requires inoculation of appropriate living host

Hosts for Bacterial and Archael Viruses

- Usually cultivated in broth or agar cultures of suitable, young, actively growing bacteria
- Broth cultures lose turbidity as viruses reproduce
- Plaques observed on agar cultures

Hosts for Animal Viruses

- Tissue (cell) cultures
  - cells are infected with virus (phage)
  - viral plaques
    - localized area of cellular destruction and lysis that enlarge as the virus replicates
- Cytopathic effects (CPEs)
  - microscopic or macroscopic degenerative changes or abnormalities in host cells and tissues
- Embryonated eggs



Plaques formed by multiplication of a poliovirus in lawn of monkey kidney cells

Hosts for Plant Viruses

- Plant tissue cultures
- Plant protoplast cultures
- Suitable whole plants
  - – may cause localized necrotic lesions or generalized symptoms of infection

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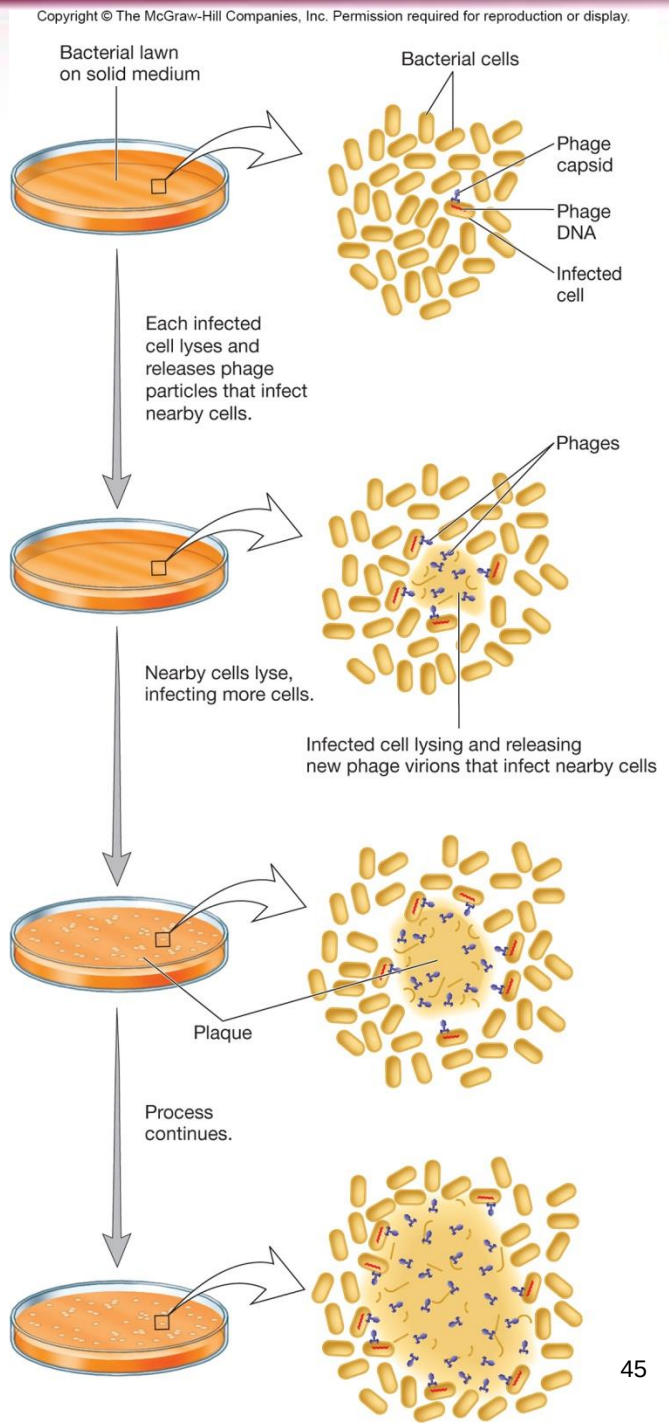
Quantification of Virus

- Direct counting – count viral particles
- Indirect counting by an observable of the virus
  - hemagglutination assay
  - plaque assays

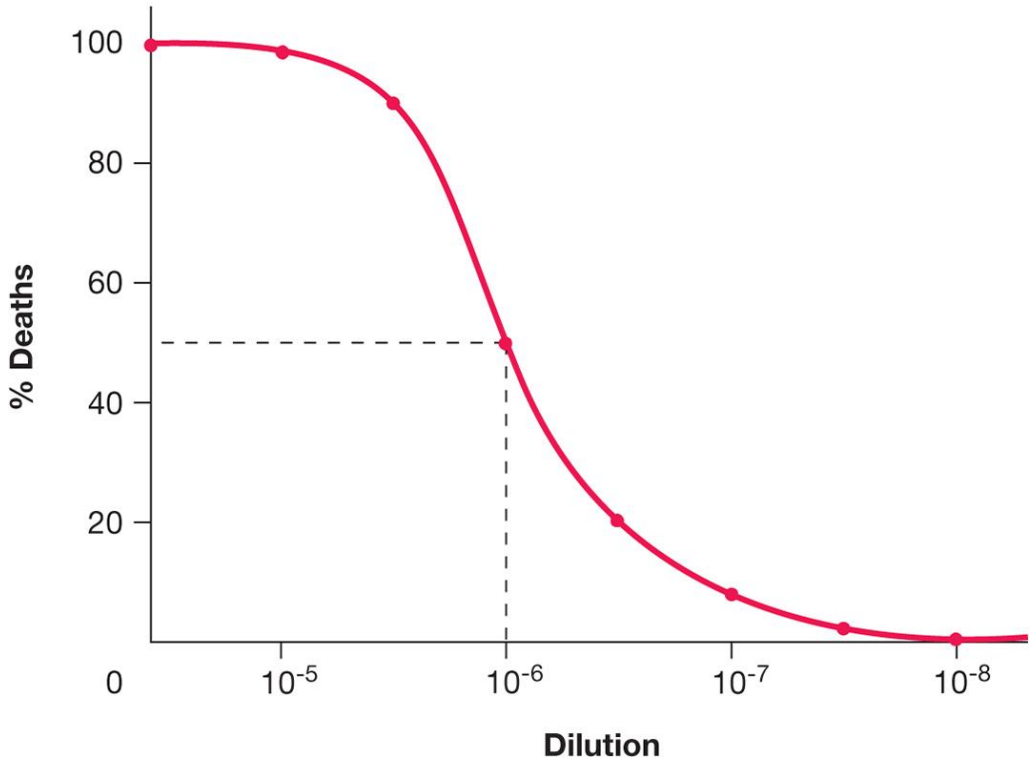


Measuring Concentration of Infectious Units

- Plaque assays
  - dilutions of virus preparation made and plated on lawn of host cells
  - number of plaques counted
  - results expressed as plaque-forming units (PFU) – plaque forming units (PFU)
    - PFU/ml = number of plaques/sample dilution

Measuring Biological Effects

- Infectious dose and lethal dose assays
  - determine smallest amount of virus needed to cause infection (ID) or death (LD) of 50% of exposed host cells or organisms (ID_{50} or LD_{50})

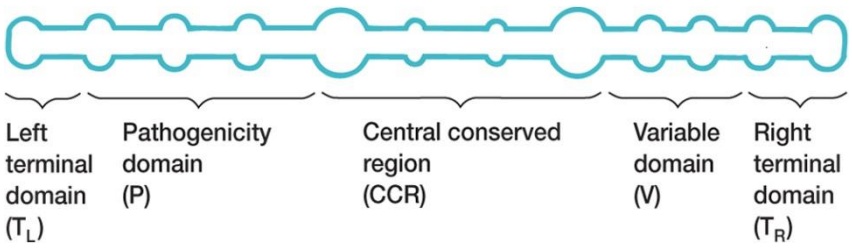


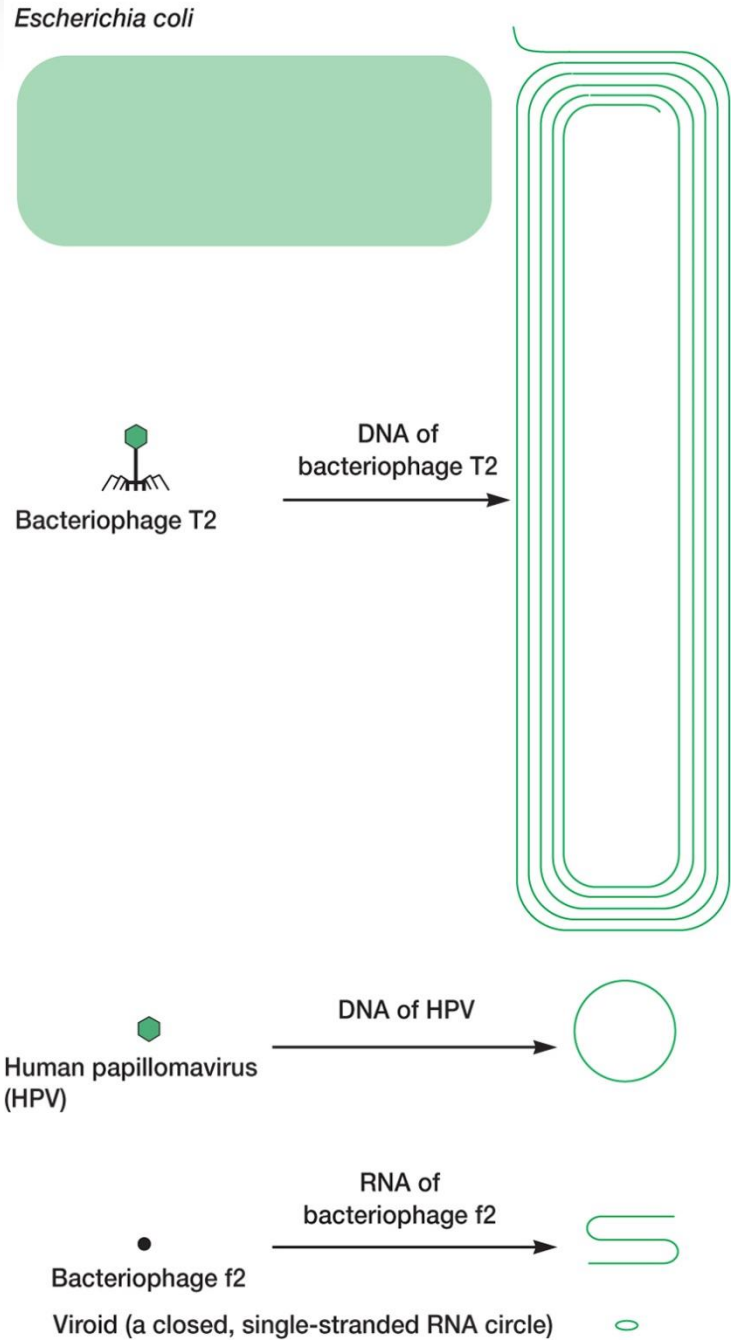
6.6 Viroids and satellites

1. Describe the structure of a viroid and discuss the practical importance of viroids.
2. Distinguish satellite viruses from satellite nucleic acids.

Viroids

- infectious agents composed of closed, circular ssRNAs
- do not encode gene products
- requires host cell DNA-dependent RNA polymerase to replicate
- cause plant diseases





Satellites

- Infectious nucleic acids (DNA or RNA)
  - Satellite viruses encode their own capsid proteins when helped by a helper virus
  - Satellite RNAs/DNAs do NOT encode their own capsid proteins
- Encode one or more gene products
- Require a helper virus for replication
  - human hepatitis D virus is satellite
  - requires human hepatitis B virus

6.7 Prions

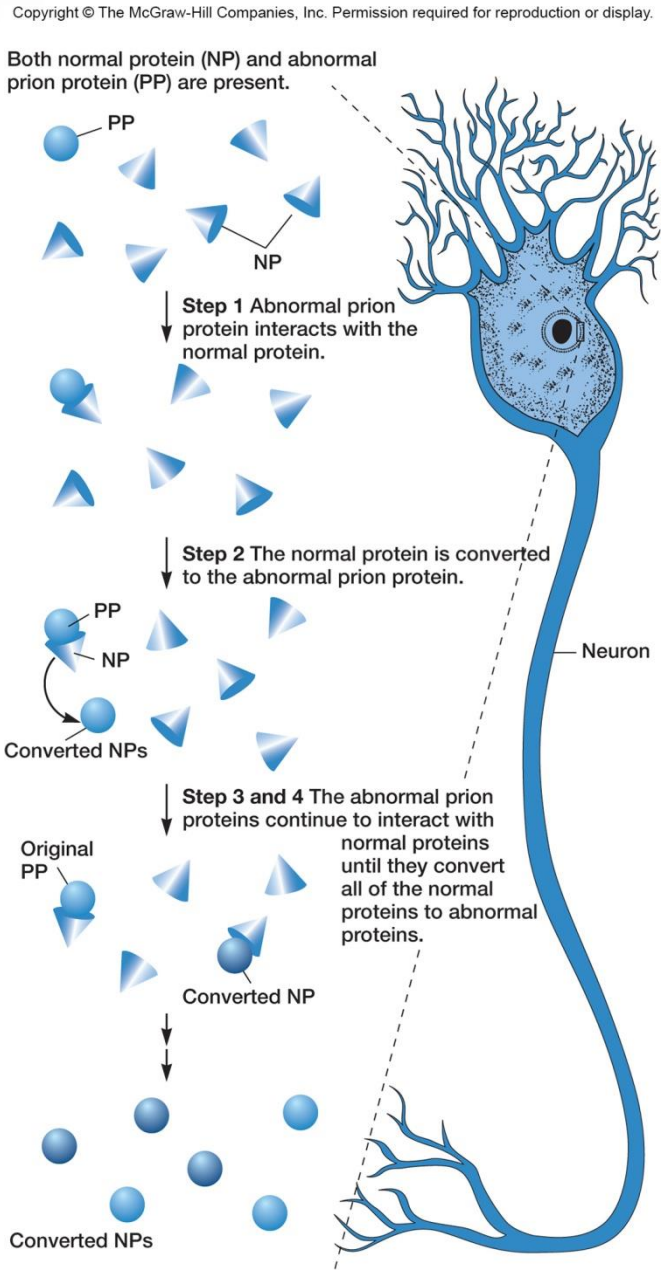
1. Describe prion structure and how prions are thought to replicate.
2. List characteristics common to all animal diseases caused by prions.
3. Name at least two human diseases caused by prions.
4. Describe the mechanisms by which a prion protein might first appear in a brain cell.

Prions – Proteinaceous Infectious Particle

- Cause a variety of degenerative diseases in humans and animals
  - scrapie in sheep
  - bovine spongiform encephalopathy (BSE) or mad cow disease
  - Creutzfeldt-Jakob disease (CJD) and variant CJD (vCJD) in humans
  - kuru in humans

Current Model of Disease Production by Prions

- PrP^{C} (prion protein) is present in "normal" form (abnormal form of prion protein is PrP^{Sc})
- PrP^{Sc} causes PrP^{C} protein to change its conformation to abnormal form
- newly produced PrP^{Sc} molecules convert more normal molecules to the abnormal form through unknown mechanism



Neural Loss

- Evidence suggests that PrP^{C} must be present for neural degeneration to occur
- Interaction of PrP^{Sc} with PrP^{C} may cause PrP^{C} to crosslink and trigger apoptosis
- PrP^{C} conversion causes neuron loss, PrP^{Sc} is the infectious agent
- All prion caused diseases
  - have no effective treatment
  - result in progressive degeneration of the brain and eventual death