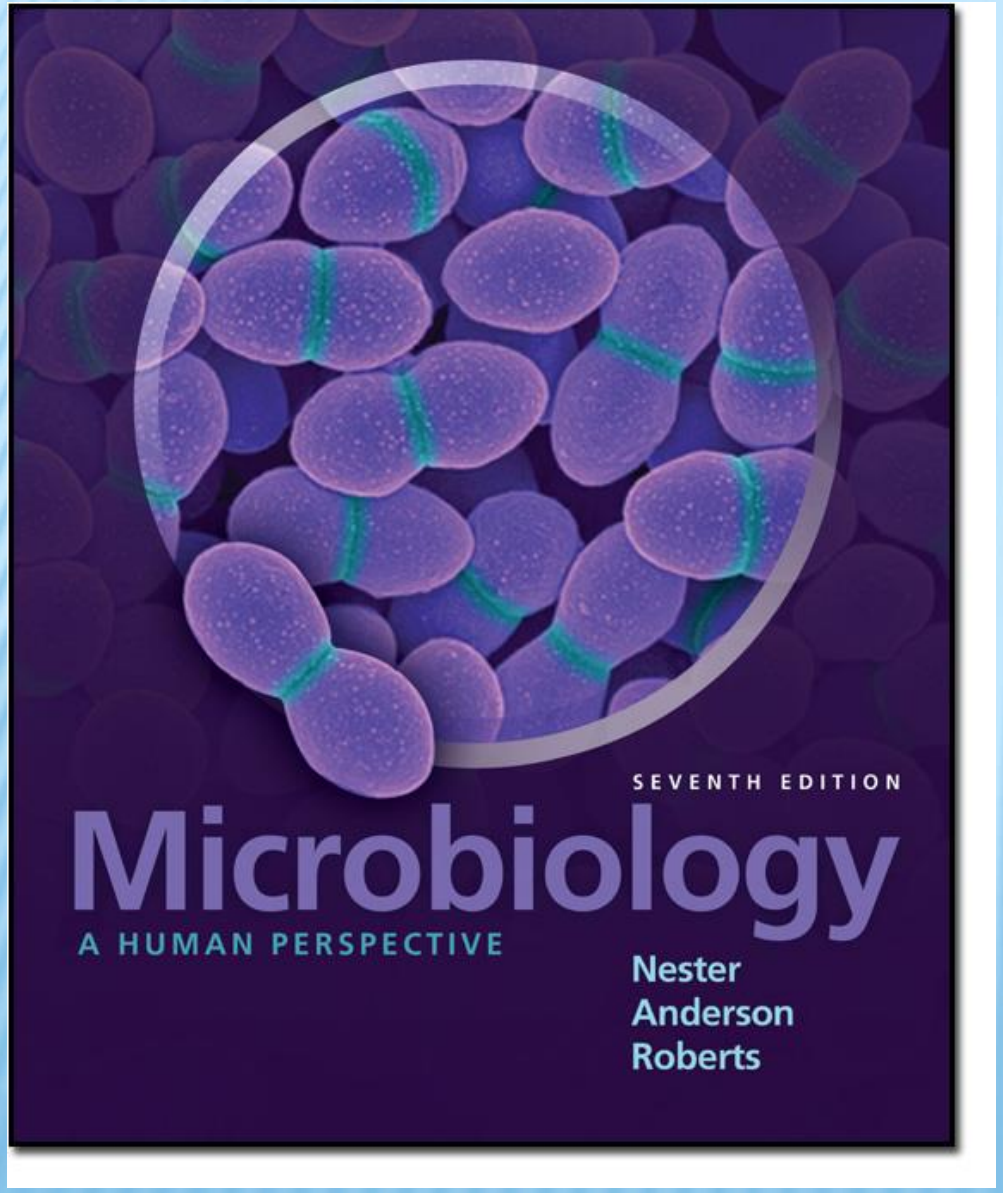


Chapter 13

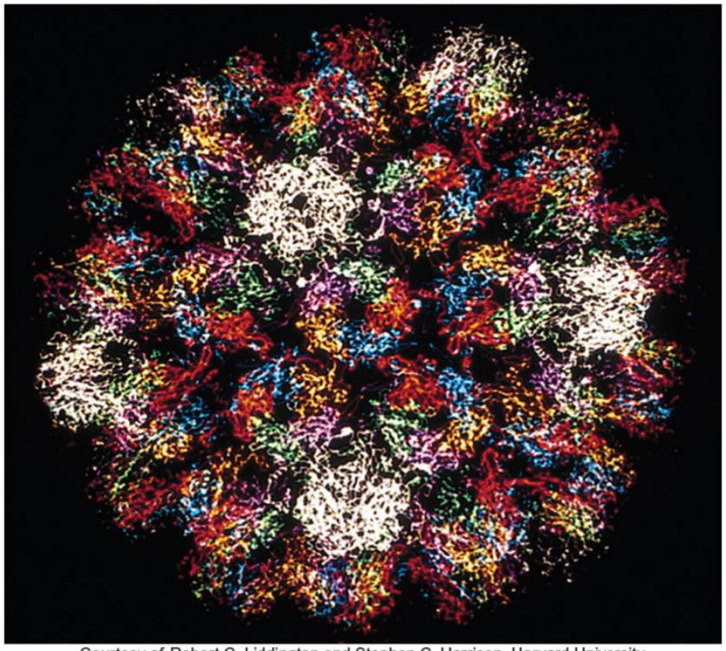
Viruses, Viroides and Prions

A GLIMPSE OF HISTORY

- Tobacco mosaic disease (1890s)
  - D. M. Iwanowsky, Martinus Beijerinck determined caused by “filterable virus” too small to be seen with light microscope, passed through filters for bacteria
  - Decade later, F. W. Twort and F. d’Herelle discovered “filterable virus” that destroyed bacteria
  - Previously, many bacteria, fungi, protozoa identified as infectious diseases
  - Virus means “poison”
  - Viruses have many features more characteristic of complex chemicals (e.g., still infective following precipitation from ethyl alcohol suspension or crystallization)

VIRUSES: OBLIGATE INTRACELLULAR PARASITES

- Viruses simply genetic information: DNA or RNA contained within protective coat
  - Inert particles: no metabolism, replication, motility
  - Genome hijacks host cell's replication machinery
  - Inert outside cells; inside, direct activities of cell
  - Infectious agents, but not alive
  - Can classify generally based on type of cell they infect: eukaryotic or prokaryotic
    - Bacteriophages (phages) infect prokaryotes
    - May provide alternative to antibiotics



Courtesy of Robert C. Liddington and Stephen C. Harrison, Harvard University

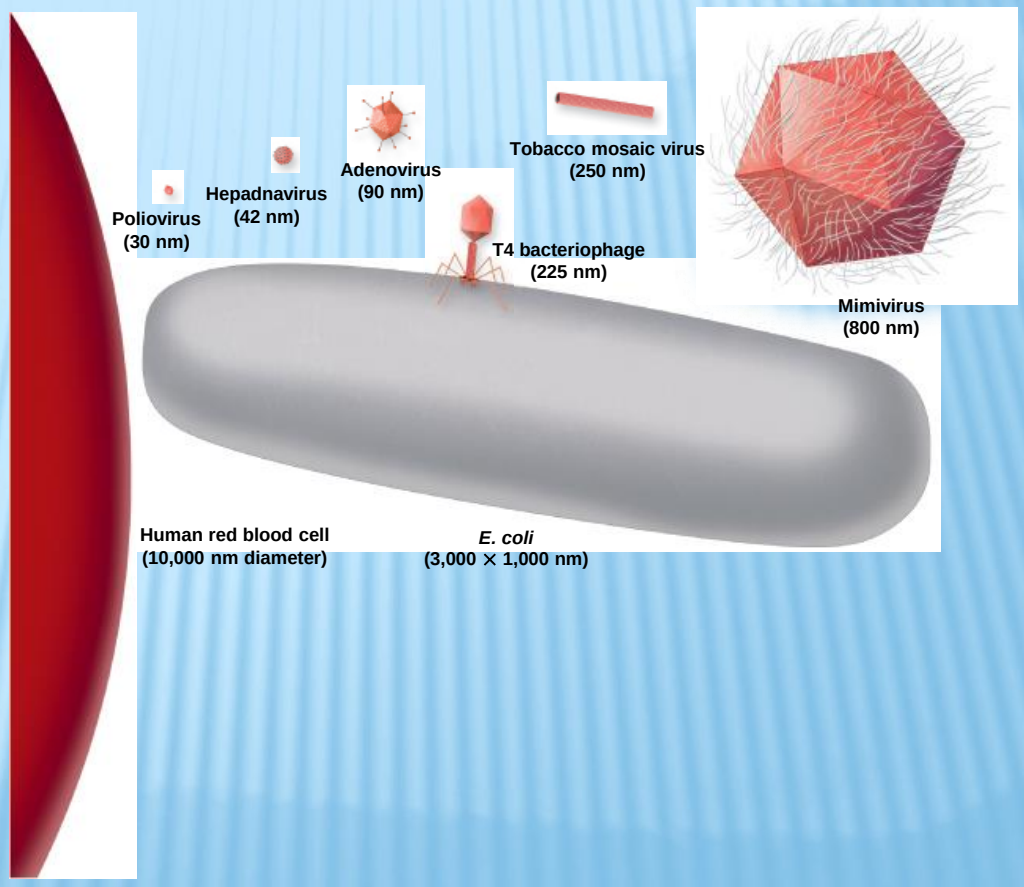
13.1. GENERAL CHARACTERISTICS OF VIRUSES

- Most viruses notable for small size
  - Smallest:

    ~10 nm

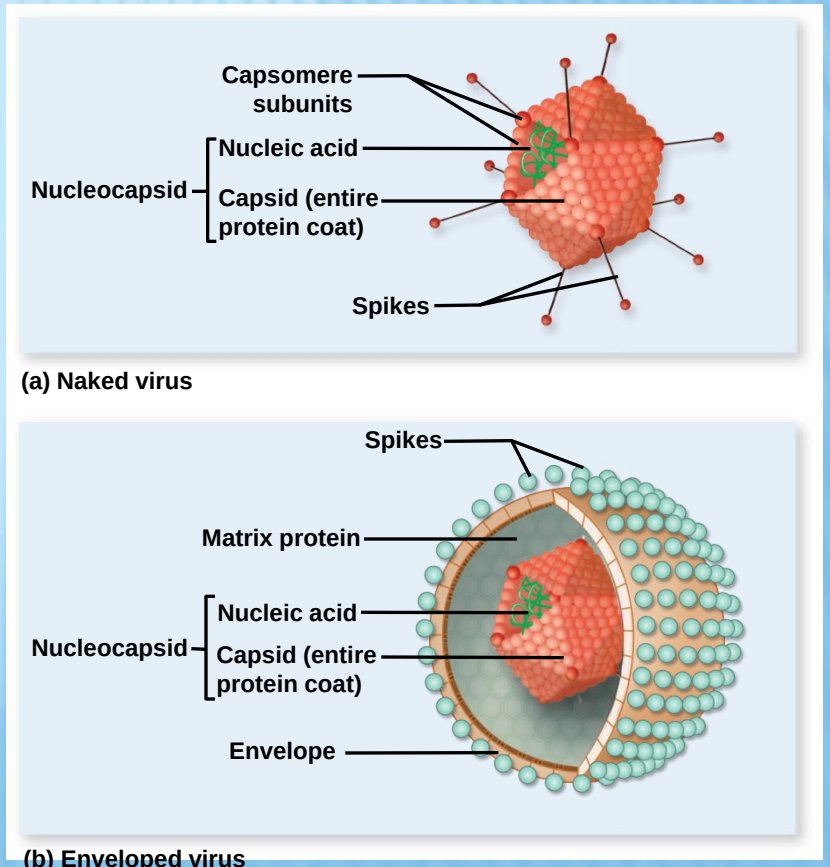
    ~10 genes
  - Largest:

    ~500 nm



13.1. GENERAL CHARACTERISTICS OF VIRUSES

- Virion (viral particle) is nucleic acid, protein coat
  - Protein coat is capsid: protects nucleic acids
    - Carries required enzymes
    - Composed of identical subunits called capsomers
  - Capsid plus nucleic acids called nucleocapsid
  - Enveloped viruses have lipid bilayer envelope
  - Matrix protein between nucleocapsid and envelope
  - Naked viruses lack envelope; more resistant to disinfectants

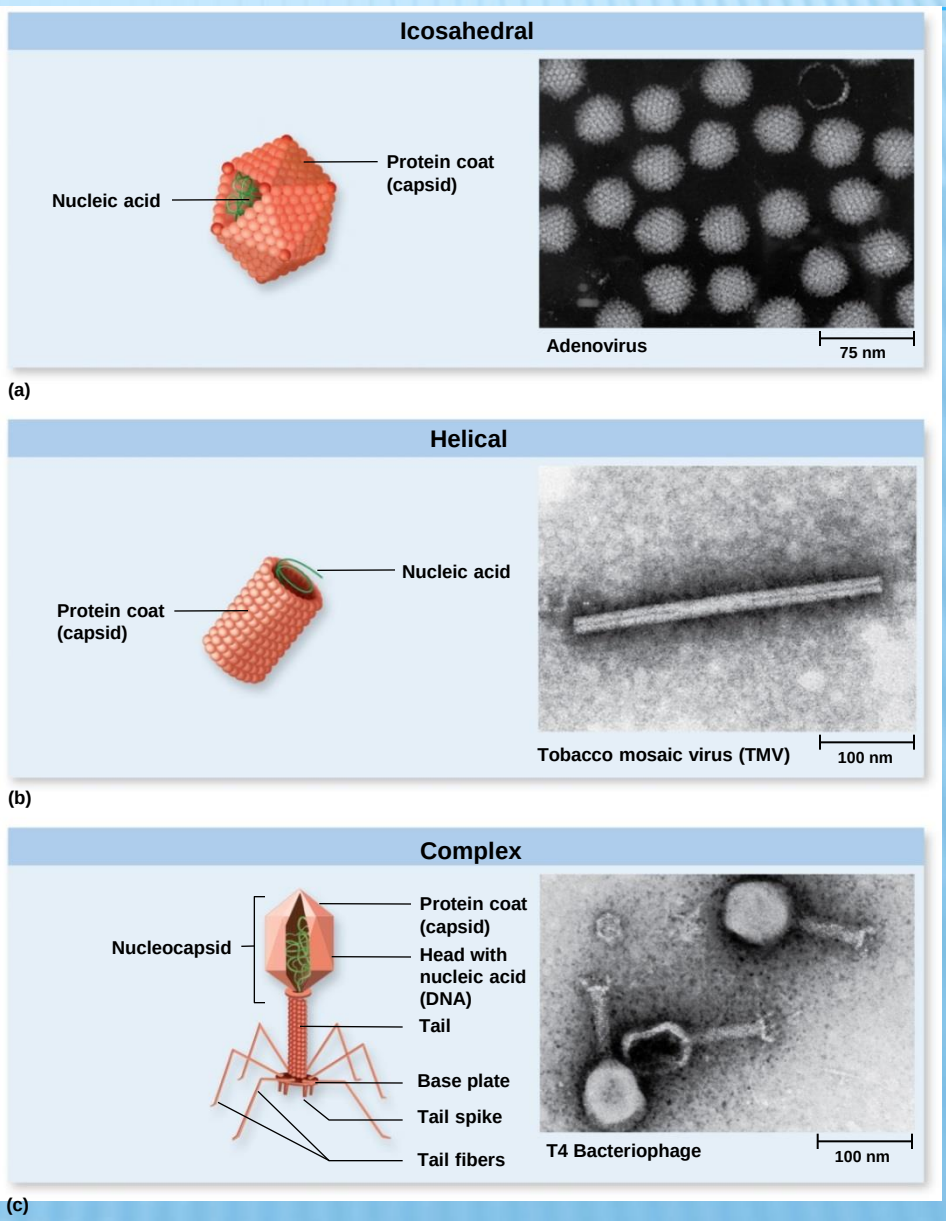


13.1. GENERAL CHARACTERISTICS OF VIRUSES

- Viral genome either DNA or RNA, never both
  - Useful for classification (i.e., DNA or RNA viruses)
  - Genome linear or circular
  - Double- or single-stranded
    - Affects replication strategy
- Viruses have protein components for attachment
  - Phages have tail fibers
  - Many animal viruses have spikes
  - Allow virion to attach to specific receptor sites
- Generally three different shapes
  - Icosahedral, helical, or complex

13.1. GENERAL CHARACTERISTICS OF VIRUSES

- Three shapes:
  - Icosahedral
  - Helical
  - Complex



13.1. GENERAL CHARACTERISTICS OF VIRUSES

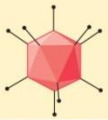
- International Committee on Viral Taxonomy (ICVT) publishes classification of viruses
- 2009 report:

  >6,000 viruses → 2,288 species → 348 genera

  → 87 families

  → 6 orders

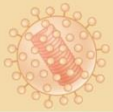
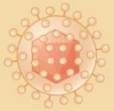
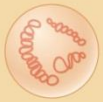
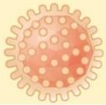
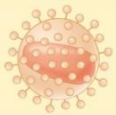
TABLE 13.1 Classification of Some Human Viruses

Nucleic Acid	Outer Covering	Family	Drawing of Virion (not to scale)	Representative Member (and disease caused)
DNA Viruses				
Double-stranded DNA	Naked	*Papillomaviridae*		Human papillomaviruses (some types cause warts; others cause cancers)
		Polyomaviridae		Merkel cell polyomavirus (Merkel skin cancer)
		Adenoviridae		Human adenoviruses (respiratory infections)
	Enveloped	*Herpesviridae*		Herpes zoster virus (chickenpox); herpes simplex viruses (cold sores, genital herpes)
		Poxviridae		Smallpox virus (smallpox)
Single-stranded DNA	Naked	*Parvoviridae*		Human parvovirus B19 (fifth disease)
RNA Viruses				
Double-stranded	Naked	*Reoviridae*		Human rotaviruses (diarrheal disease)
Single-stranded (plus strand)	Naked	*Picornaviridae*		Polioviruses (poliomyelitis); rhinovirus (colds); hepatitis A virus (hepatitis A)
		Caliciviridae		Norovirus (gastroenteritis)
	Enveloped	*Togaviridae*		Rubella virus (rubella); Chikungunya virus (Chikungunya fever)

13.1. GENERAL CHARACTERISTICS OF VIRUSES

- Key characteristics include genome structure (nucleic acid and strandedness) and hosts infected
- Other characteristics (e.g., viral shape, disease symptoms) also considered

TABLE 13.1 Classification of Some Human Viruses (*Continued*)

Nucleic Acid	Outer Covering	Family	Drawing of Virion (not to scale)	Representative Member (and disease caused)
RNA Viruses, *cont.*				
	Enveloped	*Flaviviridae*		Yellow fever virus (yellow fever); dengue virus (dengue hemorrhagic fever); hepatitis C virus (hepatitis C)
		Coronaviridae		Coronavirus (SARS)
Single-stranded (minus strand)	Enveloped	*Rhabdoviridae*		Rabies virus (rabies)
		Filoviridae		Ebola virus (hemorrhagic fever)
		Paramyxoviridae		Mumps virus (mumps); measles virus (measles)
		Orthomyxoviridae		Influenza virus (influenza)
		Bunyaviridae		Hantavirus (hantavirus pulmonary syndrome)
		Arenaviridae		Lassa virus (lassa fever)
Reverse Transcribing Viruses				
DNA	Enveloped	*Hepadnaviridae*		Hepatitis B virus (hepatitis B)
RNA	Enveloped	*Retroviridae*		Human immunodeficiency virus (AIDS)

13.1. GENERAL CHARACTERISTICS OF VIRUSES

- Virus families end in suffix *-viridae*
  - Names follow no consistent pattern
  - Some indicate appearance (e.g., *Coronaviridae* from *corona*, meaning “crown”)
  - Others named for geographic area from which first isolated (e.g., *Bunyaviridae* from Bunyamwera in Uganda, Africa)
- Genus ends in *-virus* (e.g., *Enterovirus*)
- Species name often name of disease
  - E.g., *poliovirus* causes poliomyelitis
  - Viruses commonly referred to only by species name

13.1. GENERAL CHARACTERISTICS OF VIRUSES

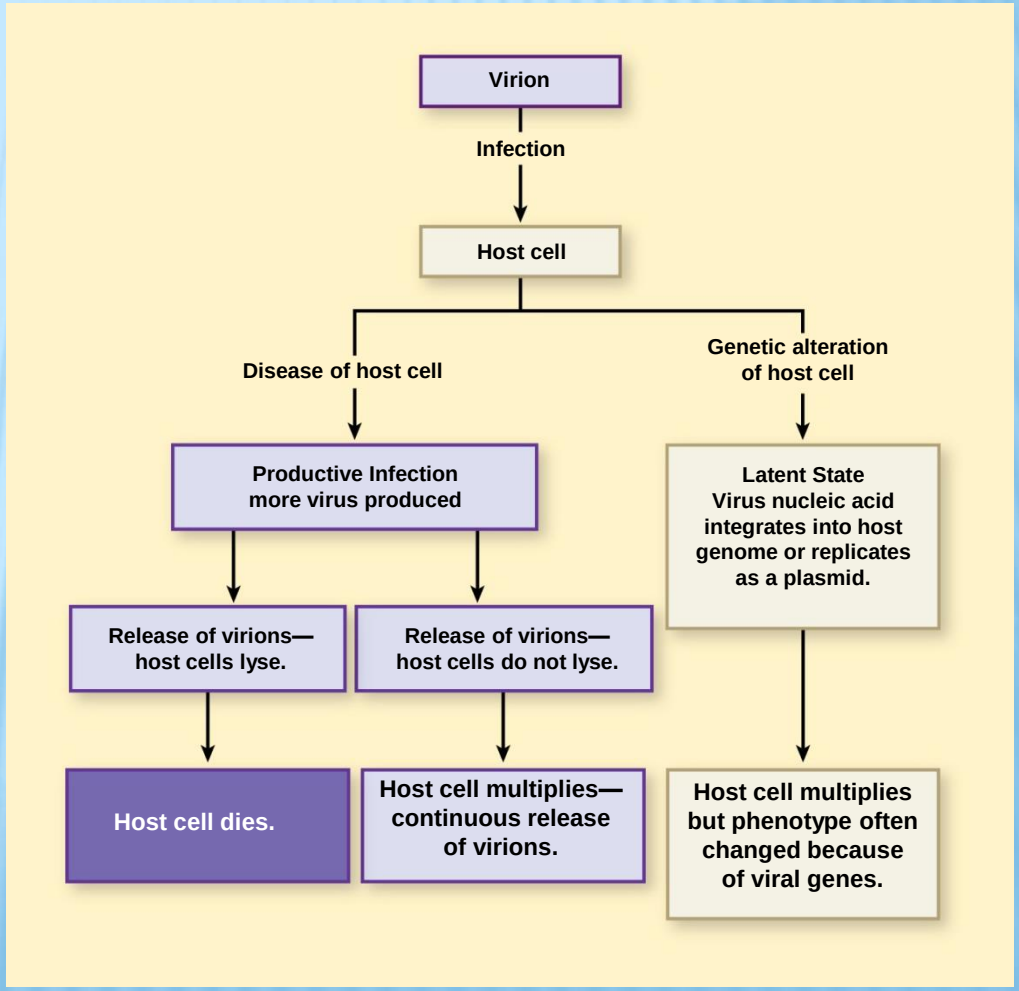
- Viruses often referred to informally
  - Groups of unrelated viruses sharing routes of infection
  - Oral-fecal route: enteric viruses
  - Respiratory route: respiratory viruses
  - Zoonotic viruses cause zoonoses (animal to human)
  - Arboviruses (from arthropod borne) are spread by arthropods; often can infect widely different species
    - Important diseases: yellow fever, dengue fever, West Nile encephalitis, La Crosse encephalitis

TABLE 13.2 Grouping of Human Viruses Based on Route of Transmission

Virus Group	Mechanism of Transmission	Common Viruses Transmitted
Enteric	Fecal-oral route	Enteroviruses (polio, coxsackie B); noroviruses; rotaviruses (diarrhea)
Respiratory	Respiratory or salivary route	Influenza; measles; rhinoviruses (colds)
Zoonotic	Vector (such as arthropods)	Sandfly fever; dengue; West Nile encephalitis
	Animal to human directly	Rabies; cowpox
Sexually transmitted	Sexual contact	Herpes simplex virus type 2 (genital herpes); HIV

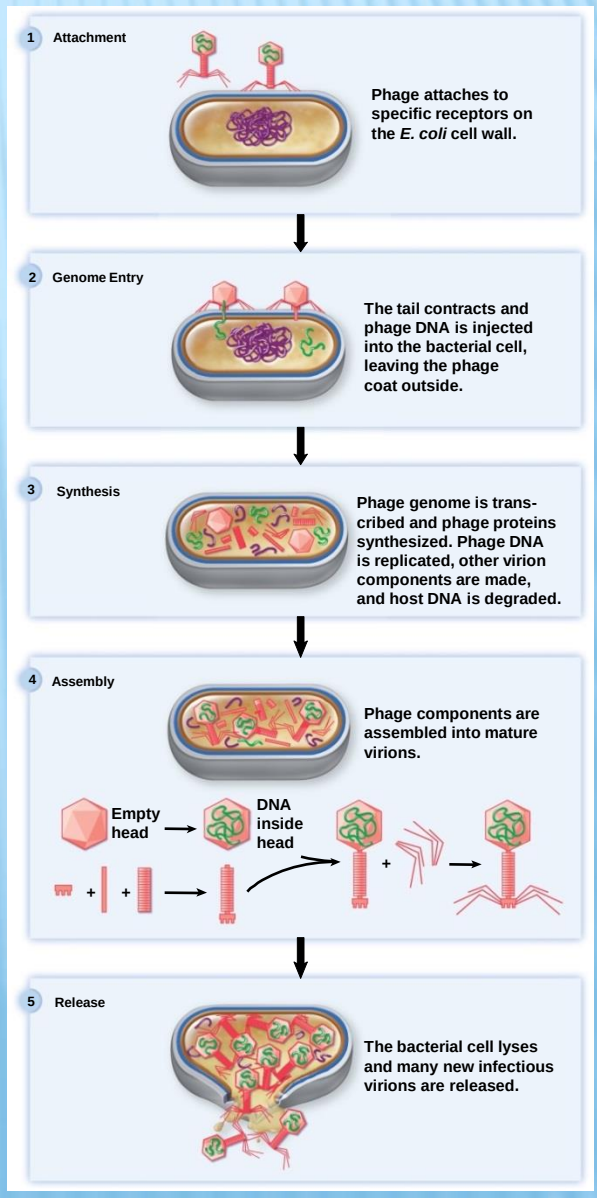
13.2. BACTERIOPHAGES

- Three general types of bacteriophages based on relationship with host
  - Lytic phages
  - Temperate phages
  - Filamentous phages



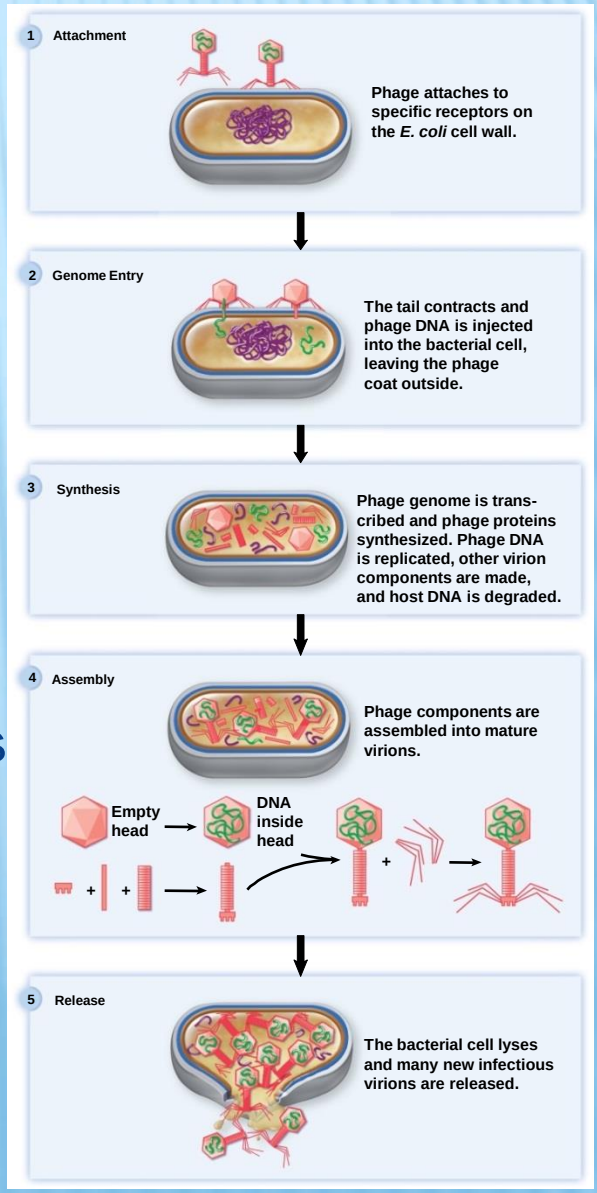
13.2. BACTERIOPHAGES

- Lytic Phage Infections
  - Lytic or virulent phages exit host
  - Cell is lysed
    - Productive infection: new particles formed
  - T4 phage (dsDNA) as model; entire process takes ~30 minutes
  - Five step process
    - Attachment
    - Genome entry
    - Synthesis
    - Assembly
    - Release



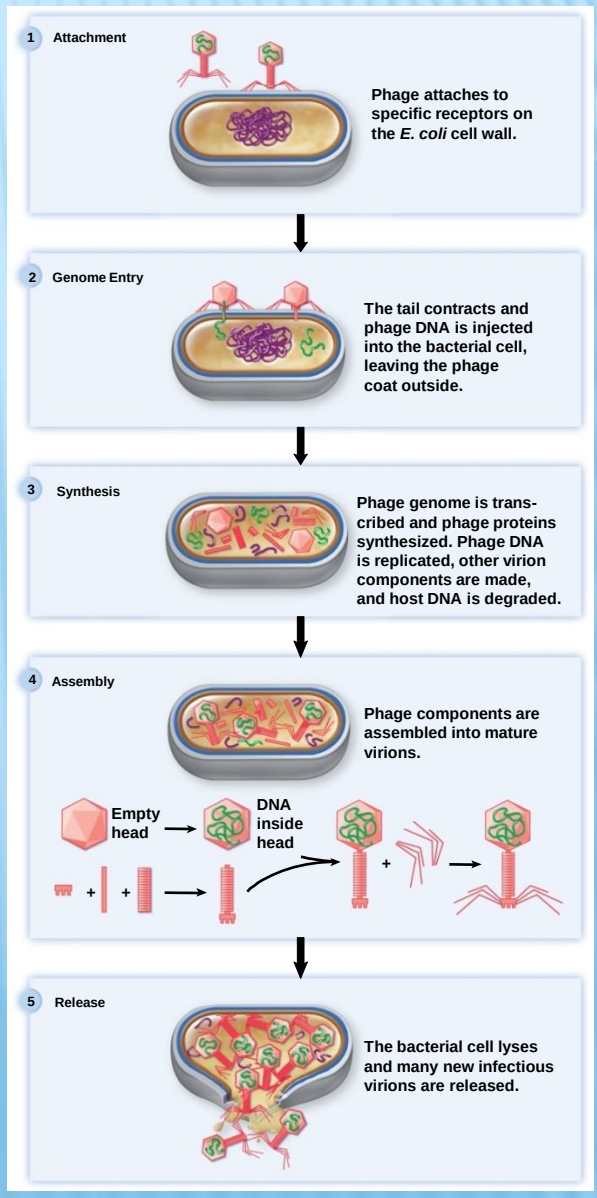
13.2. BACTERIOPHAGES

- Lytic Phage Infections (cont…)
  - Attachment
    - Phage exploits bacterial receptors
  - Genome entry
    - T4 lysozyme degrades cell wall
    - Tail contracts, injects genome through cell wall and membrane
  - Synthesis of proteins and genome
    - Early proteins translated within minutes nuclease degrades host DNA; protein modifies host's RNA polymerase to not recognize its own promoters



13.2. BACTERIOPHAGES

- Lytic Phage Infections (cont…)
  - Late proteins are structural proteins (capsid, tail); produced toward end of cycle
  - Assembly (maturation)
    - Some components spontaneously assemble, others require protein scaffolds
  - Release
    - Lysozyme produced late in infection; digests cell wall
    - Cell lyses, releases phage
    - Burst size of T4 is ~200

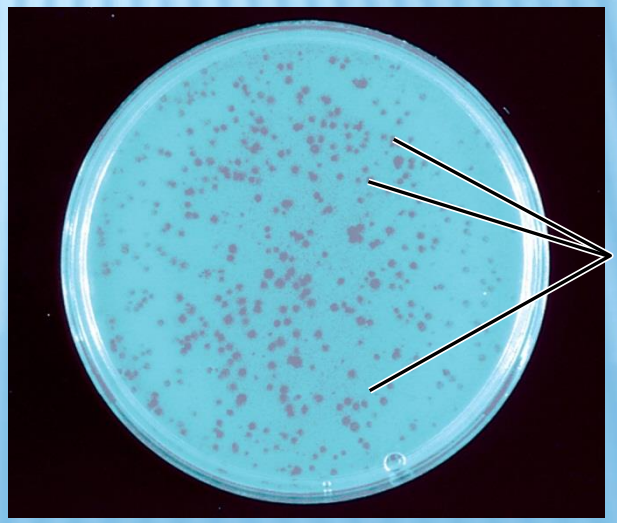


13.4. BACTERIAL DEFENSES AGAINST PHAGES

- Several approaches bacteria can take
- Preventing Phage Attachment
  - Alter or cover specific receptors on cell surface
    - May have other benefits to bacteria
    - E.g., *Staphylococcus aureus* produces protein A, which masks phage receptors; also protects against certain human host defenses
    - Surface polymers (e.g., biofilms) also mask receptor

13.5. METHODS USED TO STUDY BACTERIOPHAGES

- Viruses multiply only inside living cells
  - Must cultivate suitable host cells to grow viruses
    - Bacterial cells easier than animal cells
  - Plaque assays used to quantitate phage particles in samples: sewage, seawater, soil
  - Soft agar inoculated with bacterial host and specimen, poured over surface of agar in Petri dish
  - Bacterial lawn forms
  - Zones of clearing from bacterial lysis are plaques
  - Counting plaque forming units (PFU) yields titer

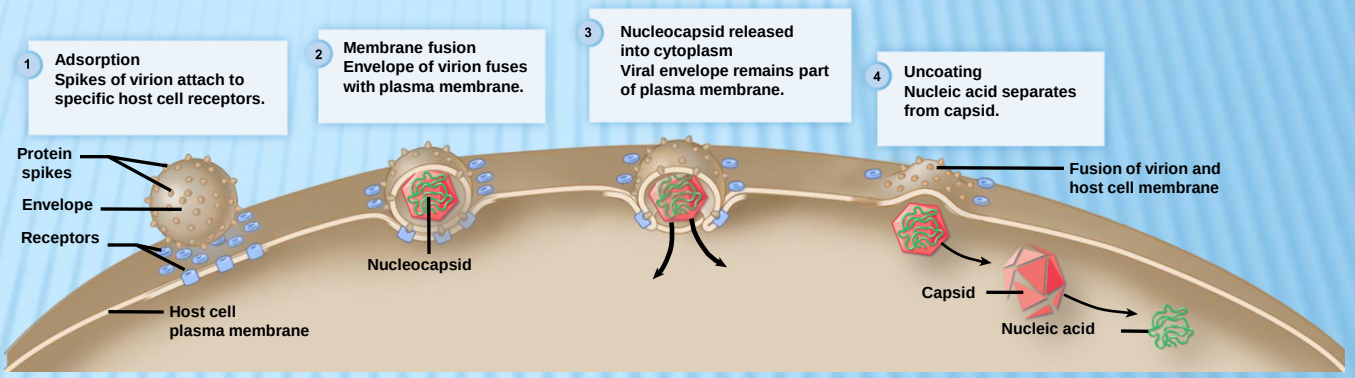


13.6. ANIMAL VIRUS REPLICATION

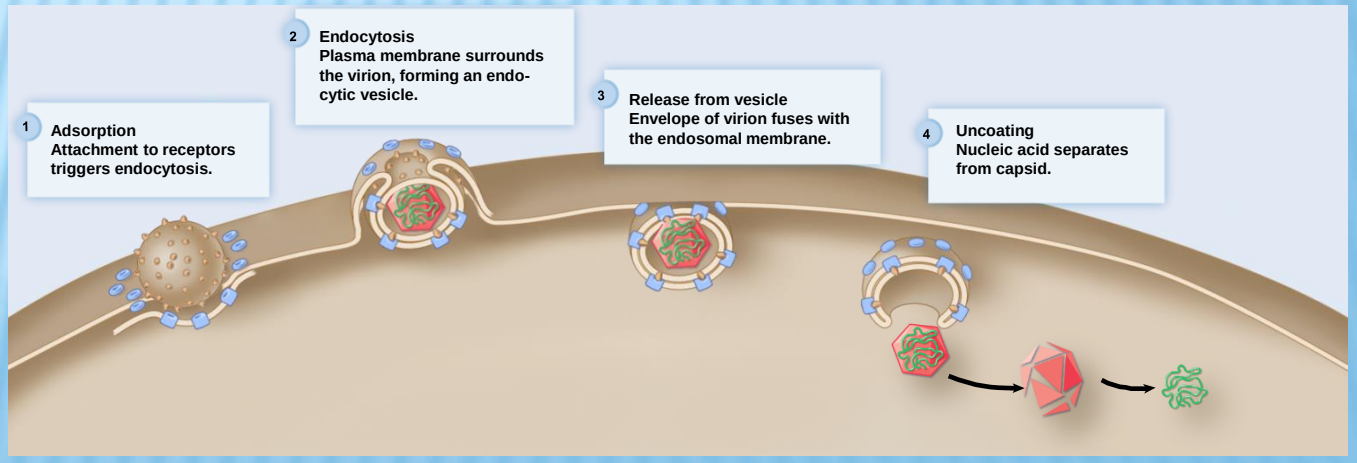
- Five-step infection cycle
  - Attachment
    - Viruses bind to receptors
    - Usually glycoproteins on plasma membrane
    - Often more than one required (e.g., HIV binds to two)
    - Normal function unrelated to viral infection
    - Specific receptors required; limits range of virus
    - E.g., dogs do not contract measles from humans

13.6. ANIMAL VIRUS REPLICATION

- Five-step infection cycle (continued…)
  - Penetration and uncoating: fusion or endocytosis
    - Naked viruses cannot fuse



(a) Entry by membrane fusion



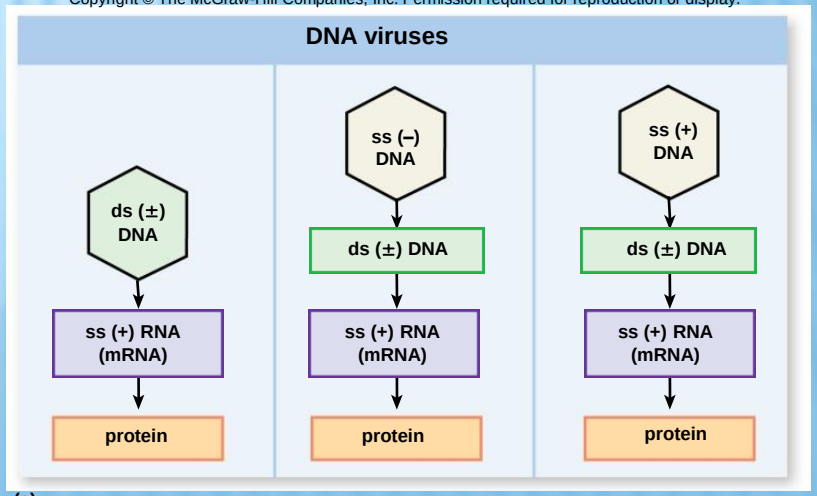
(b) Entry by endocytosis

13.6. ANIMAL VIRUS REPLICATION

- Five-step infection cycle (continued…)
  - Synthesis
    - Expression of viral genes to produce viral structural and catalytic genes (e.g., capsid proteins, enzymes required for replication)
    - Synthesis of multiple copies of genome
    - Most DNA viruses multiply in nucleus
    - Enter through nuclear pores following penetration
    - Three general replication strategies depending on type of genome of virus
      - DNA viruses
      - RNA viruses
      - Reverse transcribing viruses

13.6. ANIMAL VIRUS REPLICATION

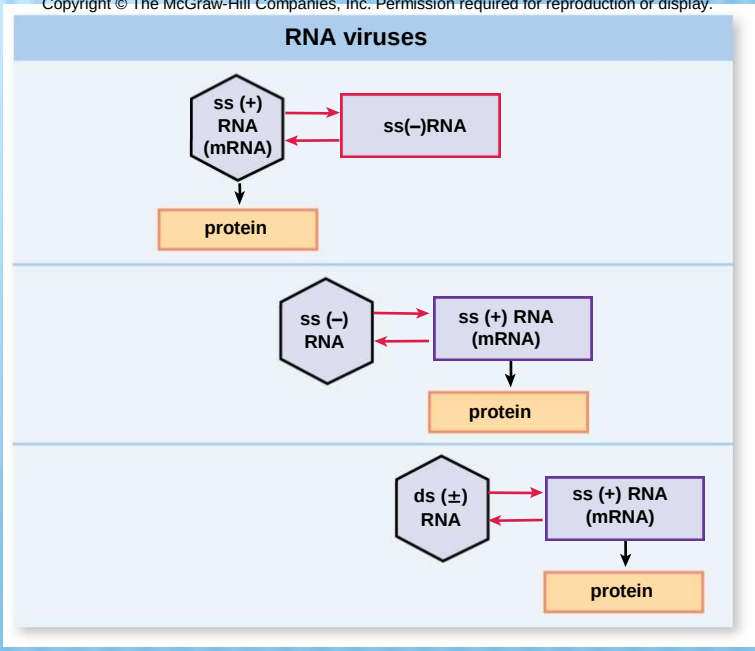
- Five-step infection cycle (continued…)
  - Replication of DNA viruses
    - Usually in nucleus
    - Poxviruses are exception: replicate in cytoplasm, encode all enzymes for DNA, RNA synthesis
    - dsDNA replication straightforward
    - ssDNA similar except complement first synthesized



(a)

13.6. ANIMAL VIRUS REPLICATION

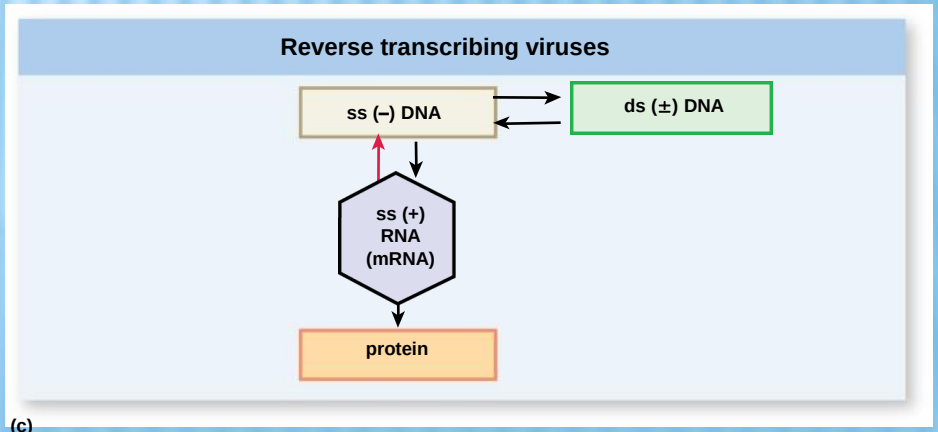
- Five-step infection cycle (continued…)
  - Replication of RNA viruses
    - Majority single-stranded; replicate in cytoplasm
    - Require virally encoded RNA polymerase (replicase), which lacks proofreading, allows antigenic drift
      - E.g., influenza viruses
    - ss (+) RNA used as mRNA
    - ss (–) RNA, dsRNA viruses carry replicase to synthesize (+) strand
    - Some RNA viruses segmented; reassortment results in antigenic shift



(b)

13.6. ANIMAL VIRUS REPLICATION

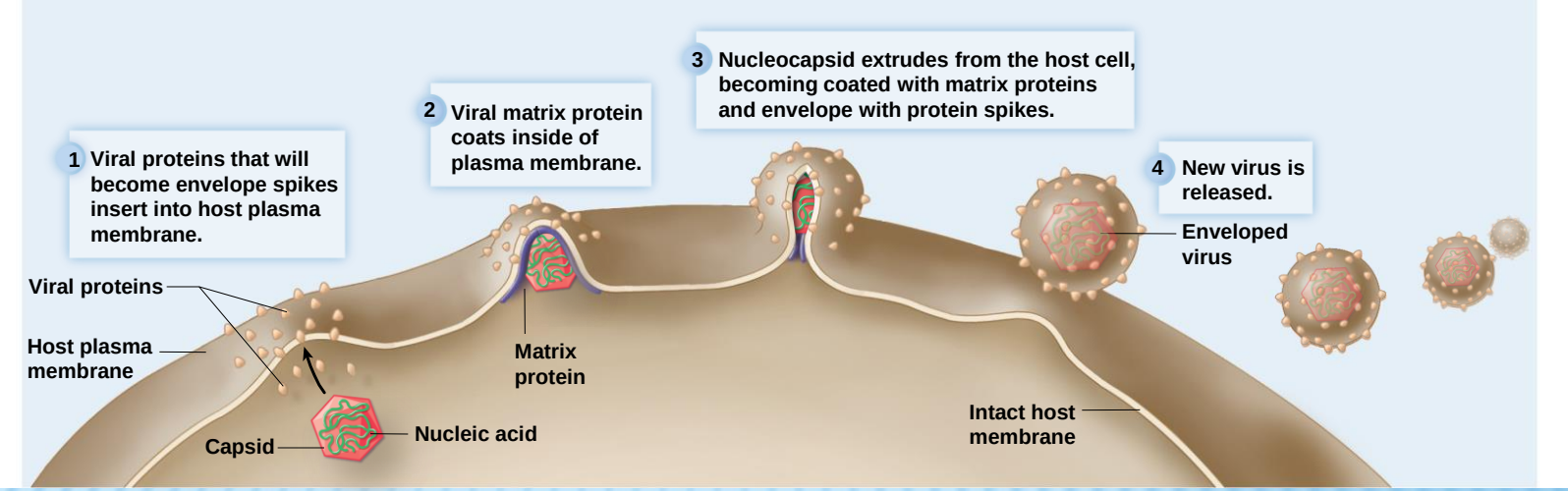
- Five-step infection cycle (continued…)
  - Replication of reverse-transcribing viruses
    - Encode reverse transcriptase: makes DNA from RNA
    - Retroviruses have ss (+) RNA genome (e.g., HIV)
    - Reverse transcriptase synthesizes single DNA strand
    - Complementary strand synthesized; dsDNA integrated into host cell chromosome
    - Can direct productive infection or remain latent
    - Cannot be eliminated



13.6. ANIMAL VIRUS REPLICATION

- Five-step infection cycle (continued…)
  - Assembly
    - Protein capsid forms; genome, enzymes packaged
    - Takes place in nucleus or in organelles of cytoplasm
  - Release
    - Most via budding
    - Viral protein spikes insert into host cell membrane; matrix proteins accumulate; nucleocapsids extruded
    - Covered with matrix protein and lipid envelope
    - Some obtain envelope from organelles
    - Naked viruses released when host cell dies, often by apoptosis initiated by virus or host

13.6. ANIMAL VIRUS REPLICATION



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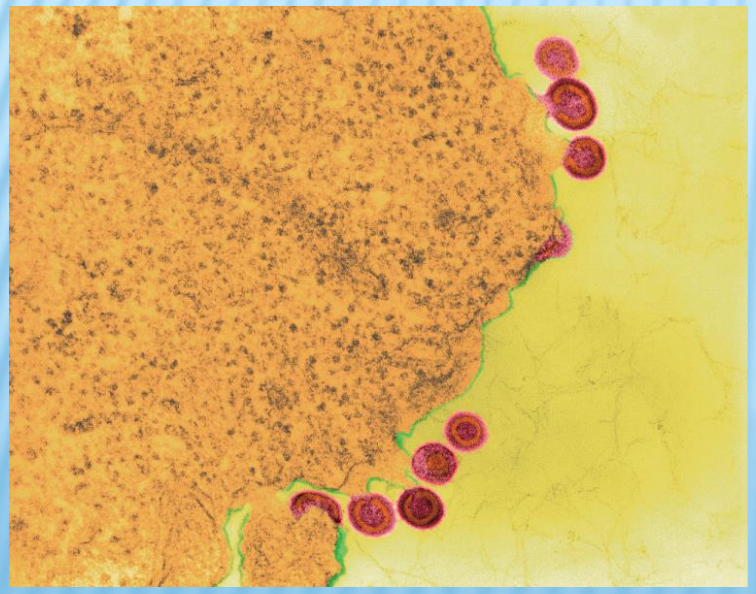
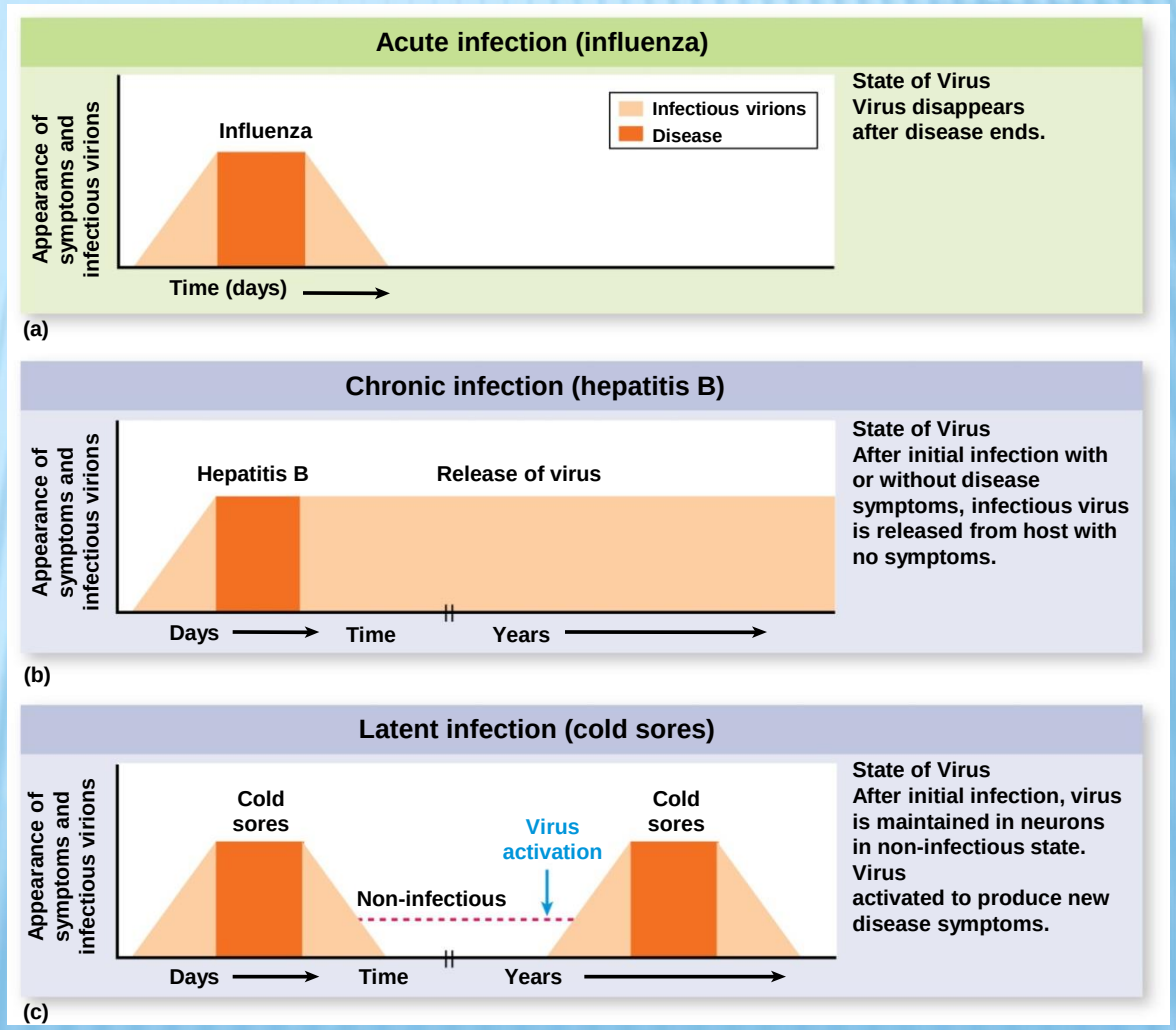


(b)

13.7. CATEGORIES OF ANIMAL VIRUS INFECTIONS

- Acute and Persistent Infections
  - Acute:
    - Rapid onset
    - Short duration
  - Persistent:
    - Continue for years or lifetime
    - May or may not have symptoms
  - Some viruses exhibit both (e.g., HIV)



13.7. CATEGORIES OF ANIMAL VIRUS INFECTIONS

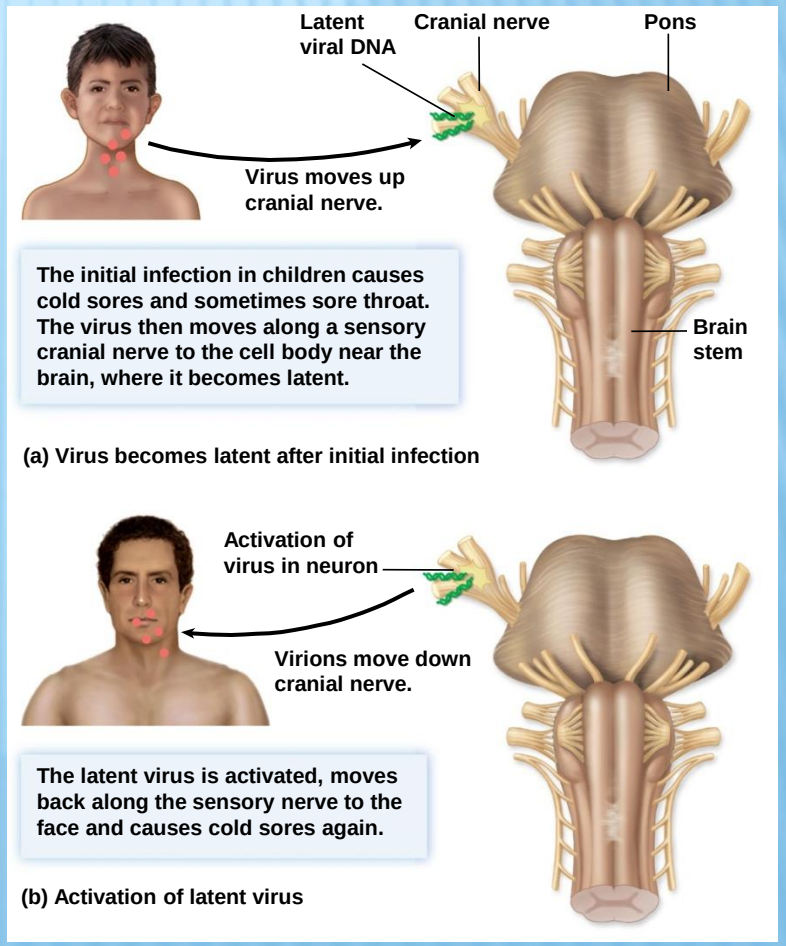
- Acute and Persistent Infections (continued…)
  - Persistent infections chronic or latent
    - Chronic infections: continuous production of low levels of virus particles
    - Latent infections: viral genome (provirus) remains silent in host cell; can reactivate

TABLE 13.4 Examples of Persistent Infections

Virus	Type of Infection	Cells Involved	Disease
Hepatitis B virus	Chronic	Hepatocytes (liver cells)	Hepatitis, cirrhosis, hepatocellular carcinoma
Hepatitis C virus	Chronic	Hepatocytes (liver cells)	Hepatitis, cirrhosis, hepatocellular carcinoma
Herpes simplex virus type 1	Latent	Neurons of sensory ganglia	Primary oral herpes and recurrent herpes simplex (cold sores)
Herpes simplex virus type 2	Latent	Neurons of sensory ganglia	Genital herpes and recurrent genital herpes
Varicella zoster (*Herpesviridae* family)	Latent	Satellite cells of sensory ganglia	Chickenpox and herpes zoster (shingles)
Cytomegalovirus (CMV; *Herpesviridae* family)	Latent	Salivary glands, kidney epithelium, leukocytes	CMV pneumonia, eye infections, mononucleosis, congenital CMV infection
Epstein-Barr virus	Latent	B cells, which are involved in antibody production	Burkitt's lymphoma
Human immunodeficiency virus (HIV)	Chronic	Activated helper T cells, macrophages	AIDS
	Latent	Memory helper T cells	

13.7. CATEGORIES OF ANIMAL VIRUS INFECTIONS

- Acute and Persistent Infections (continued…)
  - Latent infections: (cont…)
  - Provirus integrated into host chromosome or replicates separately, much like plasmid
  - Cannot be eliminated
  - Can later be reactivated



13.8. VIRUSES AND HUMAN TUMORS

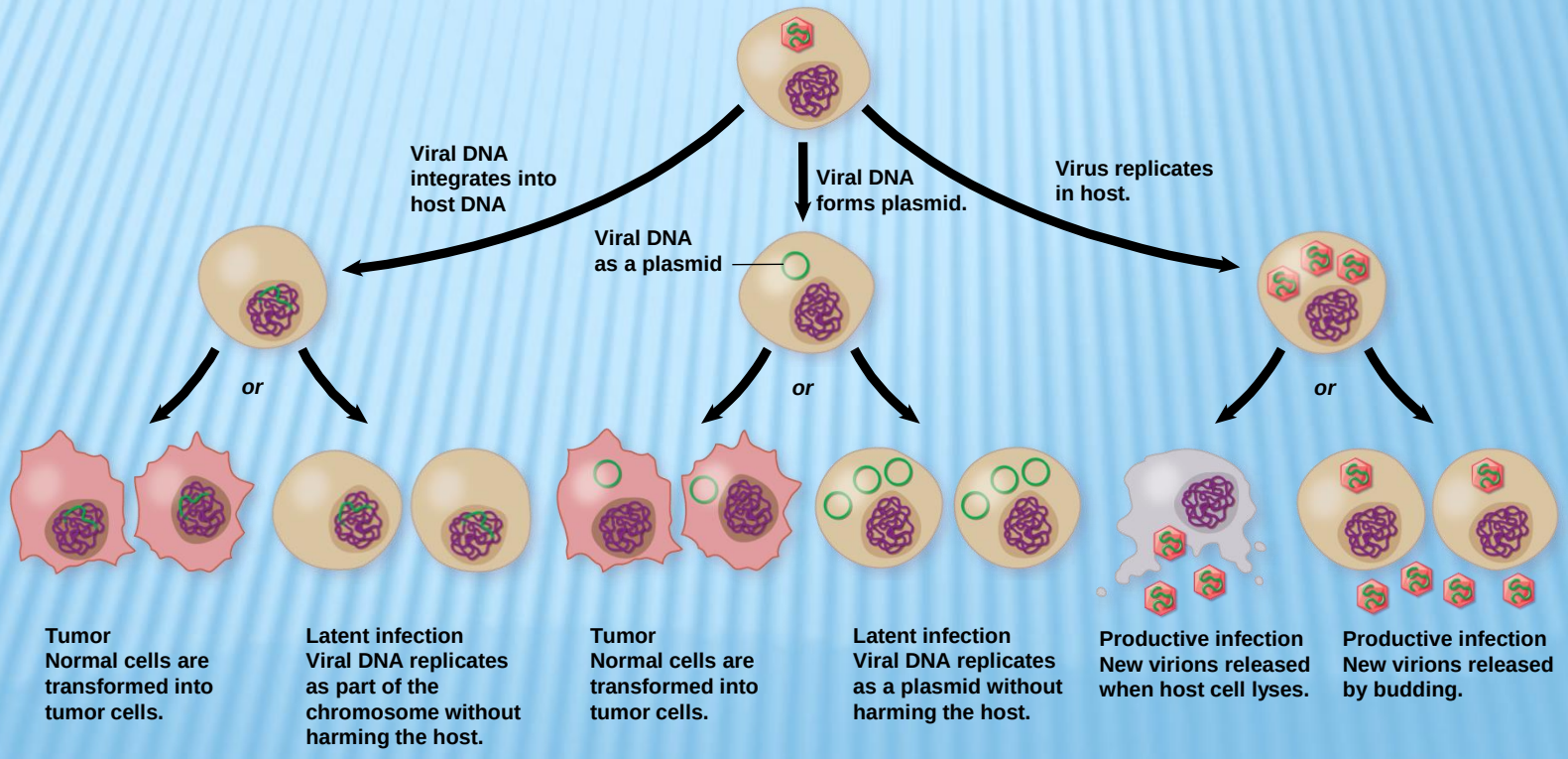
- Tumor is abnormal growth
  - Cancerous or malignant can metastasize; benign do not
  - Proto-oncogenes and tumor suppressor genes work together to stimulate, inhibit growth and cell division
  - Mutations cause abnormal and/or uncontrolled growth
    - Usually multiple changes at different sites required
  - Viral oncogenes similar to host proto-oncogenes; can interfere with host control mechanisms, induce tumors

TABLE 13.5 Viruses Associated with Cancers in Humans

Virus	Type of Nucleic Acid	Kind of Tumor
Human papillomaviruses (HPVs)	DNA	Different kinds of tumors, caused by different HPV types
Hepatitis B	DNA	Hepatocellular carcinoma
Epstein-Barr	DNA	Burkitt's lymphoma; nasopharyngeal carcinoma; B-cell lymphoma
Hepatitis C	RNA	Hepatocellular carcinoma
Human herpesvirus type 8	DNA	Kaposi's sarcoma
HTLV-1	RNA (retrovirus)	Adult T-cell leukemia (rare)

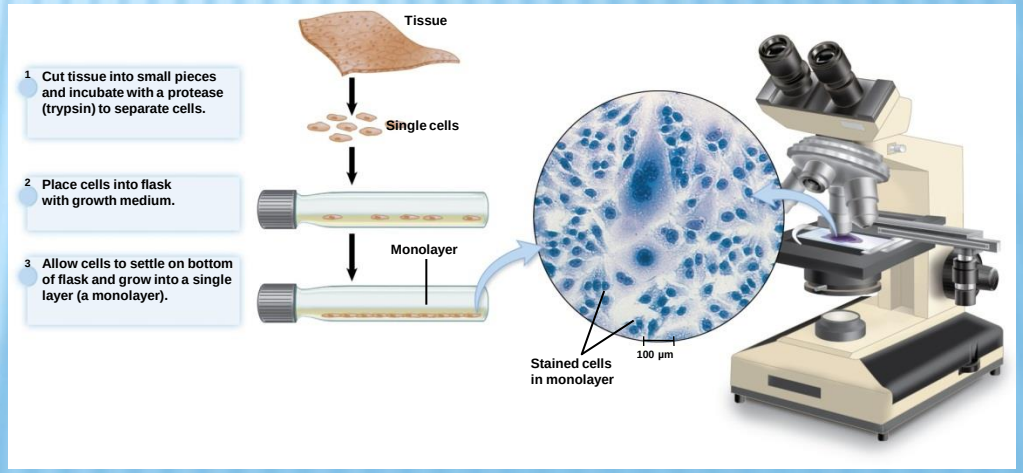
13.8. VIRUSES AND HUMAN TUMORS

- Productive infections, latent infections, tumors
  - Virus-induced tumors rare; most result from mutations



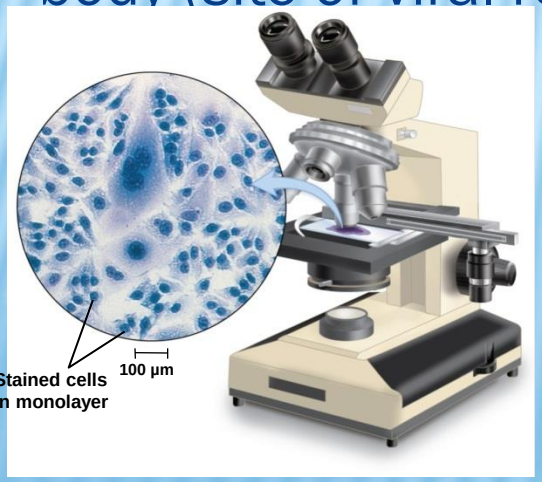
13.9. CULTIVATING AND QUANTITATING ANIMAL VIRUSES

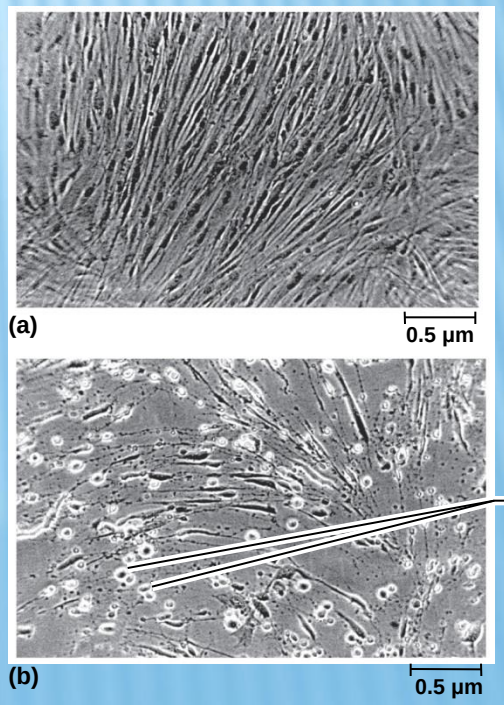
- Viruses must be grown in appropriate host
  - Historically done by inoculating live animals
  - Embryonated (fertilized) chicken eggs later used
  - Cell culture or tissue culture now commonly used
  - Can process animal tissues to obtain primary cultures
  - Drawback is cells divide only limited number of times
  - Tumor cells often used, multiply indefinitely



13.9. CULTIVATING AND QUANTITATING ANIMAL VIRUSES

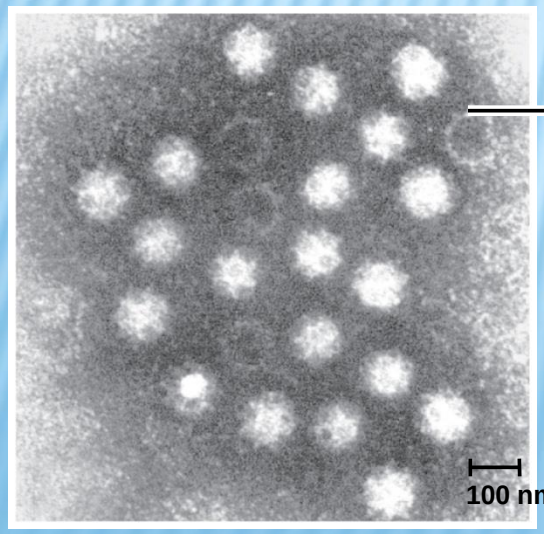
- Effects of Viral Replication of Cell Cultures
  - Many viruses cause distinct morphological alterations called cytopathic effect
  - Cells may change shape, fuse, detach from surface, lyse, fuse into giant multinuclear cell (syncytium), or form inclusion body (site of viral replication)

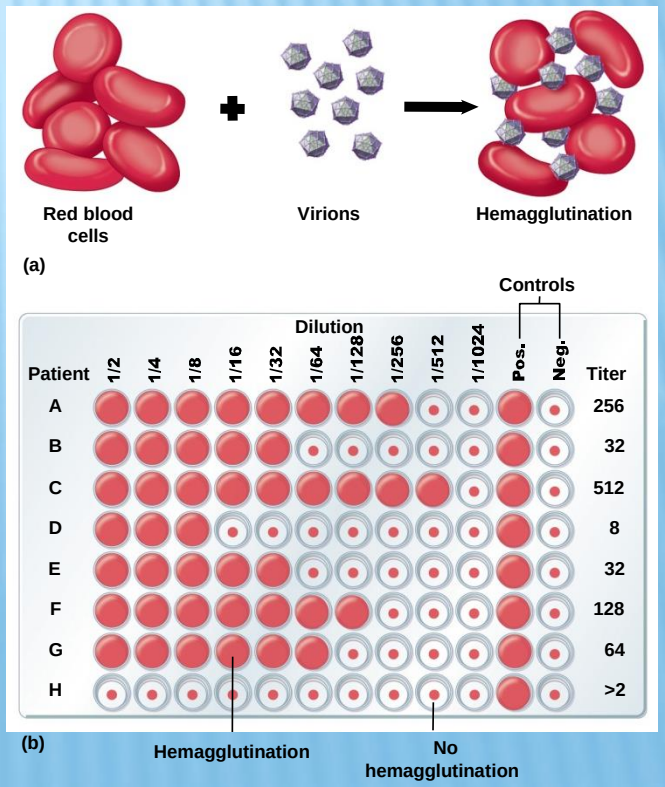




13.9. CULTIVATING AND QUANTITATING ANIMAL VIRUSES

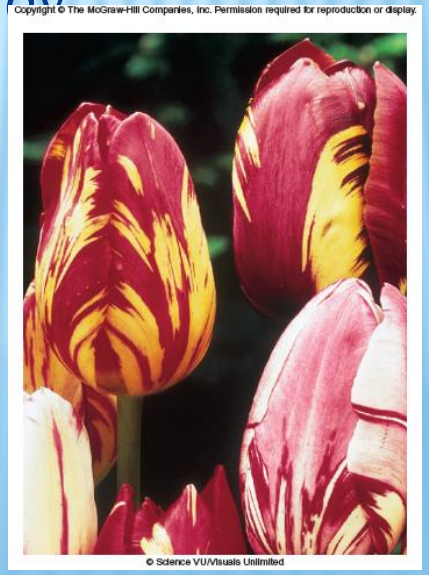
- Quantitating Animal Viruses
  - Plaque assays using monolayer of tissue culture cells
  - Direct counts via EM
  - Quantal assay: dilution yielding ID_{50} or LD_{50}
  - Hemagglutination: relative concentration

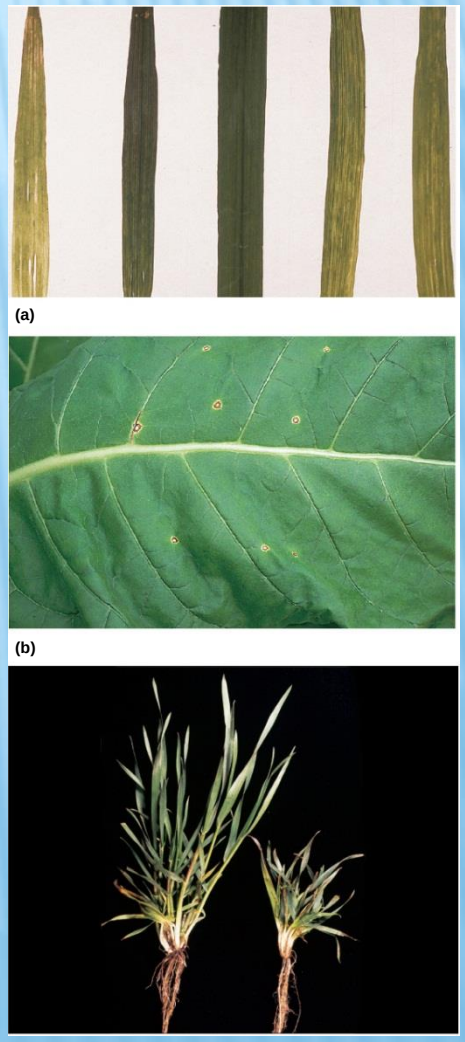




13.10. PLANT VIRUSES

- Plant viruses very common
  - Do not attach to cell receptors; enter via wounds in cell wall, spread through cell openings (plasmodesmata)
  - Plants rarely recover, lack specific immunity
  - Many viruses extremely hardy
  - Transmitted by soil; humans; insects; contaminated seeds, tubers, pollen; grafting



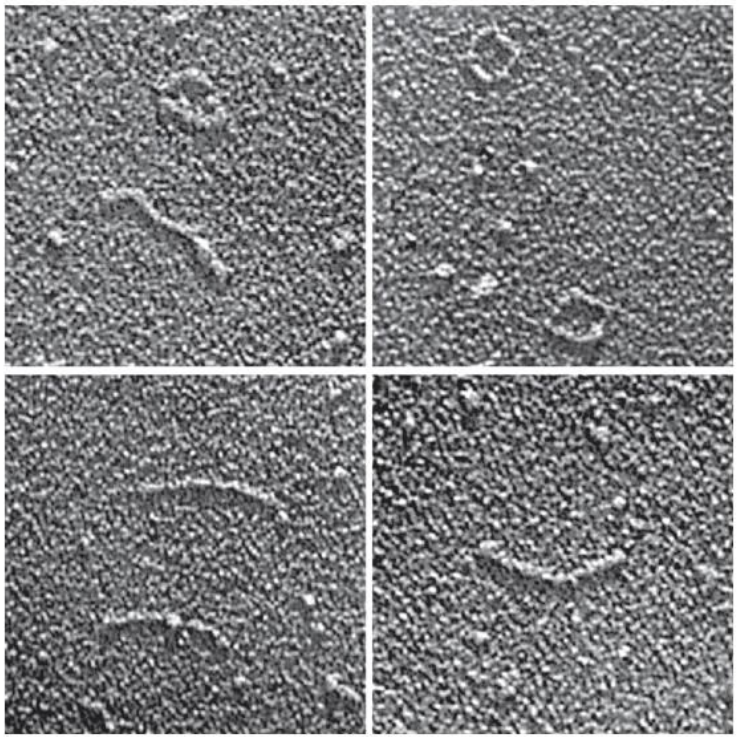

(a)

(b)

(c)

13.11. OTHER INFECTIOUS AGENTS: VIROIDS AND PRIONS

- Viroids are small single-stranded RNA molecules
  - 246–375 nucleotides, about 1/10th smallest RNA virus
  - Forms closed ring; hydrogen bonding gives ds look
  - Thus far only found in plants; enter through wound sites
  - Many questions remain:
    - How do they replicate?
    - How do they cause disease?
    - How did they originate?
    - Do they have counterparts in animals?



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13.11. OTHER INFECTIOUS AGENTS: VIROIDS AND PRIONS

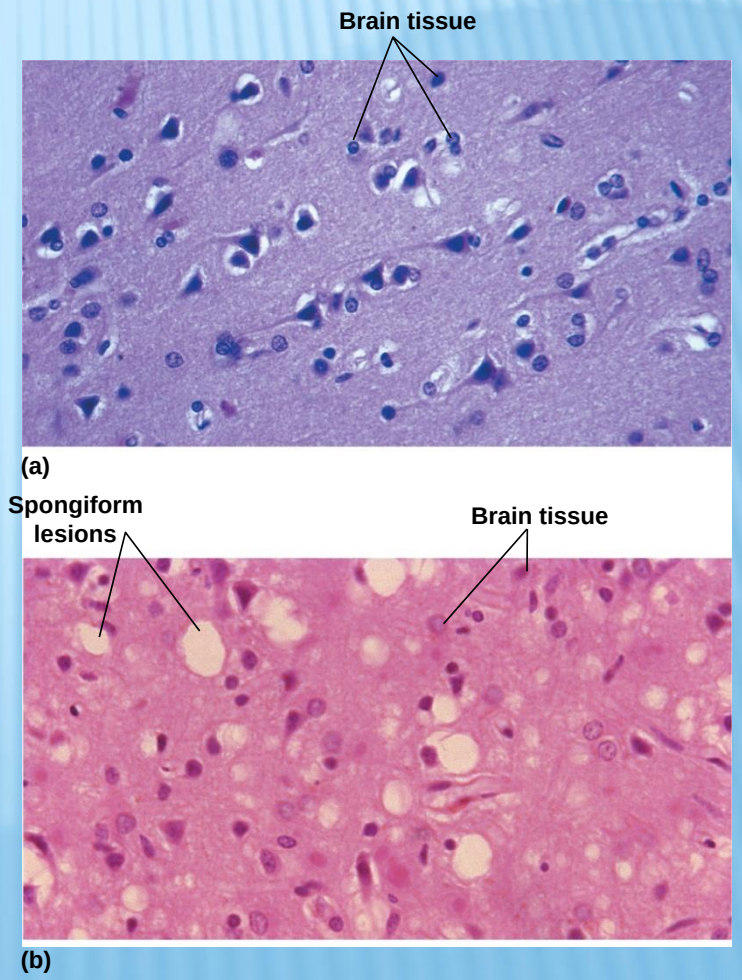
- Prions are proteinaceous infectious agents
  - Composed solely of protein; no nucleic acids
  - Linked to slow, fatal human diseases; animal diseases
  - Usually transmissible only within species
  - Mad cow disease in England killed >170 people

TABLE 13.6 Prion Diseases

Disease	Host
Scrapie	Sheep and goats
Bovine spongiform encephalopathy	Cattle
Chronic wasting disease	Deer and elk
Transmissible mink encephalopathy	Ranched mink
Exotic ungulate encephalopathy	Antelope in South Africa
Feline spongiform encephalopathy	Cats
Kuru	Humans (caused by cannibalism)
Variant Creutzfeldt-Jakob disease	Humans (caused by consumption of prion-contaminated beef)
Creutzfeldt-Jakob disease	Humans (inherited)
Gerstmann–Straussler Scheinker syndrome	Humans (inherited)
Fatal familial insomnia	Humans (inherited)

13.11. OTHER INFECTIOUS AGENTS: VIROIDS AND PRIONS

- Prions (continued…)
  - Prion proteins accumulate in neural tissue
    - Neurons die
    - Tissues develop holes
    - Brain function deteriorates
  - Characteristic appearance gives rise to general term for all prion diseases: transmissible spongiform encephalopathies



13.11. OTHER INFECTIOUS AGENTS: VIROIDS AND PRIONS

- Prions (continued…)
  - Cells produce normal form
    - PrP^{C} (prion protein, cellular)
    - Proteases readily destroy
  - Infectious prion proteins
    - PrP^{SC} (prion protein, scrapie)
    - Resistant to proteases; become insoluble, aggregate
    - Unusually resistant to heat, chemical treatments
  - Hypothesized that PrP^{SC} converts PrP^{C} folding to PrP^{SC}