Viruses & Prions

CCV
Dr. Melanie Meyer

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Viruses were first described in scientific terms as "filterable agents"

- Russian Dmitri Ivanowski, 1892, passed diseased tobacco leaf extract through filters that removed bacteria, filtrate caused disease (tobacco mosaic virus)
- In 1935 Wendell Stanley crystallized TMV

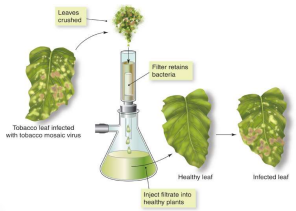

Figure 05.02A: Discovery of "filterable" viruses.

Figure 05.02B: TEM of TMV.

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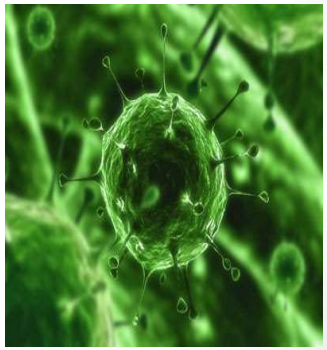
General Characteristics of Viruses

- Obligatory intracellular parasites—require living host cells in order to multiply
- Inert outside of living host cells
- Enter host→viral nucleic acids become active and viral multiplication occurs
- Especially small organisms—filterable



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Distinctive Features of Viruses

- Acellular (subcellular)
- Contain a single type of nucleic acid—either DNA or RNA (never both)
- Contain a protein coat that surrounds the nucleic acid
- Multiply inside living cells by using the synthesizing machinery of the cell
- Cause the synthesis of a specialized structure that can transfer the viral nucleic acid to other cells
- Viruses have few or no enzymes for their own metabolism—cannot synthesize ATP
- Most viruses are able to infect specific types of cells

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Host Range

= the spectrum of host cells the virus can infect

- Viruses can infect invertebrates, vertebrates, plants, protists, fungi and bacteria
- Most infect specific cell types but some can cross the host-range barrier

Ex: bacteriophage (*phages)* = viruses that infect bacteria

Phage therapy –using bacteriophages to treat bacterial infections

- Has been in use for 100 years
- Study is still underway

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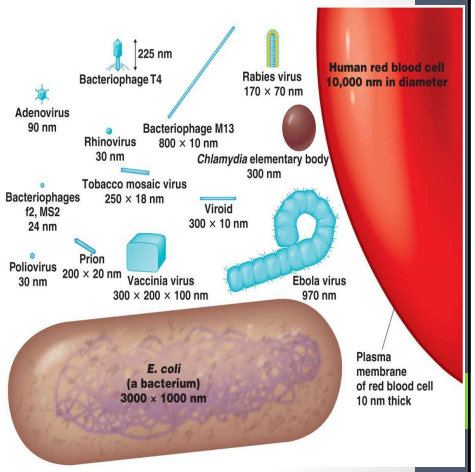
Viral Size

- Determined with the aid of an electron microscope
- Considerable variability in virus size
- Most are smaller than bacteria but with exceptions



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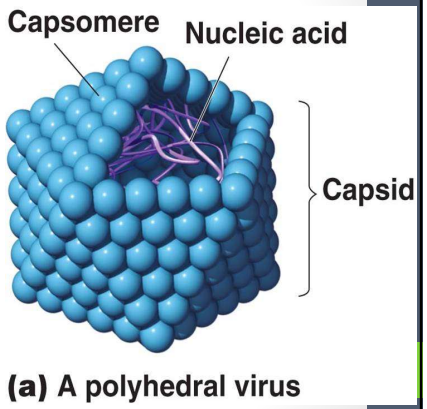
Viral Structure

Virion= a complete, fully developed, infectious viral particle composed of nucleic acid and surrounded by a protein coat outside of a host cell

- Extracelllular terminology
- Viruses are classified by their nucleic acid and by differences in the structures of their coats



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Viral Structure

Nucleic Acid:

- In contrast to prokaryotic and eukaryotic cells, in which DNA is always the primary genetic material, a virus can have either DNA *or* RNA—never both
- Nucleic acid of a virus can be single-stranded or double-stranded

TABLE 5.1 Virus Classification Based on Nucleic Acid Composition

Nucleic Acid	Virus(es) (Disease)
dsDNA	Human papillomavirus, Epstein-Barr virus, adenovirus, herpes simplex virus, varicella-zoster virus (chickenpox and shingles), *Variola* virus (smallpox)
ssDNA	Parvovirus B19 (possibly slapped-cheek disease)
ssRNA	Hepatitis A virus, poliovirus, norovirus, rubella virus, Ebola virus, influenza virus, West Nile encephalitis virus
dsRNA	Rotavirus, Colorado tick fever virus, reovirus

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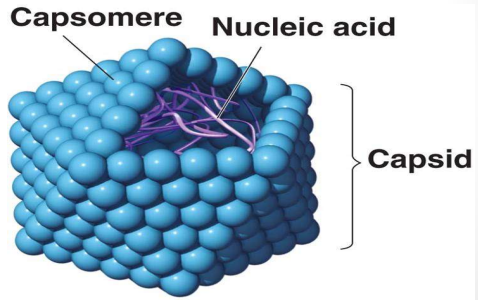
Viral Structure

Capsid—the protein coat which protects the nucleic acid of a virus

- Each capsid is composed of protein subunits called **capsomeres**



(a) A polyhedral virus

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Viral Structure

Envelope:

In some viruses, the capsid is covered by an envelope, which usually consists of some combination of lipids, proteins, and carbohydrates

- Some animal viruses are released from the host cell by an extrusion process that coats the virus with a layer of the host's plasma membrane—this layer becomes the viral envelope
- Depending on the virus, envelopes may or may not be covered by spikes

Spikes = carbohydrate-protein complexes that project from the surface of the envelope

- Some viruses attach to host cells by means of spikes

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Viral Structure

Non-enveloped Viruses (*naked viruses)*:

- Viruses whose capsids are not covered by an envelope
- The capsid of a non-enveloped virus protects the nucleic acid from nuclease enzymes in biological fluids and promotes the viruses's attachment to susceptible host cells

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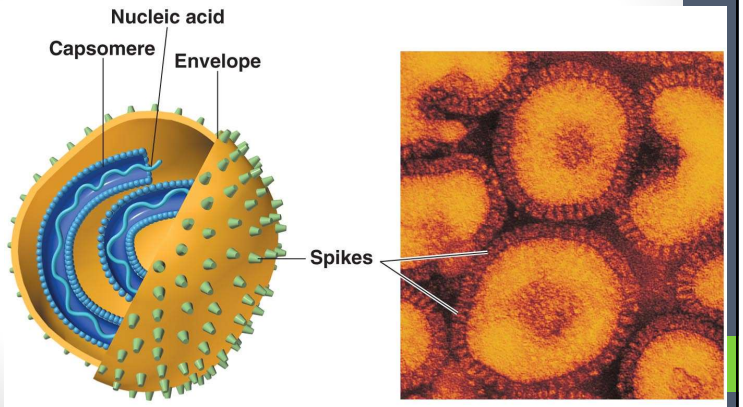
Morphology of an Enveloped Virus



(a) An enveloped helical virus

(b) *Influenzavirus*

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General Morphology

1) Helical Viruses: consist of a series of rod-shapes capsomeres that form a continuous helical tube

2) Polyhedral: many-sided

- **Icosahedral—**most polyhedral viruses are in this shape = 3-dimensional, 20-sided triangular stuctures that confer a geodesic shape to the virus

3) Complex: consist of a polyhedral head, a helical tail, and tail fibers that serve for attachment to the host cell (resemble a spacecraft)

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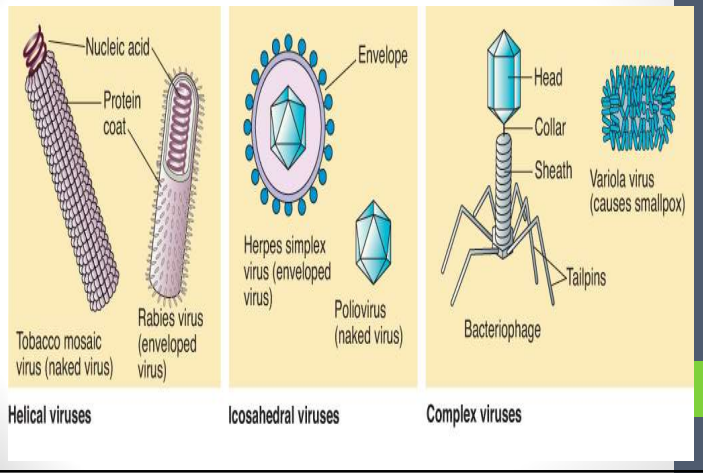
General Morphology—classification by capsid architecture



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Taxonomy of Viruses

- Family names end in -*viridae*.
- Genus names end in -*virus*.
- **Viral species**: A group of viruses sharing the same genetic information and ecological niche (host). Common names are used for species.
- Subspecies are designated by a number.

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Taxonomy of Viruses

- Herpesviridae
- *Herpesvirus*
- Human herpes virus HHV-1, HHV-2, HHV-3
- Retroviridae
- *Lentivirus*
- Human immunodeficiency virus HIV-1, HIV-2

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Viral Classification

- **Structure**: by type of nucleic acid and shape
- **Host Cell**: (microbe, plant, or animal)
  - Bacterial viruses termed **bacteriophage** or **phage**
- **Clinical**: organ or organ system
  - **Dermotropic** (skin)
  - **Viscerotropic** (internal organs)
  - **Pneumotropic** (respiratory system)

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TABLE 5.2 Clinical Classification of Viruses

Category	Tropisms (Tissue Affinities)	Diseases
Dermotropic	Skin and subcutaneous tissues	Chickenpox, shingles, measles, mumps, smallpox, rubella, herpes simplex, warts and cervical cancer (caused by papillomaviruses)
Neurotropic	Brain and central nervous system tissues	Rabies, arboviral encephalitides (eastern equine encephalitis, St. Louis encephalitis, La Crosse encephalitis, West Nile encephalitis), polio, Nipah encephalitis
Viscerotropic	Internal organs	Yellow fever, AIDS, hepatitis A and B, infectious mononucleosis, dengue fever, gastroenteritis (e.g., norovirus)
Pneumotropic	Lungs and other respiratory structures	Influenza, common cold, respiratory syncytial disease, severe acute respiratory syndrome (SARS)

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Latent Viral Infections

- Virus remains in asymptomatic host cell for long periods
  - Cold sores, shingles

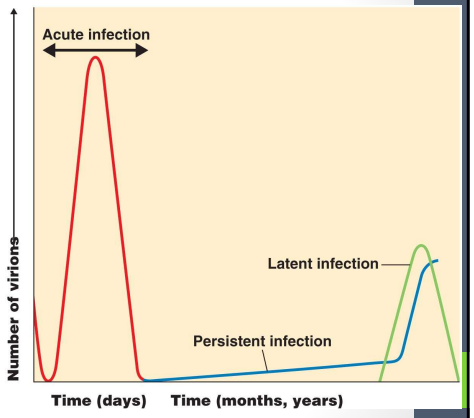

Figure 13.21

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Persistent Viral Infections

- Disease processes occurs over a long period; generally is fatal
  - Subacute sclerosing panencephalitis (measles virus)

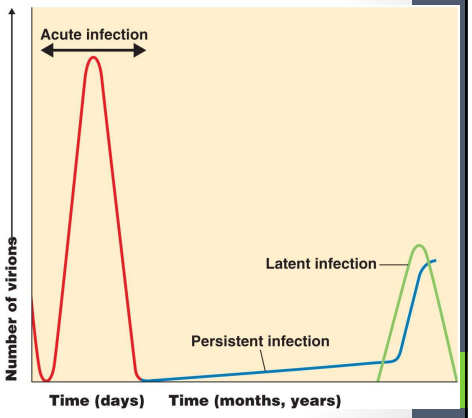

Figure 13.21

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Viral Replication—5 Stages

TABLE 5.3 Generalized Viral Replication Cycle[a]

Stage	Description
Adsorption (attachment)	Viruses attach to cell surface receptor molecules by spikes, capsids, or envelope.
Penetration	Entire viral particle or only nucleic acid enters via endocytosis or by fusion with cell membrane.
Replication	Process is complex, and details depend on particular viruses and their nucleic acid structure. Replication may occur in the nucleus or cytoplasm, viral nucleic acid replicates, and genes are expressed, leading to production of viral components.
Assembly	Components are assembled into mature viruses.
Release (exit)	Viruses are extruded from host cell by budding (HIV) or lysis of host cell membrane.

[a]Specific strategies vary with particular viruses.

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Viral Multiplication

- For a virus to multiply, it must invade a host cell and take over the host's metabolic machinery
- A single virion can give rise to several or even thousands of similar viruses in a single host cell.
- This process can drastically change the host cell and usually causes its death
- In a few viral infections, cells survive and continue to produce viruses indefinitely

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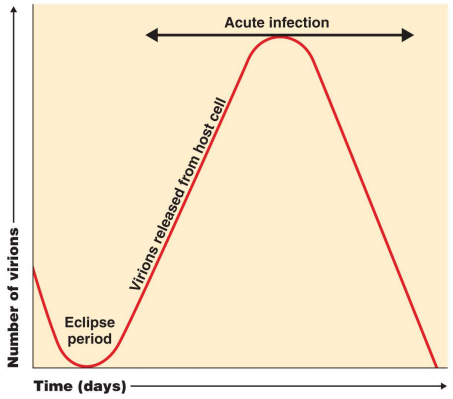
Viral One-Step Growth Curve



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Bacteriophage Replication

- **Lytic cycle**
  - Phage causes lysis and death of host cell
- **Lysogenic cycle**
  - Prophage DNA incorporated in host DNA
  - Phage conversion
  - Specialized transduction

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Two types of bacteriophage infection: **lytic** or **lysogenic**

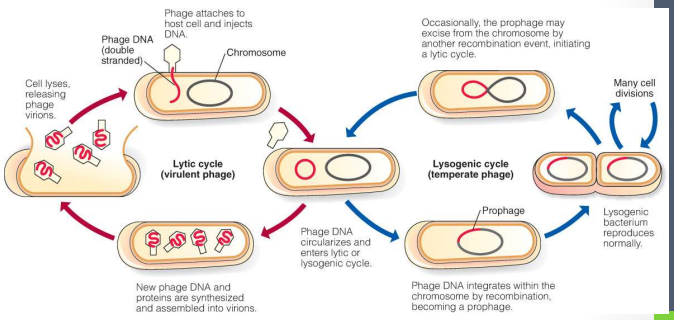

Figure 05.05: Replication cycle of a bacterial virus.

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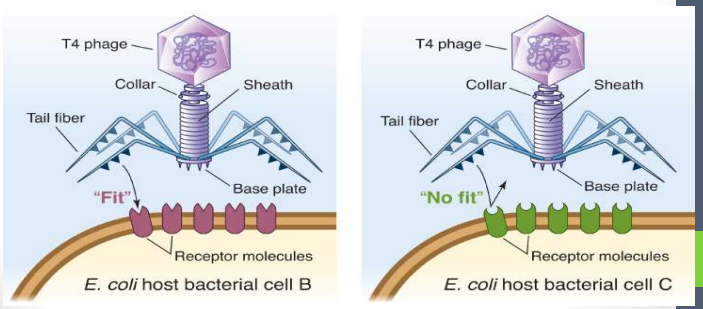
1) Adsorption (Attachment)

- Virus docks with specific cell surface **receptors**
- Recognition specificity establishes **host range**



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2) Penetration

Viruses have evolved various strategies for delivering the genome to the cytoplasm

- The nucleic acid alone enters the cell and the virion remains outside the cell (e.g., phage, polio).
- For some enveloped viruses (e.g., measles, mumps) the viral and cell membranes **fuse** to release nucleocapsid.
- For other enveloped viruses (e.g., poxviruses, influenza) the entire virion is **endocytosed**. The vesicle and viral membranes fuse to release the nucleocapsid.

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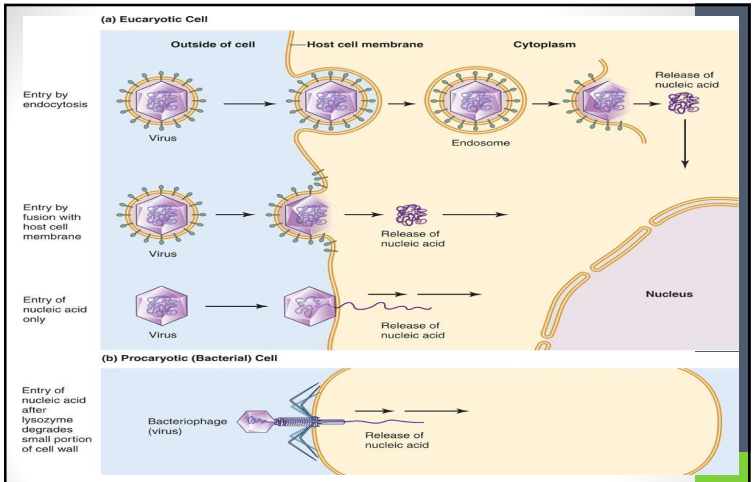

Figure 05.07: Strategies of viral penetration of cells.

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3) Viral Replication

- The replication process is dependent upon the genome type of the virus (DNA or RNA, ss or ds)
- The genome contains the blueprints for making the parts of the virus
- The various viral parts (protein and nucleic acid) are manufactured (usually) using machinery and building block molecules from the host

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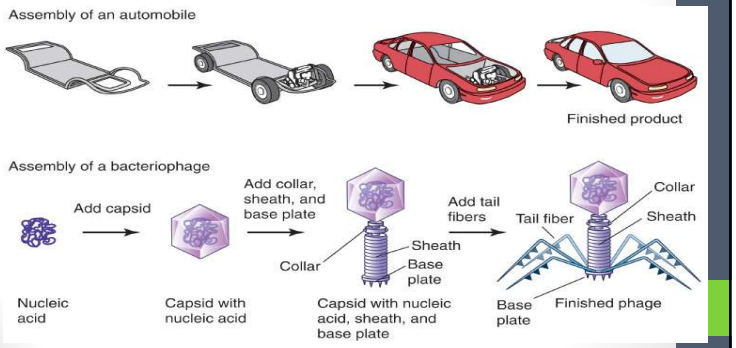
4) Assembly

- Once the parts are made (proteins, genome, etc.), they "self-assemble," in an assembly line fashion, to make new virions



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5) Release

New virions exit the cell by one of several virus-specific mechanisms

- Phage and some animal viruses rupture (lyse) the cell (the host cell dies).
- Some enveloped viruses are released by **budding**, or "endocytosis in reverse."
  - budding is gradual and does not necessarily kill the cell

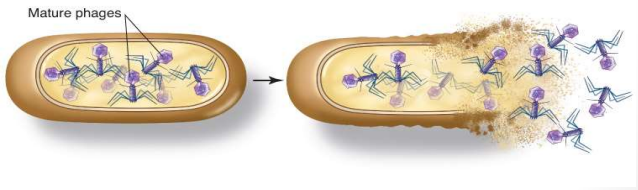

Figure 05.09: Lysis of bacterial host cell by phage.

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Budding of enveloped viruses

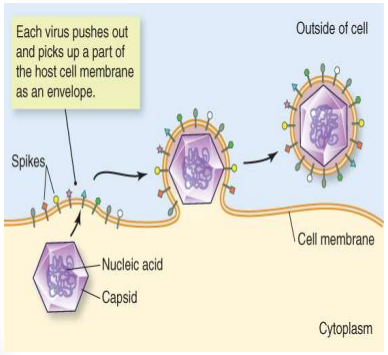

Figure 05.10A: Release by budding.

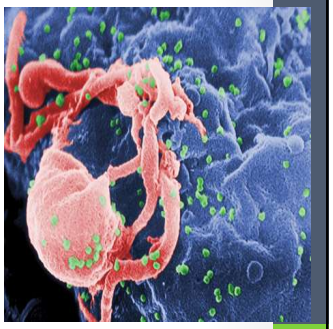

Figure 05.10B: SEM of HIV-budding from T lymphocyte.

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Studying Viruses in the Lab

- Hard to grow and study due to status as obligate intracellular parasites
- Cultivation of viruses requires the presence of living cells
- Bacteriophages are relatively easy to study due to the ease of using readily grown bacteria as the host
- Bacteriophages have served a model for animal viruses
- Mostly the T4 bacteriophase with *E. coli*

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Growing Viruses

- Animal and plant viruses may be grown in cell culture
  - Continuous cell lines may be maintained indefinitely

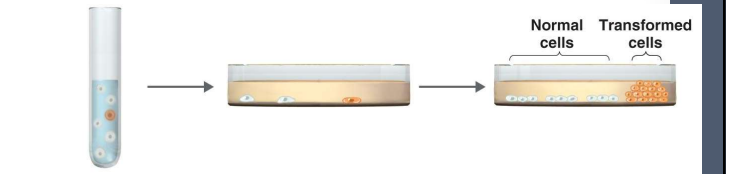

Figure 13.8

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Growing Viruses

- Animal viruses may be grown in living animals or in embryonated eggs

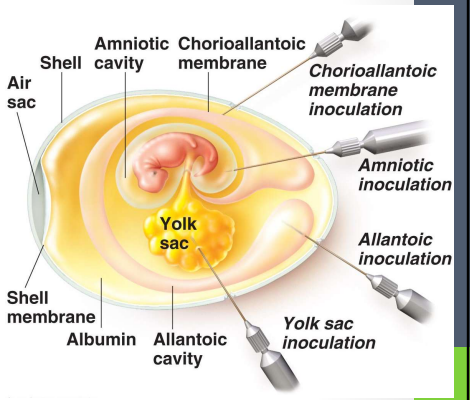

Figure 13.7

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Growing Viruses

- Bacteriophages form plaques on a lawn of bacteria

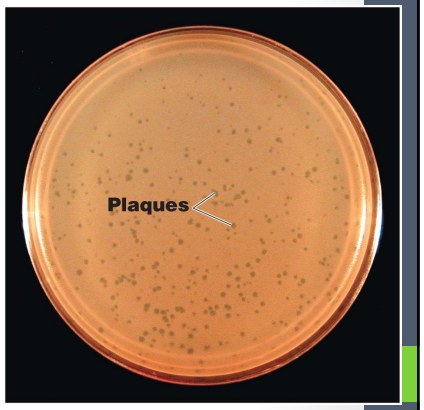

Figure 13.6

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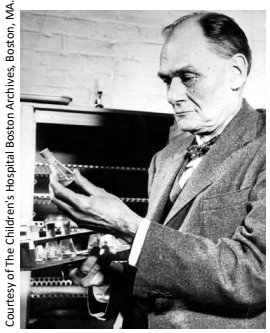
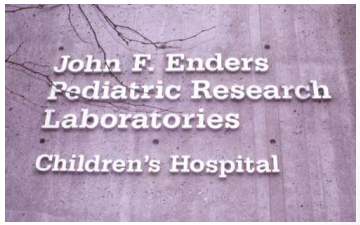
Enders, Weller and Robbins successfully cultivated poliovirus in monkey kidney cells in culture.



Courtesy of The Children's Hospital Boston Archives, Boston, MA.



Author's Photo (RIK)

Figure 05.14: John F. Enders Pediatric Research Laboratories.

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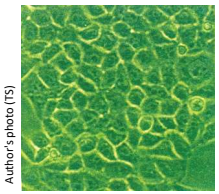
Cell culture in the ' 40s and ' 50s replaced use of live monkeys fo growth of polio virus and led to Salk and Sabin vaccines



Author's photo (TS)

Figure 05.13B: Monkey kidney cells in culture.

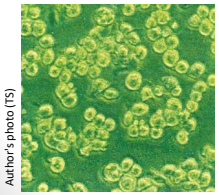


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Figure 05.13C: Monkey kidney cells in culture infected with vaccinia virus. CPEs include detachment and rounding of cells.

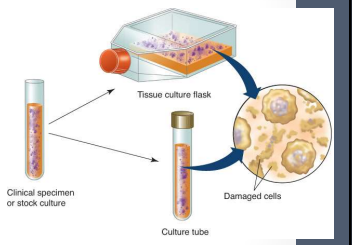

Figure 05.13A: Cultivation of viruses in cell culture.

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Diagnosis of Viral Infection

- Unlike bacteria, viruses are usually not cultured for diagnosing illness
  - –Too difficult, expensive, and time-consuming
- Diagnosis based on signs and symptoms/clinical presentation in the majority of viral infections
  - Telltale rashes are particularly useful (chickenpox, etc.)

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Host Cell Damage

- Most viruses damage or even kill their host
- Can be helpful to examine host tissue for pathological changes associated with viral infection
- **Cytopathic effect** (CPE) is characteristic of the damage caused
- Viewed microscopically, the CPE may be helpful in viral identification
  - Observation of **syncytia** formation
  - **Negri bodies** are visualized 50% of the time in brain tissues from rabid animals

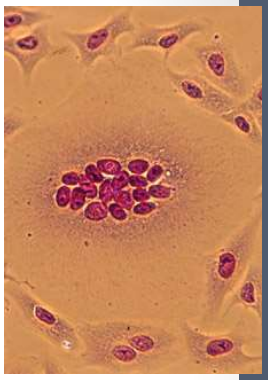

Figure 05.11: Syncytia formation (cells in culture infected with measles virus).

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Diagnosis of Viral Infection

Molecular technology is available in specialized viral diagnostic labs.

1) Enzyme-linked immunosorbent assays (ELISAs)—look for antibodies to the virus in the host's body fluid

- Some designed as rapid tests
  - Rapid influenza A & B test

2) Nucleic acid-based diagnostics

- Uses some form of **polymerase chain reaction (PCR)**
- Amplifies the genetic material to identify the virus

Figure 05.15: Rapid influenza A & B test.

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Diagnosis of Viral Infection

TABLE 5.4 Most Useful Diagnostic Procedures Used for Viral Infections

Diagnostic Approach	Virus
Microscopy	Varicella zoster, arenaviruses, poxviruses
ELISA (to detect viral antigens or antibodies)	Influenza A and B, HIV, hepatitis B and C viruses, papillomaviruses
PCR-based method	Rabies virus, rotaviruses
Cell culture	Rhinoviruses, mumps virus, rubella virus

Table 05.04: Most useful diagnostic tests used for viral infections.

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Viruses & Cancer

- Viruses have been implicated in the development of a number of cancers

Some background to better understand the relationship:

- Under normal conditions, the division of cells in a mature multicellular animal is under strict genetic control
- If something upsets genetic control, cells begin to divide uncontrollably
- Neoplasia = phenomenon of uncontrolled cell division
- Tumor = mass of neoplastic cells
- Benign or malignant
- Cancers rob normal cells of space and nutrients and cause pain→affect tissue function

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Virus and Cancer Theories

- Several theories have been proposed to explain the role viruses play in the development of cancers
- These theories revolve around the presence if *protooncogenes.*
- Protooncogenes play a role in cell division. As long as they are repressed, no cancer results.
- If they become active as *oncogenes* or if oncogene repressors are inactivated, cancer can develop.
- In most cases, several genetic changes must occur before cancer develops→ "multiple hits" to the genome
- Environmental factors contributing to the inhibition of oncogene repressors include: UV light, radiation, certain chemicals called carcinogens, and viruses

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Viruses & Cancer

Viruses cause 20-25% of human cancers in several ways:

- Some viruses carry copies of oncogenes as part of their genomes.
- Some viruses promote oncogenes already present in the host
- Some viruses interfere with normal tumor repression when they insert, as proviruses, into repressor genes

Documented virally-induce cancers in humans include:

- Burkitt's Lymphoma
- Hodgkin's disease
- Kaposi's sarcoma
- Cervical cancer

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Oncogenic Viruses

- **Oncogenic DNA viruses**
  - Adenoviridae
  - Herpesviridae
  - Poxviridae
  - Papovaviridae
  - Hepadnaviridae
- **Oncogenic RNA viruses**
  - Retroviridae
  - Viral RNA is transcribed to DNA, which can integrate into host DNA
  - HTLV-1
  - HTLV-2

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In better news…

Viruses are also being studied for their potential in treating cancer.

Oncolytic virotherapy = an emerging technology that uses engineered viruses to treat malignancies

- Viruses can be designed with biological specificity to infect cancerous cells preferentially, and to replicate in these cells exclusively.
- Malignant cells may be killed directly by overwhelming viral infection and lysis, which releases additional viral particles to infect neighboring cells and distant metastasis.
- Viral infections may also activate the immune system, unmask stealthy tumor antigens, and aid the immune system to recognize and attack neoplasms.

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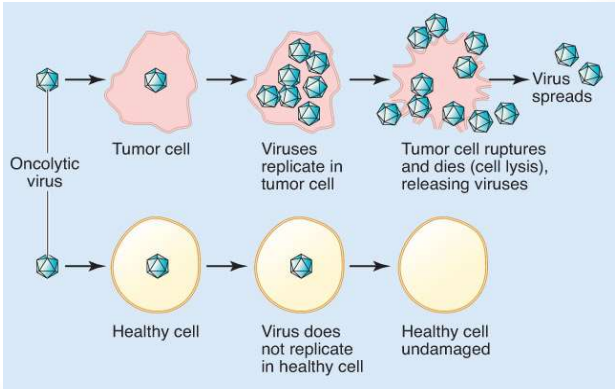
Oncolytic Virotherapy



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Phage Therapy

- The problem of antibiotic resistance in bacteria has rejuvenated interest in using phage to fight bacterial infection
  - Phage target specific bacteria, leaving beneficial ones alone
  - Phage are inexpensive, easily grown and purified

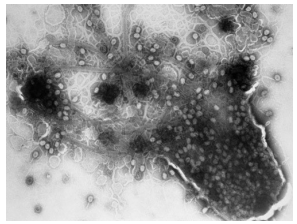

Figure 05.16A: The lytic activity of phage.

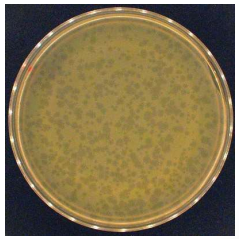

Figure 05.16B: A lawn of *E. coli* with plaques.

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Prions

=*Pro*teinaceous *In*fectious particle

- Subcellular/acellular
- Smaller than a virus
- Lacks both DNA and RNA
- Cause: bovine spongiform encephalopathy (mad cow disease) in animals and variant Creutzfeldt-Jakob disease (vCJD) in humans
- Once thought to be "slow viruses"
- Inherited and transmissible by ingestion, transplant, and surgical instruments
  - Spongiform encephalopathies: Sheep scrapie, Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia, mad cow disease
- Normal cooking or sterilization procedures do not deactivate prions
- No treatment for prion disease
- Different prions may lie behind other neuronal diseases such as Alzheimer's Dz, Parkinson's Dz, and ALS.

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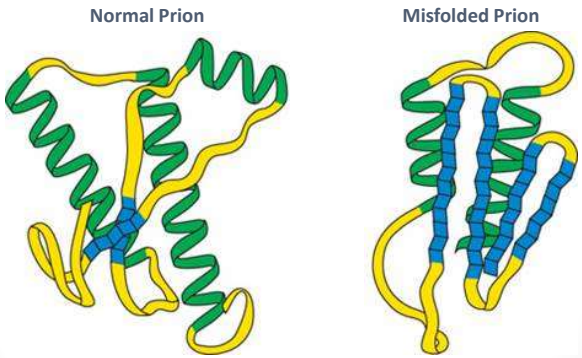
Prions



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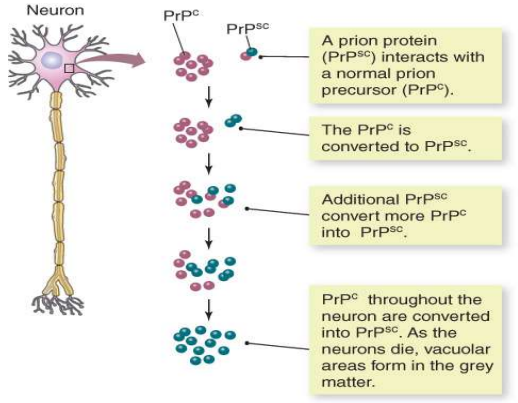
Conversion of Normal Prion to Infectious Prion



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