Unit 4: Eukaryotic Microorganisms and Viruses

I. Fungi: Yeasts; Molds; Fungal Pathogenicity; Control of Fungi.

II. Protozoa

III. Viruses: Characteristics of Viruses; Size and Shapes of Viruses; Viral Structure; Viral Classification; Viral Life Cycles; Viral Pathogenicity; Control of Viruses.

An overview of fungi
AN OVERVIEW

Fungi

A. An Overview of Fungi

Fundamental Statement for this Softchalk Lesson:

1. Fungi include yeasts, molds, and fleshy fungi.
2. Fungi are are eukaryotic organisms and possess a cell wall.
3. Most fungi are saprophytes, organisms that live off of decaying matter; a few are parasites, organisms that live off of living matter.
4. A fungal infection is called a mycosis.

Common Course Objectives

1. Recall features of fungi that distinguish them from other microbes with emphasis on dimorphism and true versus opportunistic infections.
2. Distinguish between different fungal infections based on the causative agent, modes of transmission, portal of entry, and portal of exit.

Detailed Learning Objectives

1. Name 3 groups of fungi.

2. Define mycosis.

An Overview of Fungi

Mycology is the study of fungi. Fungi include **yeasts, molds, and fleshy fungi**. They:

1. are eukaryotic;
2. have a rigid cell wall;
3. are chemoheterotrophs (organisms that require organic compounds for both carbon and energy sources);
4. obtain their nutrients by absorption;
5. obtain nutrients as saprophytes, organisms that live off of decaying matter, or as parasites, organisms that live off of living matter.

For more information: Review of prokaryotic and eukaryotic cells

Of the over 100,000 species of fungi, only about 100 species are pathogenic for animals. They play a major role in the recycling of nutrients by their ability to cause decay and are used by industry to produce a variety of useful products. However, they also cause many undesirable economic effects such as the spoilage of fruits, grains, and vegetables, as well as the destruction of unpreserved wood and leather products.

We will be concerned mainly with the yeasts and molds, especially those causing **mycoses** (fungal infections).

Quiz Group

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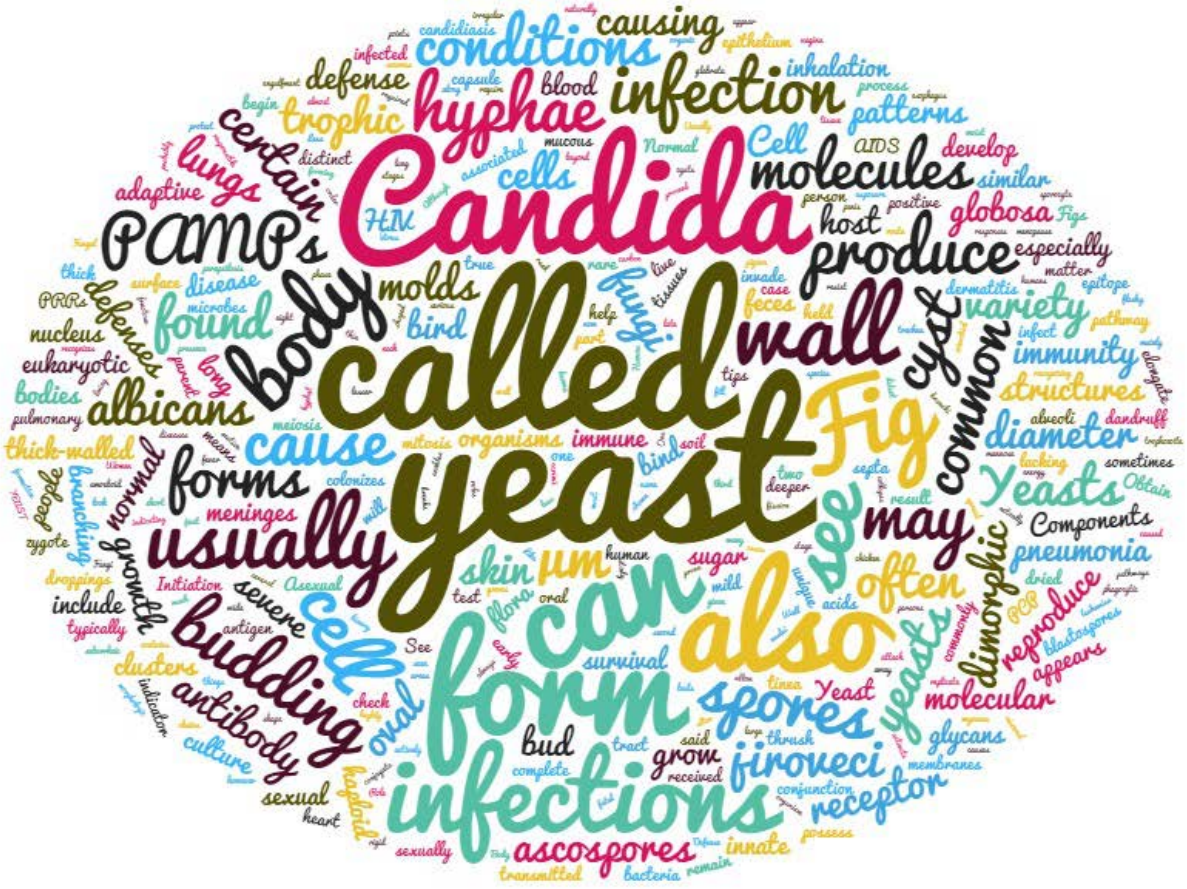
Yeasts
YEASTS

Fungi

B. Yeasts



Fundamental Statement for this Softchalk Lesson:

1. Yeasts are eukaryotic unicellular fungi
2. Some yeast are dimorphic in that they can grow as an oval, budding yeast, but under certain culture conditions, they may produce filament-like structures called hyphae similar to molds.
3. Components of the yeast cell wall that function as pathogen-associated molecular patterns or PAMPs include lipoteichoic acids, zymosan, and mannose-rich glycans.
4. These PAMPs bind to pattern-recognition receptors or PRRs on a variety of body defense cells and triggers innate immune defenses.
5. Cell wall molecules can also trigger adaptive immunity such as the production of antibody molecules against bacterial cell wall antigens.
6. Yeasts reproduce asexually by a process called budding.
7. Candida albicans is found as normal flora on the mucous membranes and in the gastrointestinal tract but is usually held in check by the body's normal microbiota and normal body defenses.
8. Candida can cause a variety of opportunistic infections in people who are debilitated, immunosuppressed, or have received prolonged antibacterial therapy, and infect the lungs, blood, heart, and meninges, especially in the compromised or immunosuppressed host.
9. Cryptococcus neoformans infections are usually mild or subclinical but, when symptomatic, usually begin in the lungs after inhalation of the yeast in dried bird feces.
10. Pneumocystis jiroveci can cause a severe pneumonia called PCP (Pneumocystis pneumonia).
11. Malassezia globosa is the most frequent cause of a superficial skin infection called tinea versicolor and also the most common cause of dandruff.

Common Course Objectives

1. Recall features of fungi that distinguish them from other microbes with emphasis on dimorphism and true versus opportunistic infections.
2. Distinguish between different fungal infections based on the causative agent, modes of transmission, portal of entry, and portal of exit.

Detailed Learning Objectives

1. Briefly describe yeasts and state how they reproduce asexually.

2. Briefly describe pseudohyphae, hyphae, blastoconidia (blastospores), and chlamydoconidia (chlamydospores) and name a yeast producing these structures.

3*. Name 3 potentially pathogenic yeasts and state an infection each causes.

(*) = Common theme throughout the course

TPS Questions

Mycology is the study of fungi. Fungi include **yeasts, molds, and fleshy fungi**. They:

1. are eukaryotic;
2. have a rigid cell wall;
3. are chemoheterotrophs (organisms that require organic compounds for both carbon and energy sources);
4. obtain their nutrients by absorption;
5. obtain nutrients as saprophytes, organisms that live off of decaying matter, or as parasites, organisms that live off of living matter.

We will be concerned mainly with the yeasts and molds, especially those causing **mycoses** (fungal infections).

In this section we will look at yeasts.

Yeasts

A. Yeast Morphology

1. Yeast **(see Fig. 1)** are **unicellular fungi** which usually appear as oval cells 1-5 µm wide by 5-30 µm long.

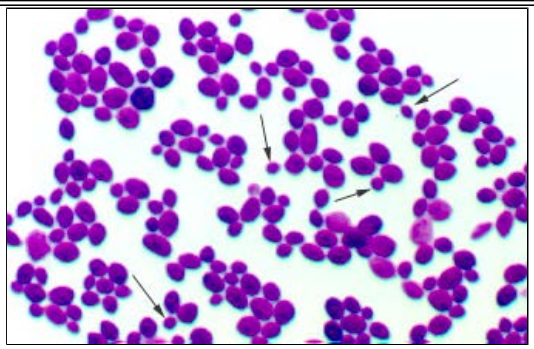
2. They have typical eukaryotic structures **(see Fig. 2 and Fig. 3)**.

3. They have a thick polysaccharide cell wall.

4. They are facultative anaerobes.

5. The yeast *Candida* is said to be dimorphic in that it can grow as an oval, budding yeast, but under certain culture conditions, the budding yeast may elongate and remain attached producing filament-like structures called pseudohyphae. *C. albicans* may also produce true **hyphae** similar to molds **(see Fig. 4)**. In this case long, branching filaments lacking complete septa form. The pseudohyphae and hyphae help the yeast to invade deeper tissues after it colonizes the epithelium. Asexual spores called **blastoconidia** (blastospores) develop in clusters along the hyphae, often at the points of branching. Under certain growth conditions, thick-walled survival spores called chlamydoconidia (chlamydospores) may also form at the tips or as a part of the hyphae **(see Fig. 5.)**

Fig. 1: Photomicrograph of the Yeast *Saccharomyces cerevisiae*



Note budding yeasts (arrows).

Fig. 2: Transmission Electron Micrograph of *Candida albicans* (Eukaryotic Cell)

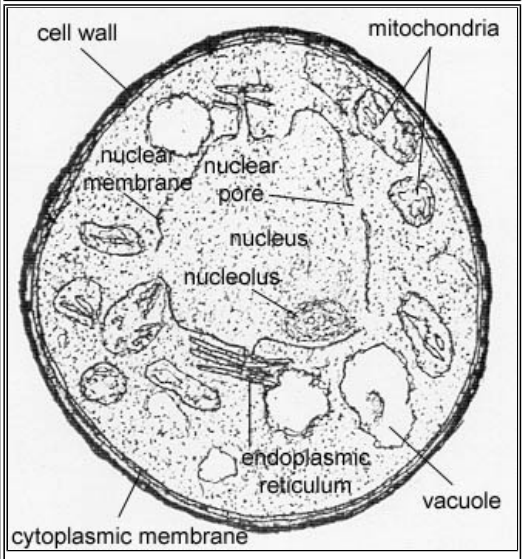
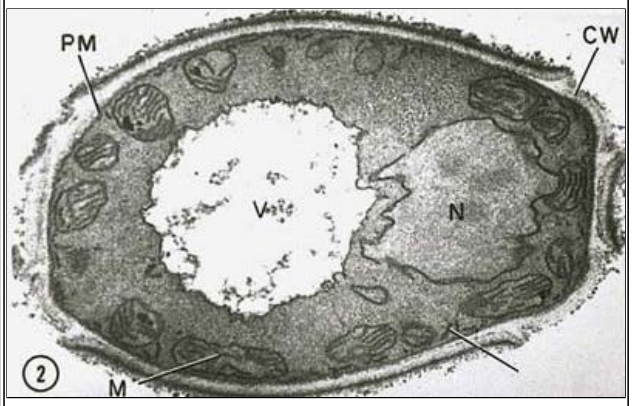


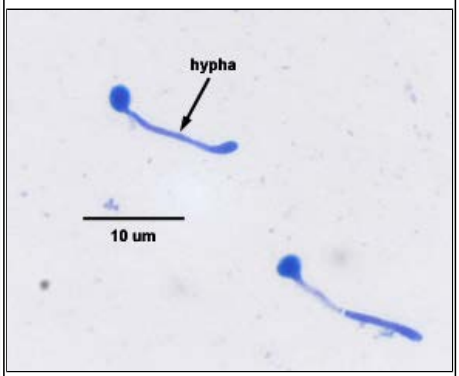
Fig. 3: Transmission Electron Micrograph of *Candida albicans*



PM = plasma membrane; M = mitochondria; N = nucleus; V = vacuole; CW = cell wall

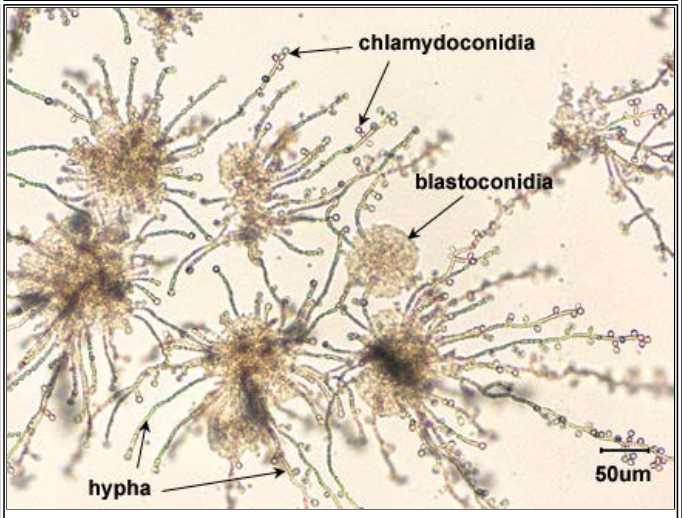
Courtesy of the Centers for Disease Control and Prevention.

Fig. 4: Photomicrograph of *Candida albicans* Producing a Hypha



Dimorphic *Candida albicans* switching from a yeast form to a filamentous form.

Fig. 5: Photomicrograph of *Candida albicans* in its Hyphal Form



Note hyphae, blastoconidia (blastospores), and chlamydoconidia (chlamydospores).

For more information: Review of prokaryotic and eukaryotic cells

Self Check

B. The Role of Fungal Cell Wall Components in Initiating Body Defense

initiation of innate immunity

In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by **recognizing molecules unique to microorganisms that are not associated with human cells**. These unique molecules are called **pathogen-associated molecular patterns or PAMPs**. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometimes referred to as microbe-associated molecular patterns or MAMPs.)

Components of the yeast cell wall that function as PAMPs include **lipoteichoic acids, and zymosan**. In addition, bacteria and other microorganisms also possess **mannose-rich glycans** (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar) that function as PAMPs. These mannose-rich glycans are common in microbial glycoproteins and glycolipids but rare in those of humans. These PAMPs bind to **pattern-recognition receptors (PRRs)** on a variety of defense cells of the body and **triggers innate immune defenses such as inflammation, fever, and phagocytosis**.

Yeast cell wall components also **activate the alternative complement pathway and the lectin pathway**, defense pathways that play a variety of roles in body defense.

Flash animation showing the release of fungal mannans from the cell walls of yeast and their subsequent binding to pattern-recognition receptors on a macrophage.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the release of fungal mannans from the cell walls of yeast and their subsequent binding to pattern-recognition receptors on a macrophage.
1) Yeasts release fungal mannans, lipoteichoic acids, glycolipids, and zymosan from their cell wall. 2) The mannans bind to a TLR- 4 receptors on defense cells such as macrophages and dendritic cells. 3) The binding of fungal mannans to TLR- 4 enables regulatory molecules within the cell - Mal, MyD88, Tram, and Trif - to trigger reactions that activate a master regulator of inflammation called NF-kappa B. Activated NF-kappa B enters the cell's nucleus and switches on genes coding for cytokines such as: a. Interleukin-1 (IL-1) and Tumor necrosis factor-alpha (TNF-alpha): enhance inflammatory responses; b. Interleukin-8 (IL-8): aids in the ability of white blood cells to leave the blood vessels and enter the tissue; a chemoattractant for phagocytes; c. Interleukin-6 (IL-6) promotes B-lymphocyte activity; and d. Interleukin-12 (IL-12): promotes T-lymphocyte activity. (5) 4) Cytokine genes are transcribed into mRNA molecules that goes to the cytoplasm to be translated into inflammatory cytokines that are subsequently secreted from the cell.

For More Information: Preview of pathogen-associated molecular patterns (PAMPs)
For more information: Preview of pattern-recognition receptors (PRRs)
For more information: Preview of Inflammation
For more information: Review of the complement pathways

Initiation of adaptive immunity

Cell wall molecules can also **trigger adaptive immunity** such as the production of antibody molecules against bacterial cell wall antigens. An **antigen** is defined as a substance that reacts with antibody molecules and antigen receptors on lymphocytes. An immunogen is an antigen that is recognized by the body as non-self and stimulates an adaptive immune response.

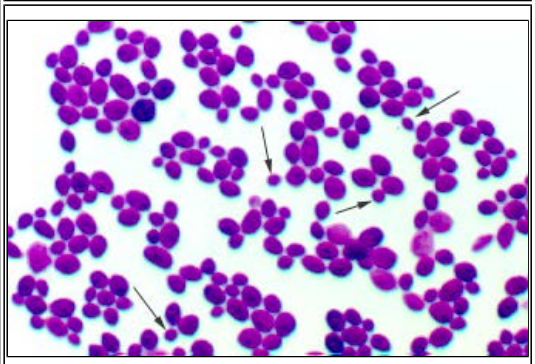
The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

For more information: Review of antigens and epitopes

C. Reproduction of yeasts

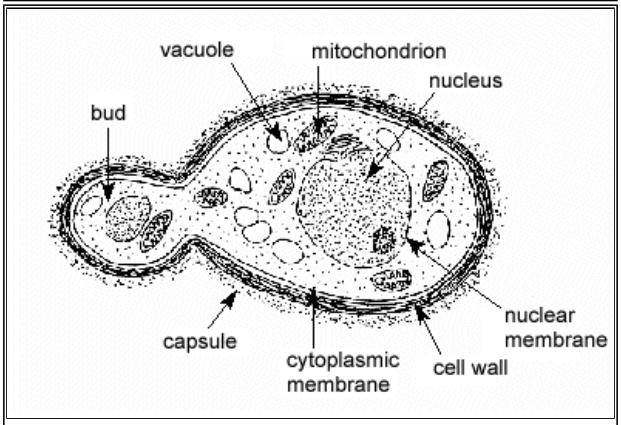
1. Yeasts reproduce **asexually** by a process called budding **(see Fig. 1 and Fig 6)**. A bud is formed on the outer surface of the parent cell as the nucleus divides. One nucleus migrates into the elongating bud. Cell wall material forms between the bud and the parent cell and the bud breaks away.

Fig. 1: Photomicrograph of budding *Saccharomyces cerevisiae*



Note budding yeast (arrows).

Fig. 6: Drawing of a Budding Yeast

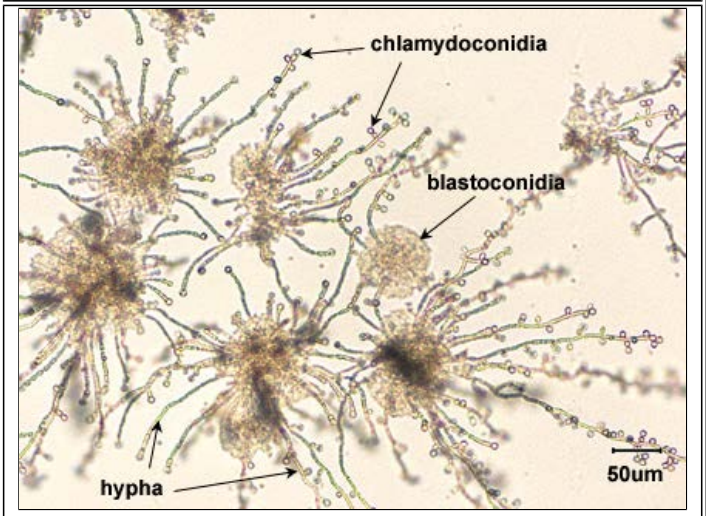


Scanning electron micrograph of budding *Saccharomyces*; courtesy of Dennis Kunkel's Microscopy.

Movie of *Saccharomyces cerevisiae* reproducing by budding
Time-lapse movie of the yeast *Saccharomyces cerevisiae* reproducing by budding. © Phillip Meaden, author. Licensed for use, ASM MicrobeLibrary

2. A few yeasts, such as *Candida albicans*, also produce clusters of asexual reproductive spores called **blastoconidia** and thick-walled survival spores called chlamydoconidia **(see Fig. 5)**.

Fig. 5: Photomicrograph of *Candida albicans* in its hyphal form.



Note hyphae, blastoconidia (blastospores), and chlamydoconidia (chlamydospores).

3. Yeasts can also reproduce **sexually** by means of sexual spores called **ascospores** which result from the fusion of the nuclei from two cells followed by meiosis. Sexual reproduction is much less common than asexual reproduction but does allow for genetic recombination.

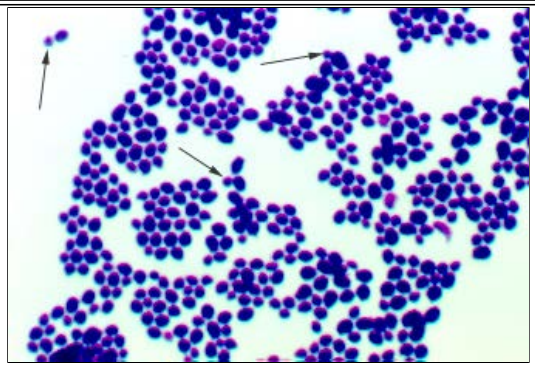
D. Yeast infections

1. ***Candida albicans***

Candida albicans **(see Fig. 7)** is found as normal flora on the mucous membranes and in the gastrointestinal tract but is usually held in check by:

a) normal flora bacteria; and
b) normal body defenses.

Fig. 7: Photomicrograph of *Candida albicans*

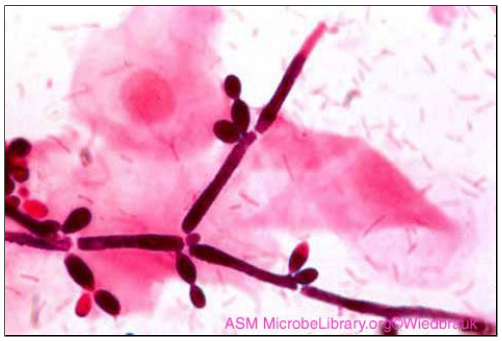


Note budding yeast (arrows).

Candida can cause a variety of opportunistic infections in people who are debilitated, immunosuppressed, or have received prolonged antibacterial therapy. Women who are diabetic, pregnant, taking oral contraceptives, or having menopause are also more prone to vaginitis. These conditions alter the sugar concentration and pH of the vagina making it more favorable for the growth of *Candida*. People who are immunosuppressed frequently develop thrush, vaginitis, and sometimes disseminated infections.

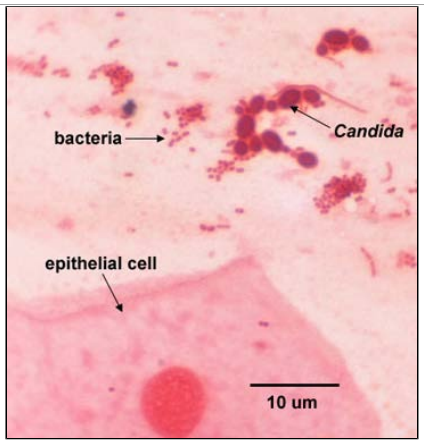
Any *Candida* infection is called **candidiasis**. *Candida* most commonly causes vaginitis, thrush (an infection of the mouth), balantitis (an infection of the foreskin and head of the penis), onychomycosis (a fungal infection of the nails), and **dermatitis** (diaper rash and other infections of moist skin). Less commonly, *Candida* can infect the **lungs, blood, heart, and meninges**, especially in the compromised or immunosuppressed host. *Candida* now causes about 10% of all cases of septicemia. Candidiasis of the esophagus, trachea, bronchi, or lungs, in conjunction with a positive HIV antibody test, is one of the indicator diseases for AIDS.

Fig. 8: Photomicrograph of a Vaginal Smear of a Person with *Candida* Vaginitis	**Fig. 9: Photomicrograph of a Mouth Smear of a Person with Thrush**



Candida albicans. Danny Wiedbrauk, author. Licensed for use, ASM MicrobeLibrary.

Note epithelial cells, rod-shaped bacteria, and *Candida albicans* in its hyphal form.



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Note budding yeast (arrows)

Fig. 10: Photograph of Balantitis caused by *Candida albicans*

Image Provided by Richard O. Detrick

Courtesy of the Centers for Disease Control and Prevention.

Balantitis is swelling of the foreskin and head of the penis.

Fig. 11: Photograph of Onchomycosis caused by *Candida albicans*

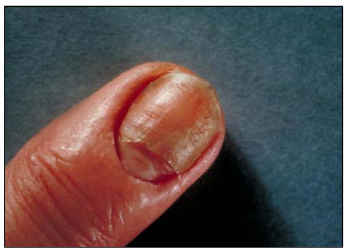


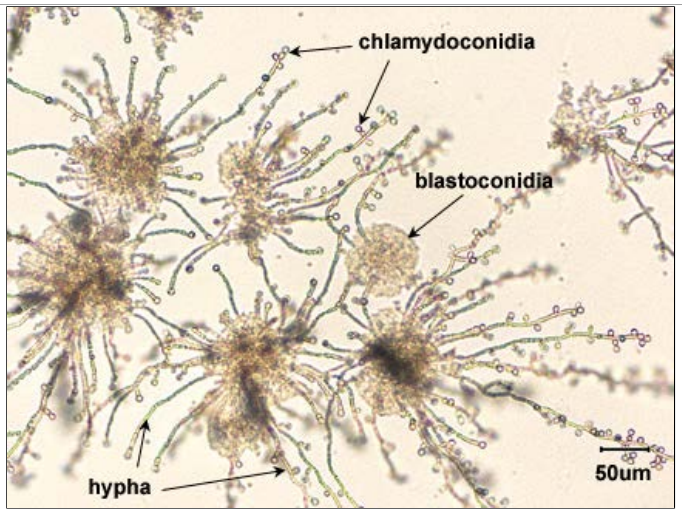
Image Provided by Sherry Brinkman

Courtesy of the Centers for Disease Control and Prevention.

Onchomycosis is a fungal infection of the nails.

Candida is said to be dimorphic, that is it has two different growth forms. It can grow as an oval, budding yeast, but under certain culture conditions, the budding yeast may elongate and remain attached producing filament-like structures called pseudohyphae. *C. albicans* may also produce true hyphae similar to molds. In this case long, branching filaments lacking complete septa form. The pseudohyphae and hyphae help the yeast to invade deeper tissues after it colonizes the epithelium. Asexual spores called **blastoconidia** are a reproductive units produced by budding in yeast. Under certain growth conditions, thick-walled survival spores called chlamydoconidia may also form at the tips or as a part of the hyphae **(see Fig. 5)**.

Fig. 5: Photomicrograph of *Candida albicans* in its hyphal form.



Note hyphae, blastoconidia (blastospores), and chlamydoconidia (chlamydospores).

The most common *Candida* species causing human infections is *C. albicans*, causing 50-60% of all *Candida* infections. *Candida glabrata* is second, causing 15-20% of *Candida* infections; *Candida parapsilosis* is third, responsible for 10-20%.

2. ***Cryptococcus neoformans***

A lesser known but often more serious pathogenic yeast is ***Cryptococcus neoformans***. Like many fungi, this yeast can also reproduce sexually and the name given to the sexual form of the yeast is ***Filobasidiella neoformans***. It appears as an oval yeast 5-6 µm in diameter, forms buds with a thin neck, and is surrounded by a thick capsule **(see Fig. 12).** It does not produce pseudohyphae and chlamydospores. The capsule enables the yeast to resist phagocytic engulfment. The yeast is dimorphic. In its sexual form, as well as in its asexual form under certain conditions, it can produce a hyphal form.

Fig. 12: Photomicrograph of an India Ink Stain of *Cryptococcus neoformans*

Note encapsulated, budding yeast.

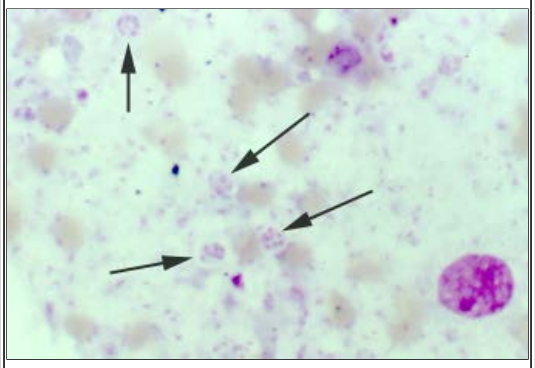
Cryptococcus infections are usually mild or subclinical but, when symptomatic, usually begin in the lungs after inhalation of the yeast in dried bird feces. It is typically associated with with pigeon and chicken droppings and soil contaminated with these droppings. *Cryptococcus*, found in soil, actively grows in the bird feces but does not grow in the bird itself. Usually the infection does not proceed beyond this pulmonary stage. Any disease by this yeast is usually called **cryptococcosis**.

Dissemination of the pulmonary infection can result in a very severe and often fatal **cryptococcal meningoencephalitis**. Cutaneous and visceral infections are also found. Although exposure to the organism is probably common, large outbreaks are rare, indicating that an immunosuppressed host is usually required for the development of severe disease. Extrapulmonary cryptococcosis, in conjunction with a positive HIV antibody test, is another indicator disease for AIDS.

3. ***Pneumocystis jiroveci***

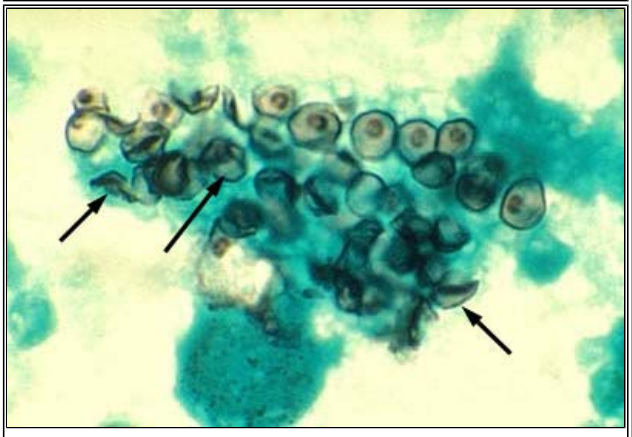
Pneumocystis jiroveci (formerly called *Pneumocystis carinii*) **(see Fig. 13 and Fig. 14)** is thought to be transmitted from person to person by the respiratory route and is almost always asymptomatic. However, in persons with highly depressed immune responses, such as people with leukemias or infected with the Human Immunodeficiency Virus (HIV), *P. jiroveci* can cause a severe pneumonia called **PCP** (*Pneumocystis* pneumonia).

Fig. 13: Photomicrograph of Cysts of *Pneumocystis jiroveci* from the Lungs of a Person with PCP



Cysts of *Pneumocystis jiroveci* in bronchoalveolar material, Giemsa stain method.The rounded cysts are 4 to 7 µm in diameter and contain 6 to 8 intracystic bodies, whose nuclei are stained by the dye. The walls of the cysts are not stained.

Fig. 14: Photomicrograph of Cysts of *Pneumocystis jiroveci* in Smear from Bronchoalveolar Lavage



Courtesy of the Centers for Disease Control and Prevention.

Cysts of *Pneumocystis jiroveci* in lung tissue, Gomori methenamine silver stain method. The walls of the cysts are stained black and often appear crescent shaped or like crushed ping-pong balls. The intracystic bodies are not visible with this stain.

P. jiroveci can be found in 3 distinct morphologic stages:

- The trophozoite (trophic form), a haploid amoeboid form 1-4 µm in diameter that replicates by mitosis and binary fission. The trophic forms are irregular shaped and often appears in clusters.
- A precystic form or early cyst. Haploid trophic forms conjugate and produce a zygote or sporocyte (early cyst).
- The cyst form, which contains several intracystic bodies or spores are 5-8 µm in diameter. It has been postulated that in formation of the cyst form (late phase cyst), the zygote undergoes meiosis and subsequent mitosis to typically produce eight haploid ascospores (sporozoites) **See Fig. 13**. As the haploid ascospores are released the cysts often collapse forming crescent-shaped bodies **(see Fig. 14)**. *P. jiroveci* is usually transmitted by inhalation of the cyst form. Released ascospores then develop into replicating trophic forms that attach to the wall of the alveoli and replicate to fill the alveoli.

In biopsies from lung tissue or in tracheobronchial aspirates, both a trophic form about 1-4 µm in diameter with a distinct nucleus and a cyst form between 5-8 µm in diameter with 6-8 intracystic bodies (ascospores) can be seen.

4. *Malassezia globosa*

Malassezia globosa is a dimorphic yeast that is the most frequent cause of a superficial skin infection called **tinea versicolor** that commonly appears as a **hypopigmentation of the infected skin**. *M. globosa* is also the most common cause of **dandruff** and seborrheic dermatitis. The yeast is naturally found on the skin.

Fig. 15: Photomicrograph of a *Malassezia* Species in its Dimorphic Form

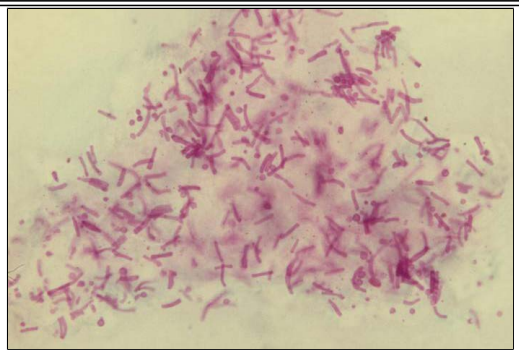


Image provided by Dr. Lucille K. Georg. Courtesy of the Centers for Disease Control and Prevention.

Note both the yeast form and the hyphal form.

Self Check

Images of tinea versicolor.

To view additional photomicrographs of *Candida*, *Filobasidiella* (*Cryptococcus*), and *Pneumocystis*, see the AIDS Pathology Tutorial at the University of Utah.

Concept Map for Fungi, Part-1:Yeasts

TPS Questions

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Candida albicans*
- *Cryptococcus neoformans*
- *Pneumocystis carinii*

Self-Quiz for Yeasts

Quiz Group

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Molds
MOLDS

Fungi

C. Molds

Fundamental Statement for this Softchalk Lesson:

1. Molds are multinucleated, filamentous fungi composed of hyphae.
2. Molds reproduce primarily by means of asexual reproductive spores.
3.The dermatophytes are a group of molds that cause superficial mycoses of the hair, skin, and nails and utilize the protein keratin that is found in hair, skin, and nails, as a nitrogen and energy source.
4. Dimorphic fungi may exhibit two different growth forms. Outside the body they grow as a mold, producing hyphae and asexual reproductive spores, but in the body they grow in a yeast-like form.
5. The appearance of a mold and the type of spores it produces is useful in its identification.
6. Mold infections include tinea or ringworm, coccidioidomycosis, histoplasmosis, blastomycosis, and Aspergillosis.

Common Course Objectives

1. Recall features of fungi that distinguish them from other microbes with emphasis on dimorphism and true versus opportunistic infections.
2. Distinguish between different fungal infections based on the causative agent, modes of transmission, portal of entry, and portal of exit.

Detailed Learning Objectives

1. Define:

   a. mold
   b. hyphae
   c. mycelium
   d. vegetative mycelium
   e. aerial mycelium.

2. Briefly describe the following fungal asexual reproductive spores:

   a. conidiospores
   b. macroconidia
   c. microconidia
   d. sporangiospores
   e. arthrospores.

4*. Define dermatophyte, list 2 genera of dermatophytes, and name 3 dermatophytic infections.

5.* Describe what is meant by the term "dimorphic fungus", name 2 systemic infections caused by dimorphic fungi, and state how they are initially contracted.

(*) = Common theme throughout the course

TPS Questions

Mycology is the study of fungi. Fungi include **yeasts, molds, and fleshy fungi**. They:

1. are eukaryotic;
2. have a rigid cell wall;
3. are chemoheterotrophs (organisms that require organic compounds for both carbon and energy sources);
4. obtain their nutrients by absorption;
5. obtain nutrients as saprophytes, organisms that live off of decaying matter, or as parasites, organisms that live off of living matter.

We will be concerned mainly with the yeasts and molds, especially those causing **mycoses** (fungal infections).

In this section we will look at molds.

Molds

A. Mold morphology

1. Molds are multinucleated, filamentous fungi composed of **hyphae.** A hypha is a branching tubular structure approximately 2-10 µm in diameter which is usually divided into cell-like units by crosswalls called **septa**. The total mass of hyphae is termed a **mycelium**. The portion of the mycelium that anchors the mold and absorbs nutrients is called the **vegetative mycelium**, composed of vegetative hyphae; the portion that produces asexual reproductive spores is the **aerial mycelium**, composed of aerial hyphae **(see Fig. 1)**.

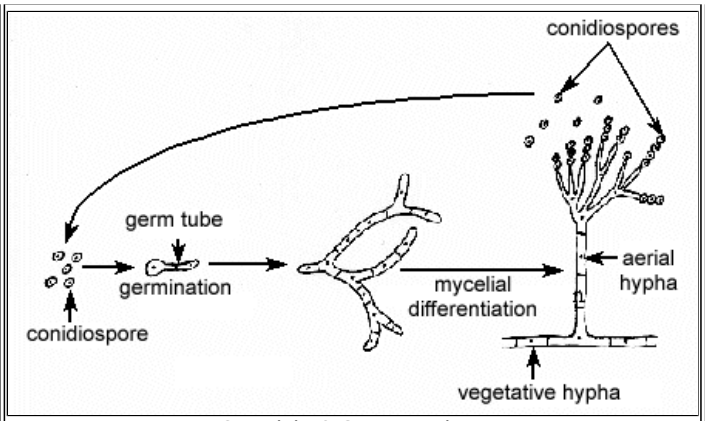
2. Molds have typical eukaryotic structures **(see Fig. 2)**.

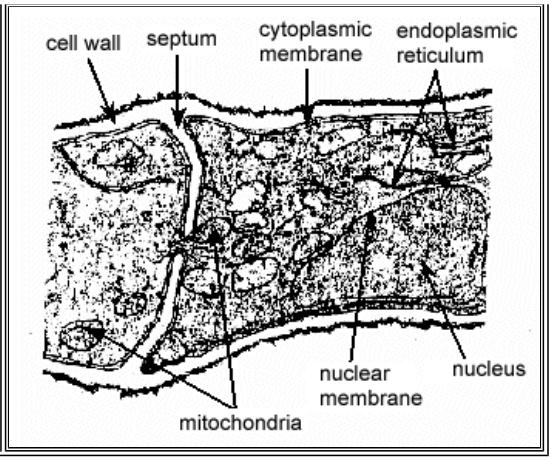
3. Molds have a cell wall usually composed of chitin, sometimes cellulose, and occasionally both.

4. Molds are obligate aerobes.

5. Molds grow by elongation at apical tips of their hyphae and thus are able to penetrate the surfaces on which they begin growing.

Fig. 1: Asexual Reproduction in Molds	**Fig. 2: Electron Micrograph of a Segment of a Mold Hypha**





For more information: Review of prokaryotic and eukaryotic cells

Self Check

B. Reproduction of molds

1. Molds reproduce primarily by means of **asexual reproductive spores**. These include the following.

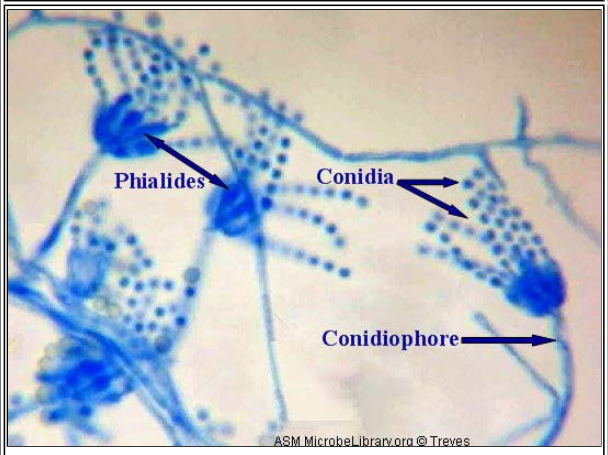
   a. conidiospores (conidia). **See Fig. 3**.

   Spores borne externally on an aerial hypha called a conidiophore; see **Fig. 4** and **Fig. 5**.

   Scanning electron micrographs of the conidiospores of *Penicillium*; courtesy of Dennis Kunkel's Microscopy.

   Scanning electron micrographs of the conidiospores of *Aspergillus*; courtesy of Dennis Kunkel's Microscopy.

Fig. 3: Photomicrograph of conidiospores of *Penicillium*



David Treves and Candace Martens, authors.
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Fig. 4: Photomicrograph of Conidiospores of *Aspergillus*

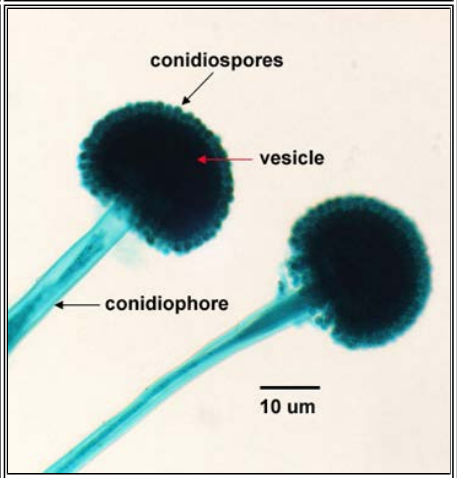
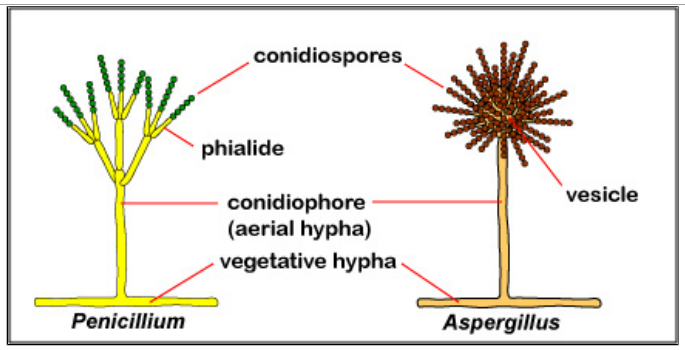


Fig. 5: Illustration of Conidiospores of *Penicillium* and *Aspergillus*



b. sporangiospores. **See Fig. 6**.

Spores borne in a sac or sporangium on an aerial hypha called a sporangiophore. **See Fig. 7**.

Scanning electron micrograph of the conidiospores of *Rhizopus*; courtesy of Dennis Kunkel's Microscopy.

Fig. 6: Photomicrograph of Sporangiospores of *Rhizopus*

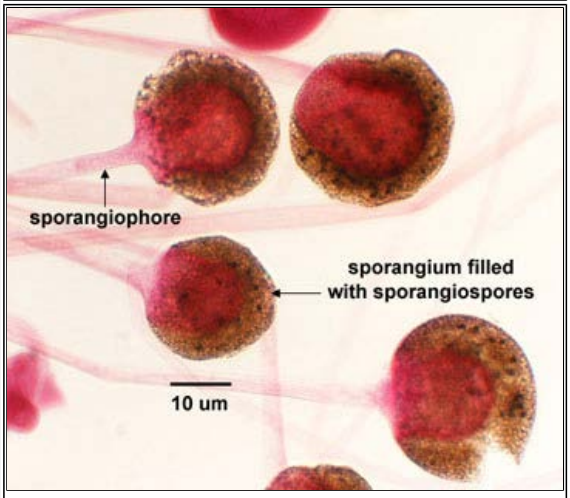
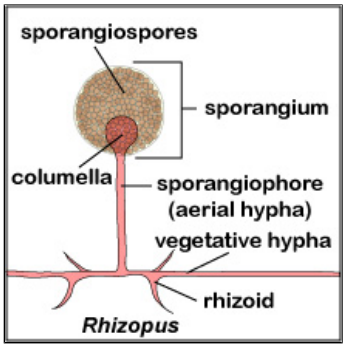


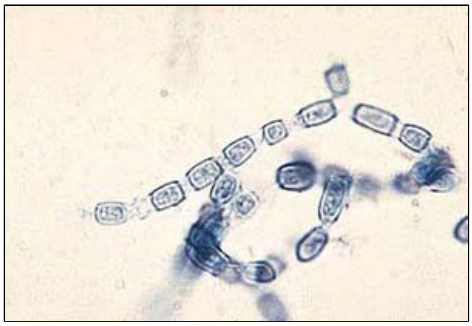
Fig. 7: Illustration of Sporangiospores within a Sporangium in *Rhizopus*



c. arthrospores. **See Fig. 8**.

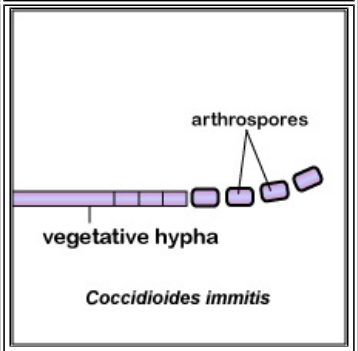
spores produced by fragmentation of a vegetative hypha **(see Fig. 9)**.

Fig. 8: Photomicrograph of Arthrospores of *Coccidioides immitis*



Courtesy of the Centers for Disease Control and Prevention.

Fig. 9: Illustration of Arthrospores



2. Molds may also reproduce **sexually** by sexual spores such as ascospores and zygospores but this is not common.

Self Check

C. Pathogenic molds

1. **Dermatophytes**

The dermatophytes are a group of molds that cause **superficial mycoses of the hair, skin, and nails** and utilize the protein **keratin**, that is found in hair, skin, and nails, as a nitrogen and energy source. Infections are commonly referred to as **ringworm** or **tinea** infections and include:

tinea capitis (infection of the skin of the scalp, eyebrows, and eyelashes)

tinea barbae (infection of the bearded areas of the face and neck)

tinea faciei (infection of the skin of the face)

tinea corporis (infection of the skin regions other than the scalp, groin, palms, and soles)

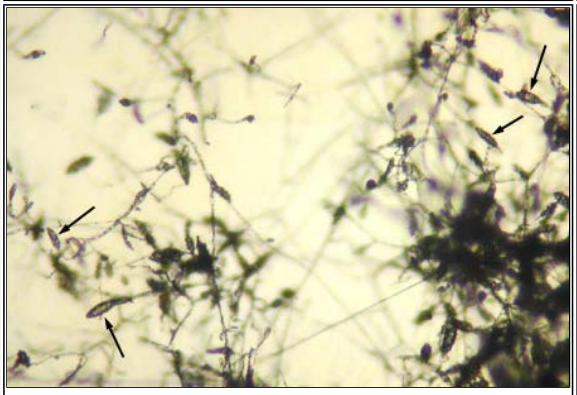
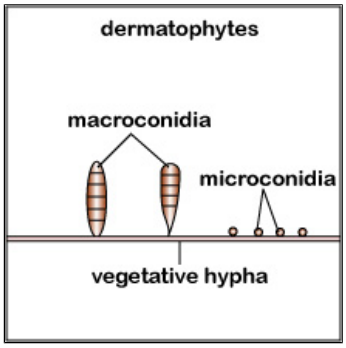
tinea cruris (infection of the groin; jock itch)

tinea unguium (onchomycosis; infection of the fingernails and toenails)

tinea pedis (athlete's foot; infection of the soles of the feet and between the toes).

The three most common dermatophytes are *Microsporum, Trichophyton*, and *Epidermophyton*. They produce characteristic asexual reproductive spores called macroconidia and microconidia (See **Fig. 10** and **Fig. 11**).

Scanning electron micrograph of the macroconidia of *Epidermophyton*; courtesy of Dennis Kunkel's Microscopy.

Fig. 10: Photomicrograph of Macroconidia and Microconidia of the Dermatophyte *Microsporum*	**Fig. 11: Illustration of Macroconidia and Microconidia of Dermatophytes**
 Copyright © Gary E. Kaiser	 Copyright © Gary E. Kaiser
Note macroconidia (arrows)	Macroconidia are large and often multinucleated; microconidia are smaller and have a different appearance.

Another tinea infection of the skin is tinea versicolor caused by the yeast *Malassezia globosa*. Tinea versicolor appears as a hypopigmentation of the infected skin. *M. globosa* is also the most common cause of dandruff.

2. **Dimorphic fungi**

Dimorphic fungi may exhibit **two different growth forms**. Outside the body they grow as a **mold**, producing hyphae and asexual reproductive spores, but in the body they grow in a **non-mycelial yeast form**. These infections appear as **systemic mycoses** and usually begin by inhaling spores from the mold form. After germination in the **lungs**, the fungus grows as a yeast . Factors such as body temperature, osmotic stress, oxidative stress, and certain human hormones activate a dimorphism-regulating histidine kinase enzyme in dimorphic molds, causing them to switch from their avirulent mold form to their more virulent yeast form.

For example:

a.***Coccidioides immitis* causes coccidioidomycosis (see Fig. 12)**, a disease endemic to the southwestern United States. An estimated 100,000 infections occur annually in the United States, but one to two thirds of these cases are subclinical.

The mold form of the fungus grows in arid soil and produces thick-walled, barrel-shaped asexual spores called **arthrospores (see Fig. 8)** by a fragmentation of its vegetative hyphae.

After inhalation, the arthrospores germinate and develop into **endosporulating spherules (see Fig. 13)** in the terminal bronchioles of the lungs. The spherules reproduce by a process called endosporulation, where the spherule produces numerous endospores (yeast-like particles), ruptures, and releases viable endospores that develop into new spherules.

Fig. 12: Illustration of the Life Cycle of the Dimorphic Fungus *Coccidioides immitis*

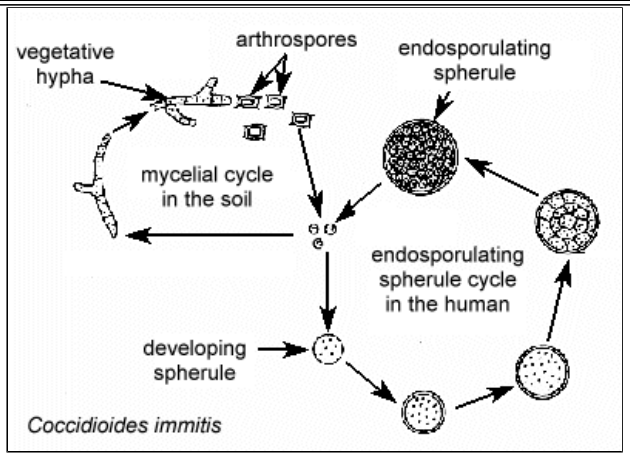
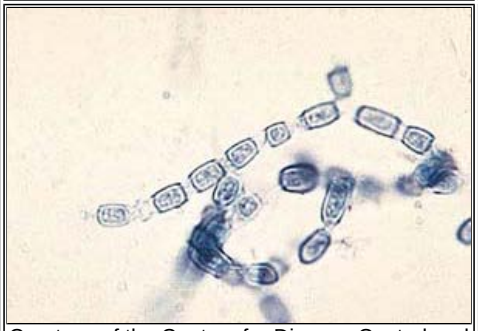
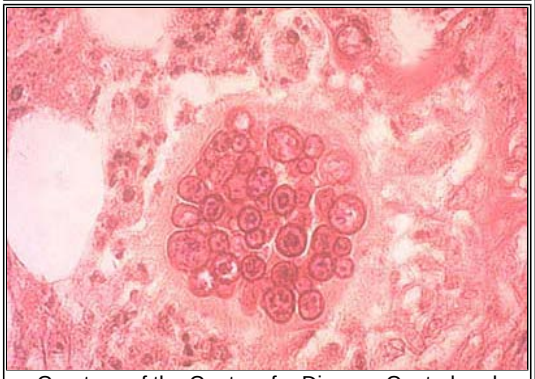


Fig. 8: Photomicrograph of Arthrospores of *Coccidioides immitis*



Courtesy of the Centers for Disease Control and Prevention.

Fig. 13: Photomicrograph of *Coccidioides immitis* in the Lung: A Spherule Containing Endospores (Yeast-Like Cells)



Courtesy of the Centers for Disease Control and Prevention.

b.***Histoplasma capsulatum* (see Fig. 14) causes histoplasmosis**, a disease commonly found in the Great Lakes region and the Mississippi and Ohio River valleys. Approximately 250,000 people are thought to be infected annually in the US, but clinical symptoms of histoplasmosis occur in less than 5% of the population. Most individuals with histoplasmosis are asymptomatic. Those who develop clinical symptoms are typically either immunocompromised or are exposed to a large quantity of fungal spores.

The mold form of the fungus often grows in bird or bat droppings or soil contaminated with these droppings and produces large **tuberculate macroconidia** and small **microconidia (see Fig. 15)**. Although birds cannot be infected by the fungus and do not transmit the disease, bird excretions contaminate the soil and enrich it for mycelial growth. Bats, however, can become infected and transmit histoplasmosis through their droppings. After inhalation of the fungal spores and their germination in the lungs, the fungus grows as a **budding, encapsulated yeast (see Fig. 16)**.

Chest X-ray of a person with histoplasmosis.

Fig. 14: Illustration of the Life Cycle of the Dimorphic Fungus *Histoplasma capsulatum*

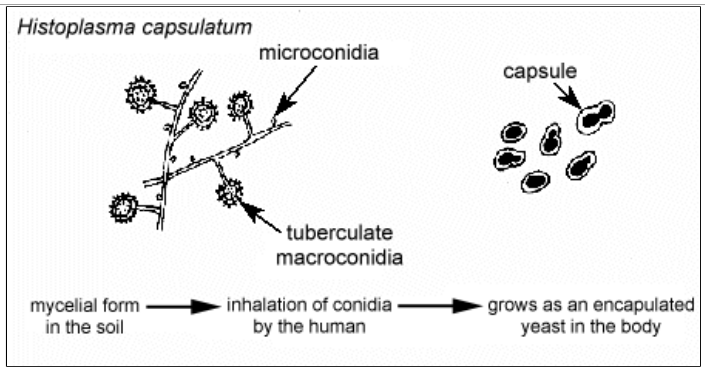


Fig. 15: Photomicrograph of the Mold Phase of *Histoplasma capsulatum* Showing Tuberculate Macroconidia and Microconidia

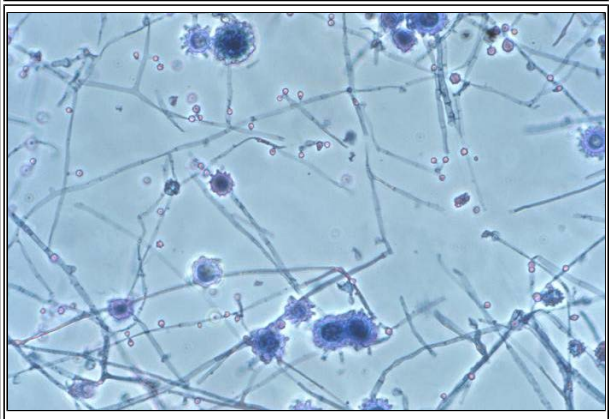


Image Provided by Dr. Libero Ajello
Courtesy of the Centers for Disease Control and Prevention.

Fig. 16: Photomicrograph of the Yeast Phase of *Histoplasma capsulatum* in the Lungs

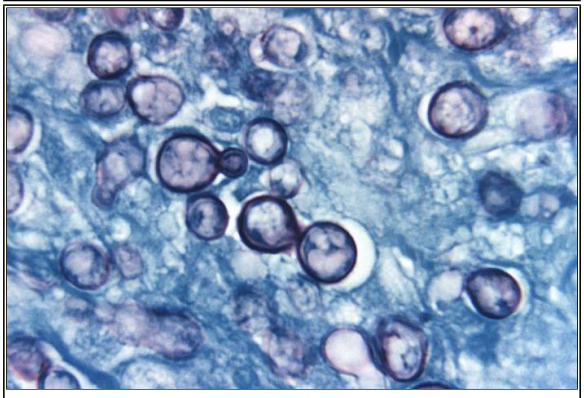
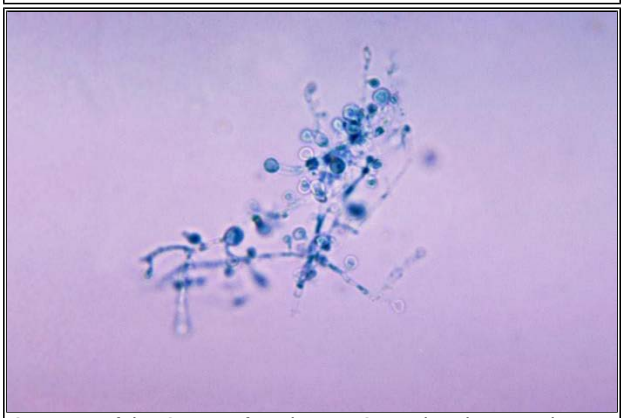


Image Provided by Dr. Libero Ajello
Courtesy of the Centers for Disease Control and Prevention.

c. ***Blastomyces dermatitidis* causes blastomycosis**, a disease commonly found around the Great Lakes region and the Mississippi and Ohio River valleys. Infection can range from an asymptomatic, self-healing pulmonary infection to widely disseminated and potentially fatal disease. Pulmonary infection may be asymptomatic in nearly 50% of patients. *Blastomyces dermatitidis* can also sometimes infect the skin.

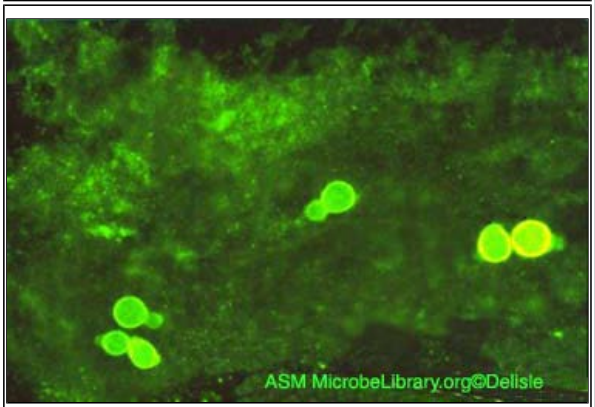
Blastomyces dermatitidis produces a mycelium with small conidiospores **(see Fig. 17)** and grows actively in bird droppings and contaminated soil. When spores are inhaled or enter breaks in the skin, they germinate and the fungus grows as a yeast **(see Fig. 18)**.having a characteristic thick cell wall. It is diagnosed by culture and by biopsy examination.

Fig. 17: Photomicrograph of the Mold Form of *Blastomyces dermatitidis* with hyphae and conidiospores



Courtesy of the Centers for Disease Control and Prevention.Dr. Leanor Haley

Fig. 18: Photomicrograph of the Yeast Phase of *Blastomyces dermatitidis*



Note the fluorescing budding yeast.

These infections usually remains localized in the lungs, but in rare cases may spread throughout the body.

As mentioned earlier, the yeast *Candida albicans* can also exhibit dimorphism.

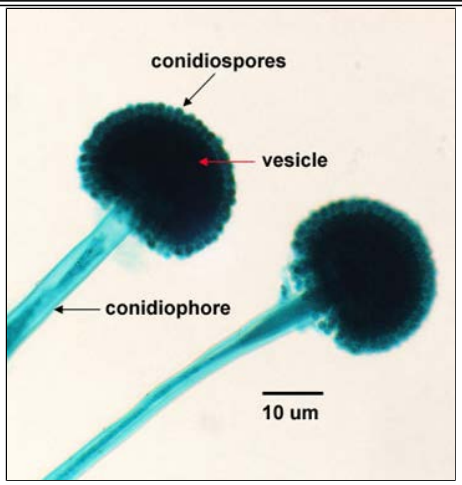
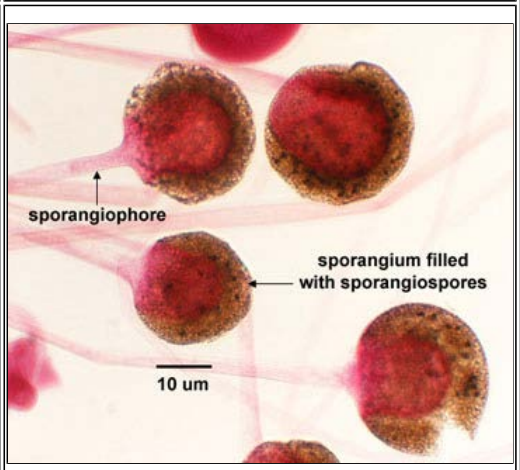
To view additional photomicrographs of *Coccidioides* and *Histoplasma*, see the AIDS Pathology Tutorial at the University of Utah.

3. **Opportunistic molds**

Certain molds once considered as non-pathogenic have recently become a fairly common cause of opportunistic lung and wound infections in the debilitated or immunosuppressed host. These include the common molds *Aspergillus* **(see Fig. 4)** and *Rhizopus* **(see Fig. 6)**.

Although generally harmless in most healthy individuals, *Aspergillus* species do cause allergic bronchopulmonary aspergillosis (ABPA), chronic necrotizing *Aspergillus* pneumonia (or chronic necrotizing pulmonary aspergillosis [CNPA]), aspergilloma (a mycetoma or fungus ball in a body cavity such as the lung), and invasive aspergillosis. In highly immunosuppressed individuals, however, *Aspergillus* may disseminate beyond the lung via the blood.

Mucormycoses are infections caused by fungi belonging to the order of Mucorales. *Rhizopus* species are the most common causative organisms. The most common infection is a severe infection of the facial sinuses, which may extend into the brain. Other mycoses include pulmonary, cutaneous, and gastrointestinal.

Fig. 4: Photomicrograph of the Conidiospores of *Aspergillus*	**Fig. 6: Photomicrograph of the Sporangiospores of *Rhizopus***
	
Copyright © Gary E. Kaiser	Copyright © Gary E. Kaiser
Causes aspergillosis.	Causes mucormycosis.

Quiz Group

Concept Map for Fungi, Part-2: Molds

TPS Questions

Medscape article on infections associated with organisms mentioned in this Learning Object.
Registration to access this website is free.

- Dermatophytic infections (tinea)
- *Coccidioides immitis*

- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- Aspergillosis
- *Rhizopus*

Self-Quiz for Molds

Quiz Group

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Back to Softchalk Lessons Table of Contents

Fungal pathogenicity
FUNGAL PATHOGENICITY

Fungi

D. Fungal Pathogenicity

Fundamental Statement for this Softchalk Lesson:

1. ***Many of the same factors that enable bacteria to colonize the body also enable fungi to colonize.***
2. ***Many of the same factors that enable bacteria to harm the body also enable fungi to cause harm.***

Common Course Objectives

1. Recall features of fungi that distinguish them from other microbes with emphasis on dimorphism and true versus opportunistic infections.

Detailed Learning Objectives

1. Name at least 3 fungal virulence factors that promote fungal colonization.
2. Name at least 2 fungal virulence factors that damage the host.

Fungal Pathogenicity

As with the bacteria, fungal virulence factors can be divided into two categories: virulence factors that promote fungal colonization of the host; and virulence factors that damage the host.

A. Virulence Factors that Promote Fungal Colonization

Virulence factors that promote fungal colonization of the host include the ability to:

1. contact host cells;
2. adhere to host cells and resist physical removal;
3. invade host cells;
4. compete for nutrients;
5. resist innate immune defenses such as phagocytosis and complement; and
6. evade adaptive immune defenses.

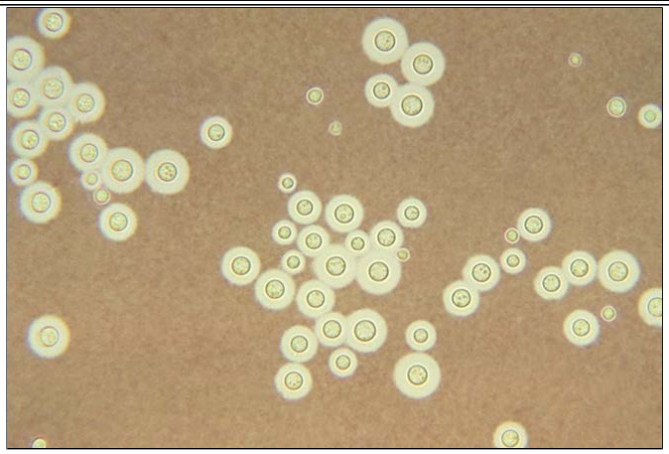
Examples of virulence factors that promote fungal colonization include:

1. **A compromised immune system is the primary predisposing factor for serious fungal infections**. A person highly immunosuppressed, such as a person taking immunosuppressive drugs to suppress transplant rejection, or a person with advancing HIV infection, or a person with other immunosuppressive disorders, becomes very susceptible to infections by fungi generally considered not very harmful to a healthy person with normal defenses.

2. As with bacteria, the ability to **adhere** to host cells with cell wall adhesins seems to play a role in fungal virulence.

3. Some fungi produce **capsules** allowing them to **resist phagocytic engulfment**, such as the yeast *Cryptococcus neoformans* and the yeast form of *Histoplasma capsulatum* **(See Fig. 1)**.

Fig. 1: Photomicrograph of an India Ink Stain of *Cryptococcus neoformans*



Courtesy of CDC

4. *Candida albicans* **stimulates the production of a cytokine called GM-CSF** and this cytokine can **suppress the production of complement by monocytes and macrophages**. This may decrease the production of the opsonin C3b as well as the complement proteins that enhance chemotaxis of phagocytes.

5. *C. albicans* also appears to be able to **acquire iron from red blood cells**.

6. *C. albicans* produces acid proteases and phospholipases that aid in the penetration and damage of host cell membranes.

7. Some fungi are **more resistant to phagocytic destruction**, e.g., *Candida albicans, Histoplasma capsulatum*, and *Coccidioides immitis*.

8. There is evidence that when the yeast form of *Candida* enters the blood it activates genes allowing it to switch from its budding form to its hyphal form. In addition, when engulfed by macrophages, it starts producing the tubular germ tubes which penetrate the membrane of the macrophage thus causing its death.

A movie of *Candida* killing a macrophage from within.

Courtesy of the Theriot Lab Website at Stanford University Medical School.

9. Factors such as body temperature, osmotic stress, oxidative stress, and certain human hormones activate a dimorphism-regulating histidine kinase enzyme in dimorphic molds, such as *Histoplasma capsulatum, Blastomyces dermatitidis*, and *Coccidioides immitis,* causing them to **switch from their avirulent mold form to their virulent yeast form**. It also t**riggers the yeast *Candida albicans* to switch from its yeast form to its more virulent hyphal form**.

B. Virulence Factors that Damage the Host

1. Like bacteria, **fungal PAMPs binding to PRRs can trigger excessive cytokine production leading to a harmful inflammatory response** that damages tissues and organs.

2. As fungi grow in the body, they can **secrete enzymes to digest cells**. These include proteases, phospholipases, and elastases. In response to both the fungus and to cell injury, **cytokines are released**. As seen earlier under Bacterial Pathogenesis, this leads to an inflammatory response and extracellular killing by phagocytes that leads to **further destruction of host tissues**.

3. Many molds secrete **mycotoxins**, especially when growing on grains, nuts and beans. These toxins may cause a variety of effects in humans and animals if ingested including loss of muscle coordination, weight loss, and tremors. Some mycotoxins are mutagenic and carcinogenic.

Aflatoxins, produced by certain *Aspergillus* species, are especially carcinogenic. A mold called *Stachybotrys chartarum* is a mycotoxin producer that has been implicated as a potential serious problem in homes and buildings as one of the causes of "sick building syndrome." Mycotoxin symptoms in humans include dermatitis, inflammation of mucous membranes, , cough, fever, headache, and fatigue.

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Candida albicans*
- *Cryptococcus neoformans*
- *Pneumocystis carinii*
- Dermatophytic infections (tinea)
- *Coccidioides immitis*
- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- Aspergillosis
- *Rhizopus*
- Mold allergy

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Back to Softchalk Lessons Table of Contents

Chemotherapeutic control of fungi
CHEMOTHERAPEUTIC CONTROL OF FUNGI

Fungi

E. Chemotherapeutic Control of Fungi

Fundamental Statement for this Softchalk Lesson:

1. Because fungi, like human cells, are eukaryotic, there are far fewer chemotherapeutic agents that are selectively toxic for fungi than there are for prokaryotic bacteria.
2. Most antifungal agents bind to or interfere with the synthesis of ergosterol, the sterol in their cytoplasmic membrane, altering membrane structure and function.

Common Course Objectives

1. Identify appropriate methods of microbial control under a given specific circumstance.
2. Recall the advantages and disadvantages of the different types of chemical control.
3. Recall the mechanism of action for each class of chemotherapeutic chemical agent and give specific examples for each class.
4. Explain the medical importance of chemotherapeutic agents.
5. Explain why selective toxicity is an important feature of a chemotherapeutic agent.

Detailed Learning Objectives

1. Briefly describe 3 different ways antifungal chemotherapeutic agents may affect fungi and give an example of an antibiotic for each way.

TPS Question

Chemotherapeutic Control of Fungi

Remember that like human cells, fungal cells are eukaryotic. Since fungal cells, unlike prokaryotic bacterial cells, are not that different from human cells, it is more difficult to find a chemotherapeutic agent that is selectively toxic for fungi, that is, will inhibit or kill fungal cells without also inhibiting or killing human cells. Some of the common antifungal chemotherapeutic agents are listed below.

1. One antibiotic, **griseofulvin** (*Fulvicin, Grifulvin, Gris-PEG*), **interferes with nuclear division by preventing the aggregation of microtubules needed for mitosis** in **superficial mycelial fungi**. It is used only for severe dermatophyte infections.

2. The antimetabolites **trimethoprim + sulfomethoxazole , trimetrexate, atovaquone, and flucytosine interfere with normal nucleic acid synthesis**. Trimethoprim/sulfomethoxazole (*Septra, Bactrim*), atovaquone (*Mepron*), and trimetrexate (*Neutrexin*) are used to treat *Pneumocystis* pneumonia. Flucytosine (*Ancobon*) is used for more serious *Candida* infections.

3. Polyene antibiotics such as **amphotericin B, pimaricin, and nystatin** are fungicidal drugs that **bind to ergosterol in the fungal cytoplasmic membrane thus altering its structure and function and causing leakage of cellular needs**. Nystatin (*Mycostatin*) is used to treat superficial *Candida* infections (thrush, vaginitis, cutaneous infections), amphotericin B (*Abelcet, Fungizone*) is used for systemic *Candida* infections, *Cryptococcus* infections, and dimorphic fungal infections.

4. The azole derivative antibiotics such as **clotrimazole, miconazole, itraconazole, fluconazole, and ketoconazole**, are fungistatic drugs used to treat many fungal infections. They **interfere with ergosterol biosynthesis and thus alter the structure of the cytoplasmic membrane as well as the function of several membrane-bound enzymes** like those involved in nutrient transport and chitin synthesis.

Clotrimazole (*Lotramin, Mycelex*), miconazole (*Monistat*), and econazole (*Spectazole*) are used to treat superficial *Candida* and dermatophyte infections; oxiconazole (*Oxistat*) and sulconazole (*Exelderm*) are used for dermatophyte infections; butaconazole (*Femstat-3*), terconazole (*Terazole*), and tioconazole (*Vagistat-1*) are used for *Candida* vaginitis; ketoconazole (*Nizoral*) and itraconazole (*Sporanox*) are used for systemic *Candida, Cryptococcus*, and dimorphic fungal infections; and fluconazole (*Diflucan*) is used for *Candida* infections. Voriconazole (*VFEND*) is a triazole is used to treat *Candida* infections such as candidemia, disseminated infections in skin and infections in abdomen, kidney, bladder wall, and wounds. It is also used for invasive aspergillosis.

5. Echinocandins, including caspofungin (*Cancidas*) and micafungin (*Mycamine*) are intravenous antifungals that inhibits glucan synthesis in fungal cell walls. It is used in the treatment of candidemia , *Candida* intra-abdominal abscesses, peritonitis, esophageal candidiasis, and pleural space infections.

6. **Naftifine** (*Naftin*) and **terbinafine** (*Lamisil*) are allylamines that **block synthesis of ergosterol** as does the topical thiocarbonate **tolnaftate**. They are used to treat dermatophyte infections.

TPS Question

For a more detailed description of any specific antimicrobial agent, see the website of RxList - The Internet Drug Index**.**

Quiz Group

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Protozoa and protozoan infections

Protozoa

Fundamental Statement for this Softchalk Lesson:

1. ***Protozoa are unicellular eukaryotic microorganisms lacking a cell wall and belonging to the Kingdom Protista.***
2. ***Protozoa reproduce asexually by fission, schizogony, or budding. Some protozoa can also reproduce sexually.***
3. ***Relatively few protozoa cause disease.***
4. ***The vegetative, reproducing, feeding form of a protozoan is called a trophozoite.***
5. ***Under certain conditions, some protozoa produce a protective form called a cyst.***
6. ***Components of protozoa that function as PAMPs include GPI-anchored proteins and mannose-rich glycans.***
7. ***These PAMPS bind to PRRs on various defense cells and trigger innate immunity.***
8. ***Protozoan molecules can also trigger adaptive immunity such as the production of antibody molecules against protozoan antigens.***
9. ***Protozoan diseases include amoebic dysentery, giardiasis, balantidiasis, cryptosporidiosis African sleeping sickness, acanthamoebiasis, toxoplasmosis, and genitourinary trichomoniasis.***
10. ***Many of the same factors that enable bacteria to colonize a host also enable protozoans to colonize a host.***
11. ***Many of the same factors that enable bacteria to harm the host enable protozoans to harm the host.***

Common Course Objectives

1. Recall features of protozoa with emphasis on their life stages.
2. Distinguish between the different parasite diseases studied based on the causative agent, modes of transmission, portal of entry, and portal of exit.
3. Discuss appropriate modes of control for the parasite diseases.

Detailed Learning Objectives

1. Briefly describe protozoa.
2. Briefly describe 3 ways protozoans may reproduce asexually.
3. Define the following:
    a. trophozoite
    b. protozoan cyst.
4. State a disease caused by each of the following protozoans and indicate their means of motility and how they are transmitted to humans:
    a. *Entamoeba histolytica*
    b. *Acanthamoeba*
    c. *Giardia lamblia*
    d. *Trichomonas vaginalis*
    e. *Trypanosoma brucei-gambiens*
    f. *Balantidium coli*
    g. *Plasmodium* species
    h. *Toxoplasma gondii*
    i. *Cryptosporidium*

TPS Question

Protozoa

A. Characteristics of Protozoa

Protozoa are unicellular eukaryotic microorganisms lacking a cell wall and belonging to the Kingdom Protista. Although there are nearly 20,000 species of protozoa, relatively few cause disease. Most inhabit soil and water.

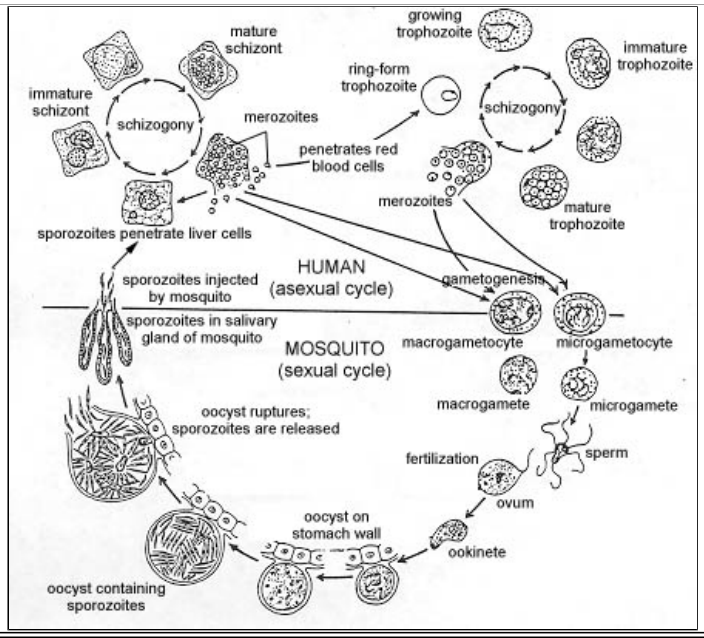
For more information: Review of prokaryotic and eukaryotic cells

Protozoa reproduce **asexually** by the following means:

1. **fission**: One cell splits into two.
2. **schizogony**: A form of asexual reproduction characteristic of certain protozoa, including sporozoa, in which daughter cells are produced by multiple fission of the nucleus of the parasite followed by segmentation of the cytoplasm to form separate masses around each smaller nucleus.
3. **budding**: Buds form around a nucleus and pinch off of the parent cell.

Some protozoa also reproduce sexually by fusion of gametes **(see Fig. 1)**. The vegetative, reproducing, feeding form of a protozoan is called a **trophozoite**. Under certain conditions, some protozoa produce a protective form called a **cyst** that enable them to survive harsh environments. Cysts allow some pathogens to survive outside their host.

Fig. 1: Illustration of the Life Cycle of *Plasmodium*, the Protozoan that causes Malaria



The protozoan reproduces asexually in the human and sexually in the mosquito.

TPS Question

B. The Role of Protozoan Cytoplasmic Membrane Components in Initiating Body Defense

1. Initiation of Innate Immunity

In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by **recognizing molecules unique to microorganisms that are not associated with human cells**. These unique molecules are called **pathogen-associated molecular patterns** or **PAMPs**. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometimes referred to as microbe-associated molecular patterns or MAMPs.)

Components of protozoa that function as PAMPs include GPI-anchored proteins (GPI = Glycosylphosphatidylinositol) and mannose-rich glycans (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar) that function as PAMPs. These mannose-rich glycans are common in microbial glycoproteins and glycolipids but rare in those of humans. These PAMPs bind to **pattern-recognition receptors** or **PRRs** on a variety of defense cells of the body and **triggers innate immune defenses such as inflammation, fever, and phagocytosis**.

Flash Animation showing the release of GPI-anchored proteins in parasites and their subsequent binding to pattern-recognition receptors on a macrophage.

1) Protozoans release GPI-anchored proteins (GPI = glycosylphosphatidylinositol) from their cytoplasmic membranes.
2) The GPI binds to a TLR-1/TLR-2 pair on defense cells such as macrophages and dendritic cells.
3) The binding of GPI to TLR-1/TLR-2 enables regulatory molecules within the cell - Mal, MyD88, Tram, and Trif - to trigger reactions that activate a master regulator of inflammation called NF-kappa B. Activated NF-kappa B enters the cell's nucleus and switches on genes coding for cytokines such as:

a. Interleukin-1 (IL-1) and Tumor necrosis factor-alpha (TNF-alpha): enhance inflammatory responses;
b. Interleukin-8 (IL-8): aids in the ability of white blood cells to leave the blood vessels and enter the tissue; a chemoattractant for phagocytes;
c. Interleukin-6 (IL-6) promotes B-lymphocyte activity; and
d. Interleukin-12 (IL-12): promotes T-lymphocyte activity. (5)

4) Cytokine genes are transcribed into mRNA molecules that goes to the cytoplasm to be translated into inflammatory cytokines that are subsequently secreted from the cell.

html5 version of animation for iPad showing the release of GPI-anchored proteins in parasites and their subsequent binding to pattern-recognition receptors on a macrophage.

For More Information: Preview of Pathogen-Associated Molecular

Patterns (PAMPs)
For More Information: Preview of Pattern-Recognition Receptors
For More Information: Preview of Inflammation

2. Initiation of adaptive immunity

Proteins associated with protozoa function as antigens and initiate adaptive immunity. An **antigen** is defined as a substance that reacts with antibody molecules and antigen receptors on lymphocytes. An **immunogen** is an antigen that is recognized by the body as non-self and stimulates an adaptive immune response.
The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR). This will be discussed in greater detail in Unit 6.

For More Information: Review of antigens and epitopes

Concept Map for Protozoa

In the next section we will briefly look at some medically important protozoa classified into phyla based on their motility.

DragNDrop Activity

B. Medically Important Protozoa

1. The Sarcomastigophora (Amoeboflagellates)

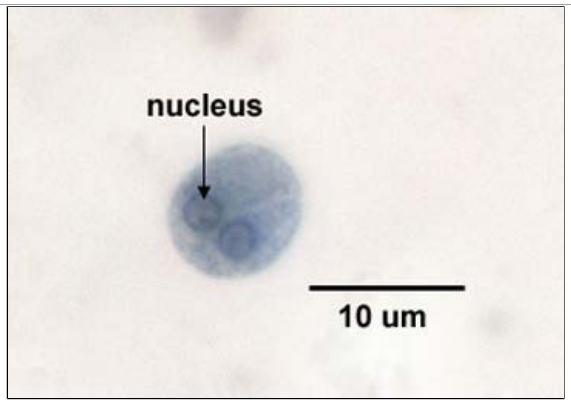
The **amoebas** (subphylum Sarcodina) move by extending lobelike projections of their cytoplasm called pseudopodia. **See Fig. 1**.

Fig. 1:Photomicrograph of *Amoeba proteus*

Note the numerous pseudopodia.

a. *Entamoeba histolytica* **(see Fig. 2)** which causes a gastrointestinal infection called **amoebic dysentery** . The organism produces protective cysts which pass out of the intestines of the infected host and are ingested by the next host. It is transmitted by the fecal-oral route.

Fig. 2: Photomicrograph of Cyst of *Entamoeba histolytica* in a Fecal Smear



Note the nuclei.

b. *Acanthamoeba* can cause **rare, but severe infections of the eye, skin, and central nervous system**. *Acanthamoeba* keratitis is an infection of the eye **(see Fig. 3)** that typically occurs in healthy persons and can result in blindness or permanent visual impairment. Granulomatous amebic encephalitis (GAE) is an infection of the brain and spinal cord typically occurring in persons with a compromised immune system. *Acanthamoeba* is found in soil, dust, and a variety of water sources including lakes, tap water, swimming pools, and heating and air conditioning units. It **typically enters the eyes and most cases are associated with contact lens use, but it can also enter cuts or wounds and be inhaled**.

Fig. 3: *Acanthamoeba* keratitis

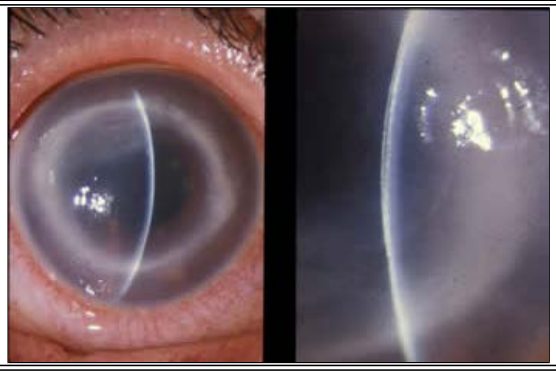


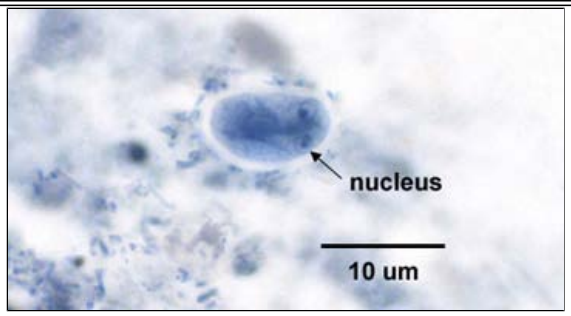
Photo courtesy of Centers for Disease Control and Prevention, Dan B. Jones, M.D.

c. *Naegleria fowleri* (sometimes called the"brain-eating amoeba"), is another amoeba that can cause a **rare but devastating infection of the brain called primary amebic meningoencephalitis** (PAM). The amoeba is commonly found in warm freshwater rivers, lakes, rivers, and hot springs, as well as in the soil. It **typically causes infections when contaminated water enters the body through the nose where it can subsequently travel to the brain**.

The **flagellates** (subphylum Mastigophora) move by means of **flagella.** Some also have an undulating membrane.

a. *Giardia lamblia* **(see Fig. 4)** can cause a gastrointestinal infection called **giardiasis**. Cysts pass out of the intestines of the infected host and are ingested by the next host. It is transmitted by the fecal-oral route.

Fig. 4: Photomicrograph of *Giardia lamblia* in a Fecal Smear



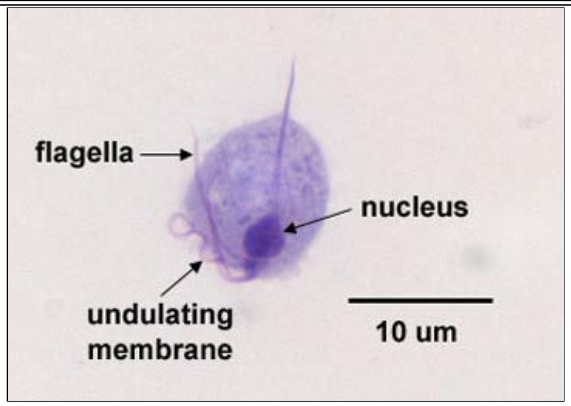
Note the two macronuclei (arrow).

Scanning electron micrograph of *Giardia* in the intestines; courtesy of Dennis Kunkel's Microscopy.

Scanning electron micrograph of *Giardia*;courtesy of CDC.

b. *Trichomonas vaginalis* **(see Fig. 5)** infects the **vagina and the male urinary tract** causing an infection called genitourinary trichomoniasis. It does not produce a cysts stage and is usually transmitted by sexual contact.

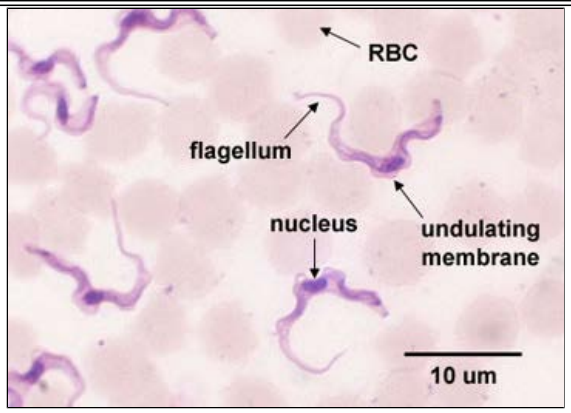
Fig. 5: Photomicrograph of *Trichomonas vaginalis* in Vaginal Discharge



Note large nucleus, flagella, and undulating membrane.

c. *Trypanosoma brucei gambiens* **(see Fig. 6)** causes **African sleeping sickness** and is transmitted by the bite of an infected Tsetse fly. The disease primarily involves the lymphatic and nervous systems of humans.

Fig. 6: Photomicrograph of *Trypanosoma gambiense* in a Blood Smear



Note nucleus, flagellum, and undulating membrane.

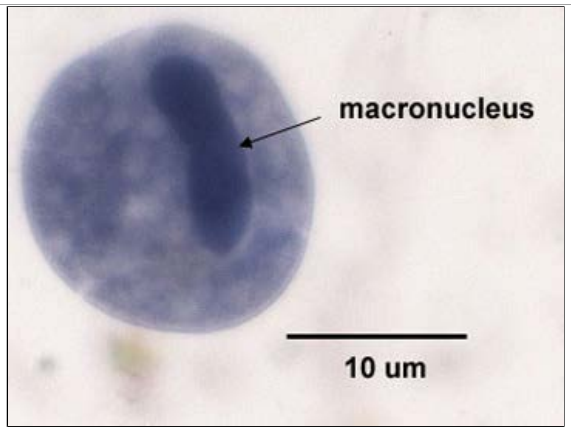
2. The Ciliophora

The **ciliates** move by means of **cilia**.

Scanning electron micrograph of *Paramecium*, a ciliated protozoan; courtesy of Dennis Kunkel's Microscopy.

a. The only pathogenic ciliate is *Balantidium coli* **(see Fig. 7)** which causes a diarrhea-type infection called balantidiasis. Cysts pass out of the intestines of the infected host and are ingested by the next host. It is transmitted by the fecal-oral route.

Fig. 7: Photomicrograph of *Balantidium coli* in a Fecal Smear



Note large dumbbell-shaped macronucleus (arrow).

3. The Apicomplexans

The apicomplexans are **not motile** in their mature forms, reproduce both asexually and sexually, and often have complex life cycles for transmission from host to host. They possess a complex of organelles called apical complexes at their apex that contain enzymes used in penetrating host tissues.

a. Species of *Plasmodium* **(see Fig. 8)** cause **malaria** and are transmitted by the bite of an infected female *Anopheles* mosquito. They reproduces asexually by schizogony in **human liver cells and red blood cells** but also reproduce sexually by gametes in the mosquito **(see Fig. 9)**. In the case of malaria caused by *P. vivax* and *P. ovale*, a dormant form or hypnozoite remains in the liver and may cause later relapses.

Fig. 8: Photomicrograph of a *Plasmodium*-Infected Red Blood Cells (arrows)

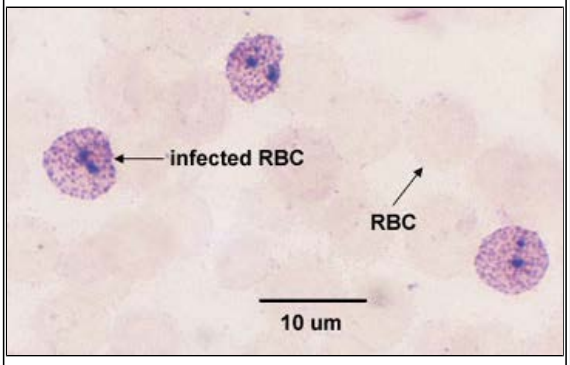
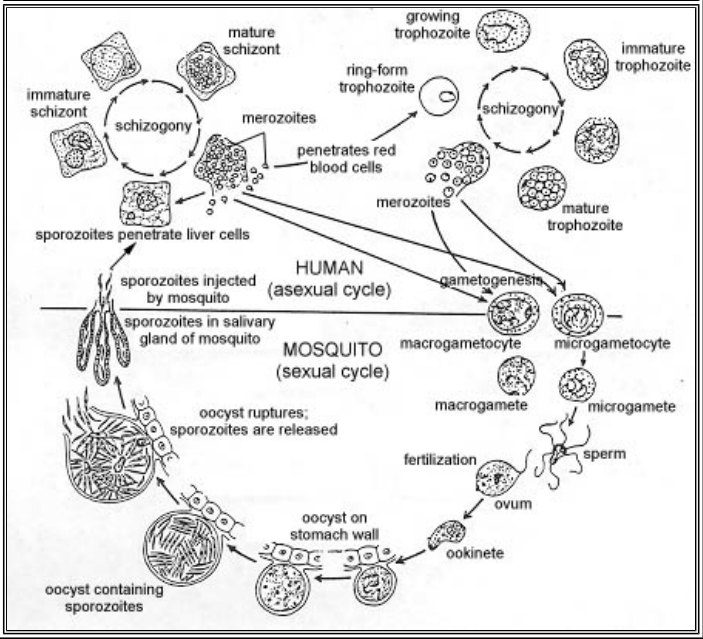


Fig. 9: Illustration of the Life Cycle of *Plasmodium*, the Protozoan that causes Malaria



b. *Toxoplasma gondii* is another intracellular apicomplexan and causes **toxoplasmosis** (see the AIDS pathology tutorial at the University of Utah). It can infect most mammals and is contracted by inhaling or ingesting cysts from the feces of infected domestic cats, where the protozoa reproduce both asexually and sexually, or by ingesting raw meat of an infected animal. Toxoplasmosis is usually **mild in people with normal immune responses but can infect the brain, heart, or lungs of people who are immunosuppressed**. It can also be transmitted **congenitally** and infect the **nervous system** of the infected child.

c. *Cryptosporidium* is an intracellular parasite that causes **a gastrointestinal infection** called **cryptosporidiosis**, although in people who are immunosuppressed it can also cause respiratory and gallbladder infections. It is transmitted by the fecal-oral route.

Movie of motile *Cryptosporidium*	***Movie of Cryptosporidium entering an epithelial cell***
Courtesy of the Sibly Lab, Washington Univerisity in St. Louis School of Medicine.	Courtesy of the Sibly Lab, Washington University in St. Louis School of Medicine.

4. Virulence Factors that Promote Colonization of Protozoans

Virulence factors that promote protozoal colonization of the host include the ability to:

1. contact host cells;
2. adhere to host cells and resist physical removal;
3. invade host cells;
4. compete for nutrients;
5. resist innate immune defenses such as phagocytosis and complement; and
6. evade adaptive immune defenses.

Examples of virulence factors that promote protozoal colonization include:

a. Some protozoa, such as *Entamoeba histolytica*,*Trichomonas vaginalis*, *Giardia lamblia*, and *Balantidium coli* use pseudopodia, flagella or cilia to swim through mucus and contact host cells.

b. Protozoa use adhesins associated with their cytoplasmic membrane to adhere to host cells, colonize, and resist flushing.

c. Some protozoa, such as the apicomplexans (*Plasmodium*, *Toxoplasma gondii,* and *Cryptosporidium*) possess a complex of organelles called apical complexes at their apex that contain enzymes used in penetrating host tissues and cells.

d. Protozoans such as Trypanosoma*brucei gambiens* and *Plasmodium* species are able to change their surface antigens during their life cycle in the human. As the protozoa change the amino acid sequence and shape of their surface antigens, antibodies and cytotoxic T-lymphocytes made against a previous shape will no longer fit and the body has to start a new round of adaptive immunity against the new antigen shape.

e. Some protozoa, such as *Entamoeba histolytica* shed their surface antigens so that antibodies made by the body against these surface antigens are tied up by the shed antigens.

Concept Map for Protozoa

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Entamoeba histolytica*
- *Acanthamoeba*
- *Giardia lamblia*
- *Trichomonas vaginalis*
- *Trypanosoma brucei gambiens*
- *Balantidium coli*
- *Plasmodium*
- *Toxoplasma gondii*
- *Cryptosporidium*

Self Check

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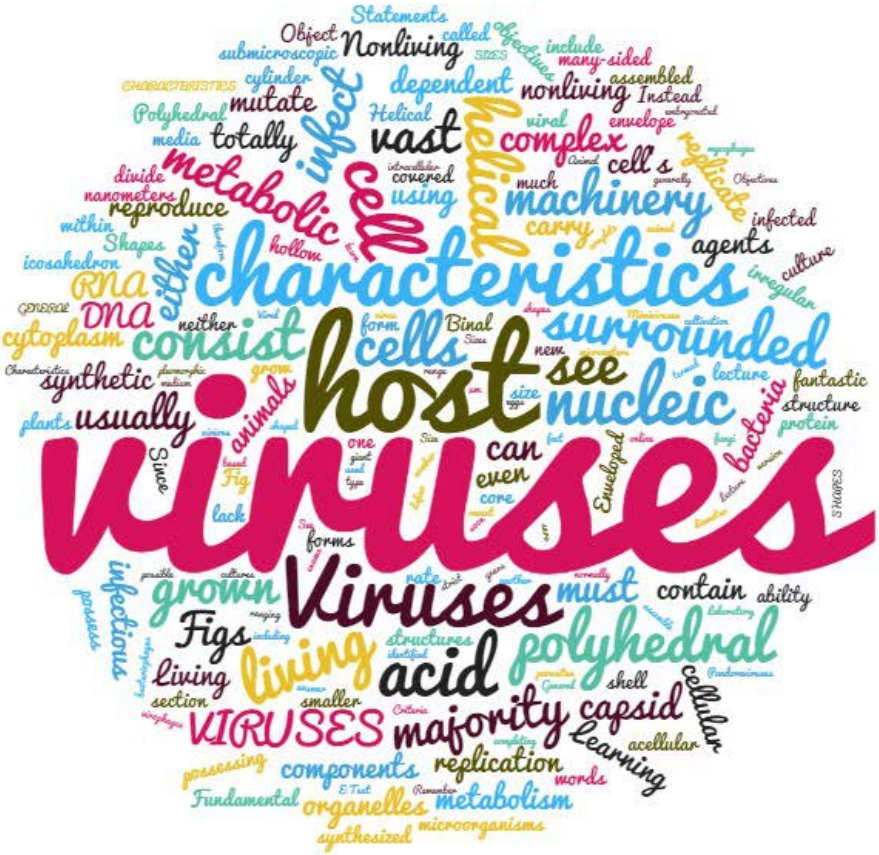
Viral characteristics, sizes, and shapes
VIRAL CHARACTERISTICS, SIZES, AND SHAPES

Viruses

A. Viral Characteristics, Sizes, and Shapes



Fundamental Statement for this Softchalk Lesson:

1. Viruses are infectious agents with both living and nonliving characteristics.
2. Living characteristics of viruses include the ability to reproduce – but only in living host cells – and the ability to mutate.
3. Nonliving characteristics include the fact that they are not cells, have no cytoplasm or cellular organelles, and carry out no metabolism on their own and therefore must replicate using the host cell's metabolic machinery.
4. Viruses can infect animals, plants, and even other microorganisms.
5. Since viruses lack metabolic machinery of their own and are totally dependent on their host cell for replication, they cannot be grown in synthetic culture media.
6. Viruses are usually much smaller than bacteria with the vast majority being submicroscopic, generally ranging in size from 5 to 300 nanometers (nm).
2. Helical viruses consist of nucleic acid surrounded by a hollow protein cylinder or capsid and possessing a helical structure.
7. Polyhedral viruses consist of nucleic acid surrounded by a polyhedral (many-sided) shell or capsid, usually in the form of an icosahedron.
8. Enveloped viruses consist of nucleic acid surrounded by either a helical or polyhedral core and covered by an envelope.
9. Binal (complex) viruses have neither helical nor polyhedral forms, have irregular shapes, or have complex structures.

Common Course Objectives

1. Recall characteristics that are present only in viruses and not in other cellular pathogens.
2. Describe what an animal virus consists of structurally and state the function of those viral parts.

Detailed Learning Objectives

1. State 2 living and 2 nonliving characteristics of viruses.

2*. List 3 criteria used to define a virus.

3. Discuss why bacteria can be cultivated on synthetic media such as nutrient broth whereas viruses cannot.

5. Define bacteriophage.

6. Compare the size of most viruses to that of bacteria.

7. List 4 shapes of viruses.

(*) = Common theme throughout the course

TPS Question

Viruses

Viral Characteristics, Sizes, and Shapes

1. General Characteristics of Viruses

Viruses are infectious agents with both living and nonliving characteristics. They can infect animals, plants, and even other microorganisms. Viruses that infect only bacteria are called bacteriophages and those that infect only fungi are termed mycophages. There are even some viruses called virophages that infect other viruses.

a. Living characteristics of viruses

1. They **reproduce** at a fantastic rate, but only in living host cells.
2. They can **mutate.**

b. Nonliving characteristics of viruses

1. They are **acellular**, that is, they contain no cytoplasm or cellular organelles.
2. They **carry out no metabolism on their own and must replicate using the host cell's metabolic machinery**. In other words, viruses don't grow and divide. Instead, new viral components are synthesized and assembled within the infected host cell.
3. The vast majority of viruses possess either DNA or RNA but not both.

c. Criteria used to define a virus

1. The vast majority of viruses contain only one type of nucleic acid: DNA or RNA, but not both.
2. Viruses are totally dependent on a host cell for replication. (They are strict intracellular parasites.)
3. Viral components must assemble into complete viruses (virions) to go from one host cell to another.

Recently, **viruses have been declared as living entities** based on the large number of protein folds encoded by viral genomes that are shared with the genomes of cells. This indicates that viruses likely arose from multiple ancient cells.

d. Laboratory cultivation of viruses

Since viruses lack metabolic machinery of their own and are totally dependent on their host cell for replication, they cannot be grown in synthetic culture media. Animal viruses are normally grown in animals, embryonated eggs, or in **cell cultures** where in animal host cells are grown in a synthetic medium and the viruses are then grown in these cells.

Quiz Group

2. Sizes and Shapes of Viruses

a. Sizes (see Fig. 1, Fig. 2, and Fig. 3C)

Viruses are usually much smaller than bacteria with the vast majority being submicroscopic. While most viruses range in size from 5 to 300 nanometers (nm), in recent years a number of giant viruses, including Mimiviruses and Pandoraviruses with a diameter of 0.4 micrometers (µm), have been identified.

To view a nice interactive illustration comparing size of cells and microbes, see the Cell Size and Scale Resource at the University of Utah.

Fig. 1: Sizes and Shapes of Viruses (Animal RNA Viruses)

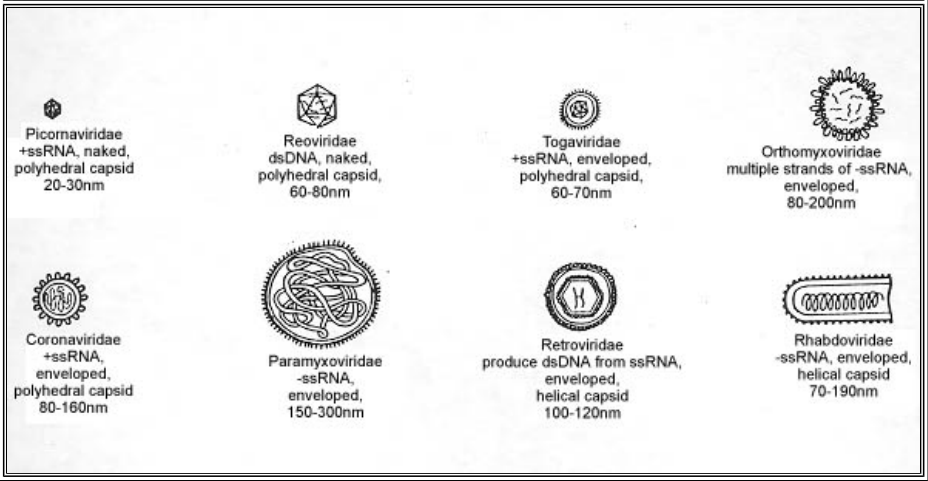


Fig. 2: Sizes and Shapes of Viruses (Animal DNA Viruses)

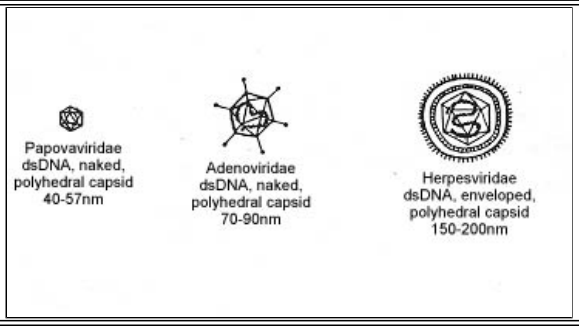
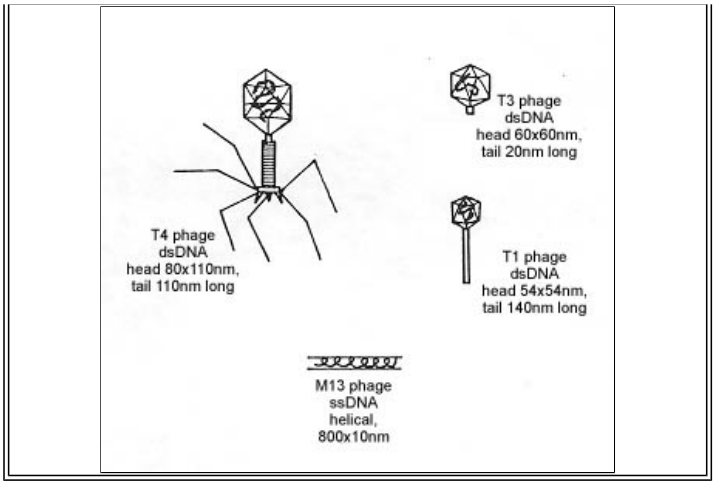


Fig. 3: Sizes and Shapes of Viruses (Bacteriophages)



b. Shapes (see Fig. 1, Fig. 2, and Fig. 3)

1. Helical viruses consist of nucleic acid surrounded by a hollow protein cylinder or capsid and possessing a helical structure **(see Fig. 4)**.
2. Polyhedral viruses consist of nucleic acid surrounded by a polyhedral (many-sided) shell or capsid, usually in the form of an icosahedron; **(see Fig. 5)**.

Fig. 4: Illustration of a Helical Virus

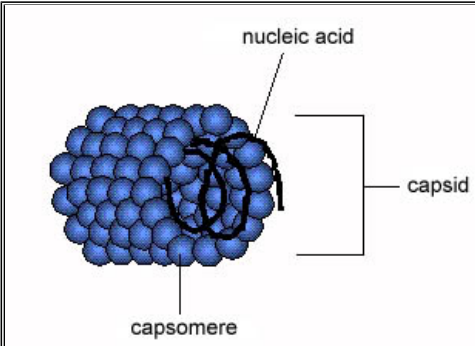
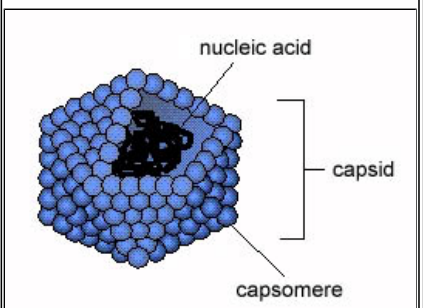


Fig. 5: Illustration of a Polyhedral Virus



Transmission electron micrograph of Adenoviruses; courtesy of CDC.

Transmission electron micrograph of Poliomyelitis viruses; courtesy of CDC.

Transmission electron micrograph of Poliomyelitis viruses; courtesy of Dennis Kunkel's Microscopy.

3. Enveloped viruses consist of nucleic acid surrounded by either a helical or polyhedral core and covered by an envelope **(see Fig. 6 and Fig. 7)**.

Fig. 6: Illustration of a Helical Enveloped Viruses

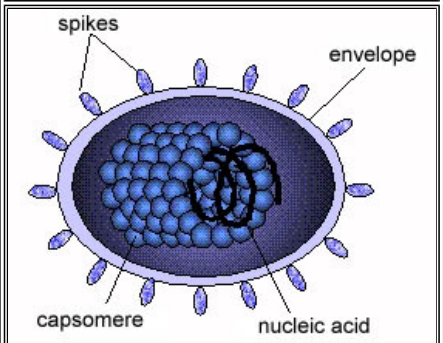
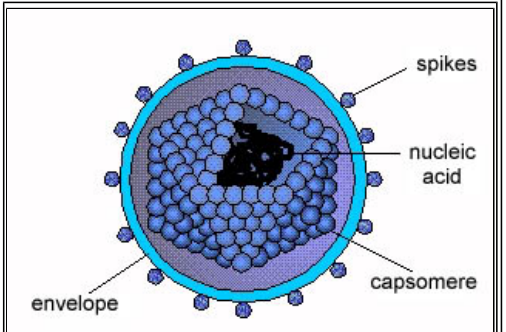


Fig. 7: Illustration of a Polyhedral Enveloped Viruses



Transmission electron micrograph of Hepatitis B viruses; courtesy of CDC.

Transmission electron micrograph of an Influenza A virus; courtesy of CDC.

Transmission electron micrograph of HIV; courtesy of CDC.

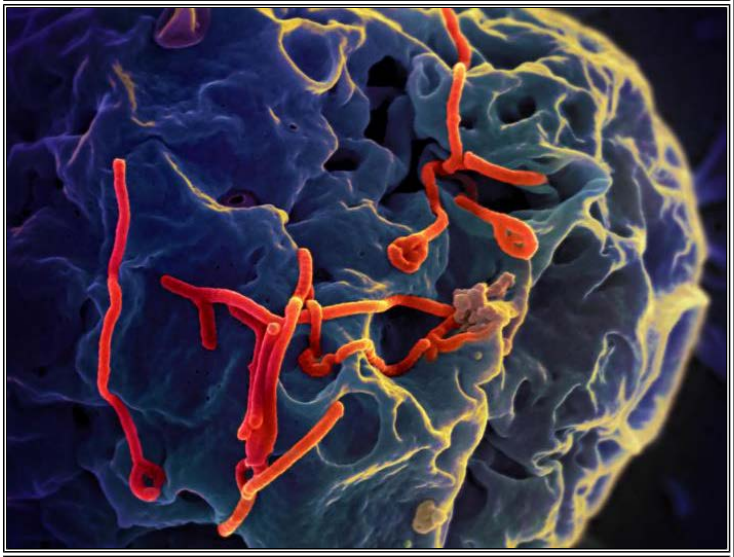
Transmission electron micrograph showing envelope and glycoprotein spikes Coronaviruses; courtesy of CDC.

Transmission electron micrograph of Herpes Simplex Viruses; courtesy of Dennis Kunkel's Microscopy.

4. Binal **(complex) viruses** have neither helical nor polyhedral forms, are pleomorphic or irregular shaped **(see fig 8)**, or have complex structures **(see Fig. 9)**.

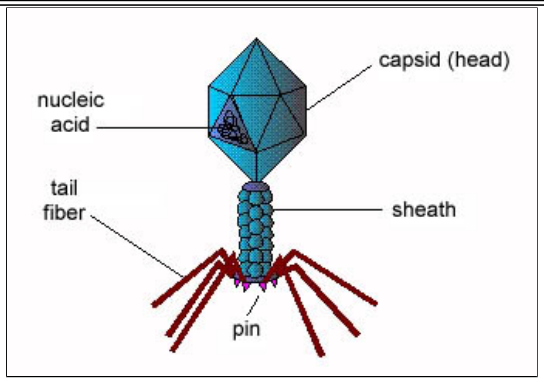
Fig. 8: Scanning Electron Micrograph of Filamentous Ebola Viruses (red)

Budding from a Host Cell



Courtesy of National Institute of Allergy and Infectious Diseases (NIAID)

Fig. 9: Illustration of a Complex Binal Virus



Transmission electron micrograph of the bacteriophage Coliphage T4; courtesy of Dennis Kunkel's Microscopy.

TPS Question

Concept Map for Sizes, Shapes, and Characteristics of Viruses

Quiz Group

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Viral structure
VIRAL STRUCTURE

Viruses

Viral Structure

Fundamental Statement for this Softchalk Lesson:

- ***1. Since viruses are not cells, they are structurally much simpler than bacteria.***
- ***2. An intact infectious viral particle - or virion - consists of a genome, a capsid, and maybe an envelope.***
- ***3. Viruses possess either DNA or RNA as their genome.***
- ***4. The genome is typically surrounded by a protein shell called a capsid composed of protein subunits called capsomeres.***
- ***5. Some viruses consist of no more than a genome surrounded by a capsid and are called nucleocapsid or naked viruses.***
- ***5. Most animal viruses also have an envelope surrounding a polyhedral or helical nucleocapsid that is typically derived from host cell membranes by a budding process and are called enveloped viruses.***
- ***6. Specific proteins or glycoproteins on the viral surface are used to attach viruses to the surface of its host cell.***

Common Course Objectives

1. Recall characteristics that are present only in viruses and not in other cellular pathogens.

2. Describe what an animal virus consists of structurally and state the function of those viral parts.

Detailed Learning Objectives

1*. Describe what an animal virus consists of structurally.

2. Define the following:

a. capsid

b. capsomere

c. nucleocapsid.

3*. Describe how most animal viruses obtain their envelope.

4. State why some bacteriophages are more complex than typical polyhedral or helical viruses.

(*) = Common theme throughout the course

TPS Question

Viruses

Viral Structure

Since viruses are not cells, they are structurally much simpler than bacteria. An intact infectious viral particle is called a virion and consists of:

1. A genome

The **viral genome** is a single or segmented, circular or linear molecule of nucleic acid functioning as the genetic material of the virus. It can be single-stranded or double-stranded **DNA or RNA** (but almost never both), and codes for the synthesis of viral components and viral enzymes for replication. It is also becoming recognized that viruses may play a critical role in evolution of life by serving as shuttles of genetic material between other organisms.

2. A capsid

The capsid, or **core**, is a protein shell surrounding the genome and is usually composed of protein subunits called capsomeres. The capsid serves to protect and introduce the genome into host cells. Some viruses consist of no more than a genome surrounded by a capsid and are called **nucleocapsid or** naked viruses **(see Fig. 1 and Fig. 2)**. **Attachment proteins** project out from the capsid and **bind the virus to susceptible host cells**.

Fig. 1: Illustration of a Helical Naked Virus

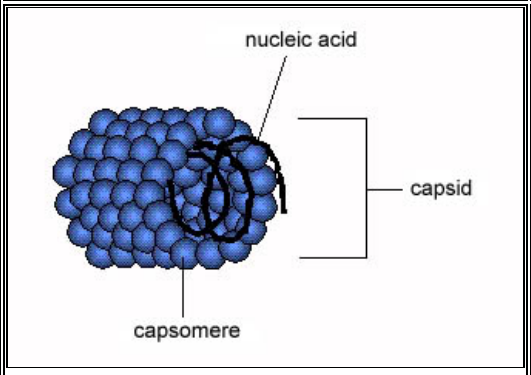
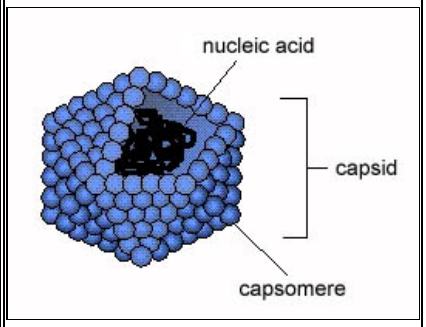


Fig. 2: Illustration of a Polyhedral Naked Virus



Transmission electron micrograph of Adenoviruses; courtesy of CDC.

Transmission electron micrograph of Poliomyelitis viruses; courtesy of CDC.

3. An envelope

Most animal viruses also have an **envelope** surrounding a polyhedral or helical nucleocapsid, in which case they are called **enveloped viruses (see Fig. 3, Fig. 4,and Fig. 5)**. The envelope is composed of phospholipids and glycoprotein and for most viruses, is **derived from host cell membranes by a process called budding** (see Fig. 6 and Fig. 7B). The envelope may come from the host cell's nuclear membrane, vacuolar membranes (packaged by the Golgi apparatus), or outer cytoplasmic membrane.

Fig. 3: Illustration of an Enveloped Helical Virus

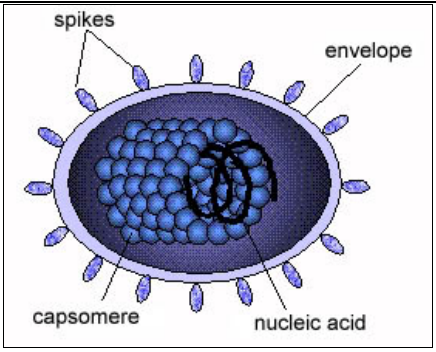


Fig. 4: Illustration of an Enveloped Polyhedral Virus

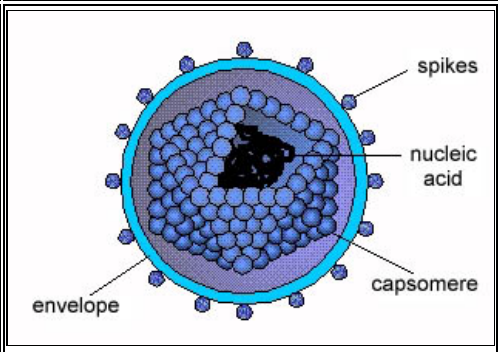


Fig. 5: Illustration of HIV

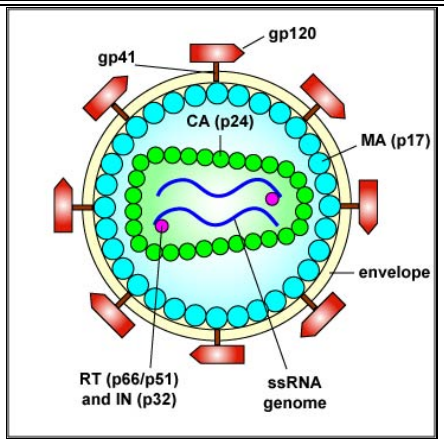


Fig. 6: Illustration of a Virus Obtaining Its Envelope from Host Cell Membrane by Budding

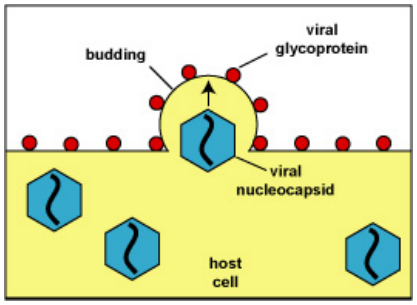


Fig. 7: Transmission Electron Micrograph of Rubella Viruses Budding from a Host Cell

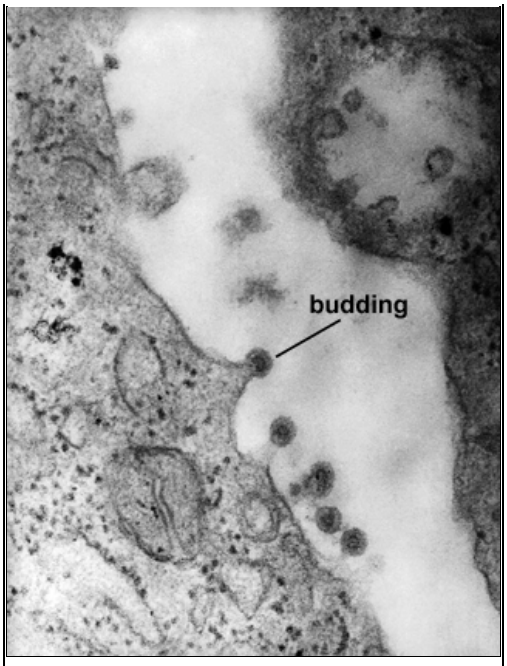


Image provided by Dr. Fred Murphy

and Sylvia Whitfield.
Courtesy of the Centers for Disease Control and Prevention.

Transmission electron micrograph of HIV budding from a T4-lymphocyte; courtesy of Dennis Kunkel's Microscopy.

Transmission electron micrograph of an Influenza A virus; courtesy of CDC.

Transmission electron micrograph of HIV; courtesy of CDC.

Although the envelope is usually of host cell origin, the virus does incorporate proteins of its own, often appearing as glycoprotein **spikes**, into the envelope. These glycoprotein spikes function in **attaching the virus to receptors on susceptible host cells**.

Transmission electron micrograph showing envelope and glycoprotein spikes (gp120) of HIV; courtesy of CDC.

Transmission electron micrograph showing envelope and glycoprotein spikes Coronaviruses; courtesy of CDC.

Bacteriophages are viruses that only infect bacteria. **Some bacteriophages are structurally much more complex than typical nucleocapsid or enveloped viruses and may possess a unique tail structure** composed of a base plate, tail fibers, and a contractile sheath **(also see Fig. 8, Fig. 9, and Fig. 10)**. Other bacteriophages, however, are simple icosahedrals or helical **(see Fig. 8)**.

Fig. 8: Sizes and Shapes of Viruses (Bacteriophages)

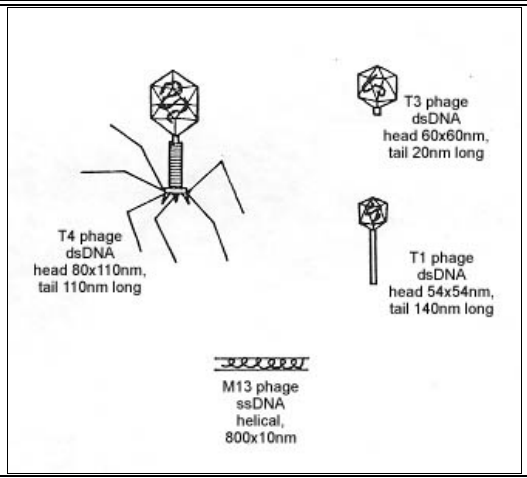
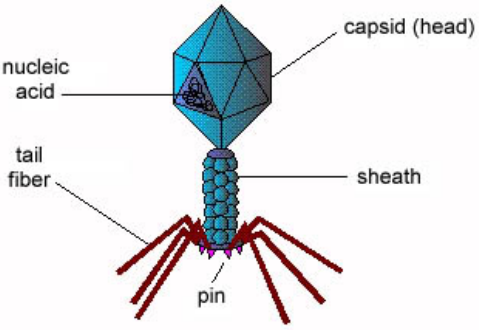
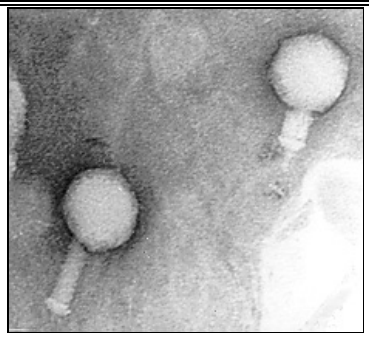


Fig. 9: Illustration of a Binal Virus, Coliphage T4	Fig. 10: Electron Micrograph of a Bacteriophage with a Contractile Sheath
 Copyright © Gary E. Kaiser	 A bacteriophage before it contracts its sheath is seen on the left. A bacteriophage after contraction of its sheath is seen on the right. Electron Micrograph Copyright © Robert Burchard Used with permission.

Transmission electron micrograph of the bacteriophage Coliphage T4; courtesy of Dennis Kunkel's Microscopy.

TPS Question

Concept Map for Viral Structure

Self-Quiz for Viral Structure

Quiz Group

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Viral classification
VIRAL CLASSIFICATION

Viruses

Viral Classification

Fundamental Statement for this Softchalk Lesson:

1. Viruses can store their genetic information in six different types of nucleic acid which are named based on how that nucleic acid eventually becomes transcribed to the viral mRNA.
2. (+) and (-) strands of nucleic acid are complementary. Copying a (+) stand gives a (-) strand; copying a (-) stand gives a (+) strand.
3. Only (+) strands of viral RNA can be translated into viral protein.
4. Regarding the enzymes involved in nucleic acid replication, the "dependent" part of the name refers what type of nucleic acid is being copied. The "polymerase" part of the name refers what type of nucleic acid is being synthesized.

Common Course Objectives

1. Recall characteristics that are present only in viruses and not in other cellular pathogens.

2. Explain how viruses are classified by genetic material and/or capsid arrangement.

Detailed Learning Objectives

1. State what criteria are used in viral classification.

2. Regarding the naming of enzymes involved in the replication of viral nucleic acid, state what the "dependent" part of the name refers to and what the "polymerase" part of the name refers to.

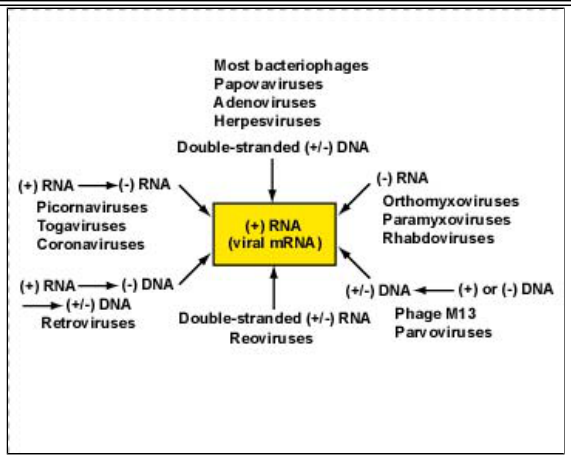
TPS Question

Viruses

Viral Classification

Viruses can store their genetic information in six different types of nucleic acid which are named based on how that nucleic acid eventually becomes transcribed to the viral mRNA **(see Fig. 1)** capable of binding to host cell ribosomes and being translated into viral proteins.

Fig. 1: Transcription of Viral Nucleic Acid into Viral mRNA

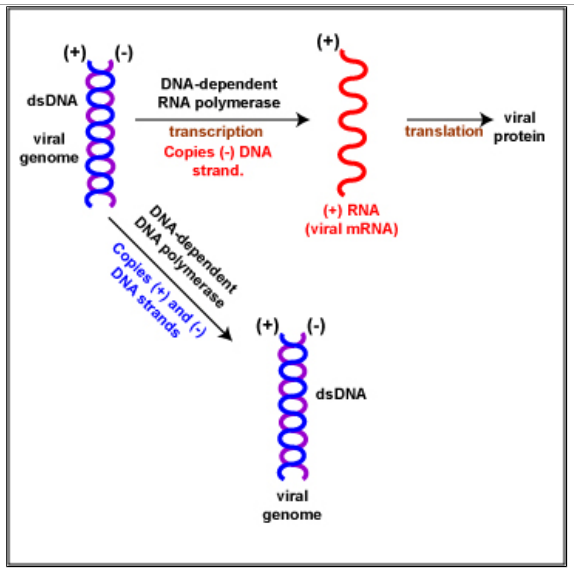


A (+) RNA can be translated into viral protein. (+) and (-) strands are complementary.

In the diagrams below, (+) and (-) represent complementary strands of nucleic acid. Copying of a (+) strand by complementary base pairing forms a (-) strand. Only a (+) viral mRNA strand can be translated into viral protein. Regarding the enzymes involved in nucleic acid replication, the "dependent" part of the name refers what type of nucleic acid is being copied. The "polymerase" part of the name refers what type of nucleic acid is being synthesized, e.g., DNA-dependent RNA-polymerase would synthesize a strand of RNA complementary to a strand of DNA. These six forms of viral nucleic acid are:

a. **(+/-) double-stranded DNA (see Fig. 2)** . To replicate the viral genome, DNA-dependent DNA polymerase enzymes copy both the (+) and (-) DNA strands producing dsDNA viral genomes. To produce viral mRNA molecules, DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes. Examples include most bacteriophages, Papovaviruses, Adenoviruses, and Herpesviruses.

Fig. 2: Replication of a Double-Stranded DNA Viral Genome and production of Viral mRNA

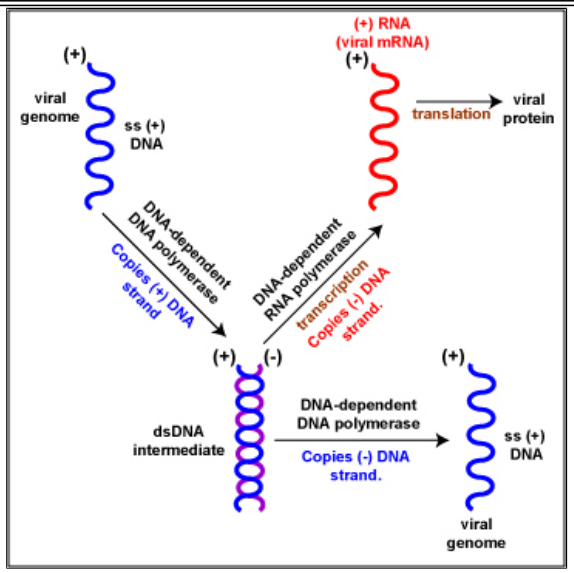


To replicate the viral genome, DNA-dependent DNA polymerase enzymes copy both the (+) and (-) DNA strands producing dsDNA viral genomes.

To produce viral mRNA molecules. DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes.

b. **(+) single-stranded DNA (see Fig. 3)**. To replicate the viral genome, DNA-dependent DNA polymerase enzymes copy the (+) DNA strand of the genome producing a dsDNA intermediate. DNA-dependent DNA polymerase enzymes then copy the (-) DNA strand into ss (+) DNA genomes. To produce viral mRNA molecules, DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes. Examples include Phage M13 and Parvoviruses.

Fig. 3: Replication of a Single-Stranded DNA Viral Genome and Production of Viral mRNA

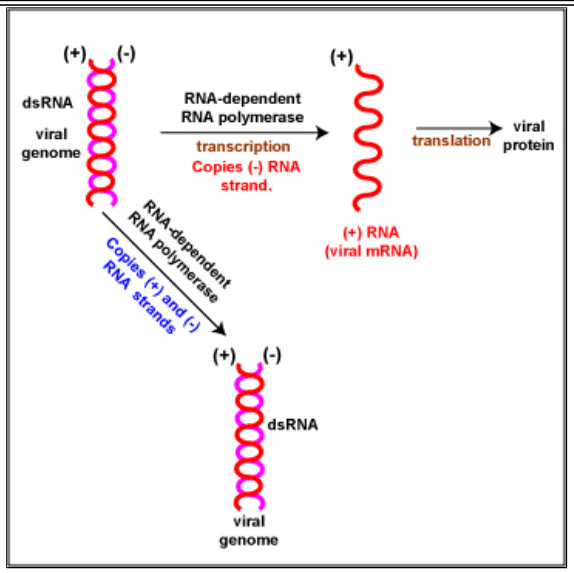


To replicate the viral genome, DNA-dependent DNA polymerase enzymes copy the (+) DNA strand of the genome producing a dsDNA intermediate. DNA-dependent DNA polymerase enzymes then copy the (-) DNA strand into ss (+) DNA genomes.

To produce viral mRNA molecules. DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes.

c. **(+/-) double-stranded RNA (see Fig. 4)** . To replicate the viral genome, RNA-dependent RNA polymerase enzymes copy both the (+) RNA and (-) RNA strands of the genome producing a dsRNA genomes. To produce viral mRNA molecules, RNA-dependent RNA polymerase enzymes copy the (-) RNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes. Reoviruses are an example.

Fig. 4: Replication of a Double-Stranded RNA Viral Genome and Production of Viral mRNA

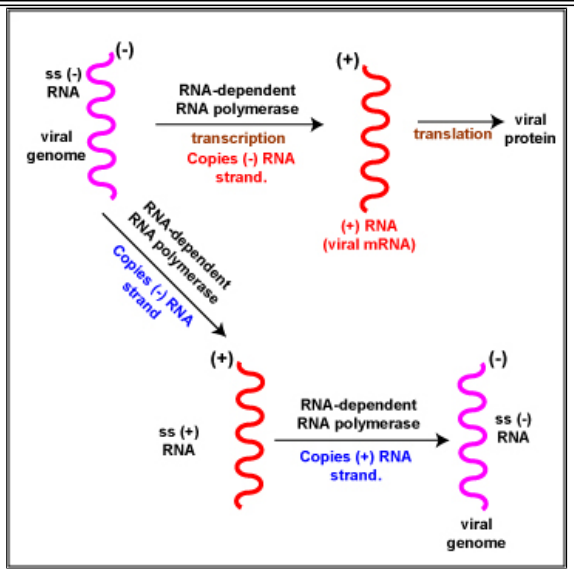


To replicate the viral genome, RNA-dependent RNA polymerase enzymes copy both the (+) RNA and (-) RNA strands of the genome producing a dsRNA genomes.

To produce viral mRNA molecules. RNA-dependent RNA polymerase enzymes copy the (-) RNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes.

d. **(-) RNA (see Fig. 5)**. To replicate the viral genome, RNA-dependent RNA polymerase enzymes copy the (-) RNA genome producing ss (+) RNA. RNA-dependent RNA polymerase enzymes then copy the (+) RNA strands producing ss (-) RNA viral genome. The (+) mRNA strands also function as viral mRNA and can then be translated into viral proteins by host cell ribosomes. Examples include Orthomyxoviruses, Paramyxoviruses, Rhabdoviruses.

Fig. 5: Replication of a Single-Stranded Minus RNA Viral Genome and Production of Viral mRNA

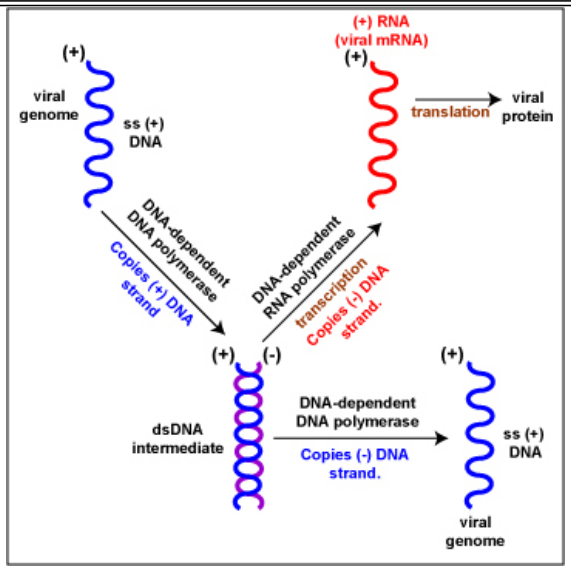


To replicate the viral genome, RNA-dependent RNA polymerase enzymes copy the (-) RNA genome producing ss (+) RNA. RNA-dependent RNA polymerase enzymes then copy the (+) RNA strands producing ss (-) RNA viral genome.

The (+) mRNA strands also function as viral mRNA and can then be translated into viral proteins by host cell ribosomes.

e. **(+) RNA (see Fig. 6)**. To replicate the viral genome, RNA-dependent RNA polymerase enzymes copy the (+) RNA genome producing ss (-) RNA. RNA-dependent RNA polymerase enzymes then copy the (-) RNA strands producing ss (+) RNA viral genome. To produce viral mRNA molecules. RNA-dependent RNA polymerase enzymes copy the (-) RNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes. Examples include Picornaviruses, Togaviruses, and Coronaviruses.

Fig. 6: Replication of a Single-Stranded Plus RNA Viral Genome and Production of Viral mRNA

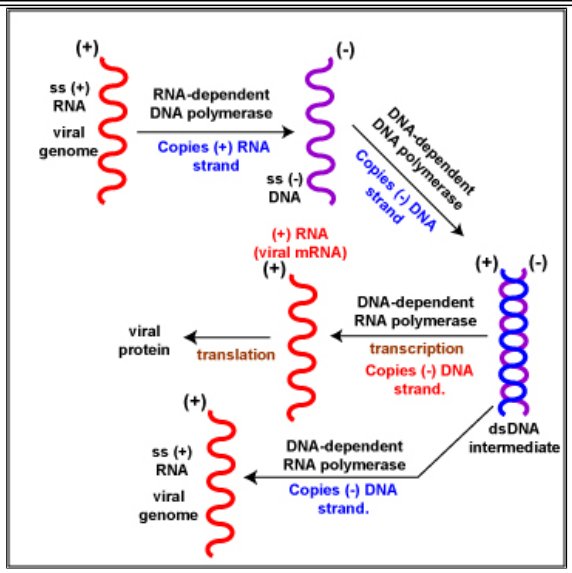


To replicate the viral genome, RNA-dependent RNA polymerase enzymes copy the (+) RNA genome producing ss (-) RNA. RNA-dependent RNA polymerase enzymes then copy the (-) RNA strands producing ss (+) RNA viral genome.

To produce viral mRNA molecules. RNA-dependent RNA polymerase enzymes copy the (-) RNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes.

f. **(+) RNA Retroviruses (see Fig. 7)**. To replicate the viral genome, reverse transcriptase enzymes (RNA-dependent DNA polymerases) copy the (+) RNA genome producing ss (-) DNA strands. DNA-dependent DNA polymerase enzymes then copy the (-) DNA strands to produce a dsDNA intermediate. DNA-dependent RNA polymerase enzymes then copy the (-) DNA strands to produce ss (+) RNA genomes. To produce viral mRNA molecules, DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes. Retroviruses, such as HIV-1, HIV-2, and HTLV-1 are examples.

Fig. 7: Replication of a Single-Stranded Plus RNA Viral Genome and Production of Viral mRNA by way of Reverse Transcriptase



To replicate the viral genome, reverse transcriptase enzymes (RNA-dependent DNA polymerases) copy the (+) RNA genome producing ss (-) DNA strands. DNA-dependent DNA polymerase enzymes then copy the (-) DNA strands to produce a dsDNA intermediate. DNA-dependent RNA polymerase enzymes then copy the (-) DNA strands to produce ss (+) RNA genomes.

To produce viral mRNA molecules. DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral

proteins by host cell ribosomes.

TPS Question

For more information: Review of Deoxyribonucleic Acid DNA

For more information: Review of DNA replication

For More Information: Transcription

For More Information: Translation

For More Information: RNA

Table of Selected Viral Families, Viruses, and Species Affected

The Table below describes some of the medically important viruses.

Table 2-1

Classification of Viruses

1. single-stranded DNA; naked; polyhedral capsid

- **Viral family**: Parvoviridae
- **Size**: 18-25nm
- **Examples and diseases:** parvoviruses (roseola, fetal death, gastroenteritis; some depend on coinfection with adenoviruses)

2. double-stranded, DNA; naked; polyhedral capsid

- **Viral family**: Papovaviridae; circular dsDNA
- **Size**: 40-57nm
- **Examples and diseases**: human papilloma viruses (HPV; benign warts and genital warts; genital and rectal cancers)

- **Viral family**: Adenoviridae; dsDNA
- **Size**: 70-90nm
- **Examples and diseases**: adenoviruses (respiratory infections, gastroenteritis, infectious pinkeye, rashes, meningoencephalitis)

3. double-stranded, circular DNA; enveloped; complex

- **Viral family**: Poxviridae
- **Size**: 200-350nm
- **Examples and diseases**: smallpox virus (smallpox), vaccinia virus (cowpox), molluscipox virus (molluscum contagiosum-wartlike skin lesions)

4. double-stranded DNA; enveloped; polyhedral capsid

- **Viral family**: Herpesviridae
- **Size**: 150-200nm
- **Examples and diseases**: herpes simplex 1 virus (HSV-1; most oral herpes; herpes simplex 2 virus (HSV-2; most genital herpes), herpes simplex 6 virus (HSV-6; roseola), varicella-zoster virus (VZV; chickenpox and shingles), Epstein-Barr virus (EBV; infectious mononucleosis and lymphomas), cytomegalovirus (CMV; birth defects and infections of a variety of body systems in immunosuppressed individuals)

- **Viral family**: Hepadnaviridae
- **Size**: 42nm
- **Examples and diseases**: hepatitis B virus (HBV; hepatitis B and liver cancer)

5. (+)single-stranded RNA; naked; polyhedral capsid

- **Viral family**: picornaviridae
- **Size**: 28-30nm
- **Examples and diseases**: enteroviruses (poliomyelitis), rhinoviruses (most frequent cause of the common cold), Noroviruses (gastroenteritis), echoviruses (meningitis), hepatitis A virus (HAV; hepatitis A)

6. (+)single-stranded RNA; enveloped; usually a polyhedral capsid

- **Viral family**: Togaviridae
- **Size**: 60-70nm
- **Examples and diseases**: arboviruses (eastern equine encephalitis, western equine encephalitis), rubella virus (German measles)

- **Viral family**: Flaviviridae
- **Size**: 40-50nm
- **Examples and diseases**: flaviviruses (yellow fever, dengue fever, St. Louis encephalitis), hepatitis C virus (HCV; hepatitis C)

- **Viral family**: Coronaviridae
- **Size**: 80-160nm
- **Examples and diseases**: coronaviruses (upper respiratory infections and the common cold; SARS)

7. (-)single-stranded RNA; enveloped; pleomorphic

- **Viral family**: Rhabdoviridae; bullet-shaped
- **Size**: 70-189nm
- **Examples and diseases**: rabies virus (rabies)

- **Viral family**: Filoviridae; long and filamentous
- **Size**: 80-14,000nm
- **Examples and diseases**: Ebola virus, Marburg virus (hemorrhagic fevers)

- **Viral family**: Paramyxoviridae; pleomorphic
- **Size**: 150-300nm
- **Examples and diseases**: paramyxoviruses (parainfluenza, mumps); measles virus (measles)

8. (-) strand; multiple strands of RNA; enveloped

- **Viral family**: Orthomyxoviridae
- **Size**: 80-200nm
- **Examples and diseases**: influenza viruses A, B, and C (influenza)

- **Viral family**: Bunyaviridae
- **Size**: 90-120nm
- **Examples and diseases**: California encephalitis virus (encephalitis); hantaviruses (Hantavirus pulmonary syndrome, Korean hemorrhagic fever)

- **Viral family**: Arenaviridae
- **Size**: 50-300nm
- **Examples and diseases**: arenaviruses (lymphocytic choriomeningitis, hemorrhagic fevers)

9. produce DNA from (+) single-stranded RNA using reverse transcriptase; enveloped; bullet-shaped or polyhedral capsid

- **Viral family**: Retroviridae
- **Size**: 100-120nm
- **Examples and diseases**: HIV-1 and HIV-2 (HIV infection/AIDS); HTLV-1 and HTLV-2 (T-cell leukemia)

10. dsRNA; naked; polyhedral capsid

- **Viral family**: Reoviridae
- **Size**: 60-80nm
- **Examples and diseases**: reoviruses (mild respiratory infections, infant gastroenteritis); Colorado tick fever virus (Colorado tick fever)

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- Fifth Disease
- Parvovirus Infection
- Human Papilloma Virus
- Adenoviruses
- Smallpox
- Poxviruses
- Herpes Sinplex
- Varicella-Zoster Virus
- Infectious Mononucleosis
- Cytomegalovirus
- Hepatitis B
- Enteroviruses
- Poliomyelitis
- Rhinoviruses
- Norwalk Virus
- Echoviruses
- Hepatitis A
- Viral Encephalitis
- Rubella
- Yellow Fever
- Dengue Fever
- Hepatitis C
- Severe Acute Respiratory Syndrome (SARS)
- Rabies
- Ebola Virus
- Mumps
- Measles
- Influenza
- Hantavirus Pulmonary Syndrome
- Hemorrhagic Fevers
- HIV Infection and AIDS
- Human T-Cell Lymphotropic Viruses
- Reoviruses

Self Quiz for Viral Classification

Quiz Group

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Viral activation of innate and adaptive immunity
Viral Activation of Immune Responses

Viral Activation of Immune Responses

Fundamental Statement for this Softchalk Lesson:

1. Innate immunity is an antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to almost any microbe. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.
2. Viral nucleic acids functions as a pathogen-associated molecular pattern (PAMP).
3. Binding of viral PAMPs to host cell pattern-recognition receptors (PRRs) triggers the synthesis and secretion of anti-viral cytokines called type-1 interferons that block viral replication within infected host cells.
4. Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to react with and remove a specific antigen. This is the immunity one develops throughout life.
5. An antigen is a molecular shape that reacts with antigen receptors on lymphocytes to initiate an adaptive immune response.
6. The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes

and T-lymphocytes are called epitopes.
7. Opsonizing antibodies made against viral surface antigens can bind viruses to phagocytes for more efficient phagocytosis; antibodies made against viral surface proteins can block adsorption of viruses to host cell receptors.
8. Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigens. These defense cells help to remove infected cells and cancer cells displaying foreign epitopes.
9. One of the body's major defenses against viruses, intracellular bacteria, and cancers is the destruction of infected cells and tumor cells by cytotoxic T-lymphocytes or CTLs, effector cells derived from naïve T8-lymphocytes during cell-mediated immunity.
10. When the TCR and CD8 of the CTL binds to the MHC-I/epitope on the surface of the virus-infected cell or tumor cell, this triggers the release of cytotoxic perforins/granzymes/ granulysin granules from the CTL that lead to apoptosis, a programmed cell suicide of that cell to which the CTL has bound.
11. NK cells (Natural Killer cells) recognize infected cells displaying stress-induced proteins and not displaying MHC-I molecules and kill these cells by inducing apoptosis.
12. During apoptosis, the cell breaks into membrane-bound apoptotic fragments that are subsequently removed by macrophages.

Common Course Objectives

1. Explain how various viral structures can contribute to the initiation of immune defenses.

2. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

3. Describe how activated cytotoxic T-cells kill target cells.

Detailed Learning Objectives

1*. Compare and contrast innate immunity and adaptive immunity.

2*. Define antigen and epitope.

3*. Define humoral immunity and cell-mediated immunity.

4*. Briefly describe how opsonizing antibodies can promote phagocytosis of viruses and how antibodies made against viral surface antigens can block adsorption of viruses to host cell receptors.

5.* In terms of the role of cytotoxic T-lymphocytes (CTLs) in body defense:

a. Describe how they can react with and destroy virus-infected cells, cells containing intracellular bacteria, and cancer cells. (Indicate the role of following: TCR, CD4, MHC-I, and peptides from viral antigens.)

b. State the mechanism by which cytotoxic T-lymphocytes kill the cells to which they bind.

(*) = Common theme throughout the course

(**) = More depth and common theme

Viral Activation of Immune Responses

The body has two immune systems: the innate immune system and the adaptive immune system.

1. **Innate immunity** is an antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to almost any microbe. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.

2. **Adaptive (acquired) immunity** refers to antigen-specific defense mechanisms that take several days to become protective and are designed to react with and remove a specific antigen. This is the immunity one develops throughout life.

Viral Activation of Innate Immunity

In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by **recognizing molecules unique to microorganisms that are not associated with human cells**. These unique molecules are called pathogen-associated molecular patterns or PAMPs. For example, most viral genomes contain a high frequency of unmethylated cytosine-guanine dinucleotide sequences (a cytosine lacking a methyl or CH_3 group and located adjacent to a guanine). Mammalian DNA has a low frequency of cytosine-guanine dinucleotides and most are methylated. In addition, most viruses produce unique double-stranded viral RNA, and some viruses produce uracil-rich single-stranded viral RNA during portions of their life cycle. These forms of viral nucleic acids are common PAMPs associated with viruses. These PAMPs bind to pattern-recognition receptors or PRRs called toll-like receptors or TLRs found within the endosomes of phagocytic cells. Viral RNA can also bind to cytoplasmic PRRs called RIG-1 (retinoic acid-inducible gene-1)and MDR-5 (melanoma differentiation-associated gene-5).

Most of the PRRs that bind to viral components trigger the synthesis of cytokines called Type-I interferons that block viral replication within infected host cells. The TLRs for viral components are found in the membranes of the phagosomes used to degrade viruses during phagocytosis. As viruses are engulfed by phagocytes, the viral PAMPS bind to TLRs located within the phagolysosomes (endosomes). The TLRs for viral components include:

1. TLR-3 binds double-stranded viral RNA;
2. TLR-7 binds uracil-rich single-stranded viral RNA such as in HIV;
3. TLR-8 binds single-stranded viral RNA;
4. TLR-9 binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes.
5. RIG-1 (retinoic acid-inducible gene-1) and MDA-5 (melanoma differentiation-associated gene-5), are cytoplasmic sensors that both viral double-stranded and single-stranded RNA molecules produced in viral-infected cells.

Animation showing toll-like receptors (TLRs) recognizing viral double-stranded RNA.
Copyright © Gary E. Kaiser
html5 version of animation showing toll-like receptors (TLRs) recognizing viral double-stranded RNA
In order to protect against infection, one of the things the

body must initially do is detect the presence of microorganisms. The body does this by recognizing molecules unique to microorganisms that are not associated with human cells. These unique molecules are called pathogen-associated molecular patterns or PAMPS and they bind to pattern recognition receptors called toll-like receptors (TLRs) found on host defense cells. For example, most viral genomes contain a high frequency of unmethylated cytosine-guanine dinucleotide sequences (a cytosine lacking a methyl or CH_3 group and located adjacent to a guanine). Mammalian DNA has a low frequency of cytosine-guanine dinucleotides and most are methylated. In addition, most viruses produce unique double-stranded viral RNA, and some viruses produce uracil-rich single-stranded viral RNA during portions of their life cycle. The binding of these unique viral molecules bind to the endodsomal TLRs of defense cells such as macrophages and dendritic cells triggers the production of antiviral cytokines called type I interferons that are able to block viral replication.

a. TLR-3 - binds double-stranded viral RNA;
b. TLR-7 - binds single-stranded viral RNA, such as in HIV, rich in guanine/uracil nucleotide pairs;
c. TLR-8 - binds single-stranded viral RNA;
d. TLR-9 - binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes but uncommon or masked in human DNA and RNA.

Interferons induce uninfected cells to produce enzymes capable of degrading mRNA. These enzymes remain inactive until the uninfected cell becomes infected with a virus. At this point, the enzymes are activated and begin to degrade both viral and cellular mRNA. This not only blocks viral protein synthesis, it also eventually kills the infected cell.

For more information: Preview of Pathogen-Associated Molecular Patterns (PAMPs)

For more information: Preview of Pattern-Recognition Receptors (PRRs)

Viral Activation of Adaptive Immunity

Proteins and glycoproteins associated with the viral envelope and/or the viral capsid function as antigens and initiate adaptive immunity. An **antigen** is defined as a molecular shape that reacts with antibody molecules and with antigen receptors on

lymphocytes. We recognize those molecular shapes as foreign or different from our body's molecular shapes because they fit specific antigen receptors on our **B-lymphocytes** and **T-lymphocytes**, the cells that carry out adaptive immunity.

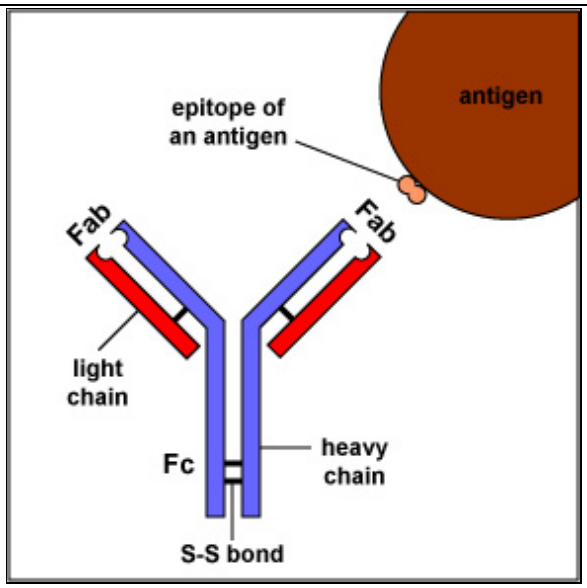
The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called **epitopes**. An epitope is typically a group of 5-15 amino acids with a unique shape that makes up a portion of a protein antigen (see Fig. 6A) , or 3-4 sugar residues branching off of a polysaccharide antigen (see Fig. 6B). A single microorganism has many hundreds of different shaped epitopes that our lymphocytes can recognize as foreign and mount an adaptive immune response against.The body recognizes an antigen as foreign when epitopes of that antigen bind to **B-lymphocytes** and **T-lymphocytes** by means of epitope-specific receptor molecules having a shape complementary to that of the epitope. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. **Humoral immunity**: Humoral immunity involves the production of **antibody** molecules in response to an antigen and is mediated by B-lymphocytes. Through a variety of mechanisms, these antibodies are able to remove or neutralize microorganisms and their toxins after binding to their epitopes. For example, antibodies made against viral surface antigens can prevent viruses from adsorbing to host cell receptors thus blocking viral replication or function as opsonizing antibodies to attach viruses to phagocytes for enhanced attachment.

   As will be seen in Unit 6, one of the major defenses against free viruses is the immune defenses' **production of antibody molecules against the virus**. The "tips" of the antibody (the Fab portion; **see Fig. 1**) have shapes that have a complementary shape to portions of viral attachment proteins and glycoproteins called epitopes found on the viral surface. When antibodies react with these attachment proteins, they **block viral adsorption to host cell receptors** and, therefore, block viral replication.

Fig. 1: Structure of the Antibody IgG



The Fab portion of the antibody has specificity for binding an epitope of an antigen. The Fc portion directs the biological activity of the antibody. In the case of IgG, the Fc portion can bind to phagocytes for enhanced attachment (opsonization) as well as activate the classical complement pathway. Antibodies are composed of 4 protein chains: 2 identical heavy chains and 2 identical light chains. Disulfide (S-S) bonds join

the protein chains together.

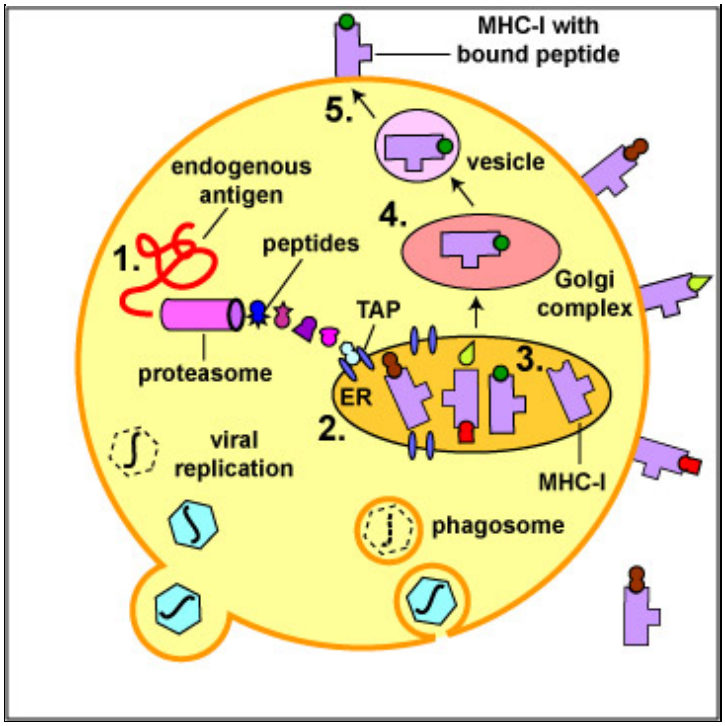
Flash animation showing neutralization of viruses by antibodies.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing neutralization of viruses by antibodies.
The Fab portion of the antibodies made against epitopes of the virus attachment site blocks the virus from adsorbing to the receptor site on the host cell membrane. As a result, the virus can not penetrate and replicate.

In addition, Antibodies such as IgG function as opsonins and **stick viruses to phagocytes**.

Flash animation showing opsonization of viruses by antibodies.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing opsonization of viruses by antibodies.
The Fab portion of IgG binds to epitopes of the viral surface. The Fc portion can now attach the virus to Fc receptors on phagocytes for enhanced attachment. Once attached to the phagocyte by way of IgG, the virus can be engulfed more efficiently, placed in a phagosome, and destroyed by lysosomes. C3b and C4b from the activated complement pathways are also able to attach viruses to phagocytes.

2. **Cell-mediated immunity**: Cell-mediated immunity involves the production of **cytotoxic T-lymphocytes**, activated **macrophages**, activated **NK cells**, and **cytokines** in response to an antigen and is mediated by T-lymphocytes. These defense cells help to remove infected cells and cancer cells displaying foreign epitopes. Virus-infected host cells naturally **bind viral epitopes to a host molecule called** MHC-I **and place the MHC-1 with bound viral epitope on the surface of the infected cell (see Fig. 2) where they can be recognized by CTLs having a T-cell receptors on its surface with a complementary shape**.

Fig. 2: Binding of Peptide Epitopes from Viruses to MHC-I Molecules

Endogenous antigens are those being produced within cells of the body. Examples include:

a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

The body marks infected cells and tumor cells for destruction by placing peptide epitopes from these endogenous antigens on their surface by way of MHC-I molecules. Cytotoxic T-lymphocytes (CTLs) are then able to recognize peptide/MHC-I complexes by means of their T-cell receptors (TCRs) and CD8 molecules and kill the cells to which they bind.

1. Endogenous antigens, such as viral proteins, pass through proteasomes where they are degraded into a series of peptides.
2. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
3. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
4. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by CTLs by way of TCRs and CD8 molecules having a complementary shape.

In this way the CTL can kill the infected cell by apoptosis, a programmed cell suicide.

Flash animation of a CTL triggering apoptosis by way of perforins and granzymes.

html5 version of a CTL triggering apoptosis by way of perforins and granzymes.

Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. (Alternately, the granzymes and perforins may enter by endocytosis and the perforins then promote the release of the granzymes from the endocytic vesicle into the cytoplasm.)

Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

Flash animation showing CTL-induced apoptosis of a virus-infected cell.

html5 version of animation for iPad showing CTL-induced apoptosis of a virus-infected cell.

Killing of the infected cell or tumor cell by apoptosis involves a variety of mechanisms:

- Certain granzymes can activate the caspase enzymes that lead to apoptosis of the infected cell. The caspases are proteases that destroy the protein structural scaffolding of the cell - the cytoskeleton - and degrade both the target cell's nucleoprotein and microbial DNA within the cell.
- Granzymes cleave a variety of other cellular substrates that contribute to cell death.
- The perforin molecules may also polymerize and form pores in the membrane of the infected cell, similar to those produced by MAC. This can increase the permeability of the infected cell and contribute to cell death. If enough perforin pores form, the cell might not be able to exclude ions and water and may undergo cytolysis. A granule called granulysin can also alter the

permeability of both miocrobial and host cell membranes.

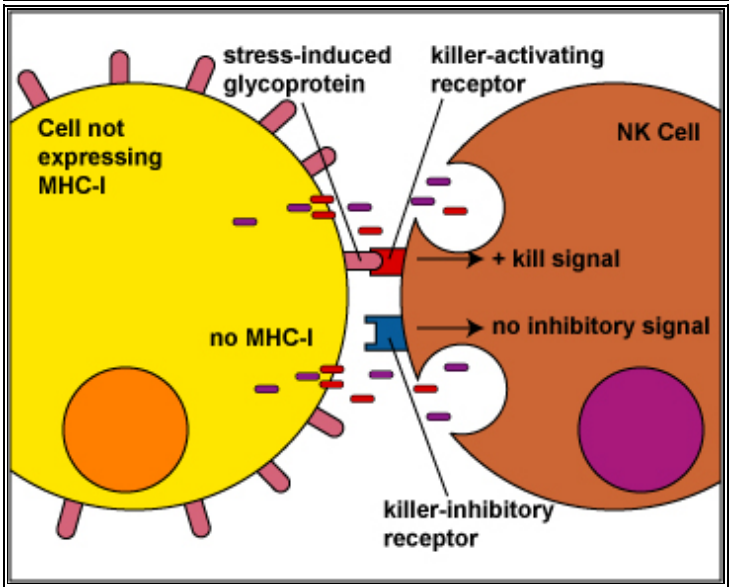
This animations shows destruction of both the cytoskeleton and nucleoprotein of the infected cell. As the infected cell breaks up into apoptotic fragments, the fragments are subsequently removed by phagocytes. This reduces inflammation and also prevents the release of viruses that have assembled within the infected cell and their spread into uninfected cells.

Animation illustrating the MHC-I system marking an infected cell for destruction and its subsequent killing by CTLs.

Courtesy of HHMI's Biointeractive.

Another defense cell that is able to kill virus-infected cells is the NK cell. NK cells **recognize infected cells displaying stressed-induced proteins and not displaying MHC-I molecules on their surface and kill these cells (see Fig. 3)**.

Fig. 3: NK Cell Interacting with a Virus-Infected Cell or a Mutant Cell Not Expressing MHC-I Molecules



Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the

killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell kills the cell to which it has bound.

MHC-I molecules are the molecules on host cells that display viral epitopes to cytotoxic T-lymphocytes (CTLs). Some viruses suppress the production of MHC molecules by host cells, preventing CTLs from recognizing the infected cell as foreign and killing it. NK cells, however, can recognize cells not displaying MHC-I and kill them anyway.

Flash animation showing a NK cell interacting with a normal body cell.

html5 version of animation for iPad showing a NK cell interacting with a normal body cell.

NK cells appear to use a duel receptor system in determining whether to kill or not kill human cells. When cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules are produced and are put on the surface of that cell. The first NK cell receptor, called the killer-activating receptor, recognizes these stress-induced molecules. This interaction sends a positive signal which enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal. This second receptor, called the killer-inhibitory receptor, recognizes MHC-I molecules that are also usually present on all nucleated human cells. If MHC-I molecules are expressed on the cell, the killer-inhibitory receptor sends a negative signal that overrides the kill signal and prevents the NK cell from killing that cell.

Flash animation showing a NK cell interacting with a virus-infected cell or tumor cell not expressing MHC-I molecules.

html5 version of animation for iPad showing a NK cell interacting with a virus-infected cell or tumor cell not expressing MHC-I molecules.

Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

Innate immunity will be discussed in greater detail in Unit 5; adaptive immunity will be discussed in greater detail in Unit 6.

Self Quiz for Viral Activation of Immune Responses

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Viroids and prions
OTHER ACELLULAR INFECTIOUS AGENTS: VIROIDS AND PRIONS

Viruses

Other Acellular Infectious Agents: Viroids and Prions

Fundamental Statement for this Softchalk Lesson:

1. Viroids are small, circular, single-stranded molecules of infectious RNA that cause several plant diseases.
2. Prions are infectious protein particles responsible for a group of transmissible and/or inherited neurodegenerative diseases as a result of prion protein misfolding.
3. Diseases including Creutzfeldt-Jakob disease Gerstmann-Straussler-syndrome, and mad cow disease.
4. There is growing evidence that other probable protein misfolding diseases initiated by prions include Alzheimer's disease, Hunington's disease, Parkinson's disease, amyotrophic lateral sclerosis, and certain cancers.

Common Course Objectives

1. Recall characteristics that are present only in viruses and not in other cellular pathogens.

Detailed Learning Objectives

1. Define viroid and name an infection caused by a viroid.

2. Define prion and name 3 protein misfolding diseases that appear to be initiated by prions.

Other Acellular Infectious Agents: Viroids and Prions

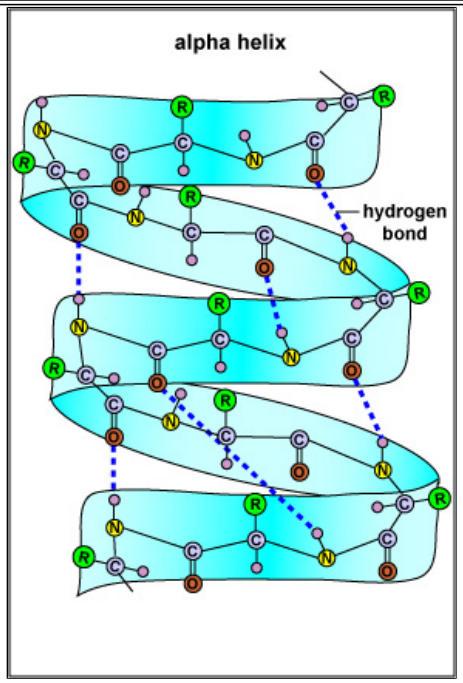
Viroids are even more simple than viruses. They are small, circular, single-stranded molecules of **infectious RNA** lacking even a protein coat. They are the cause of a few plant diseases such as potato spindle-tuber disease,cucumber pale fruit, citrus exocortis disease, and cadang-cadang (coconuts).

Prions are **infectious protein particles** responsible for a group of **transmissible and/or inherited neurodegenerative diseases** including Creutzfeldt-Jakob disease, kuru, and Gerstmann-Straussler- syndrome in humans, as well as scrapie in sheep and goats, and bovine spongiform encephalopathy (mad cow disease) in cattle and in humans (where it is called new variant Creutzfeldt–Jakob disease humans). The infections are often referred to as transmissible spongiform encephalopathies.

Most evidence indicates that the infectious prion proteins are modified (misfolded) forms of normal proteins coded for by a host gene in the brain. It is thought that the normal prion protein, expressed on stem cells in the bone marrow and on cells that will become neurons, plays a role in the maturation of neurons. In the case of the disease scrapie, the normal prion protein in an animal without the disease has alpha-helices in the proteins secondary structure **(see Fig 1)** while the scrapie prion protein in diseased animals has beta-sheets for the secondary structure **(see Fig. 2)**. When the scrapie prion protein contacts the normal protein it causes it to change its configuration to the scrapie beta-sheet form. This suggests that the conversion of a normal prion protein into an infectious prion protein may be catalyzed by the prion protein itself upon entering the brain. Inherited forms may be a result of point mutations that make the prion protein more susceptible to a change in its protein structure.

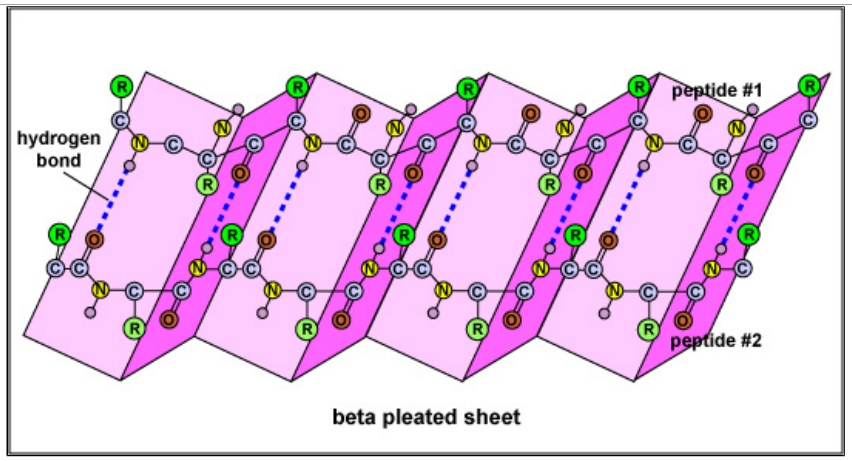
There is growing evidence that **other probable protein misfolding diseases initiated by prions** include Alzheimer's disease, Hunington's disease, Parkinson's disease, frontotemporal dementias, amyotrophic lateral sclerosis, and certain cancers.

Fig. 1: Secondary Structure of a Protein or Polypeptide: Alpha Helix



The secondary structure of a protein or polypeptide is due to hydrogen bonds forming between an oxygen atom of one amino acid and a nitrogen atom of another. There are two possible types of secondary structure: an alpha helix and a beta sheet. In the case of an alpha helix, the hydrogen bonding causes the polypeptide to twist into a helix. With a beta sheet the hydrogen bonding enables the polypeptide to fold back and forth upon itself like a pleated sheet.

Fig. 2: Secondary Structure of a Protein or Polypeptide: Beta Sheet



The secondary structure of a protein or polypeptide is due to hydrogen bonds forming between an oxygen atom of one amino acid and a nitrogen atom of another. There are two possible types of secondary structure: an alpha helix and a beta sheet. In the case of an alpha helix, the hydrogen bonding causes the polypeptide to twist into a helix. With a beta sheet the hydrogen bonding enables the polypeptide to fold back and forth upon itself like a pleated sheet.

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- Prion-Related Diseases
- Kuru
- Variant Creutzfeldt-Jakob Disease and Bovine Spongiform Encephalopathy

Quiz Group

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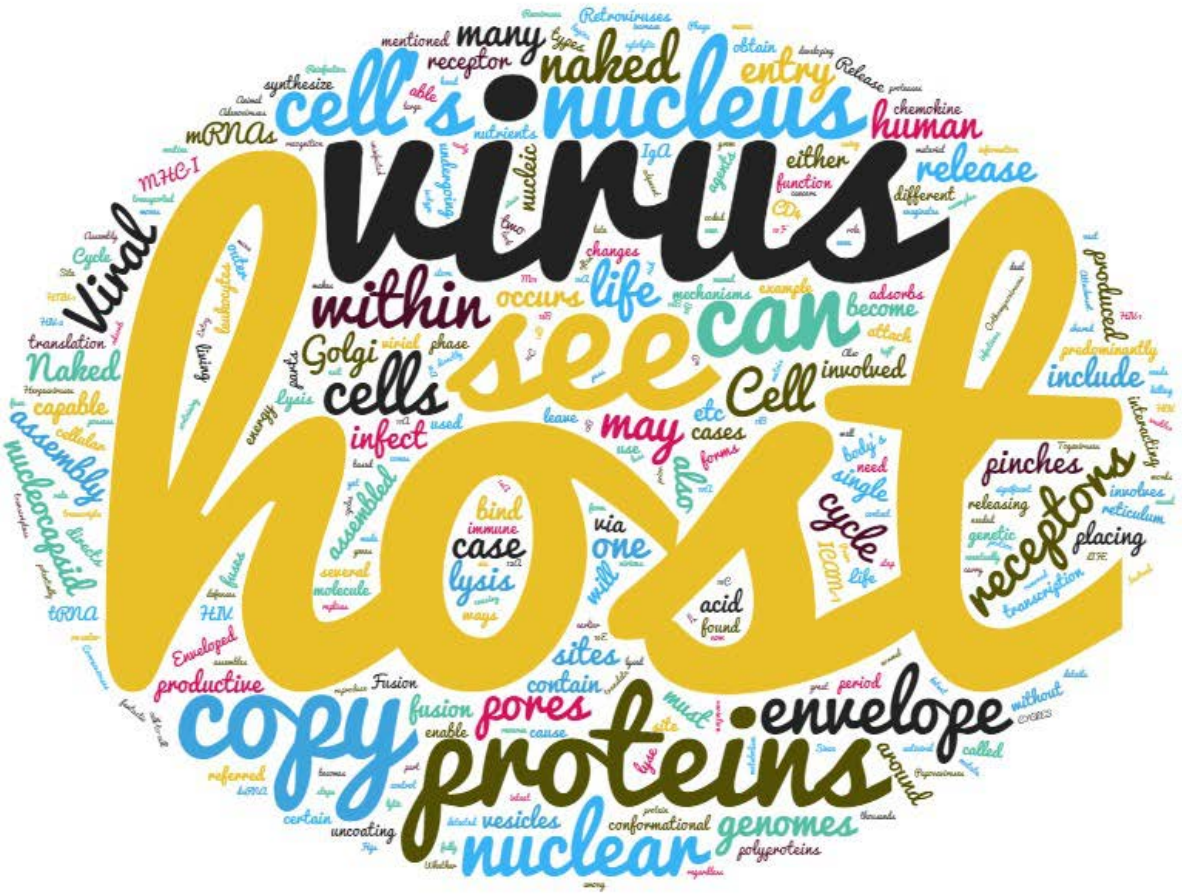
The productive (lytic) life cycle of animal viruses
THE PRODUCTIVE (LYTIC) LIFE CYCLE OF ANIMAL VIRUSES

Viruses

The Productive (Lytic) Life Cycle of Animal Viruses



Fundamental Statement for this Softchalk Lesson:

1. For a virus to infect a host cell, that cell must have receptors for the virus on its surface and also be capable of supporting viral replication.
2. Adsorption involves the binding of attachment sites on the viral surface with receptor sites on the host cell cytoplasmic membrane.
3. Once adsorbed, many viruses enter the host cell by endocytosis, whereby the host cell cytoplasmic membrane invaginates and pinches off, placing the virus in an endocytic vesicle. Some viruses enter by a fusion process whereby part of the virus fuses with the host cell enabling the remainder of the virus to enter the host cell's cytoplasm.
4. Following entry, the virus moves to the site of replication within the host cell. Most RNA viruses replicate in the host cell's cytoplasm; most DNA viruses replicate in the host cell's nucleus.
5. During replication, the viral genome directs the host cell's metabolic machinery (ribosomes, tRNA, nutrients, energy, enzymes, etc.) to synthesize viral enzymes and viral parts. The viral genome has to both replicate itself and become transcribed into viral mRNA molecules. The viral mRNA can then be transcribed by the host cell into viral structural components and enzymes need for replication and assembly of the virus.
6. During maturation, the capsid is assembled around the viral genome.
7. Prior to or during release, enveloped viruses obtain their envelopes from host cell membranes by budding. Budding occurs either at the outer cytoplasmic membrane, the nuclear membrane, or at the membranes of the Golgi apparatus.
8. Viruses obtaining their envelopes from the membranes of the nucleus, the endoplasmic reticulum, or the Golgi apparatus are then released by exocytosis via transport vesicles; viruses obtaining their envelope from the cytoplasmic membrane are released during the budding process.
9. Naked viruses are predominantly released by host cell lysis.
10.As many as 10,000 to 50,000 animal viruses may be produced by a single infected host cell.

Common Course Objectives

1. Recall characteristics that are present only in viruses and not in other cellular pathogens.
2. Describe what an animal virus consists of structurally and state the function of those viral parts.
3. Describe the stages of the productive (lytic) life cycle by a generalized animal virus.
4. Explain why viruses are more restricted in their host and cell type.

Detailed Learning Objectives

1**. When given information about a virus in terms of how it penetrates the host cell, whether it has a DNA or RNA genome, and how it is released, describe how an enveloped virus accomplishes each of the steps of the productive life cycle listed below. (Tailor the life cycle to that virus.)

A. viral attachment or adsorption to the host cell

B. viral entry into the host cell

C. viral movement to the site of replication within the host cell

D. viral replication within the host cell

E. viral assembly or maturation within the host cell

F. viral release from the host cell

2**. When given information about a virus in terms of how it penetrates the host cell, whether it has a DNA or RNA genome, and how it is released, describe how a naked virus accomplishes each of the steps of the productive life cycle listed below. (Tailor the life cycle to that virus.)

A. viral attachment or adsorption to the host cell

B. viral entry into the host cell

C. viral movement to the site of replication within the host cell

D. viral replication within the host cell

E. viral assembly or maturation within the host cell

F. viral release from the host cell

(*) = Common theme throughout the course

(**) = More depth and common theme

TPS Questions

The Productive (Lytic) Life Cycle of Animal Viruses

Viruses that infect animal cells replicate by what is called the **productive life cycle**. The productive life cycle is also often referred to as the **lytic life cycle**, even though not all viruses cause lysis of their host cell during their replication.Some viruses, such as HIV and the herpes viruses are able to become **latent** in certain cell types. A few viruses **increase the risk of certain cancers**.

We will now look at the life cycles of viruses that infect animal cells. For many animal viruses, the details of each step in their life cycle have not yet been fully characterized, and among the viruses that have been well studied there is great deal of variation. What follows is a generalized productive life cycle for animal viruses consisting of the following steps: adsorption, viral entry, viral movement to the site of replication and release of the viral genome from the remainder of the virus, viral replication, viral assembly, and viral release.

1. Viral Attachment or Adsorption to the Host Cell

Adsorption **(see Fig. 1A and Fig. 1B)** involves the **binding of attachment sites on the viral surface with receptor sites on the host cell cytoplasmic membrane**.

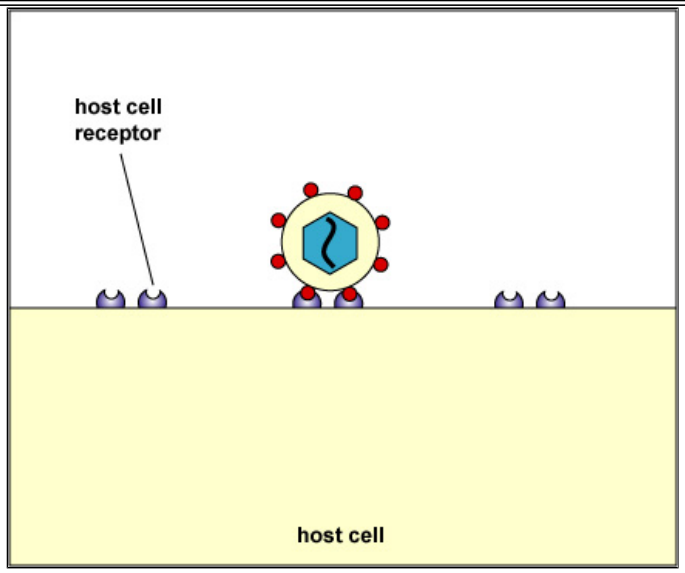
For a virus to infect a host cell, that cell must have receptors for the virus on its surface and also be capable of supporting viral replication. These host cell receptors are normal surface molecules involved in routine cellular function, but since a portion of a molecule on the viral surface resembles the chemical shape of the body's

molecule that would normally bind to the receptor, the virus is able to attach to the host cell's surface.

For example:

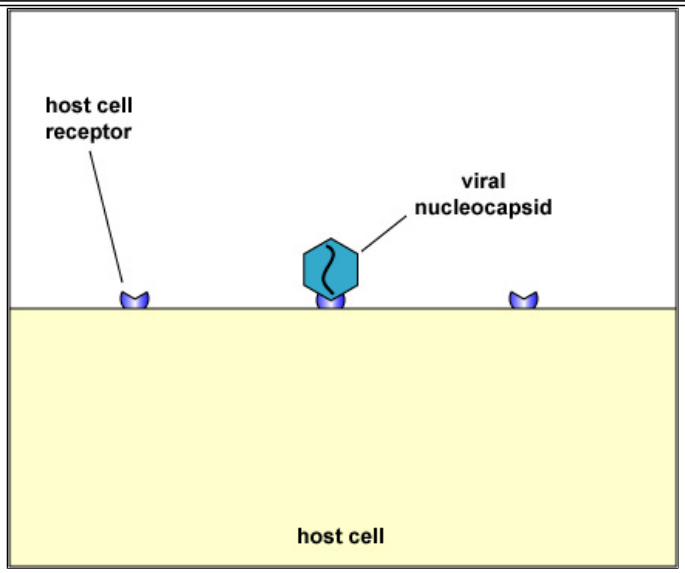
- Most human rhinoviruses that cause the common cold bind to intercellular adhesion molecules (ICAM-1) found on cells of the nasal epithelium. These ICAM-1 molecules are used normally for the recruitment of leukocytes into the respiratory tract.
- The human immunodeficiency viruses (HIV) adsorbs to first CD4 molecules and then chemokine receptors found on the surface of human T4-lymphocytes and macrophages. CD4 molecules are normally involved in immune recognition while chemokine receptors play a role in initiating inflammation and recruiting leukocytes.
- Human cytomegaloviruses (CMV) adsorb to MHC-I molecules. MHC-I molecules on human cells enable T8-lymphocytes to recognize antigens during adaptive immunity.
- The hepatitis B virus (HBV) adsorbs to IgA receptors on human cells. These receptors normally bind the antibody isotype IgA for transport across cells.

Fig. 1A: Adsorption of an Enveloped Virus to a Susceptible Host Cell



Attachment sites on the viral envelope bind to corresponding host cell receptors.

Fig. 1B: Adsorption of a Naked Virus to a Susceptible Host Cell



Attachment sites on the viral capsid bind to corresponding host cell receptors.

Flash animation showing adsorption of an enveloped virus.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing adsorption of an enveloped virus.
Adsorption involves the binding of attachment sites on the viral surface with receptor sites on the host cell cytoplasmic membrane.

Flash animation showing adsorption of a naked virus.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing adsorption of a naked virus.
Adsorption involves the binding of attachment sites on the viral surface with receptor sites on the host cell cytoplasmic membrane.

2. Viral Entry into the Host Cell

a. **Enveloped viruses**

Enveloped viruses enter the host cell in one of two ways:

1. In some cases, the **viral envelope may fuse with the host cell cytoplasmic membrane** and the **nucleocapsid is released into the cytoplasm (see Slideshow Figs. 2A, 2B and 2C).**

Slideshow Activity

Flash animation showing entry of an enveloped virus by envelope fusion.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing entry of an enveloped virus by envelope fusion.
In the case of some viruses, the viral envelope may fuse with the host cell cytoplasmic membrane and the nucleocapsid is released into the cytoplasm.

2. Usually they enter by **endocytosis**, whereby the host cell cytoplasmic membrane invaginates and pinches off, placing the virus in an endocytic vesicle **(see Slideshow Figs. 3A, 3B,3C, and 3D)**.

Slideshow Activity

Flash animation showing entry of an enveloped virus by endocytosis.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing entry of an enveloped virus by endocytosis.
Many enveloped viruses enter by endocytosis, whereby the host cell cytoplasmic membrane invaginates and pinches off, placing the virus in an endocytic vesicle.

Janet Iwasa, Gaël McGill (Digizyme) & Michael Astrachan (XVIVO).

b. **Naked viruses**

Naked viruses enter the cell in one of two ways:

1. In some cases, interaction between the viral capsid and the host cell cytoplasmic membrane causes a **rearrangement of capsid proteins allowing the viral nucleic acid to pass through the membrane** into the cytoplasm **(see Slideshow Figs. 4A, 4B, 4C, and 4D)**.

Slideshow Activity

Flash animation showing entry of a naked virus by capsid reconfiguration.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing entry of a naked virus by capsid reconfiguration.
In the case of naked viruses entering by capsid reconfiguration, interaction between the viral capsid and the host cell cytoplasmic membrane causes a rearrangement of capsid proteins allowing the viral nucleic acid to pass through the membrane into the cytoplasm.

2. Most naked viruses enter by **receptor-mediated endocytosis** whereby the host cell cytoplasmic membrane invaginates and pinches off, placing the virus in an endocytic vesicle **(see Slideshow Figs. 5A, 5B, 5C, and 5D)**.

Slideshow Activity

Flash animation showing penetration of a naked virus by endocytosis.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing penetration of a naked virus by endocytosis.
Most naked viruses enter by receptor-mediated endocytosis whereby the host cell cytoplasmic membrane invaginates and pinches off, placing the virus in an endocytic vesicle

3. Viral Movement to the Site of Replication within the Host Cell and Release of the Viral Genome from the Remainder of the Virus.

In the case of viruses that enter by endocytosis, the endocytic vesicles containing the virus move within the host cell. During this process the pH of the endocytic vesicle typically decreases and this enables the virus to leave the endocytic vesicle. Viruses exit the endocytic vesicle through a variety of mechanisms, including:

a. Fusion of the viral envelope with the membrane of the endocytic vesicle enabling the viral nucleocapsid to enter the cytoplasm of the host cell **(see Slideshow Figs. 7A, 7B, and 7C)**.

Slideshow Activity

Flash animation showing fusion of the viral envelope with the membrane of the endocytic vesicle.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing fusion of the viral envelope with the membrane of the endocytic vesicle.
In this example, once the virus enters the host cell by endocytosis, the viral envelope fuses with the endocytic vesicle. The nucleocapsid then enters the cytoplasm.

b. Lysis of the endocytic vesicle releasing the viral nucleocapsid into the cytoplasm of the host cell **(see Slideshow Figs. 7D , and 7E)**.

Slideshow Activity

Flash animation showing lysis of the endocytic vesicle.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing lysis of the endocytic vesicle.
In this example, once the virus enters the host cell by endocytosis, the endocytic vesicle is lysed and the nucleocapsid enters the cytoplasm.

c. The viral capsid undergoing conformational changes that forms pores in the endocytic vesicle enabling the virial genome to enter the cytoplasm of the host cell **(see Slideshow Figs. 8A, 8B, and 8C)**.

Slideshow Activity

Flash animation showing viral capsid undergoing conformational changes that forms pores in the endocytic vesicle and enable the virial genome to enter the cytoplasm.
Copyright © Gary E. Kaiser

html5 version of animation for iPad showing viral capsid undergoing conformational changes that forms pores in the endocytic vesicle and enable the virial genome to enter the cytoplasm.
In this example, the viral capsid undergoes conformational changes that forms pores in the endocytic vesicle enabling the virial genome to enter the cytoplasm of the host cell.

Before viruses can replicate within the infected host cell, the viral genome needs to released from the remainder of the virus. This process is sometimes referred to as uncoating.

In the case of most viruses with an RNA genome, the viral RNA genome is released from the capsid and enters the cytoplasm of the host cell **(see Slideshow Figs. 9A , and 9B)** where replication generally occurs.

Slideshow Activity

Flash animation showing release of the viral genome from the capsid (uncoating).
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing release of the viral genome from the capsid (uncoating).
In this example, the viral genome is released from the viral capsid and enters the cytoplasm of the host cell. This occurs in most viruses with an RNA genome.

In the case of most viruses with a **DNA genome**, the viral genome enters the nucleus of the host cell through one the mechanisms shown below. Most larger DNA viruses use either a or b to enter the nucleus. Method c is used by some very small DNA whose capsid is small enough to be carried through the nuclear pores.

a. The viral DNA genome is released from the capsid, enters the cytoplasm of the host cell, and subsequently enters the nucleus of the host cell through the pores in the nuclear membrane **(see Slideshow Figs. 10A and 10B)**.

Slideshow Activity

Flash animation showing a viral DNA genome entering the nucleus of the host cell through the pores in the nuclear membrane.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a viral DNA genome entering the nucleus of the host cell through the pores in the nuclear membrane.
In the case of most viruses with a DNA genome, the genome enters the nucleus of the host cell. In this example, the DNA genome is released from the capsid, enters the cytoplasm of the host cell, and subsequently enters the nucleus of the host cell through the pores in the nuclear membrane .

b. The capsid of the viruses interacts with the nuclear membrane of the host cell enabling the viral DNA genome to enter the nucleus of the host cell via the pores in the nuclear membrane **(see Slideshow Figs. 10C and 10D)**.

Slideshow Activity

Flash animation showing a viral capsid interacting with the nuclear membrane of the host cell enabling the viral DNA to enter the nucleus.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a viral capsid interacting with the nuclear membrane of the host cell enabling the viral DNA to enter the nucleus.
In the case of most viruses with a DNA genome, the genome enters the nucleus of the host cell. In this example, the capsid of the viruses interacts with the nuclear membrane of the host cell,l enabling the viral DNA to enter the nucleus of the host cell via the pores in the nuclear membrane.

c. The nucleocapsid of a small DNA virus enters the nucleus of the host cell and the capsid is subsequently removed releasing the viral DNA genome into the

nucleoplasm **(see Slideshow Figs. 10E and 10F)**.

Slideshow Activity

Flash animation showing a viral nucleocapsid entering the nuclear membrane of the host cell.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a viral nucleocapsid entering the nuclear membrane of the host cell.
In the case of most viruses with a DNA genome, the genome enters the nucleus of the host cell. In the case of some small DNA viruses whose nucleocapsid is small enough to be passed through the nuclear pores of the host cell's nuclear membrane, the entire nucleocapsid enters the nucleus of the host cell. The capsid is subsequently removed releasing the viral DNA genome into the nucleoplasm.

This uncoating begins the **eclipse period**, the period during which no intact virions can be detected within the cell. After uncoating and during the replication stage the virus is not infectious.

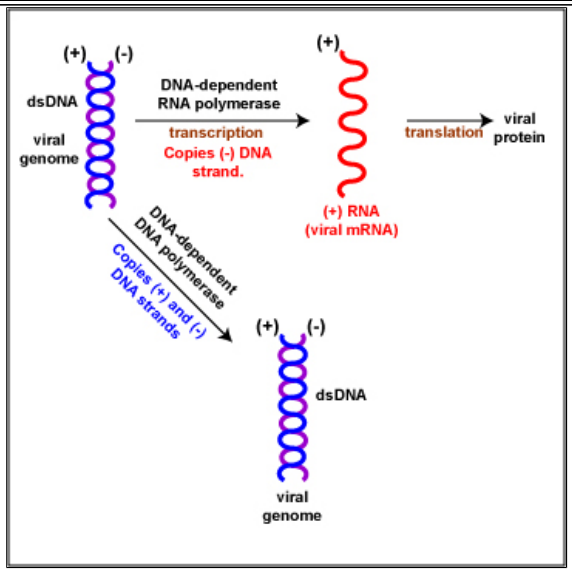
4. Viral Replication within the Host Cell

The viral genome **directs the host cell's metabolic machinery** (ribosomes, tRNA, nutrients, energy, enzymes, etc.) **to synthesize viral enzymes and viral parts**. **The viral genome has to both replicate itself and become transcribed into viral mRNA molecules**. The viral mRNA can then be translated by the host cell's ribosomes into viral structural components and enzymes need for replication and assembly of the virus.

As mentioned earlier under Viral Classification, viruses can store their genetic information in six different types of nucleic acid which are named based on how that nucleic acid eventually becomes transcribed to the viral mRNA:

a. **(+/-) double-stranded DNA (see Fig. 11A)**. To replicate the viral genome, DNA-dependent DNA polymerase enzymes (usually provided by the cell) copy both the (+) and (-) DNA strands producing dsDNA viral genomes. To produce viral mRNA molecules, host cell-DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes. Examples include most bacteriophages, Papovaviruses, Adenoviruses, and Herpesviruses.

Fig. 11A: Replication of a Double-Stranded DNA Viral Genome and production of Viral mRNA



Copyright © Gary E. Kaiser

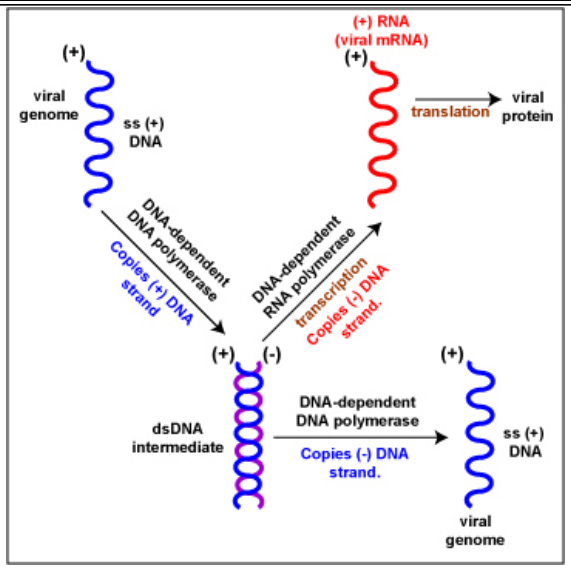
To replicate the viral genome, DNA-dependent DNA polymerase enzymes copy both the (+) and (-) DNA strands producing dsDNA viral genomes.

To produce viral mRNA molecules. DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes.

b. **(+) single-stranded DNA (see Fig. 11B)**. To replicate the viral genome, DNA-dependent DNA polymerase enzymes (usually provided by the cell) copy the (+) DNA strand of the genome producing a dsDNA intermediate. DNA-dependent DNA polymerase enzymes (again, usually provided by the cell) then copy the (-) DNA strand into ss (+) DNA genomes. To produce viral mRNA molecules, host cell-DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes. Examples include Phage M13 and Parvoviruses.

Fig. 11B: Replication of a Single-Stranded DNA Viral Genome and Production of

Viral mRNA



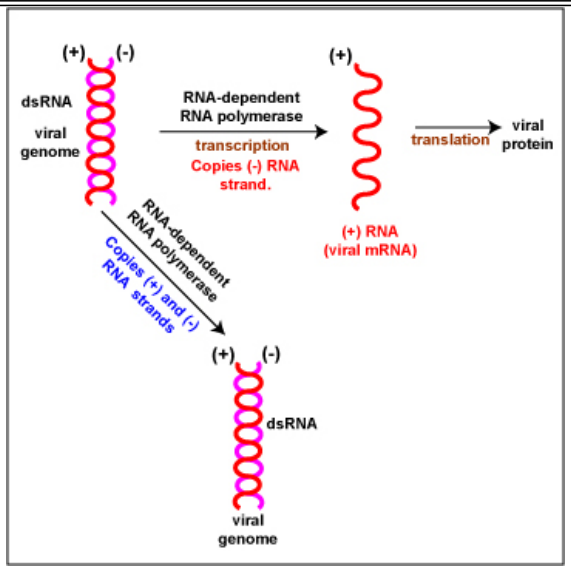
Copyright © Gary E. Kaiser

To replicate the viral genome, DNA-dependent DNA polymerase enzymes copy the (+) DNA strand of the genome producing a dsDNA intermediate. DNA-dependent DNA polymerase enzymes then copy the (-) DNA strand into ss (+) DNA genomes.

To produce viral mRNA molecules. DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes.

c. **(+/-) double-stranded RNA (see Fig. 11C)** . To replicate the viral genome, viral RNA-dependent RNA polymerase enzymes (replicase) copy both the (+) RNA and (-) RNA strands of the genome producing a dsRNA genomes. To produce viral mRNA molecules, viral RNA-dependent RNA polymerase enzymes (transcriptase) copy the (-) RNA strand into (+) viral mRNA. The (+) viral mRNA can then be transitable into viral proteins by host cell ribosomes. Reoviruses are an example.

Fig. 11C: Replication of a Double-Stranded RNA Viral Genome and Production of Viral mRNA



Copyright © Gary E. Kaiser

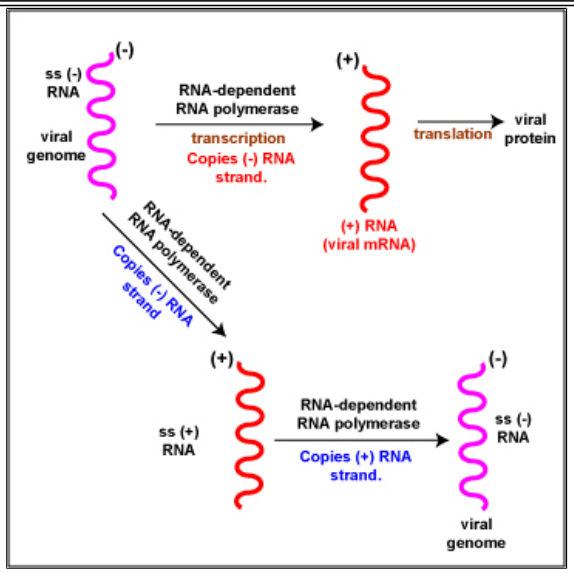
To replicate the viral genome, RNA-dependent RNA polymerase enzymes copy both the (+) RNA and (-) RNA strands of the genome producing a dsRNA genomes.

To produce viral mRNA molecules. RNA-dependent RNA polymerase enzymes copy the (-) RNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes.

d. **(-) RNA (see Fig. 11D)**. To replicate the viral genome, viral RNA-dependent RNA polymerase enzymes (transcriptase) copy the (-) RNA genome producing ss (+) RNA. Transcriptase must be carried into the cell with the virion. Viral RNA-dependent RNA polymerase enzymes (replicase) then copy the (+) RNA strands producing ss (-) RNA viral genome. The (+) mRNA strands also function as viral mRNA and can then be translated into viral proteins by host cell ribosomes.

Examples include Orthomyxoviruses, Paramyxoviruses, Rhabdoviruses.

Fig. 11D: Replication of a Single-Stranded Minus RNA Viral Genome and Production of Viral mRNA

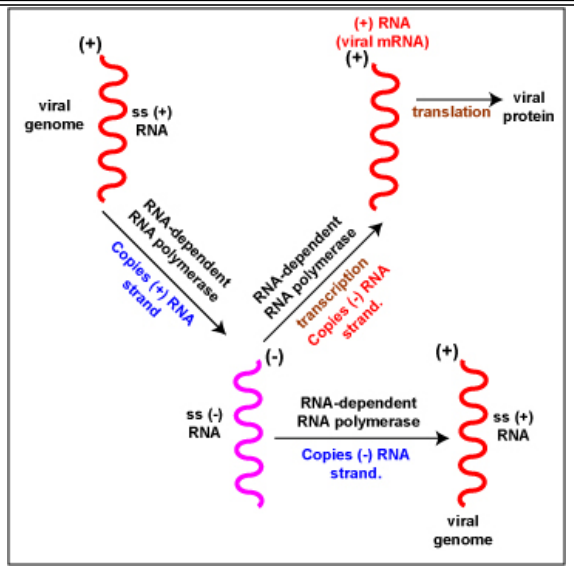


To replicate the viral genome, RNA-dependent RNA polymerase enzymes copy the (-) RNA genome producing ss (+) RNA. RNA-dependent RNA polymerase enzymes then copy the (+) RNA strands producing ss (-) RNA viral genome.

The (+) mRNA strands also function as viral mRNA and can then be translated into viral proteins by host cell ribosomes.

e. **(+) RNA (see Fig. 11E)**. To replicate the viral genome, viral RNA-dependent RNA polymerase enzymes (replicase) copy the (+) RNA genome producing ss (-) RNA. Viral RNA-dependent RNA polymerase enzymes (replicase) then copy the (-) RNA strands producing ss (+) RNA viral genome. To produce viral mRNA molecules. RNA-dependent RNA polymerase enzymes (replicase) copy the (-) RNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes. Examples include Picornaviruses, Togaviruses, and Coronaviruses.

Fig. 11E: Replication of a Single-Stranded Plus RNA Viral Genome and Production of Viral mRNA

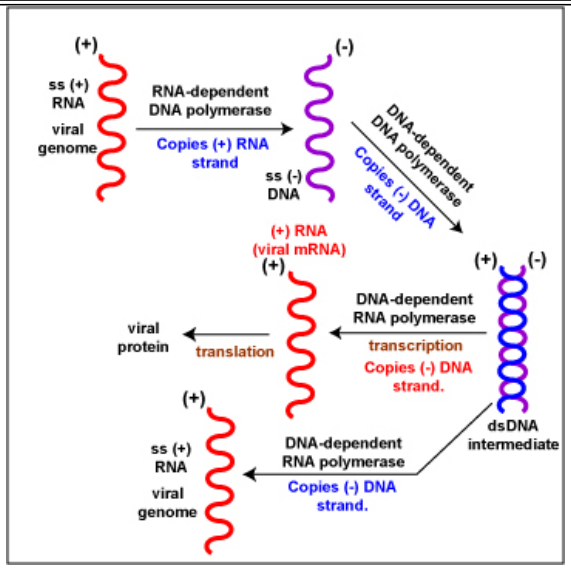


To replicate the viral genome, RNA-dependent RNA polymerase enzymes copy the (+) RNA genome producing ss (-) RNA. RNA-dependent RNA polymerase enzymes then copy the (-) RNA strands producing ss (+) RNA viral genome.

To produce viral mRNA molecules. RNA-dependent RNA polymerase enzymes copy the (-) RNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes.

f. (+) RNA Retroviruses (see Fig. 11F). To replicate the viral genome, viral reverse transcriptase enzymes (RNA-dependent DNA polymerases) copy the (+) RNA genome producing ss (-) DNA strands. Viral reverse transcriptase can also function as a DNA-dependent DNA polymerase enzymes and will copy the (-) DNA strands to produce a dsDNA intermediate. Reverse transcriptase must be carried into the cell with the viron. The viral DNA will move to the nucleus where it integrates into the cell's DNA using the viral enzyme integrase which also must be carried into the host cell with the virion. Once in the host cell's DNA, host cell DNA-dependent RNA polymerase enzymes then copy the ds (-) DNA strands to produce ss (+) RNA genomes. To produce viral mRNA molecules, host cell DNA-dependent RNA polymerase enzymes copy the ds (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes. Retroviruses, such as HIV-1, HIV-2, and HTLV-1 are examples.

Fig. 11F: Replication of a Single-Stranded Plus RNA Viral Genome and Production of Viral mRNA by way of Reverse Transcriptase



To replicate the viral genome, reverse transcriptase enzymes (RNA-dependent DNA polymerases) copy the (+) RNA genome producing ss (-) DNA strands. DNA-dependent DNA polymerase enzymes then copy the (-) DNA strands to produce a dsDNA intermediate. DNA-dependent RNA polymerase enzymes then copy the (-) DNA strands to produce ss (+) RNA genomes.

To produce viral mRNA molecules. DNA-dependent RNA polymerase enzymes copy the (-) DNA strand into (+) viral mRNA. The (+) viral mRNA can then be translated into viral proteins by host cell ribosomes.

As the host cell's ribosomes attach to the viral mRNA molecules, **the mRNAs are translated into viral structural proteins and viral enzymes**. During the early phase of replication, proteins needed for the replication of the viral genome are made and the genome makes thousands of replicas of itself. During the late phase of replication, viral structural proteins (capsid and matrix proteins, envelope glycoproteins, etc.) and the enzymes involved in maturation are produced.

Some viral mRNAs are monocistronic, that is, they contain genetic material to translate only a single protein or polypeptide. Other viral mRNAs are polycistronic. They contain transcripts of several genes and are translated into one or more large polyproteins. These polyproteins are subsequently cut into individual functional proteins by viral enzymes called proteases.

In the case of most RNA viruses, replication and assembly occurs in the host cell's cytoplasm. With DNA viruses, most replication and assembly occurs in the nucleus of the host cell. The viral genome enters the nucleus of the host cell and here is transcribed into viral mRNA. The viral mRNA molecules then leave the nucleus through the pores in the nuclear membrane and are translated into viral proteins by the host cell's ribosomes in the cytoplasm. Most of these viral proteins then re-enter the nucleus where the virus assembles around the replicated genomes.

Transmission electron micrograph of Herpes simplex viruses in the nucleus of an infected host cell; courtesy of CDC.

Also during replication, viral envelope proteins and glycoproteins coded by the viral genome are incorporated into the host cell's cytoplasmic membrane **(see Slideshow Figs. 12A and 12B)** or nuclear membrane.

Slideshow Activity

Flash animation showing viral replication.

html5 version of animation for iPad showing showing viral replication.

The viral genome directs the host cell's metabolic machinery (ribosomes, tRNA, nutrients, energy, enzymes, etc.) to synthesize viral enzymes and viral parts. The viral genome is transcribed into viral mRNA that goes to the host cell's ribosomes where it is translated into viral structural proteins and viral enzymes. During the early phase of replication, the viral genome replicates thousands of times. During the late phase of replication, viral structural proteins (capsid and matrix proteins, envelope glycoproteins, etc.) and the enzymes involved in maturation are produced. Also during this time, envelope glycoproteins coded by the viral genome of enveloped viruses are incorporated into the host cell's membranes.

In the case of most RNA viruses, replication and assembly occurs in the host cell's cytoplasm. With DNA viruses, the viral genome enters the nucleus of the host cell and here is transcribed into viral mRNA. The viral mRNA molecules then leave the nucleus through the pores in the nuclear membrane and are translated into viral proteins by the host cell's ribosomes in the cytoplasm. Most of these viral proteins then re-enter the nucleus where the virus assembles around the replicated genomes.

For more information: Review of transcription

For more information: Review of translation

Whether a virus has an RNA or a DNA genome is significant when it comes to developing antiviral agents to control viruses. In the case of RNA viruses, all of the enzymes used in genome replication and transcription are viral encoded enzymes different from those of the host cell so these enzymes can potentially be targeted. On the other hand, DNA viruses use the host cell's RNA transcription machinery and DNA replication machinery so these enzymes, shared by the virus and the host cell, cannot be targeted without killing the host cell. Since all viruses use the host cell's translation machinery regardless of genome type, translation can not be targeted in any viruses.

For more information: Preview of antiviral agents

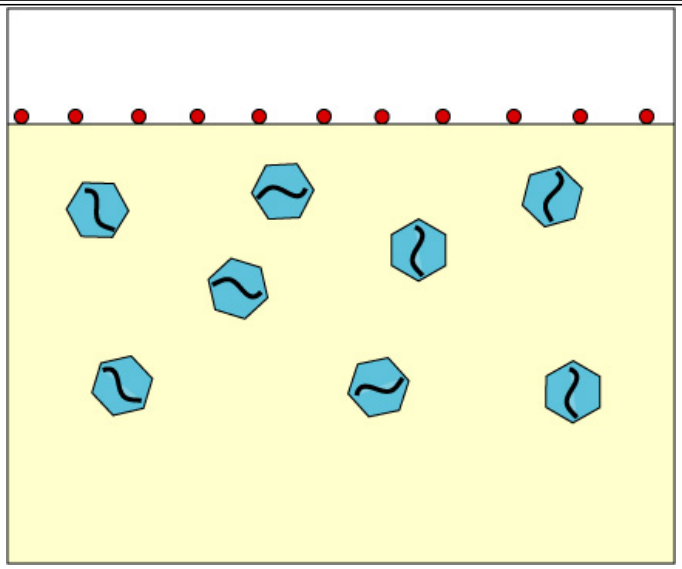
5. Viral Assembly or Maturation within the Host Cell

During maturation , the **capsid is assembled around the viral genome**.

Maturation of an enveloped virus: **see Fig. 13A**.

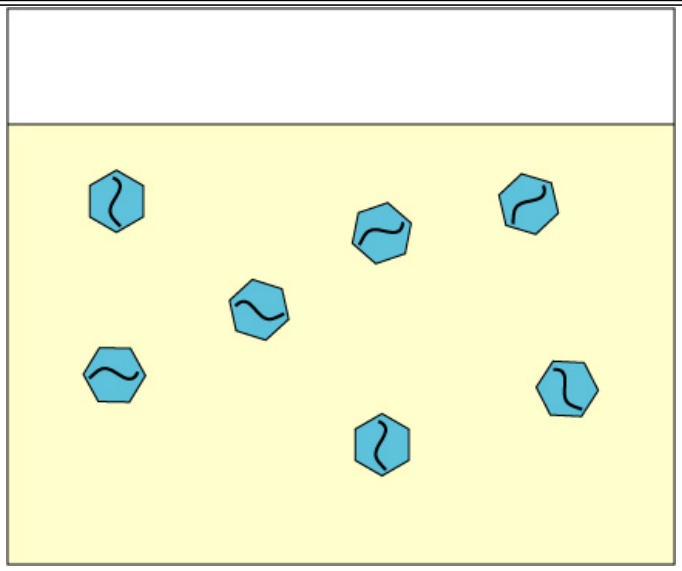
Maturation of a naked virus: **see Fig. 13B**.

Fig. 13A: Maturation of an Enveloped Virus



During maturation, envelope glycoproteins are transported to the cytoplasmic membrane of the host cell and the capsid assembles around the viral genome.

Fig. 13B: Maturation of a Naked Virus



During maturation, the capsid assembles around the viral genome.

Flash animation showing maturation of an enveloped virus that will be released by budding.

html5 version of animation for iPad showing maturation of an enveloped virus that will be released by budding.

During maturation, the capsid is assembled around the viral genome. In addition, viral-coded envelope glycoproteins are inserted in the host cell's membranes by the Golgi apparatus. The viral envelope will be added later as the virus buds from the cytoplasmic membrane of the host cell during the release stage.

Flash animation showing maturation of an enveloped virus that will be released by exocytosis.

html5 version of animation for iPad showing maturation of an enveloped virus that will be released by exocytosis.

During maturation, the capsid is assembled around the viral genome. During this time, viral coded envelope glycoproteins are inserted in the host cell's membranes by the Golgi apparatus. Viruses that will be released by exocytosis obtain their envelopes by budding from the nuclear membrane, the endoplasmic reticulum, and/or from the Golgi complex. These enveloped viruses are then packaged in exocytic vesicles for release from the host cell by exocytosis.

Flash animation showing maturation of a naked virus.

html5 version of animation for iPad showing maturation of a naked virus.

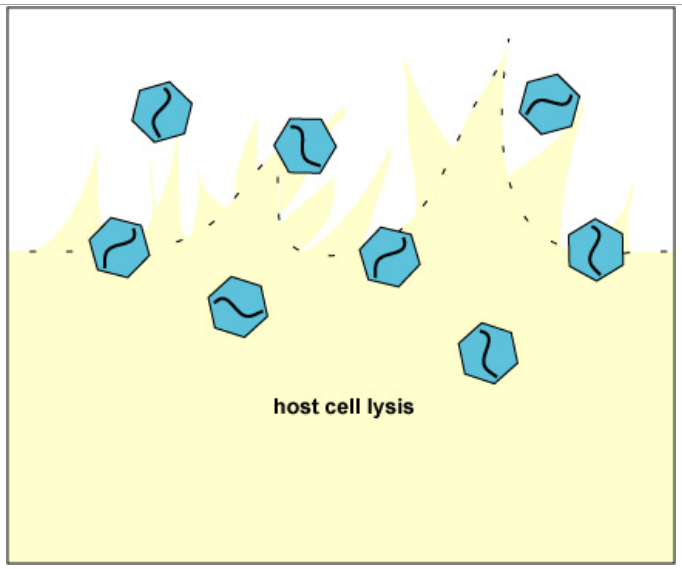
During maturation, the capsids assemble around the viral genomes.

6. Viral Release from the Host Cell

a. Naked viruses

Naked viruses are predominantly released by host cell lysis **(see Fig. 14)**. While some viruses are cytolytic and lyse the host cell more or less directly, in many cases it is the body's immune defenses that lyse the infected cell.

Fig. 14: Release of Naked Virus by Host Cell Disintegration.



The host cell membrane disintegrates and the virus is released.

Flash animation showing release of a naked virus by cell lysis.
html5 version of animation for iPad showing release of a naked virus by cell lysis.
Naked viruses are predominantly released by host cell lysis. While some viruses are cytolytic and lyse the host cell more or less directly, in many cases it is the body's immune defenses that lyse the infected cell.

b. Enveloped viruses

With **enveloped viruses**, the host cell may or may not be lysed. The viruses **obtain their envelopes from host cell membranes by budding**. As mentioned above, prior to budding, viral proteins and glycoproteins are incorporated into the host cell's membranes. During budding the host cell membrane with incorporated viral proteins and glycoproteins evaginates and pinches off to form the viral envelope. Budding occurs either at the outer cytoplasmic membrane, the nuclear membrane, or at the membranes of the endoplasmic reticulim or the Golgi apparatus.

1. **Viruses obtaining their envelope from the cytoplasmic membrane are released during the budding process (see Slideshow Figs. 15A and 15B)**.

Slideshow Activity

Flash animation showing release of an enveloped virus by budding.
html5 version of animation for iPad showing release of an enveloped virus by budding.
Viruses obtaining their envelope from the host cell's cytoplasmic membrane are released during the budding process.

Transmission electron micrograph of Rubella viruses budding from an infected host cell; courtesy of CDC.

2. **Viruses obtaining their envelopes from the membranes of the nucleus, the endoplasmic reticulum, or the Golgi apparatus are then released by exocytosis via transport vesicles (see Slideshow Figs. 16A and 6B)**.

Slideshow Activity

Flash Animation showing release of an enveloped virus by exocytosis.
html5 version of animation for iPad showing release of an enveloped virus by exocytosis.
Some viruses obtain their envelopes by budding from the nuclear membrane or budding from other internal

membranes. These viruses are then packaged in transport vesicles for release from the host cell by exocytosis.

Transmission electron micrograph of Coronaviruses in the endoplasmic reticulum of an infected host cell; courtesy of CDC.

Some viruses, capable of causing cell fusion, may be transported from one cell to adjacent cells without being released, that is, they are **transmitted by cell-to-cell** contact whereby an infected cell fuses with an uninfected cell **(see Slideshow Figs. 17A, 17B, and 17C)**.

Slideshow Activity

7. Reinfection

As many as **10,000 to 50,000** animal viruses may be produced by a single infected host cell.

TPS Questions

Flash Animation showing a summary animation of the life cycle of an enveloped virus.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a summary animation of the life cycle of an enveloped virus.
This virus is entering by fusion of it's envelope with the host cell cytoplasmic membrane, replicating in the cytoplasm, and being released by budding from the host cell's cytoplasmic membrane.

Flash Animation showing a summary animation of the life cycle of a naked virus.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a summary animation of the life cycle of a naked virus.
This virus is entering by endocytosis, replicating in the cytoplasm, and being released by host cell lysis.

Flash Animation Showing All Viral Life Cycle Animations on this Page.

Nice YouTube animation with simplistic explanation of the replication of influenza viruses. Created for NPR by medical animator, David Bolinsky
.

Great animation of the productive life cycle of the dengue virus. Dengue fever is a mosquito-borne viral infection found mainly in tropical areas. Often asymptomatic and self-limiting but when symptoms do appear, they can include joint and muscle pain, headache, and a rash that may become hemorrhagic. The virus replicates in macrophages. This is an RNA virus that enters by endocytosis, gets its envelope by budding into the endoplasmic reticulum, and is packaged by the Golgi apparatus and

released by exocytosis.

Courtesy of HHMI's Biointeractive

Concept Map for Productive Life Cycle of a Naked Animal Virus

Concept Map for Productive Life Cycle of an Enveloped Animal Virus

Self Quiz for the Productive Life Cycle of Animal Viruses

Quiz Group

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Viral latency
THE PRODUCTIVE LIFE CYCLE OF ANIMAL VIRUSES WITH POSSIBLE LATENCY

Viruses

The Productive Life Cycle of Animal Viruses with Possible Latency

Fundamental Statement for this Softchalk Lesson:

1. Some viruses, such as the herpes viruses and the retroviruses are able to remain latent within infected host cells for long periods of time without replicating or causing harm.
2. Some of these viruses remain latent within the cytoplasm of the host cell while others are able to insert or integrate their DNA into the host cell's chromosomes.
3. When viral DNA is incorporated into the host cell's DNA, it is called a provirus.
4. Herpes viruses include HSV-1, HSV-2, EBV, VZV, and CMV; retroviruses include HIV.

Common Course Objectives

1. Recall characteristics that are present only in viruses and not in other cellular pathogens.
2. In terms of viral life cycles, explain what is meant by latency and give examples of viruses that typically become latent in the body.

Detailed Learning Objectives

1*. State the major difference between the productive life cycle of animal viruses and the latent life cycle.

2. Define provirus.

3. Name 3 herpes viruses that may have a latent cycle, state in what cell types they become latent, and name the diseases each cause.

(*) = Common theme throughout the course

TPS Questions

The Productive Life Cycle of Animal Viruses with Possible Latency

Some animal viruses, such as the **herpes viruses** and a group of viruses known as the **retroviruses,** are able to **remain latent within infected host cells for long periods of time without replicating or causing harm**. Some of these viruses **remain latent within the cytoplasm** of the host cell while others are able to insert or **integrate their DNA into the host cell's chromosomes**. When the viral DNA is incorporated into the host cell's DNA, it is called a **provirus**.

In many instances, viral latency, as well as viral persistence, is thought to be due to a process **called RNA interference (RNAi)** where small non-coding regulatory RNAs (ncRNAs) such as microRNAs (miRNAs) regulate gene expression. **Certain viruses that infect humans are able to establish persistent infection by using their own miRNAs and/or miRNAs produced by their human host**.

For More Information: Review of Enzyme Regulation

For example, viral and/or host miRNAs may **bind to certain viral messenger RNA (mRNA) molecules and block translation of viral proteins required for rapid viral replication**, or they **may bind to the mRNA of human genes that produce proteins used in viral replication**. The resulting low viral levels may then minimize immune responses against that virus. In addition, these miRNAs **may directly affect host immune defenses by turning off the production of antiviral cytokines or by blocking apoptosis of infected host cells**. Examples include the herpesviruses, retroviruses, and anelloviruses.

Herpes viruses, for example, are often **latent in some cell types but productive in others**. Herpes viruses include herpes simplex type 1 (**HSV-1**) which usually causes fever blisters or oral herpes, herpes simplex type 2 (**HSV-2**) which usually causes genital herpes, Epstein-Barr virus (**EBV**) which causes infectious mononucleosis and plays a role in certain cancers, varicella-zoster virus (**VZV**) which causes chickenpox and shingles, and cytomegalovirus (**CMV**) which causes a variety of infections in immunosuppressed persons and is also a leading cause of birth defects.

For more on HSV and CMV, see the AIDS Pathology Tutorial at the University of Utah.

In the case of **HSV-1, HSV-2, and VZV,** primary infection causes the virus to replicate within epithelial cells. However, some of the viruses enter and migrate down neurons where they **become latent in the body of sensory neurons**. Subsequent activation of the latently infected neurons by a variety of extracellular stimuli enables the viruses to migrate back up the nerve cell and replicate again in the epithelial cells.

Herpesviruses use both host and viral miRNAs to **switch between the productive life cycle in infected epithelial cells** whereby large numbers of viruses are produced and the infected host cells are killed (as in the case of fever blisters) **and the persistent latent state in nerve cells** where low levels of viruses are produced and the infected host cells are not killed by apoptosis.

The Enemy Within: How viruses wield tiny molecules of RNA to help them persist in our bodies for years, decades, and sometimes an entire life span.

Courtesy of The Scientist.com

With **EBV**, the virus is **productive in epithelial cells but latent in B-lymphocytes**.

Scanning electron micrograph of HSV; courtesy of Dennis Kunkel's Microscopy.

Animations of the various stages of replication of herpes simplex viruses

Courtesy of Dr. Edward K. Wagner

TPS Questions

In the case of **HIV**, the viral genome eventually becomes a **provirus**. After integration, the **HIV proviral DNA can exist in either a latent or productive state**, which is determined by genetic factors of the viral strain, the type of cell infected, and the production of specific host cell proteins.

The majority of the proviral DNA is integrated into the chromosomes of activated T4-lymphocytes. These generally comprise between 93% and 95% of infected cells and are productively infected, not latently infected. However, a **small percentage of HIV-infected memory T4-lymphocytes persists in a resting state because of a latent provirus**. Subsequent activation of the host cell by extracellular stimuli, however, causes the needed proteins to be made and the virus again replicates via the productive life cycle. These memory T4-lymphocytes, along with infected monocytes, macrophages, and dendritic cells, **provide stable reservoirs of HIV** capable of escaping host defenses and antiretroviral chemotherapy.

In the next section we will now look at the life cycle of HIV.

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- Herpes Sinplex
- Varicella-Zoster Virus
- Infectious Mononucleosis
- Cytomegalovirus
- HIV Infection and AIDS

Self Quiz for the Productive Life Cycle of Animal Viruses with Possible Latency

Quiz Group

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The life cycle of HIV
THE LIFE CYCLE OF HIV

Viruses

The Life Cycle of HIV

Fundamental Statement for this Softchalk Lesson:

1. During adsorption, an envelope glycoprotein on the surface of HIV called gp120 must adsorbs to both a CD4 molecule and then a chemokine receptor found on the surface of only certain types of certain human cells such as T4-lymphocytes, monocytes, macrophages, and dendritic cells.
2. Following adsorption, glycoprotein gp41 enabling the viral envelope to fuse with the host cell membrane, allowing the nucleocapsid of the virus enters the host cell's cytoplasm.
3. During uncoating, the single-stranded RNA genomes within the capsid of the virus are released into the cytoplasm and HIV now uses the enzyme reverse transcriptase to make a single-stranded DNA copy of its single-stranded RNA genome. The reverse transcriptase then makes a complementary DNA strand to form a double-stranded viral DNA intermediate.
4. A viral enzyme called integrase then binds to the double-stranded viral DNA intermediate, transports it through the pores of the host cell's nuclear membrane, and inserts into one of the host cell's chromosomes to form a provirus.
5. Following activation of the provirus, molecules of mostly polycistronic mRNA are transcribed off of the proviral DNA strand, go through the nuclear pores into the rough endoplasmic reticulum where it is translated by host cell's ribosomes HIV structural proteins, enzymes, glycoproteins, and regulatory proteins.
6. Polyproteins translated from polycistronic mRNAs must be cleaved into function proteins by HIV protease enzymes.
7. The two HIV envelope glycoproteins gp41 and gp120 are transported to the plasma membrane of the host cell where gp41 anchors the gp120 to the membrane of the infected cell. HIV obtains its envelope from the plasma membrane by budding.
8. Most maturation occurs either during the budding of the virion from the host cell or after its release from the cell.

Common Course Objectives

1. Recall characteristics that are present only in viruses and not in other cellular pathogens.
2. In terms of viral life cycles, explain what is meant by latency and give examples of viruses that typically become latent in the body.
3. Describe what an animal virus consists of structurally and state the function of those viral parts.
4. Describe the stages of lytic by a generalized animal virus.
5. Explain why viruses are more restricted in their host and cell type.

Detailed Learning Objectives

1**.Describe how the retrovirus HIV-1 accomplishes each of the following steps during its life cycle. (Include the following key words in your description: gp120, CD4, chemokine receptors, gp41, capsid, RNA genome, reverse transcriptase, double-stranded DNA intermediate, provirus, polyproteins, proteases, and budding.)

A. viral attachment or adsorption to the host cell

B. viral entry into the host cell

C. viral movement to the site of replication within the host cell and production of a provirus.

D. viral replication within the host cell

E. viral assembly or maturation within the host cell and release from the host cell

2*. Name 3 types of cells HIV primarily infects and briefly explain why.

(*) = Common theme throughout the course

(**) = More depth and common theme

TPS Questions

The Life Cycle of HIV

Structure of the human immunodeficiency virus (HIV)

HIV **(see Fig. 1A, 1B and 1C)** has an envelope derived from host cell membranes during replication. Associated with the envelope are two HIV-encoded glycoproteins, gp120 and gp41. Underneath the envelope is a protein matrix composed of p17. Inside the virus is a capsid or core made of the protein p24. The nucleocapsid also contains p6, p7, reverse transcriptase (p66/p51), integrase (p32), protease (p10), and 2 molecules of single-stranded RNA, the viral genome **(see Fig. 2)**.

Fig. 1A: Illustration of the Structure of HIV

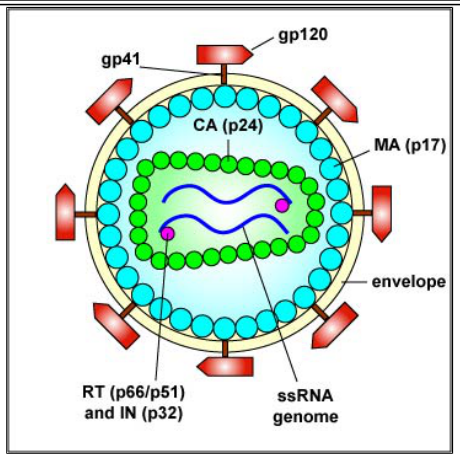


Fig. 1B: Transmission Electron Micrograph of HIV-1	Fig. 1C: Transmission Electron Micrograph of HIV-1 Stained to Show Surface Glycoproteins

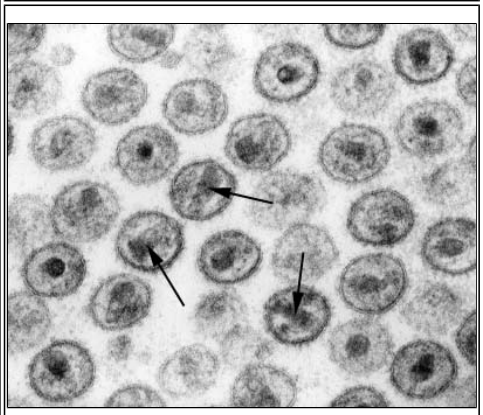
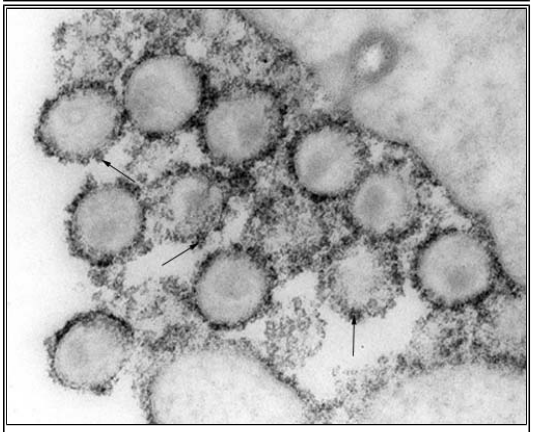
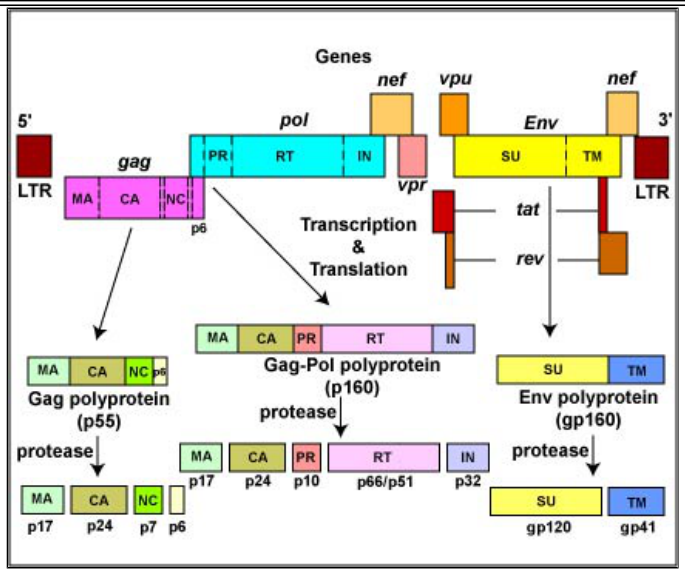

	

Image provided by Dr. Edwin P. Ewing, Jr. Courtesy of the Centers for Disease Control and Prevention.	Image provided by Dr. Edwin P. Ewing, Jr. Courtesy of the Centers for Disease Control and Prevention.
The nucleocapsid (arrows) can be seen within the envelope.	Glycoproteins (arrows) are seen on the viral envelope

To view further electron micrographs of HIV, see the AIDS Pathology Tutorial at the University of Utah.

Fig. 2: Transcription and Translation of the Genome of HIV



The *gag* and *pol* genes are transcribed as a unit and translated into two polyproteins Gag-Pol (p160) and Gag (p55). HIV proteases then cleave the Gag polyprotein (p55) into HIV matrix proteins (MA; p17), capsid proteins (CA; p24), and nucleocapsid proteins (NC, p7). The Gag-Pol polyprotein (p160) will be cleaved by HIV proteases to become HIV matrix proteins (MA; p17), capsid proteins (CA; p24), proteinase molecules (protease or PR; p10), reverse transcriptase molecules (RT; p66/p51), and integrase molecules (IN; p32). Likewise, the *env* gene is transcribed and translated into ENV polyprotein (gp160) that is cleaved by proteases into SU (surface glycoprotein; gp120) and TM (transmembrane glycoprotein; gp41).

HIV Genes: *Gag* (group antigen; codes for matrix antigen p17, capsid antigen p24, and nucleocapsid antigen); *Pol* (polymerase; codes for reverse transcriptase, protease, and integrase); *Env* (envelope; codes for surface glycoprotein gp120 and transmembrane glycoprotein gp41); *Tat* (transactivating protein; regulates transcription of integrated DNA of HIV); *Rev* (regulator of viral expression; passage of RNA transcripts out of the nucleus); *Nef* (negative factor; needed for full pathogenecity of HIV); *Vif* (viral infectivity gene; may play a role in viral

assembly); *Vpu* (blocks transport of CD4 to the host cell surface to aid in viral release); *vpr* (assists transport of dsDNA intermediate into host and arrests infected cells in the G2 phase of the cell cycle).

Attachment or Adsorption to the Host Cell

Initially, HIV uses a cellular protein called cyclophilin that is a component of its envelope to bind a low affinity host cell receptor called heparin. This first interaction (not shown in the illustrations or animations) enables the virus to initially make contact with the host cell.

In order to infect a human cell, however, an envelope glycoprotein on the surface of HIV called gp120 must adsorbs to both a CD4 molecule and then a chemokine receptor found on the surface of only certain types of certain human cells.

1. **T4-helper lymphocytes** (also called T4-cells and $CD4^+$ cells)
2. **monocytes**
3. **macrophages**
4. **dendritic cells**

For more information: Preview of dendritic cells and macrophages

For more information: Preview of T4-lymphocytes

Chemokines are cytokines that promote an inflammatory response by pulling white blood cells out of the blood vessels and into the tissue to fight infection. **Different white blood cells have receptors on their surface for different chemokines.** The **chemokine receptors are now thought to determine the type of $CD4^+$ cell HIV is able to infect**.

First, a portion or domain of the HIV surface glycoprotein gp120 binds to its primary receptor, a CD4 molecule on the host cell. This induces a change in shape that enables the chemokine receptor binding domains of the gp120 to interact with a host cell chemokine receptor. The chemokine receptor functions as the viral co-receptor.

This interaction brings about another conformational change that exposes a previously buried portion of the transmembrane glycoprotein gp41 called the fusion peptide that enables the viral envelope to fuse with the host cell membrane **(see Slideshow Figs. 3A, 3B, and 3C)**.

Slideshow Activity

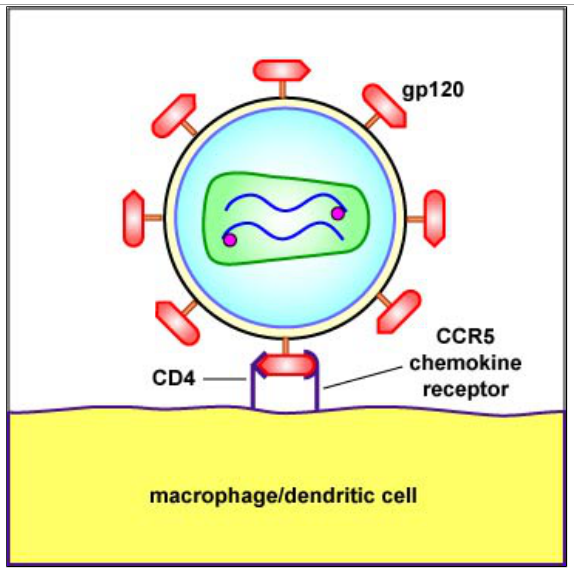
GIF animation showing adsorption of HIV.

Transmission electron micrograph showing envelope and glycoprotein spikes (gp120) of HIV; courtesy of CDC.

Scanning electron micrograph showing HIV infecting a T4-lymphocyte; courtesy of CDC.

Most strains of HIV are referred to as M-tropic or T-tropic. The gp120 of M-tropic HIV **(see Fig. 4)** is able to adsorb to the CD4 molecules and the CCR5 chemokine receptors found on **$CD4^+$** macrophages, immature dendritic cells, and memory T4-lymphocytes. (M-tropic HIV are also called R5 viruses since they adsorb to the chemokine receptor CCR5.) M-tropic HIV require only low levels of CD4 molecules expressed on the surface of the host cell for infection. **M-tropic HIV** are thought to spread the infection. These strains appear to be slower-replicating and less virulent than the later T-tropic strains and do not cause the formation of syncytias. HIV initially replicates to high levels within macrophages without destroying them. (The T-tropic HIV, found later in HIV infection, are faster-replicating, more virulent, and lead to syncytia formation.)

Fig. 4: Illustration of M-tropic HIV



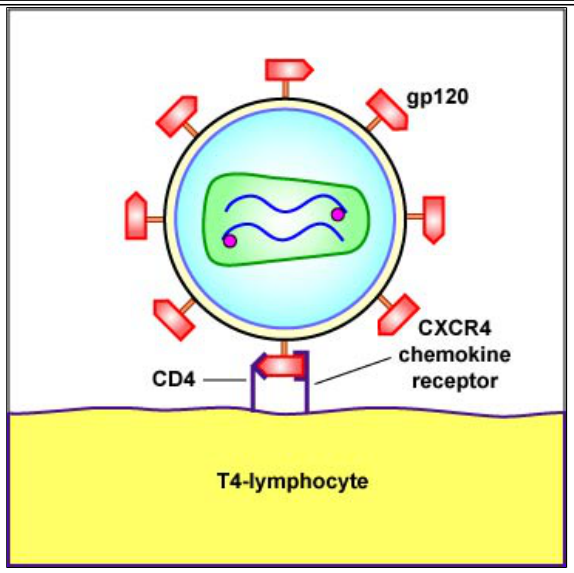
In early phase HIV infection, initial viruses are M-tropic. Their envelope glycoprotein gp120 is able to bind to CD4 molecules and chemokine receptors called CCR5 found on macrophages.

As time goes by, mutation in the gene coding for gp120 enables some of the HIV to become dual tropic and able to infect both macrophages via the CCR5 chemokine receptor found on these cells, and T4-lymphocytes via the CCR5 and CXCR4 chemokine receptors found on these cells. (Duel-tropic HIV are also called R5X4 viruses since they adsorb to both the chemokine receptors CCR5 and CXCR4.)

Later during the course of HIV infection, most of the viruses have mutated their gp120 to become T- tropic **(see Fig. 5)** and infect primarily **mature dendritic cells and T4-lymphocytes** by way of CD4 and the CXCR4 co-receptors found on these cells. (T-tropic HIV are also called X4 viruses since they adsorb to the chemokine receptor CXCR4.) T-tropic HIV require high levels of CD4 molecules expressed on the surface of the host cell for infection. As mentioned, these T-tropic strains of HIV are faster-replicating and more virulent, and cause formation of syncytias and begin the cycles of T4-lymphocyte destruction.

HIV infecting microglia cells in the brain appear to bind to a CD4 molecule and a chemokine receptor called CCR3 found on these macrophage-like cells.

Fig. 5: Illustration of T-tropic HIV



In late phase HIV infection, most of the viruses are T-tropic, having gp120 capable of binding to CD4 and CXCR4 found on T4-lymphocytes.

Viral Entry into the Host Cell

As mentioned above under adsorption, the binding of a portion or domain of the HIV surface glycoprotein gp120 to a CD4 molecule on the host cell induces a change in shape that brings the chemokine receptor binding domains of the gp120 into proximity with the host cell chemokine receptor. This, in turn, **brings about a conformational change that exposes a previously buried portion of the transmembrane glycoprotein gp41 enabling the viral envelope to fuse with the host cell membrane (see Fig. 6A and Fig. 6B)**. After fusion of the viral envelope with the host cell cytoplasmic membrane, the **genome-containing protein core** of the virus enters the host cell's cytoplasm. (Occasionally the virus enters by endocytosis, after which the viral envelope fuses with the endocytic vesicle releasing the genome-containing core into the cytoplasm.)

Slideshow Activity

GIF animation showing entry of HIV into a host cell.

Viral Movement to the Site of Replication within the Host Cell and Production of a Provirus

During uncoating, the single-stranded RNA genomes within the core or capsid of the virus are released into the cytoplasm. HIV now uses the enzyme **reverse transcriptase**, associated with the viral RNA genome, to make a **DNA copy of the RNA genome**. (Normal transcription in nature is when the DNA genome is transcribed into mRNA which is then translated into protein. In HIV reverse transcription, RNA is reverse-transcribed into DNA.)

Reverse transcriptase has three enzyme activities:

a. It has RNA-dependent DNA polymerase activity that copies the viral (+) RNA into a (-) viral complementary DNA (cDNA);

b. It has ribonuclease activity that degrades the viral RNA during the synthesis of cDNA; and

c. It has DNA-dependent DNA polymerase activity that copies the (-) cDNA strand into a (+) DNA to form a double-stranded DNA intermediate.

As the cDNA is being synthesized off of the RNA template the ribonuclease activity degrades the viral RNA genome. The reverse transcriptase then makes a complementary DNA strand to form a **double-stranded viral DNA intermediate (see Slideshow Figs. Fig. 7A, 7B, 7C, and 7D)**.

Slideshow Activity

GIF animation showing the action of reverse transcriptase.

A viral enzyme called integrase then binds to the double-stranded viral DNA intermediate, transports it through the pores of the host cell's nuclear membrane, and **inserts it into one of the host cell's chromosomes to form a provirus (see Slideshow Figs. 8A and 8B)**.

Slideshow Activity

GIF animation showing provirus formation.

After integration, the **HIV proviral DNA can exist in either a latent or productive state**, which is determined by genetic factors of the viral strain, the type of cell infected, and the production of specific host cell proteins.

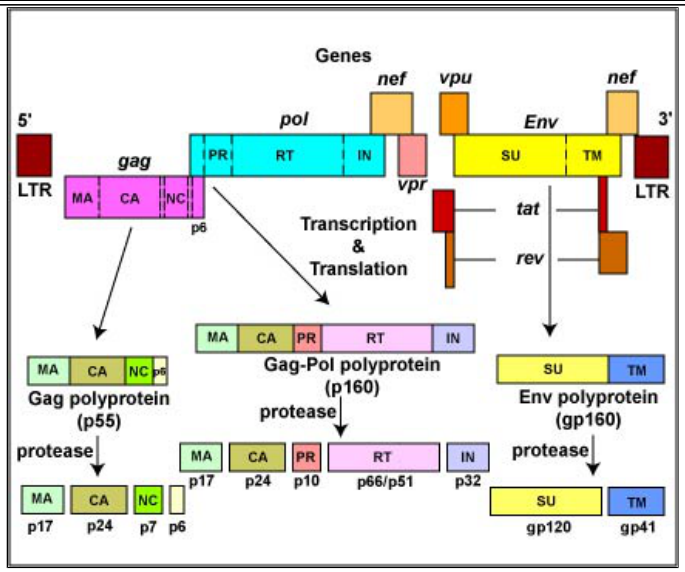
The majority of the proviral DNA is integrated into the chromosomes of activated T4-lymphocytes. These generally comprise between 93% and 95% of infected cells and are productively infected, not latently infected. However, a **small percentage of HIV-infected memory T4-lymphocytes persists in a resting state because of a latent provirus**. These, along with infected monocytes, macrophages, and dendritic cells, **provide stable reservoirs of HIV** capable of escaping host defenses and antiretroviral chemotherapy.

Replication of HIV within the Host Cell

The vast majority of T4-lymphocytes, which are productively infected, immediately begin producing new viruses. In the case of the small percentage of infected, resting memory T4-lymphocytes, before replication can occur, the HIV provirus must become activated. This is accomplished by such means as antigenic stimulation of the infected T4-lymphocytes or their activation by factors such as cytokines, endotoxins, and superantigens.

Following activation of the provirus, **molecules of (+) mRNA are transcribed off of the (-) proviral DNA strand** by the enzyme RNA polymerase II. Once synthesized, **HIV mRNA** goes through the nuclear pores into the rough endoplasmic reticulum to the host cell's ribosomes where it is **translated into HIV structural proteins, enzymes, glycoproteins, and regulatory proteins (see Fig. 2)**.

Fig. 2: Transcription and Translation of the Genome of HIV



Copyright © Gary E. Kaiser

The *gag* and *pol* genes are transcribed as a unit and translated into two polyproteins Gag-Pol (p160) and Gag (p55). HIV proteases then cleave the Gag polyprotein (p55) into HIV matrix proteins (MA; p17), capsid proteins (CA; p24), and nucleocapsid proteins (NC, p7). The Gag-Pol polyprotein (p160) will be cleaved by HIV proteases to become HIV matrix proteins (MA; p17), capsid proteins (CA; p24), proteinase molecules (protease or PR; p10), reverse transcriptase molecules (RT; p66/p51), and integrase molecules (IN; p32). Likewise, the *env* gene is transcribed and translated into ENV polyprotein (gp160) that is cleaved by proteases into SU (surface glycoprotein; gp120) and TM (transmembrane glycoprotein; gp41).

HIV Genes: *Gag* (group antigen; codes for matrix antigen p17, capsid antigen p24, and nucleocapsid antigen); *Pol* (polymerase; codes for reverse transcriptase, protease, and integrase); *Env* (envelope; codes for surface glycoprotein gp120 and transmembrane glycoprotein gp41); *Tat* (transactivating protein; regulates transcription of integrated DNA of HIV); *Rev* (regulator of viral expression; passage of RNA transcripts out of the nucleus); *Nef* (negative factor; needed for full pathogenecity of HIV); *Vif* (viral infectivity gene; may play a role in viral assembly); *Vpu* (blocks transport of CD4 to the host cell surface to aid in viral release); *vpr* (assists transport of dsDNA intermediate into host and arrests infected cells in the G2 phase of the cell cycle).

A 9 kilobase mRNA is formed that is used for three viral functions:

a. Synthesis of Gag polyproteins (p55). These polyproteins will eventually be cleaved by HIV proteases to become HIV matrix proteins (MA; p17), capsid proteins (CA; p24), and nucleocapsid proteins (NC, p7). **See Slideshow Figs. 9A and Fig. 9B.**

Slideshow Activity

b. Synthesis of Gag-Pol polyproteins (p160). These polyproteins will eventually be cleaved by HIV proteases to become HIV matrix proteins (MA; p17), capsid proteins (CA; p24), proteinase molecules (protease or PR; p10), reverse transcriptase molecules (RT; p66/p51), and integrase molecules (IN; p32). **See Slideshow Figs. 10A and 10B.**

Slideshow Activity

c. During maturation, these RNA molecules also become the genomes of new HIV virions.

The 9kb mRNA can also be spliced to form a 4kb mRNA and a 2kb mRNA. The 4kb mRNA is used to:

a. Synthesize the Env polyproteins (gp160). These polyproteins will eventually be cleaved by proteases to become HIV envelope glycoproteins gp120 and gp41. **See Slideshow Figs. 11A and 11B.**

b. Synthesize 3 regulatory proteins called *vif, vpr*, and *vpu*.

Slideshow Activity

The 2kb mRNA is used to synthesize 3 regulatory proteins known as *tat, rev*, and *naf*.

GIF Animation showing translation of HIV mRNA.

For More Information: Review of Transcription

For More Information: Review of Translation

Viral Assembly or Maturation within the Host Cell and Release from the Host Cell

Assembly of HIV virions begins at the plasma membrane of the host cell. Maturation occurs either during the budding of the virion from the host cell or after its release from the cell.

Transmission electron micrograph of HIV budding from a T4-lymphocyte; courtesy of Dennis Kunkel's Microscopy.

Prior to budding, the Env polyprotein (gp160) goes through the endoplasmic reticulum and is transported to the Golgi complex where it is cleaved by a protease (proteinase) and processed into the two HIV envelope glycoproteins gp41 and gp120. These are transported to the plasma membrane of the host cell where gp41 anchors the gp120 to the membrane of the infected cell. **See Slideshow Figs. 12A, Fig. 12B, Fig. 12C, and Fig. 12D.**

Slideshow Activity

GIF animation showing maturation of gp41 and gp120.

The Gag (p55) and Gag-Pol (p160) polyproteins also associate with the inner surface of the plasma membrane along with the HIV genomic RNA as the forming virion begins to bud from the host cell.

During maturation, HIV proteases (proteinases) will cleave the remaining polyproteins into individual functional HIV proteins and enzymes such as matrix proteins (MA; p17), capsid proteins (CA; p24), reverse transcriptase molecules (RT; p66/p51), and integrase molecules (IN; p32). **See Slideshow Figs. 13A, 13B, 13C, and 13D.**

Slideshow Activity

a. The Gag polyproteins (p55) will be cleaved by HIV proteases to become HIV matrix proteins (MA; p17), capsid proteins (CA; p24), and nucleocapsid proteins (NC, p7 and p6).

b. The Gag-Pol polyproteins (p160) will be cleaved by HIV proteases to become HIV matrix proteins (MA; p17), capsid proteins (CA; p24), proteinase molecules (protease or PR; p10), reverse transcriptase molecules (RT; p66/p51), and integrase molecules (IN; p32).

The various structural components then assemble to produce a mature HIV virion.

GIF animation showing maturation of of HIV.

Reinfection and Summary of HIV Life Cycle

Free viruses now infect new susceptible body cells. HIV can **also be transmitted by cell-to-cell contact**. This can occur when an infected cell with gp120 on its cytoplasmic membrane attaches to CD4 molecules and chemokine receptors on the surface of an uninfected cell. The cells then fuse **(see Slideshow Figs. 14A and Fig. 14B)**.

Slideshow Activity

Excellent Animation of the HIV Life Cycle

Courtesy of HHMI's Biointeractive

TPS Questions

Concept Map for the Life Cycle of HIV

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- HIV Infection and AIDS

Self Quiz for the Life Cycle of HIV

Quiz Group

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HIV infection
NATURAL HISTORY OF A TYPICAL HIV INFECTION

Viruses

The Natural History of a Typical HIV Infection

Fundamental Statement for this Softchalk Lesson:

1. The median incubation period for AIDS is around 10 years.
2. During early or acute HIV infection the virus primarily infects and destroys memory T4-lymphocytes which express the chemokine receptor CCR5 and are very abundant in mucosal lymphoid tissues. Here HIV also encounters the dendritic cells located throughout the epithelium of the skin and the mucous membranes.
3. The dendritic cells detach from the epithelium, enter lymph vessels, and are carried to regional lymph nodes where they are now able to present antigens of HIV to naive T-lymphocytes in order to induce adaptive immune responses.
4. The virus transitions from the acute phase to the chronic phase characterized by viral dissemination, viremia, and induction of adaptive immune responses.
5. The viremia allows the viruses to spread and infect T4-helper lymphocytes, macrophages, and dendritic cells found in peripheral lymphoid tissues.
6. During the chronic phase of HIV infection, the lymph nodes and the spleen become sites for continuous viral replication and host cell destruction whereby a steady state-infection generally persists where T4-lymphocyte death and T4-lymphocyte replacement by the body are in equilibrium.
7. The enormous turnover of T4-lymphocytes eventually exhausts the lymphopoietic system and it becomes unable to replace the T4-cells being destroyed eventually leading to immunodeficiency.
8. Progression to AIDS is marked by a viral load that progressively increases in number while the immune system weakens as a result of the destruction of increasing numbers of T4-lymphocytes and the inability of the body to continually replace these destroyed cells.
9. As a result of immunosuppression, the person becomes susceptible to a variety of opportunistic infections and secondary cancers.

Common Course Objectives

1. Recall characteristics that are present only in viruses and not in other cellular pathogens.
2. In terms of viral life cycles, explain what is meant by latency and give examples of viruses that typically become latent in the body.
3. Explain why viruses are more restricted in their host and cell type.

Detailed Learning Objectives

1*. State the median incubation period for AIDS and, in terms of viral load, exhaustion of the lymphopoietic system, and immune responses, briefly describe what marks the progression to AIDS.

2. Briefly describe the following:

a. early or acute HIV infection
b. chronic HIV infection
c. AIDS

(*) = Common theme throughout the course

TPS Questions

The Natural History of a Typical HIV Infection

According to WHO estimates from 2004, HIV has now infected 50 to 60 million people worldwide. The virus has killed over 22 million children adults and has left 14 million children orphaned. Worldwide, over 42 million people are currently living with HIV infection/AIDS - approximately 70% of these live in Africa, 20% in Asia. Around 3 million people die each year of AIDS and it is estimated that each day 14,000 people in the world become newly infected with HIV.

The **median incubation period for AIDS is around 10 years**. During **early or acute HIV infection** the virus primarily **infects and destroys memory T4-lymphocytes which express the chemokine receptor CCR5** and are very abundant in mucosal lymphoid tissues. Here **HIV also encounters the dendritic cells** located throughout the epithelium of the skin and the mucous membranes where in their immature form called Langerhans cells they are attached by long cytoplasmic processes. The envelope glycoproteins gp41 and gp120 of HIV contain mannose-rich glycans that bind to mannan-binding proteins (pattern recognition receptors; also called lectin receptors) on the dendritic cells.

Upon capturing antigens through pinocytosis and phagocytosis and becoming activated by pro-inflammatory cytokines, **the dendritic cells detach from the epithelium, enter lymph vessels, and are carried to regional lymph nodes**. By the time they enter the lymph nodes, the dendritic cells have matured and are now able to present antigens of HIV to naive T-lymphocytes located in the the lymph nodes in order to **induce adaptive immune responses**.

At this point the infection has **transitioned from the acute phase to the chronic phas**e. The chronic phase of HIV infection is characterized by viral dissemination, viremia, and induction of adaptive immune responses. The viremia allows the viruses to **spread and infect T4-helper lymphocytes, macrophages, and dendritic cells found in peripheral lymphoid tissues**.

During the chronic phase of HIV infection, the lymph nodes and the spleen become sites for continuous viral replication and host cell destruction. During most of this phase, **the immune system remains active and competent and there are few clinical symptoms. A steady state-infection generally persists where T4-lymphocyte death and T4-lymphocyte replacement by the body are in equilibrium**. In a person infected with HIV, somewhere between one and two billion of these T4-cells die each day as a result of HIV infection and must be replaced by the body's lymphopoietic system in the bone marrow. It is estimated that 10 billion virions are produced and cleared in an infected individual each day. However, **the enormous turnover of T4-lymphocytes eventually exhausts the lymphopoietic system and it becomes unable to replace the T4-cells being destroyed**. A variety of mechanisms then eventually lead to immunodeficiency.

Mechanisms of HIV-induced immunodeficiency include:

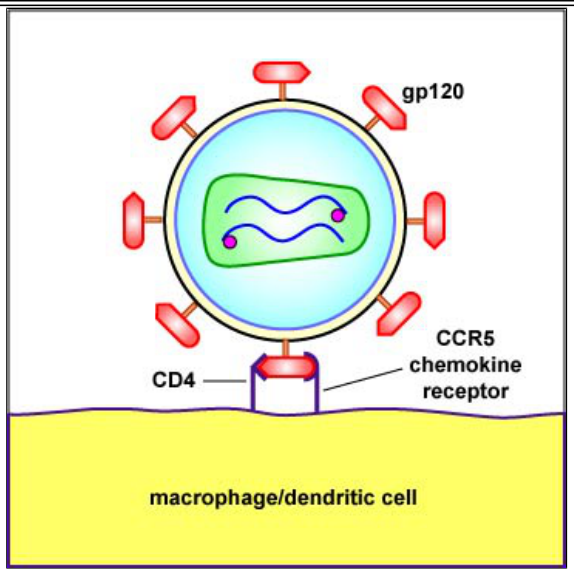
- Direct HIV-induced cytopathic effect on infected T4-lymphocytes. This can occur through:
  - Increased cell permeability as a result of gp41 expression in the host cell membrane and viral release by budding;
  - Inhibition of host cell protein synthesis as a result of viral replication within the infected cell; and
  - Fusion of infected T4-cells with numerous uninfected T4-cells resulting in syncytia formation.
- Killing of HIV-infected T4-cells by cytotoxic T-lymphocytes or CTLs.
- Killing of HIV-infected T4-cells by antibody-dependent cytotoxicity or ADCC.
- Apoptosis of T4-cells as a result of chronic activation by HIV and by cytokines.
- Shedding of gp120 molecules by HIV. This subsequently triggers a series of events that cause the adaptive immune system to become less and less effective, primarily by altering the normal balance of immunoregulatory T_H1 and T_H2 cells in the body.
- Impaired function of HIV infected macrophages and dendritic cells.

These mechanisms will be discussed in greater detail in Unit 6 under secondary immunodeficiency.

For more information: Preview of secondary immunodeficiency

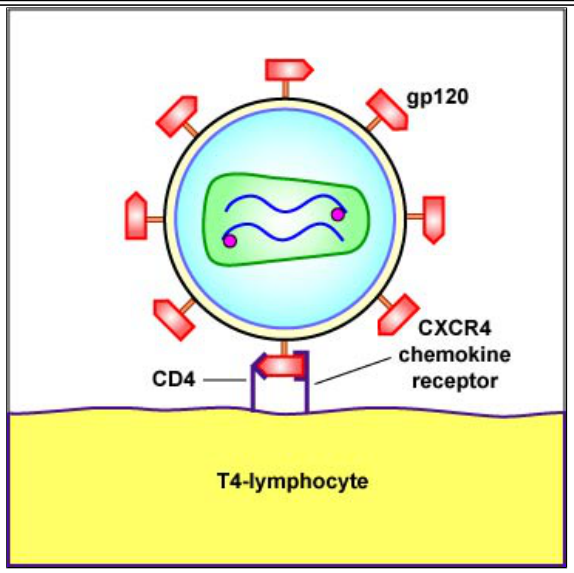
To further complicate problems, during the replication of HIV the **reverse transcriptase of HIV exhibits a high error rate as it transcribes the RNA genome into DNA**. As a result, HIV readily mutates to become more immunoresistant, more drug resistant, and able to change the preferred cell type it is able to infect, , e. g., M-tropic to T-tropic as shown in **Fig. 1 and Fig. 2**.

Fig. 1: Illustration of M-tropic HIV



In early phase HIV infection, initial viruses are M-tropic. Their envelope glycoprotein gp120 is able to bind to CD4 molecules and chemokine receptors called CCR5 found on macrophages.

Fig. 2: Illustration of T-tropic HIV



In late phase HIV infection, most of the viruses are T-tropic, having gp120 capable of binding to CD4 and CXCR4 found on T4-lymphocytes.

Progression to AIDS is marked by a viral load that progressively increases in number while the immune system weakens as a result of the destruction of increasing numbers of T4-lymphocytes and the inability of the body to continually replace these destroyed cells. As will be seen in Unit 5, the loss of T4-helper lymphocytes **leads to a marked decline in cells called cytotoxic T-lymphocytes (CTLs)**, the primary cells the body's immune responses use to destroy

virus-infected cells. Once a person progresses to full-blown AIDS he or she becomes susceptible to a variety of opportunistic infections by:

- bacteria such as *Mycobacterium avium* complex (MAC), *Salmonella*, and *Nocardia*;
- protozoa such as *Cryptosporidium* and *Toxoplasma*;
- viruses such as cytomegalovirus (CMV), herpes simplex viruses types 1 and 2 (HSV-1, HSV-2), and varicella zoster virus (VZV);
- *Candida, Cryptococcus, Coccidioides, Histoplasma*, and *Pneumocystis*.

There is also an increased incidence of tumors, such Epstein-Barr virus-associated B-cell lymphomas, other lymphomas, cervical cancer, and Kaposi's sarcoma. Wasting syndrome and encephalopathy are also common.

TPS Questions

Highly active anti-retroviral therapy (HAART) with a combination of reverse transcriptase inhibitors and protease inhibitors, as will be discussed later in Unit 4 under "Control of Viruses," has had relatively good success in **both improving T4-lymphocyte levels and reducing the levels of HIV in the body** - sometimes to undetectable levels. However, even with undetected levels of HIV, **most infected persons continue to harbor relatively small amounts of replication-competent HIV, most likely in the resting T4-memory cells** produced as a normal part of the immune responses. These infected T4-memory cells probably persist for years after antiretroviral therapy has reduced viral load below the limit of laboratory detection and could represent a pool that can keep HIV infection going or reactivate the infection. Macrophages and dendritic cells may also serve as a reservoir for HIV.

For more information: Preview of antiviral chemotherapeutic agents

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- HIV Infection and AIDS

Self Quiz for the Natural History of a Typical HIV Infection

Quiz Group

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Viruses and cancer
THE ROLE OF VIRUSES IN TUMOR PRODUCTION

Viruses

The Role of Viruses in Tumor Production

Fundamental Statement for this Softchalk Lesson:

1. Viruses are responsible for about 12% of the world's cancers.
2. Up to 80% of these human viral-associated cancers are cervical cancer (associated with the human papilloma virus or HPV) and liver cancer (associated with the hepatitis B virus or HBV and the hepatitis C virus or HCV).
3. The Epstein-Barr virus (EBV), the Kaposi sarcoma-associated herpesvirus (KSHV), the human immunodeficiency virus (HIV), and human T-lymphotropic virus type I (HTLV-I) also increase the risk of certain cancers.
4. The development of tumors is a multistep process depending on the accumulation of mutations altering a number of genes.
5. Most virus-associated cancers have long latency periods of several decades and only a small percentage of the people infected with the virus actually develop the cancer. This indicates other factors promoting changes in cellular genes are also involved.

Detailed Learning Objectives

1. Describe how certain viruses may contribute to the development of tumors by altering proto-oncogenes or tumor-suppressor genes.

2. Name 3 viruses that have been implicated in human cancers.

TPS Questions

The Role of Viruses in Tumor Production

Some viruses can also play a role in converting normal host cells into tumor cells. These viruses are capable of **viral transformation**, that is, they **transform normal cells into malignant cells**. In fact, five viruses, hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), Epstein-Barr virus (EBV), and human T-lymphotropic virus type I (HTLV-I) are thought to contribute to over 12% of the world's cancers. Up to 80% of these human viral-associated cancers are cervical cancer (associated with HPV) and liver cancer (associated with HBV and HCV).

The **hepatitis B virus (HBV)** is a DNA virus that may potentially cause chronic hepatitis in those infected. There is a strong link between chronic infection with HBV and hepatocellular carcinoma, which typically appears after 30-50 years of chronic liver damage and liver cell replacement. Chronic carriers of HBV have a 300 times greater risk of eventually developing liver cancer. Around 90% of individuals infected at birth and 10% of individuals infected as adults become chronic carriers of HBV. There are about one million chronic carriers of HBV in the US. Worldwide, HBV is responsible for 60% of all liver cancer cases.

The **hepatitis C virus (HCV)** is a RNA virus that may also cause chronic hepatitis in those infected. As with HBV, there is a strong link between chronic infection with HCV and liver cancer, typically appearing after 30-50 years of chronic liver damage and liver cell replacement. Around 85% of individuals infected with HCV become chronic carriers and there are approximately four million chronic carriers of HCV in the US. Worldwide, HCV is responsible for 22 % of all liver cancer cases.

The **human papilloma viruses (HPV)** are responsible for warts. While warts are generally considered as benign tumors, some sexually-transmitted strains of HPV (HPV-16 and 18 are definitely carcinogenic in humans; HPV-31 and 33 are probably carcinogenic), have been implicated in cervical and vulvar cancer, rectal cancer, and squamous cell carcinoma of the penis. In these tumor cells the viral DNA is usually found integrated in host cell chromosomes. In the US, HPVs are associated with 82% of the deaths due to cervical cancer each year, as well as a million precancerous lesions.

The Epstein-Barr virus (EBV), a herpes virus, normally causes benign proliferations such as infectious mononucleosis and hairy leukoplakia of the tongue. However, it can contribute to non-Hodgkin's lymphoma in AIDS patients and post-transplantation lymphoproliferative diseases, appears to be an essential factor for posterior nasopharyngeal cancer in some individuals, can be a co-factor for Burkitt's lymphoma, and contributes to smooth-muscle tumors in immunosuppressed children.

The **Kaposi sarcoma-associated herpesvirus (KSHV)** is a herpesvirus transmitted from person to person primarily through saliva, but can also be transmitted sexually, primarily among men who have sex with men. In addition, it can be spread via blood and transmitted from an infected mother to a child. Healthy individuals infected with the virus show no signs or symptoms. KSHV has been linked to several cancers, including Kaposi sarcoma and two rare lymphomas.

The **human immunodeficiency virus (HIV)**, an immunosuppressive virus responsible for AIDS, is spread through unprotected sexual activity, infected drug needles, during pregnancy from mother to child, and through infected breast milk. It is thought that a weakened immune system increases a person's risk of getting several cancers caused by other viruses, including non-Hodgkin and Hodgkin lymphomas, anogenital cancers, Kaposi sarcoma, possibly oral cancers, and liver cancer. It also increases the risk of other types of cancers, including eye cancer, non-melanoma skin cancer, and possibly lung cancer.

The retrovirus **human T-lymphotropic virus type I (HTLV-I)** can induce a rare adult T-lymphocyte leukemia-lymphoma.

The development of tumors is a **multistep process** depending on the **accumulation of mutations** altering a number of genes. The altered genes then function collectively to cause malignant growth.

Proliferation of normal cells is regulated by **growth-promoting proto-oncogenes** and counterbalanced by **growth-restricting tumor suppressor genes**. **Mutations that increase the activities of proto-oncogenes to create oncogenes and/or decrease the activities of tumor suppressor genes can lead to growth of tumors**. It is now known that many tumors require both activation of oncogenes from proto-oncogenes and inactivation of tumor suppressor genes for their development.

Viruses are thought to play a role in cancer development both indirectly and directly. Indirectly, the viruses may induce immunosuppression so that cancer cells are not removed by immune responses, as in the case of HIV/AIDS, or they may cause long term damage to tissues resulting in large scale cell regeneration which increases the chances of natural mutation in proto-oncogenes and tumor suppressor genes, as in the case of HBV and HCV. Directly, by integrating into the host cell's chromosomes, some viruses may alter the normal function of the proto-oncogenes and tumor suppressor genes, as is seen with HPV and HBV.

However, most virus-associated cancers have long latency periods of several decades and only a small percentage of the people infected with the virus actually develop the cancer. This indicates **other factors promoting changes in cellular genes are also involved**. For example, in the case of cervical cancer and HPV, two variants of a tumor suppressor gene known as *p53* are known. One form of the *p53* gene produces a suppressor protein that is much more susceptible to degradation by an oncoprotein called E6 which is produced by carcinogenic strains of HPV.

TPS Questions

Concept Map for the Role of Some Viruses in Tumor Production

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- Hepatitis B
- Hepatitis C
- Human Papilloma Virus
- Infectious Mononucleosis
- Human T-Cell Lymphotropic Viruses
- Hepatic Carcinoma
- Cervical Cancer

Quiz Group

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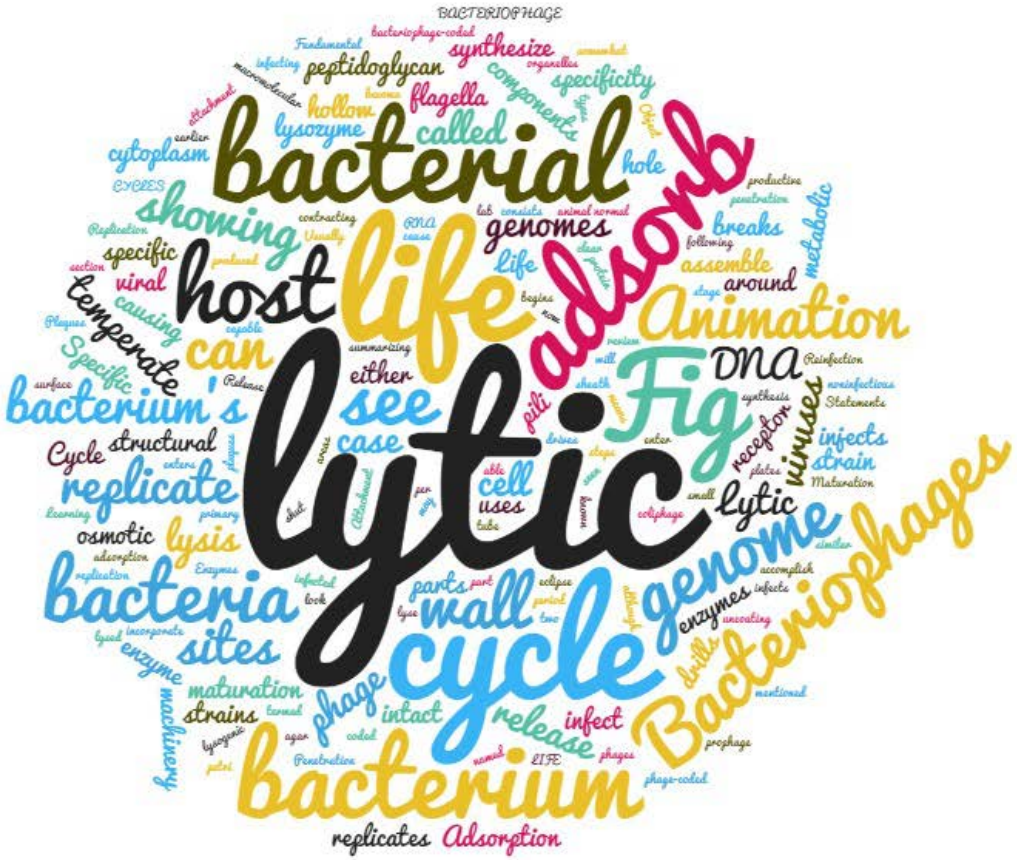
The lytic life cycle of bacteriophage
THE LYTIC LIFE CYCLE OF BACTERIOPHAGES

Viruses

The Lytic Life Cycle of Bacteriophages



Fundamental Statement for this Softchalk Lesson:

1. Bacteriophages are viruses that only infect bacteria.
2. Bacteriophages that replicate through the lytic life cycle are called lytic bacteriophages, and are so named because they lyse the host bacterium as a normal part of their life cycle.
3. Bacteriophages capable of a lysogenic life cycle are termed temperate phages. and can either replicate by means of the lytic life cycle and cause lysis of the host bacterium, or, can incorporate their DNA into the bacterium's DNA and become a non-infectious prophage.
4. Bacteriophages that replicate through the lytic life cycle are called lytic bacteriophages,
5. Adsorption is the attachment sites on the phage adsorb to receptor sites on the host bacterium.
6. Specific strains of bacteriophages can only adsorb to specific strain of host bacteria (viral specificity).
7. In the case of bacteriophages that adsorb to the bacterial cell wall, a bacteriophage enzyme "drills" a hole in the bacterial wall and the bacteriophage injects its genome into the bacterial cytoplasm.
8. The bacteriophage replicates its genome and uses the bacterium's metabolic machinery to synthesize bacteriophage enzymes and bacteriophage structural components.
9. During maturation, the bacteriophage parts assemble around the phage genomes.
10. A phage-coded lysozyme breaks down the bacterial peptidoglycan causing osmotic lysis and release of the intact bacteriophages.

Common Course Objectives

1. Recall characteristics that are present only in viruses and not in other cellular pathogens.
2. Describe what an animal virus consists of structurally and state the function of those viral parts.
3. Explain why viruses are more restricted in their host and cell type.
4. Describe the lytic and lysogenic cycle of phages

Detailed Learning Objectives

1. Name the 2 types of bacteriophage life cycles and state what the bacteriophage capable of each is called.

2. Describe the steps involved in the lytic life cycle of bacteriophages.

3. Define the following:

   a. lytic bacteriophage
   b. eclipse period

TPS Questions

Bacteriophages

Bacteriophages are viruses that only infect bacteria. There are two primary types of bacteriophages: **lytic bacteriophages** and **temperate bacteriophages**.

1. Bacteriophages that replicate through the **lytic life cycle** are called **lytic bacteriophages**, and are so named because they lyse the host bacterium as a normal part of their life cycle.

2. Bacteriophages capable of a **lysogenic life cycle** are termed **temperate phages**. When a temperate phage infects a bacterium, it can either replicate by means of the lytic life cycle and cause lysis of the host bacterium, or, it can incorporate its DNA into the bacterium's DNA and become a noninfectious prophage.

We will now look at the lytic life cycle of bacteriophages.

The Lytic Life Cycle of Bacteriophages

Bacteriophages are viruses that only infect bacteria **(see Fig. 1A and 1B)**. Bacteriophages that replicate through the **lytic life cycle** are called **lytic bacteriophages**.

Fig. 1A: Illustration of Sizes and Shapes of Bacteriophages

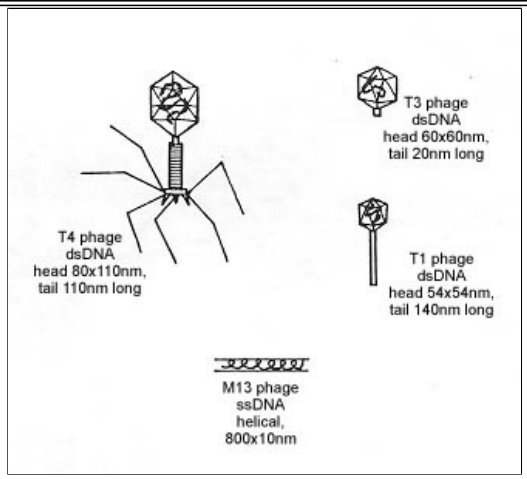
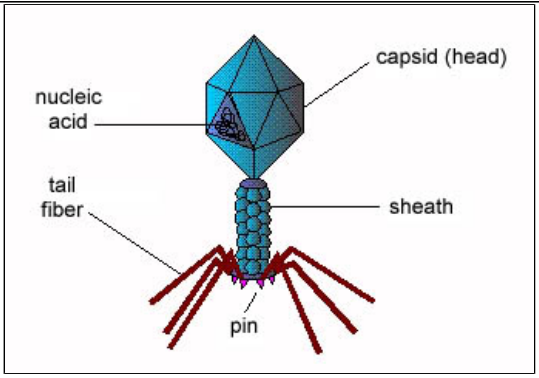


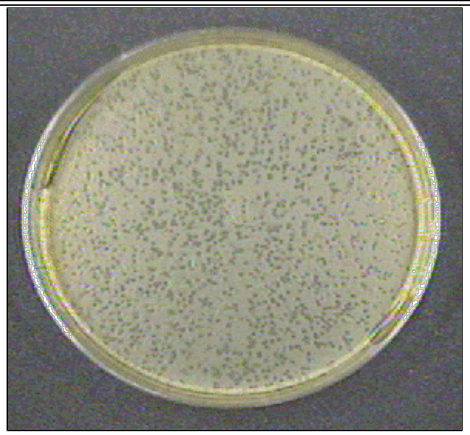
Fig. 1B: Illustration of Coliphage T4



Scanning electron micrograph of the lytic bacteriophage coliphage T4; courtesy of Dennis Kunkel's Microscopy.

After infecting bacteria with lytic bacteriophages in the lab, plaques can be seen on the petri plates. Plaques **(see Fig. 2)** are small clear areas on the agar surface where the host bacteria have been lysed by lytic bacteriophages.

Fig. 2: Plaques on an agar surface after infecting *Escherichia coli* with Coliphage T- 4

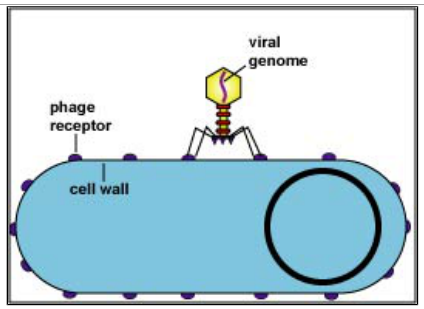


The lytic life cycle is the equivalent of the productive life cycle of animal viruses and consists of the following steps:

1. Adsorption

Attachment sites on the bacteriophage adsorb to receptor sites on the host bacterium (see Fig. 4). Most bacteriophages adsorb to the bacterial cell wall, although some are able to adsorb to flagella or pili. Specific strains of bacteriophages can only adsorb to specific strain of host bacteria. This is known as **viral specificity**.

Fig. 4: Illustration of Adsorption during the Lytic Life Cycle of a Lytic Bacteriophage



The bacteriophage binds to receptors on the bacterial cell wall.

Flash animation showing adsorption of a lytic bacteriophage.

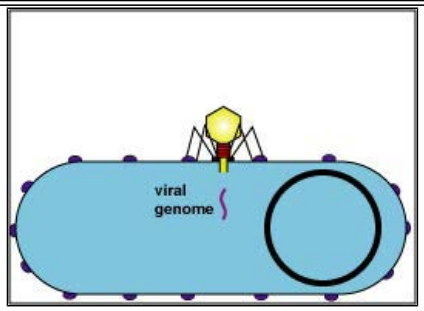
html5 version of animation for iPad showing adsorption of a lytic bacteriophage.

Attachment sites on the virus bind to corresponding receptors on the host cell wall.

2. Penetration

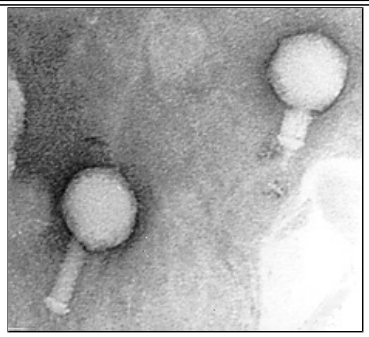
In the case of bacteriophages that adsorb to the bacterial cell wall, a bacteriophage enzyme "drills" a hole in the bacterial wall and the **bacteriophage injects its genome into the bacterial cytoplasm (see Fig. 5)**. Some bacteriophages accomplish this by contracting a sheath **(see Fig. 6)** which drives a hollow tube into the bacterium. This begins the eclipse period. The genomes of bacteriophages which adsorb to flagella or pili enter through these hollow organelles. In either case, only the phage genome enters the bacterium so there is no uncoating stage.

Fig. 5: Illustration of Penetration during the Lytic Life Cycle of a Lytic Bacteriophage



The bacteriophage injects its genome into the the cytoplasm of the host bacterium.

Fig. 6: Electron Micrograph of a Bacteriophage with a Contractile Sheath



Flash animation showing penetration of a lytic bacteriophage.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing penetration of a lytic bacteriophage.
The bacteriophage injects its genome into the bacterium's cytoplasm.

Animation illustrating the role of tail fibers in the adsorption of Coliphage T7

Movie S5: Originally published in *Science* Express on January 10 2013 by Hu, Margolin, Molineux, and Liu

3D animation illustrating adsorption and penetration of Coliphage T4

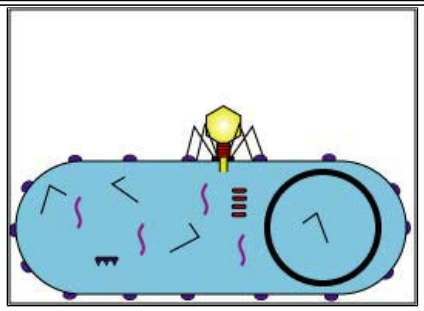
Courtesy of Dr. Michael Rossmann, Purdue University

From Seyet, LLC

3. Replication

Enzymes coded by the bacteriophage genome shut down the bacterium's macromolecular (protein, RNA, DNA) synthesis. **The bacteriophage replicates its genome and uses the bacterium's metabolic machinery to synthesize bacteriophage enzymes and bacteriophage structural components (see Fig. 7 and Fig. 8)**.

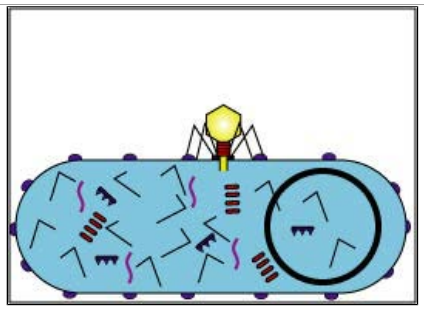
Fig. 7: Early Replication during the Lytic Life Cycle of a Lytic Bacteriophage



Copyright © Gary E. Kaiser

The bacteriophage genome replicates and bacteriophage components begin to be produced by way of the metabolic machinery of the host bacterium.

Fig. 8: Late Replication during the Lytic Life Cycle of a Lytic Bacteriophage



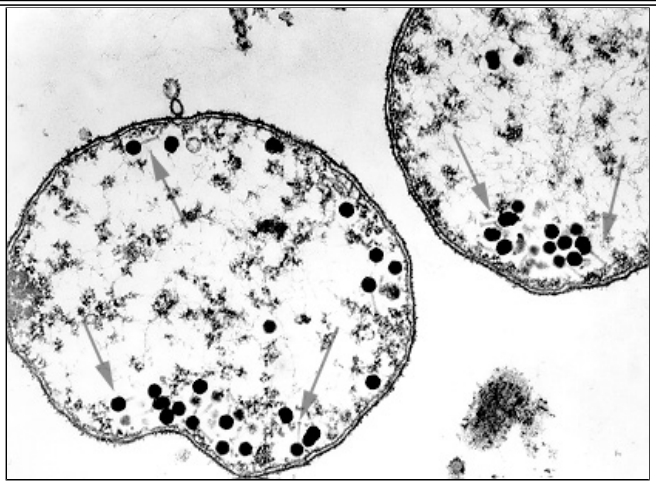
The production of bacteriophage components and enzymes progresses.

Flash animation showing replication of a lytic bacteriophage.
html5 version of animation for iPad showing replication of a lytic bacteriophage.
The viral genome directs the host cell's metabolic machinery (ribosomes, tRNA, nutrients, energy, enzymes, etc.) to synthesize viral enzymes and viral parts.

4. Maturation

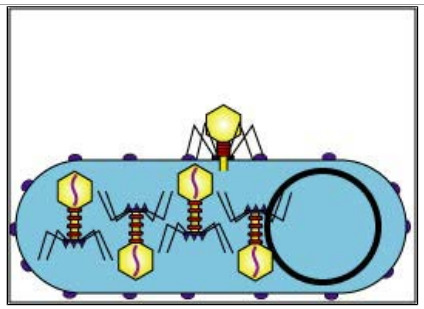
The phage parts assemble around the genomes **(see Fig. 8 and Fig. 9)**.

Fig. 8: Electron Micrograph of Bacteria Infected with Bacteriophages



Most of the phages (arrows) have assembled.

Fig. 9: Illustration of Maturation during the Lytic Life Cycle of a Lytic Bacteriophage



The bacteriophage components assemble.

Flash animation showing maturation of a lytic bacteriophage.

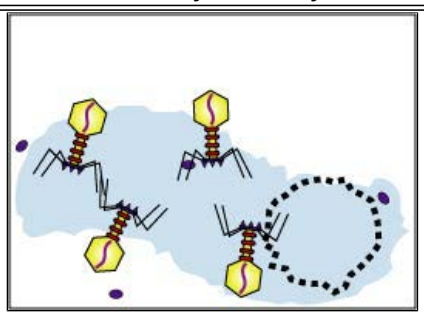
html5 version of animation for iPad showing maturation of a lytic bacteriophage.

The capsids assemble around the viral genomes as the viral tails assemble.

5. Release

Usually, a bacteriophage-coded **lysozyme breaks down the bacterial peptidoglycan causing osmotic lysis** and release of the intact bacteriophages **(see Fig. 10)**.

Fig. 10: Illustration of release of lytic bacteriophages from a bacterium by host cell lysis



A bacteriophage-coded enzyme breaks down the peptidoglycan in the bacterial cell wall causing osmotic lysis.

Flash animation showing release of a lytic bacteriophage.

html5 version of animation for iPad showing release of a lytic bacteriophage.

A bacteriophage-coded enzyme breaks down the peptidoglycan in the bacterial cell wall causing osmotic lysis.

6. Reinfection

From 50 to 200 bacteriophages may be produced per infected bacterium.

Flash Animation summarizing the lytic life cycle of a lytic bacteriophage.

TPS Questions

Concept Map for the Lytic Life Cycle of Bacteriophages

Self Quiz for the Lytic Life Cycle of Bacteriophages

Quiz Group

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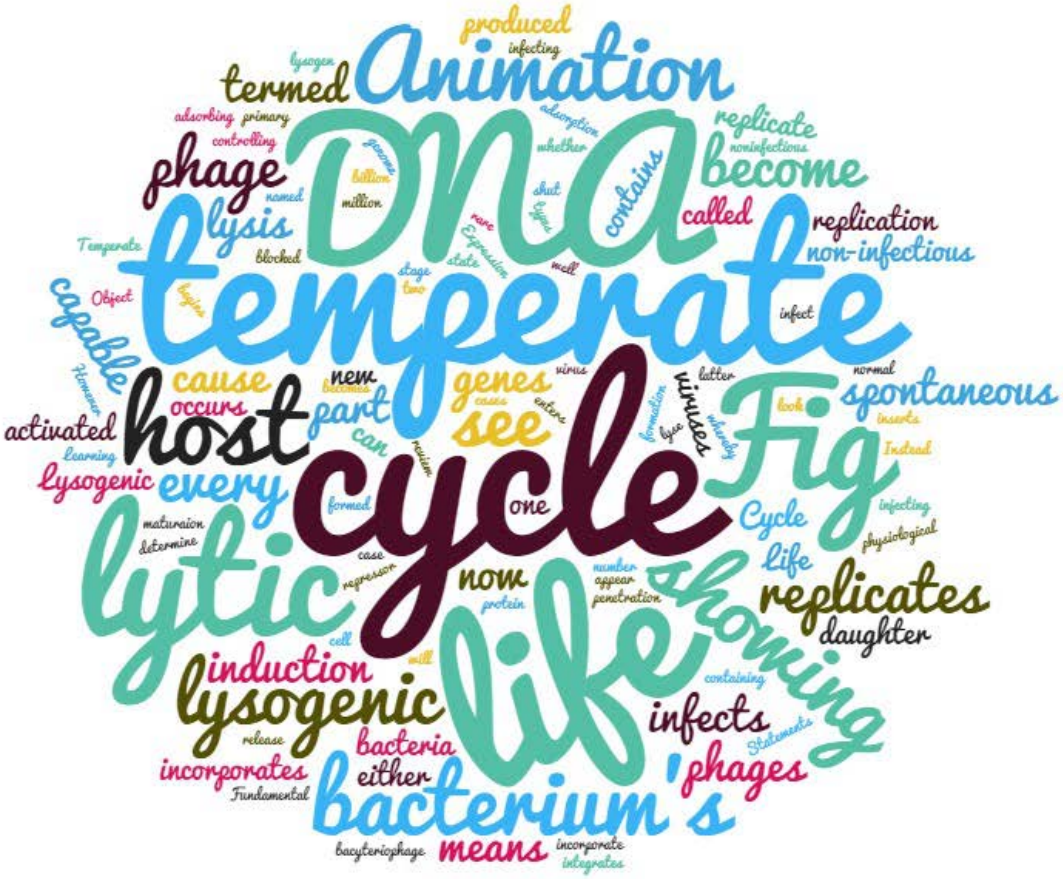
The lysogenic life cycle of bacteriophage
THE LYSOGENIC LIFE CYCLE OF BACTERIOPHAGES

Viruses

The Lysogenic Life Cycle of Bacteriophages



Fundamental Statement for this Softchalk Lesson:

1. Bacteriophages are viruses that only infect bacteria.
2. Bacteriophages that replicate through the lytic life cycle are called lytic bacteriophages, and are so named because they lyse the host bacterium as a normal part of their life cycle.
3. Bacteriophages capable of a lysogenic life cycle are termed temperate phages. and can either replicate by means of the lytic life cycle and cause lysis of the host bacterium, or, can incorporate their DNA into the bacterium's DNA and become a non-infectious prophage.
4. Bacteriophages capable of a lysogenic life cycle are termed temperate phages.
5. When a temperate bacteriophage infects a bacterium, it either replicates by means of the lytic life cycle and cause lysis of the host bacterium, or, incorporates its DNA into the bacterium's DNA and become a non-infectious prophage whereby the bacteriophage DNA replicates as a part of the bacterium's DNA so that every daughter bacterium now contains the prophage.
6. In rare cases spontaneous induction occurs. The bacteriophage genes become activated and new bacteriophages are produced by the lytic life cycle.

Common Course Objectives

1. Recall characteristics that are present only in viruses and not in other cellular pathogens.
2. Describe what an animal virus consists of structurally and state the function of those viral parts.
3. Explain why viruses are more restricted in their host and cell type.
4. Describe the lytic and lysogenic cycle of phages

Detailed Learning Objectives

1. Describe the lysogenic life cycle of temperate phages (including spontaneous induction).
2. Define the following:

   a. temperate phage
   b. lysogen
   c. prophage

TPS Questions

The Lysogenic Life Cycle of Bacteriophages

Bacteriophages are viruses that only infect bacteria. There are two primary types of bacteriophages: **lytic bacteriophages** and **temperate bacteriophages**.

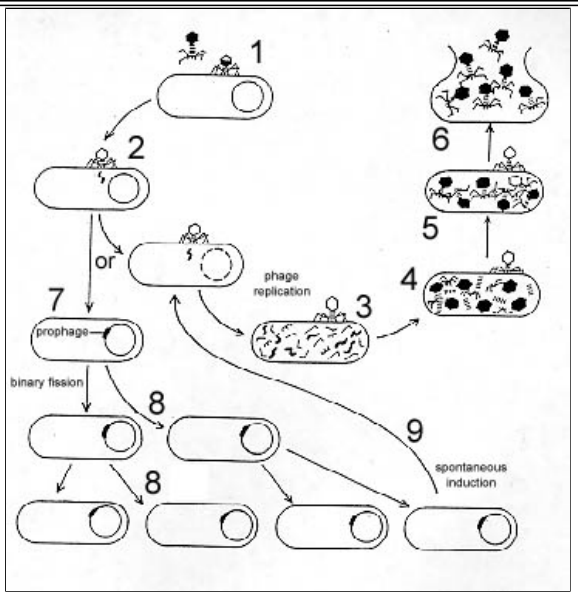
1. Bacteriophages that replicate through the **l**ytic life cycle are called lytic bacteriophages, and are so named because they lyse the host bacterium as a normal part of their life cycle.

2. Bacteriophages capable of a lysogenic life cycle are termed **temperate bacteriophages**. When a temperate phage infects a bacterium, it can either replicate by means of the lytic life cycle and cause lysis of the host bacterium, or, it can incorporate its DNA into the bacterium's DNA and become a noninfectious prophage.

We will now look at the lysogenic life cycle of bacteriophages.

Bacteriophages capable of a lysogenic life cycle are termed **temperate bacteriophages**. When a temperate bacteriophage infects a bacterium, it can either **replicate by means of the lytic life cycle** and cause lysis of the host bacterium, **or**, it can **incorporate its DNA into the bacterium's DNA** and become a noninfectious prophage **(see Fig. 1)**.

Fig. 1: Illustration of the Lysogenic Life Cycle of a Temperate Bacteriophage



1. Adsorption: phage adsorbs to receptors on the bacterial cell wall.

2. Penetration: phage injects its genome into the bacterium's cytoplasm.

3. Early replication: phage genome replicates and phage components begin to be produced.

4. Late replication: production of phage components progresses.

5. Maturation: phage components assemble.

6. Release: phage-coded lysozyme breaks down peptidoglycan causing lysis of the host bacterium.

OR

7. Phage inserts its genome into the bacterial nucleoid to become a prophage.

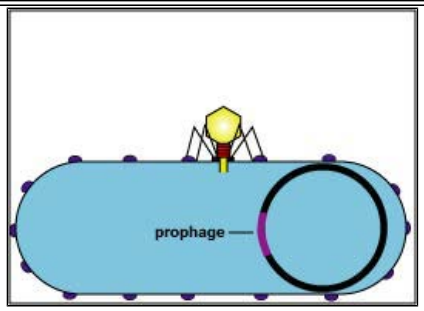
8. As the bacterium replicates, the prophage replicates as a part of the nucleoid.

9. Rare spontaneous induction: phage replicates via the lytic life cycle.

In the latter case, the cycle begins by the bacteriophage adsorbing to the host bacterium or lysogen and injecting its genome as in the lytic life cycle **(see Slideshow Figs. 2 and 3)**. However, the bacteriophage **does not shut down** the host cell. Instead, **the bacteriophage DNA inserts or integrates into the host bacterium's DNA (see Fig. 4)**. At this stage the virus is called a **prophage**. Expression of the bacteriophage genes controlling bacteriophage replication is blocked by a repressor protein, and the phage DNA replicates as a part of the bacterium's DNA so that every daughter bacterium now contains the prophage **(see Fig. 5)**.

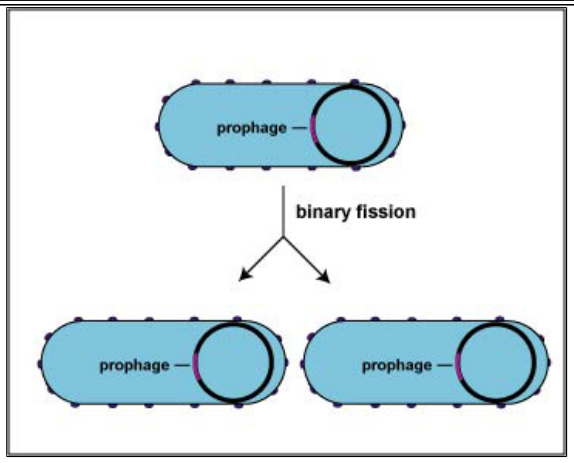
Slideshow Activity

Fig. 4: Illustration of Prophage Formation during the Lysogenic Life Cycle of a Temperate Bacteriophage



The bacteriophage inserts its genome into the chromosome of the bacterium to become a prophage.

Fig. 5: Illustration of Maintaining the Prophage during the Lysogenic Life Cycle of a Temperate Bacteriophage



As the bacterium replicates, the prophage replicates as a part of the chromosome of the bacterium.

Flash animation showing adsorption of a temperate bacteriophage.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing adsorption of a temperate bacteriophage.
Attachment sites on the virus bind to corresponding receptors on the host cell wall.

Flash animation showing penetration of a temperate bacteriophage.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing penetration of a temperate bacteriophage.
The bacteriophage injects its genome into the bacterium's cytoplasm.

Flash animation showing prophage formation.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing prophage formation.
The bacteriophage DNA inserts or integrates into the host bacterium's DNA to form a prophage.

The number of viruses infecting the bacterium as well as the physiological state of the bacterium appear to determine whether the temperate bacteriophage enters the lytic cycle or becomes a prophage.

In about one out of every million to one out of every billion bacteria containing a prophage, spontaneous induction occurs. The **bacteriophage genes are activated and new bacteriophages are produced by the lytic life cycle (see Slideshow Figs. 6, 7, 8, 9, and 10)**.

Slideshow Activity

Flash animation showing spontaneous induction.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing spontaneous induction.
In about one out of every million to one out of every billion bacteria containing a prophage, spontaneous induction occurs. The bacteriophage genes are activated and new bacteriophages are produced as in the lytic life cycle.

Flash animation showing replication of a temperate bacteriophage.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing replication of a temperate bacteriophage.
The viral genome directs the host cell's metabolic machinery (ribosomes, tRNA, nutrients, energy, enzymes, etc.) to synthesize viral enzymes and viral parts.

Flash animation showing maturation of a temperate bacteriophage.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing maturation of a temperate bacteriophage.
The capsids assemble around the viral genomes as the viral tails assemble.

Flash animation showing release of a temperate bacteriophage.
Copyright © Gary E. Kaiser

html5 version of animation for iPad showing release of a temperate bacteriophage.

A bacteriophage-coded enzyme breaks down the peptidoglycan in the bacterial cell wall causing osmotic lysis.

TPS Questions

Flash animation summarizing the lysogenic life cycle of a temperate bacteriophage.

Concept Map for the Lysogenic Life Cycle of Bacteriophages

Self Quiz for the Lysogenic Life Cycle of Bacteriophages

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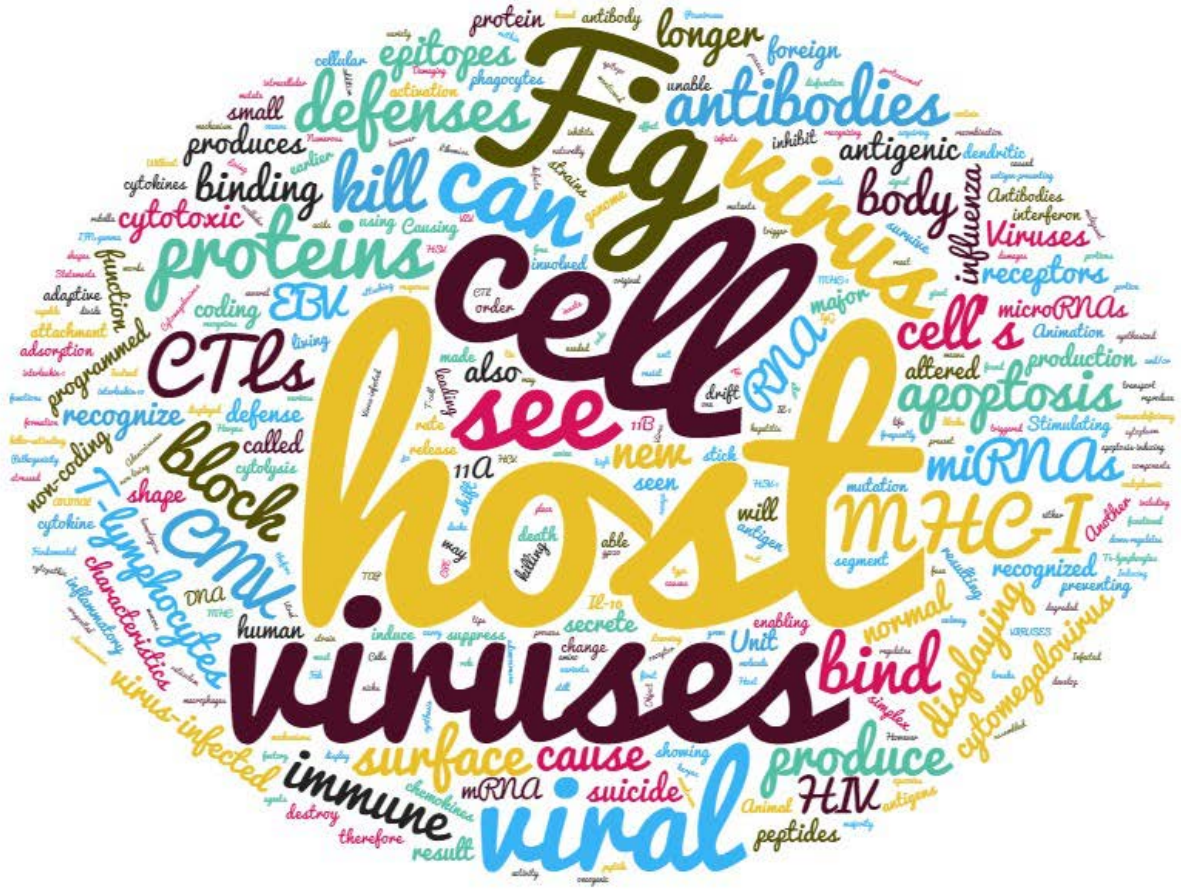
Viral pathogenicity
PATHOGENICITY OF ANIMAL VIRUSES

Viruses

Pathogenicity of Animal Viruses



Fundamental Statement for this Softchalk Lesson:

1. Alteration of host cell function and/or death of the host cell occurs as a result of viruses using an infected host cell as a factory for manufacturing viruses.
2. The body's immune defenses recognize infected host cells as foreign and destroy infected cells.
3. The body's adaptive immune defenses produce antibodies against viruses that block viral adsorption to host cells or result in opsonization of the virus.
4. The body's adaptive immune defenses produce cytotoxic T-lymphocytes (CTLs) against viruses that bind to infected host cells and induce cell suicide (apoptosis).
5. The body's innate immune defenses produce NK cells that can induce apoptosis of stressed, virus-infected host cells.
6. Viruses can develop resistance to antibodies and cytotoxic T-lymphocytes by altering the order of the amino acids and, therefore, the shape of viral antigens so the antibodies and CTLs no longer fit.
7. Viruses can alter infected host cells in such a way that NK cells no longer kill them.
8. Some viruses block apoptosis of infected host cells enabling the infected host cell to survive and produce new viruses.

Common Course Objectives

1. Describe how viruses may harm the host and give relevant examples.

2. Give relevant examples illustrating how viruses can resist immune defenses.

Detailed Learning Objectives

1. Briefly describe at least 4 ways viruses can damage infected host cells.
2. Briefly describe at least 3 different ways viruses can evade host immune defenses.

TPS Questions

Pathogenicity of Animal Viruses

How Viruses Damage Infected Host Cells.

Animal viruses may cause cytopathic effect or CPE that damages infected host cells in a variety of means, including:

1. Inhibiting normal host cell DNA, RNA, or protein synthesis. This can cause structural or functional defects in the infected host cell leading to cytolysis or altered cell functions.

2. Causing nicks or breaks in the host cell's chromosomes, as seen in congenital rubella syndrome.

3. Viral proteins and glycoproteins changing the antigenic surface of the host cell's cytoplasmic membrane resulting in its being recognized as foreign and destroyed by the body's immune defenses **(see Slideshow Figs. 1A and 1B and Slideshow Figs. 2A and 2B).** This will be discussed further in Unit 6.

Slideshow Activity Slideshow Activity

4. Depleting the host cell of cellular materials essential for life or normal function.

5. Stimulating body cells to release inflammatory cytokines and chemokines.

For more information: Review of cytokines

6. Stimulating body cells to release inflammatory vasoactive peptides, bradykinins, histamines, etc. resulting in vasodilation and increased mucous secretion.

For more information: Review of inflammation

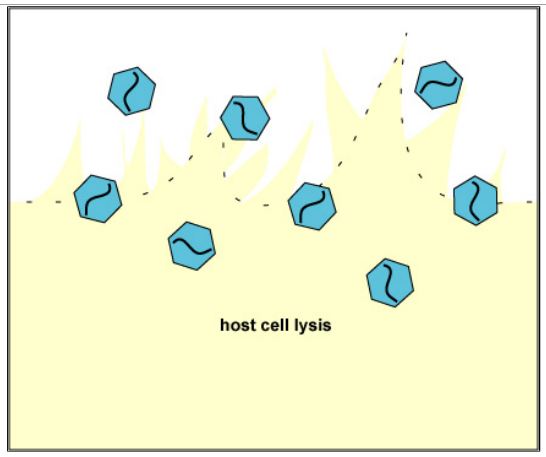
7. Inducing adjacent host cells to fuse together forming giant multinucleated cells or syncytias **(see Slideshow Figs. 3A, 3B, 3C, and 3D)** as seen with cytomegalovirus (CMV), varicella-zoster virus (VZV), and HIV.

Slideshow Activity

8. Playing a role in normal cells becoming malignant (cell transformation by oncogenic viruses).

9. Causing cytolysis of the infected host cell **(see Fig. 4)**.

Fig. 4: Release of Naked Virus by Host Cell Disintegration

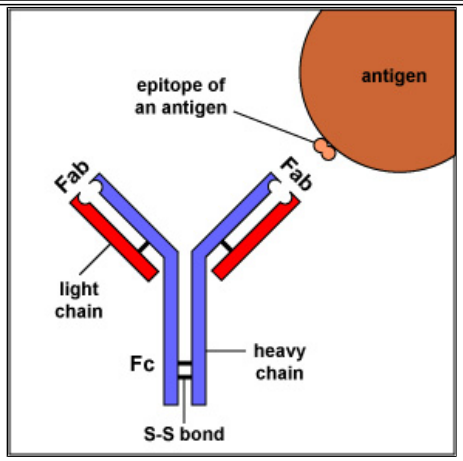


The host cell membrane disintegrates and the virus is released.

How Viruses Evade Host Immune Defenses

1. As will be seen in Unit 6, one of the major defenses against free viruses is the immune defenses' **production of antibody molecules against the virus**. The "tips" of the antibody (the Fab portion; **see Fig. 5**) have shapes that have a complementary shape to portions of viral attachment proteins and glycoproteins called epitopes found on the viral surface. When antibodies react with these attachment proteins, they **block viral adsorption to host cell receptors** and, therefore, block viral replication.

Fig. 5: Structure of the Antibody IgG



The Fab portion of the antibody has specificity for binding an epitope of an antigen. The Fc portion directs the biological activity of the antibody. In the case of IgG, the Fc portion can bind to phagocytes for enhanced attachment (opsonization) as well as activate the classical complement pathway. Antibodies are composed of 4 protein chains: 2 identical heavy chains and 2 identical light chains. Disulfide (S-S) bonds join the protein chains together.

Flash animation showing neutralization of viruses by antibodies.

html5 version of animation for iPad showing opsonization of viruses by antibodies.

The Fab portion of the antibodies made against epitopes of the virus attachment site blocks the virus from adsorbing to the receptor site on the host cell membrane. As a result, the virus can not penetrate and replicate.

In addition, Antibodies such as IgG function as opsonins and **stick viruses to phagocytes**.

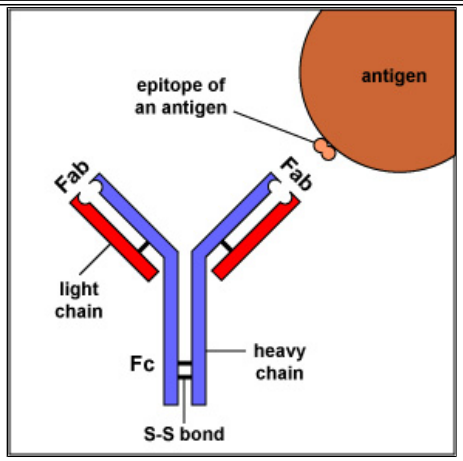
Flash animation showing opsonization of viruses by antibodies.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing opsonization of viruses by antibodies.
The Fab portion of IgG binds to epitopes of the viral surface. The Fc portion can now attach the virus to Fc receptors on phagocytes for enhanced attachment. Once attached to the phagocyte by way of IgG, the virus can be engulfed more efficiently, placed in a phagosome, and destroyed by lysosomes. C3b and C4b from the activated complement pathways are also able to attach viruses to phagocytes.

The influenza viruses undergo what is called **antigenic drift and antigenic shift**.

- With **antigenic drift**, mutations cause a gradual change in the hemagglutinin antigen that adsorbs to receptors on host cells.
- **Antigenic shift** is caused by a human influenza virus acquiring a new genome segment from an influenza virus capable of infecting other animals such as a ducks or swine. This new genome segment causes a major change in the hemagglutinin antigen.

Antibodies made against the original human influenza virus can no longer bind to the new strain of virus or stick the virus to phagocytes (see Fig. 5 and Fig. 6)

Fig. 5: Structure of the Antibody IgG



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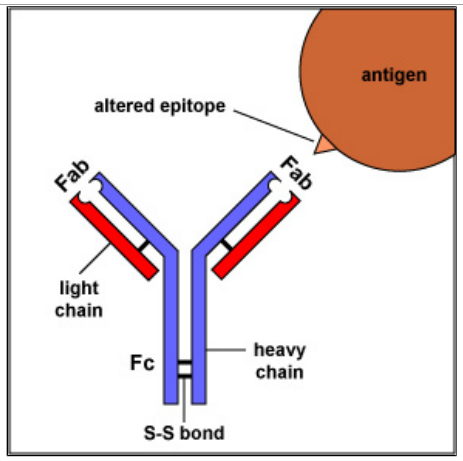
The Fab portion of the antibody has specificity for binding an epitope of an antigen. The Fc portion directs the biological activity of the antibody. In the case of IgG, the Fc portion can bind to phagocytes for enhanced attachment (opsonization) as well as activate the classical complement pathway. Antibodies are composed of 4 protein chains: 2 identical heavy chains and 2 identical light chains. Disulfide (S-S) bonds join the protein chains together.

Fig. 6: Producing an Altered Epitope that the Antibody No Longer Fits

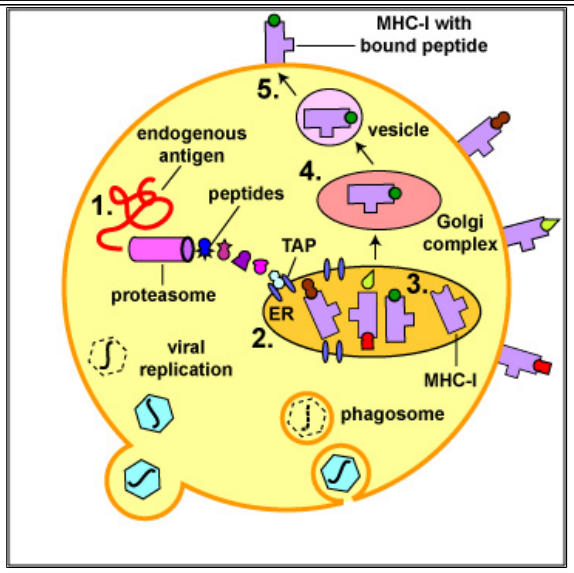


Viruses can evade antibody defense by producing an altered epitope that previous antibodies no longer fit.

- Likewise HIV, because of **its high rate of mutation and its intracellular recombination with other strains of HIV**, as mentioned earlier in this unit, produces altered gp120 to which antibodies made against the earlier strains of HIV can no longer bind.
- The hepatitis C virus (HCV) frequently **through mutation produces viral variants ("escape mutants")** to resist antibodies.

2. Another major defense against viruses, as we will see in Unit 6, is the killing of virus-infected host cells by cytotoxic T-lymphocytes **(CTLs)**. Virus-infected host cells naturally **bind viral epitopes to a host molecule called** MHC-I **and place the MHC-1 with bound viral epitope on the surface of the infected cell (see Fig. 7) where they can be recognized by CTLs having a T-cell receptors on its surface with a complementary shape**.

Fig. 7: Binding of Peptide Epitopes from Viruses to MHC-I Molecules



Endogenous antigens are those being produced within cells of the body. Examples include:

a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

The body marks infected cells and tumor cells for destruction by placing peptide epitopes from these endogenous antigens on their surface by way of MHC-I molecules. Cytotoxic T-lymphocytes (CTLs) are then able to recognize peptide/MHC-I complexes by means of their T-cell receptors (TCRs) and CD8 molecules and kill the cells to which they bind.

1. Endogenous antigens, such as viral proteins, pass through proteasomes where they are degraded into a series of peptides.
2. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
3. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
4. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by CTLs by way of TCRs and CD8 molecules having a complementary shape.

In this way the CTL can kill the infected cell by apoptosis, a programmed cell suicide **(see Slideshow Figs. 8A and Fig. 8B)**.

Flash animation of a CTL triggering apoptosis by way of perforins and granzymes.

html5 version of a CTL triggering apoptosis by way of perforins and granzymes.

Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. (Alternately, the granzymes and perforins may enter by endocytosis and the perforins then promote the release of the granzymes from the endocytic vesicle into the cytoplasm.)

Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

Flash animation showing CTL-induced apoptosis of a virus-infected cell.

html5 version of animation for iPad showing CTL-induced apoptosis of a virus-infected cell.

Killing of the infected cell or tumor cell by apoptosis involves a variety of mechanisms:

- Certain granzymes can activate the caspase enzymes that lead to apoptosis of the infected cell. The caspases are proteases that destroy the protein structural scaffolding of the cell - the cytoskeleton - and degrade both the target cell's nucleoprotein and microbial DNA within the cell.
- Granzymes cleave a variety of other cellular substrates that contribute to cell death.
- The perforin molecules may also polymerize and form pores in the membrane of the infected cell, similar to those produced by MAC. This can increase the permeability of the infected cell and contribute to cell death. If enough perforin pores form, the cell might not be able to exclude ions and water and may undergo cytolysis. A granule called granulysin can also alter the permeability of both miocrobial and host cell membranes.

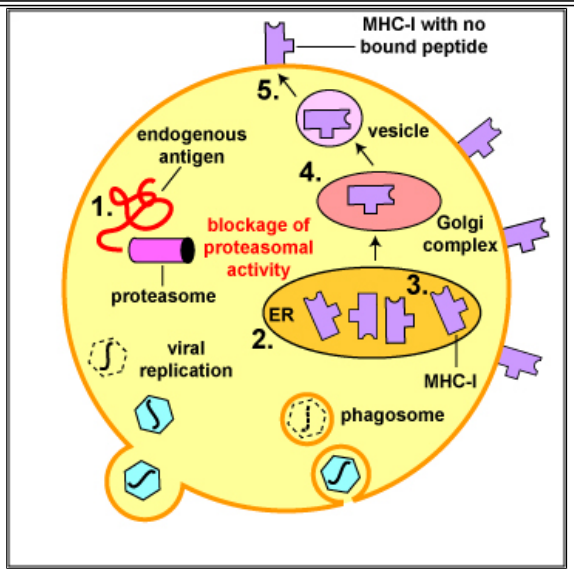
This animations shows destruction of both the cytoskeleton and nucleoprotein of the infected cell. As the infected cell breaks up into apoptotic fragments, the fragments are subsequently removed by phagocytes. This reduces inflammation and also prevents the release of viruses that have assembled within the infected cell and their spread into uninfected cells.

Animation of a virus-infected cell being marked as foreign and subsequently killed by CTLs

Courtesy of HHMI's Biointeractive.

- Epstein-Barr virus (EBV) and cytomegalovirus (CMV) inhibit proteasomal activity so that viral proteins are not degraded into viral peptides. **(see Fig. 8)**

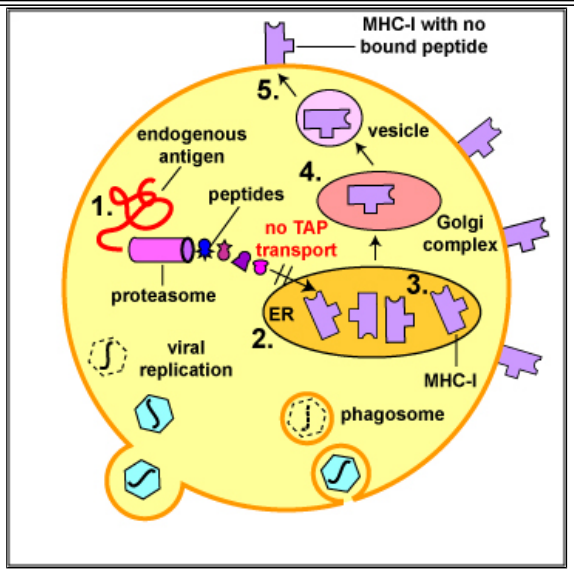
Fig. 8: Inhibition of Proteasome Activity



Epstein-Barr virus (EBV) and cytomegalovirus (CMV) inhibit proteasomal activity so that viral proteins are not degraded into viral peptides.

- Herpes simplex viruses (HSV) can block the TAP transport of peptides into the endoplasmic reticulum **(see Fig. 9)**.

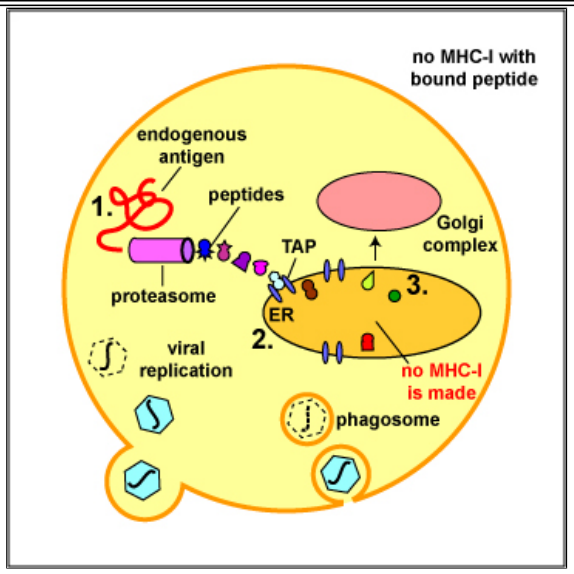
Fig. 9: Blockage of TAP Transport of Peptides into the Endoplasmic Reticulum



Herpes simplex viruses (HSV) can block the TAP transport of peptides into the endoplasmic reticulum.

- Numerous viruses, such as the cytomegalovirus (CMV) and adenoviruses **can block the formation of** MHC-I molecules **by the infected cell**. As a result, **no viral peptide is displayed on the infected cell and the** CTLs **are no longer able to recognize that the cell is infected and kill it (see Fig. 10)**.

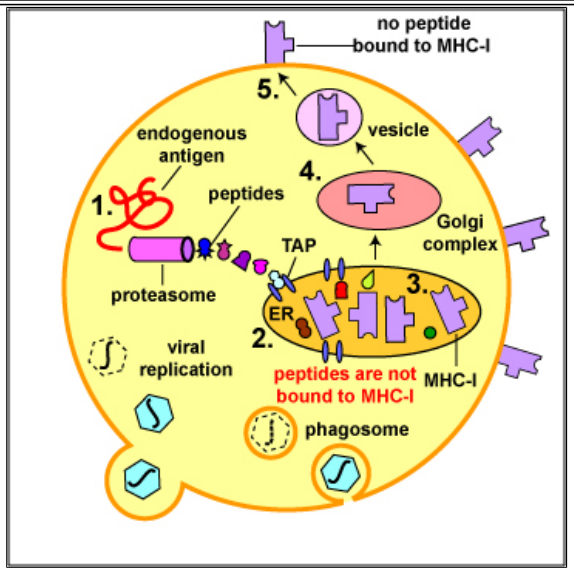
Fig. 10: Suppression of the Synthesis of MHC-I Molecules



Numerous viruses, such as the cytomegalovirus (CMV) and adenoviruses can block the formation of MHC-I molecules by the infected cell. As a result, no viral peptide is displayed on the infected cell and the CTLs are no longer able to recognize that the cell is infected and kill it.

- Epstein-Barr virus (EBV) **down regulates several host proteins involved in attaching viral epitopes to MHC-I molecules and displaying them on the host cell's surface (see Fig. 11)**.

Fig. 11: Blockage of the Binding of Peptide Epitopes from Viruses to MHC-I Molecules



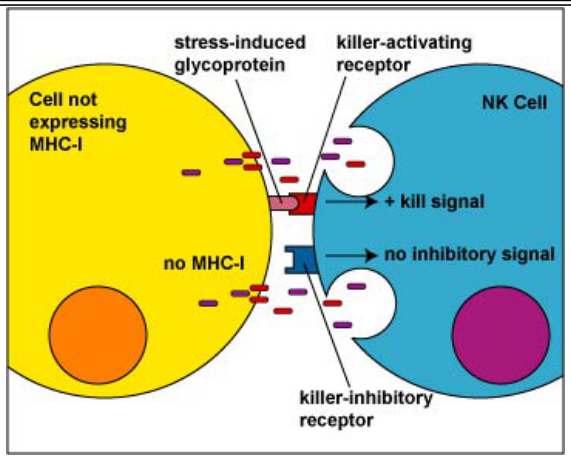
Epstein-Barr virus (EBV) down regulates several host proteins involved in attaching viral epitopes to MHC-I molecules and displaying them on the surface of the host cell. As a result, no viral peptide is displayed on

the infected cell and the CTLs are no longer able to recognize that the cell is infected and kill it.

- Adenoviruses and Epstein-Barr Virus (EBV) code for proteins that **blocks apoptosis**, the programmed cell suicide mechanism triggered by various defense mechanisms in order to destroy virus-infected cells.

3. Another defense cell that is able to kill virus-infected cells is the NK cell. NK cells **recognize infected cells displaying stressed-induced proteins and not displaying MHC-I molecules on their surface and kill these cells (see Fig. 12)**.

Fig. 12: NK Cell Interacting with a Virus-Infected Cell or a Mutant Cell Not Expressing MHC-I Molecules



Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell kills the cell to which it has bound.

MHC-I molecules are the molecules on host cells that display viral epitopes to cytotoxic T-lymphocytes (CTLs). Some viruses suppress the production of MHC molecules by host cells, preventing CTLs from recognizing the infected cell as foreign and killing it. NK cells, however, can recognize cells not displaying MHC-I and kill them anyway.

Flash animation showing a NK cell interacting with a normal body cell.

html5 version of animation for iPad showing a NK cell interacting with a normal body cell.

NK cells appear to use a duel receptor system in determining whether to kill or not kill human cells. When cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules are produced and are put on the surface of that cell. The first NK cell receptor, called the killer-activating receptor, recognizes these stress-induced molecules. This interaction sends a positive signal which enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal. This second receptor, called the killer-inhibitory receptor, recognizes MHC-I molecules that are also usually present on all nucleated human cells. If MHC-I molecules are expressed on the cell, the killer-inhibitory receptor sends a negative signal that overrides the kill signal and prevents the NK cell from killing that cell.

Flash animation showing a NK cell interacting with a virus-infected cell or tumor cell not expressing MHC-I molecules.

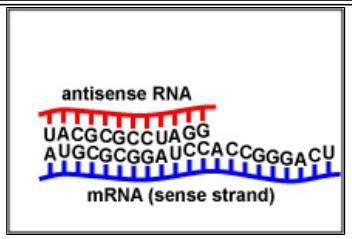
html5 version of animation for iPad showing a NK cell interacting with a virus-infected cell or tumor cell not expressing MHC-I molecules.

Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that

lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

- The cytomegalovirus (CMV) can also trigger its host cell to produce **altered** MHC-I molecules **that are unable to bind viral epitopes**, and, therefore, are **not recognized by** CTLs. However, **NK cells are also unable to kill this infected cell** because it is still displaying "MHC-I molecules" on its surface.
- CMV also produces microRNAs (miRNAs), small non-coding RNA molecules that down-regulates the production of stress-induced proteins that the killer-activating receptor of NK cells first recognizes. The miRNAs do this by binding to the host cell's mRNA coding for stress-induced proteins **(see Fig. 13)**. Without this binding there is no kill signal by the NK cell.

Fig. 13: Antisense RNA (micro RNA or miRNA)



When an antisense RNA (microRNA or miRNA) that is complementary to a mRNA coding for a particular protein or enzyme binds to the mRNA by complementary base pairing, that mRNA cannot be translated and the protein or enzyme is not made.

GIF animation showing antisense RNA.

4. Some viruses cause infected host cells to **secrete molecules that bind and tie up** cytokines, preventing them from binding to normal cytokine receptors on host cells.

- Poxviruses cause infected host cells to secrete molecules that bind interleukin-1 (IL-1) and interferon-gamma (IFN-gamma).
- Cytomegaloviruses (CMV) cause infected host cells to secrete molecules that bind chemokines.

5. Some viruses **suppress immunocompetent cells**.

- Epstein-Barr virus (EBV) produces a protein that is homologous to the cytokine interleukin-10 (IL-10). IL-10 inhibits the activation of dendritic cells and macrophages, antigen-presenting cells that are needed to present antigens to T-lymphocytes for their activation. EBV also produces microRNAs (miRNAs), small non-coding RNA molecules that inhibit an interferon response by infected cells. The miRNAs do this by binding to the host cell's mRNA coding for interferon **(see Fig. 13)**.
- The human immunodeficiency virus (HIV) infects immunocompetent dendritic cells and T4-lymphocytes leading to their death or dysfunction.

6. Some viruses block apoptosis of infected host cells enabling the infected host cell to survive and produce new viruses.

- Cytomegalovirus (CMV) and herpes simplex type 1 virus (HSV-1) produce microRNAs (miRNAs), small non-coding RNA molecules that block protein involved in apoptosis, a programmed cell suicide. The miRNAs do this by binding to the host cell's mRNA coding for apoptosis-inducing proteins **(see Fig. 13)**.

TPS Questions

Concept Map for Viral Pathogenicity

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- HIV Infection and AIDS
- Varicella-Zoster Virus
- Infectious Mononucleosis
- Cytomegalovirus
- Hepatitis B
- Hepatitis C
- Rubella
- Influenza
- Adenoviruses

Self Quiz for Pathogenicity of Animal Viruses

Quiz Group

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Back to Softchalk Lessons Table of Contents

Bacteriophage-induced alteration of bacteria
BACTERIOPHAGE-INDUCED ALTERATION OF BACTERIA

Viruses

Bacteriophage-Induced Alteration of Bacteria

Fundamental Statement for this Softchalk Lesson:

1. Lytic bacteriophages usually cause the host bacterium to lyse.
2. The added genetic information provided by the DNA of a prophage may enable a bacterium to possess new genetic traits.
3. Some bacteria become virulent only when infected themselves with a specific temperate bacteriophage. The added genetic information of the prophage allows for coding of protein exotoxin or other virulence factors.
4. Examples include the diphtheria exotoxin, streptococcal pyrogenic exotoxin (Spe), the botulism exotoxins, the cholera exotoxin, and the shiga toxin.

Common Course Objectives

1. In terms of viral life cycles, explain what is meant by latency and give examples of viruses that typically become latent in the body.

2. Explain why the lysogenic cycle can enhance the virulence of infected bacteria and recall specific examples of enhanced virulence after lysogeny.

Detailed Learning Objectives

1.*Describe the process of lysogenic conversion and give 2 examples of exotoxins that result from lysogenic conversion.

(*) = Common theme throughout the course

TPS Questions

Bacteriophage-Induced Alteration of Bacteria

1. Lytic bacteriophages usually cause the host bacterium to lyse **(see Fig. 1)**.

2. Lysogenic conversion **by prophages**

The added genetic information provided by the DNA of a prophage **(see Fig. 2)** may enable a bacterium to possess new genetic traits. For example, some bacteria become virulent only when infected themselves with a specific temperate bacteriophage. **The added genetic information of the prophage allows for coding of protein** exotoxin **or other virulence factors**. The following bacterial exotoxins are a result of lysogenic conversion by a prophage:

a. the diphtheria exotoxin of the bacterium *Corynebacterium diphtheriae;*

b. the Streptococcal pyrogenic exotoxin (Spe) produced by rare invasive strains and scarlet fever strains of *Streptococcus pyogenes*;

c. The neurotoxin produced by *Clostridium botulinum*;

d. exfoliatin, an exotoxin that causes scalded skin syndrome, produced by *Staphylococcus aureus*;

e. the cholera exotoxin produced by *Vibrio cholerae;* and

f. the shiga toxins produced by *E. coli* O157:H7.

Fig. 1: Plaques on an agar surface after lysis of *Escherichia coli* by Coliphage T- 4

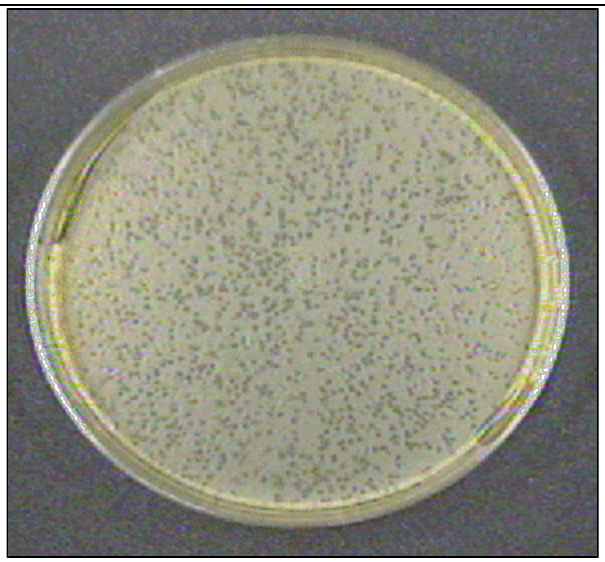
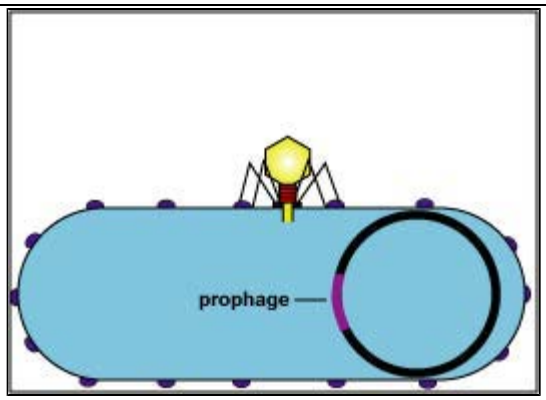


Fig. 2: Illustration of Prophage Formation during the Lysogenic Life Cycle of a Temperate Bacteriophage



The bacteriophage inserts its genome into the nucleoid of the bacterium to become a prophage.

GIF animation summarizing the lysogenic life cycle of a temperate bacteriophage.

TPS Questions
For more information: Review of type-I toxins
For more information: Review of type-II toxins
For more information: Review of type-III toxins

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Corynebacterium diphtheriae*
- *Vibrio cholerae*
- enterotoxogenic *E. coli*
- *Staphylococcus aureus*
- *Clostridium botulinum*
- *Staphylococcus aureus* and *Streptococcus pyogenes*: Toxic shock syndrome

Quiz Group

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Antiviral agents
ANTIVIRAL AGENTS

Viruses

Antiviral Agents

Fundamental Statement for this Softchalk Lesson:

1. Relatively few antiviral chemotherapeutic agents are currently available and they are only somewhat effective against just a few limited viruses.
2. Many antiviral agents resemble normal DNA nucleosides molecules and work by inhibiting viral DNA synthesis.
3. Some antiviral agents are protease inhibitors that bind to a viral protease and prevent it from cleaving the long polyprotein from polycistronic genes into proteins essential to viral structure and function.
4. Some antiviral agents are entry inhibitors that prevent the virus from either binding to or entering the host cell.
5. Antiviral agents are available for only a few viruses, including certain influenza viruses, herpes viruses, cytomegaloviruses, hepatitis C viruses, and HIV.
6. Certain interferon cytokines have been produced by recombinant DNA technology and several are used for certain severe viral infections.

Common Course Objectives

1. Describe the mechanism of the antivirals discussed in lecture.

Detailed Learning Objectives

1*. State why antibiotics are of no use against viruses and what we must rely on to control viruses.

2. State the viruses the following antiviral agents are used against:

a. amantadine, rimantidine, zanamivar, and oseltamivir
b. acyclovir, famciclovir, penciclovir, and valacyclovir
c. foscarnet, gancyclovir, cidofovir, valganciclovir, and fomivirsen
d. AZT (ZDV), didanosine, zalcitabine, stavudine, lamivudine, emtricitabine, tenofovir, and abacavir
e. nevirapine, delavirdine, and efavirenz
f. saquinavir, ritonavir, idinavir, nelfinavir, amprenavir, atazanavir, fosamprenavir, ritonavir
g. telaprevir, boceprevir, simeprevir, sofosbuvir

3. Compare how the following drugs exhibit their antiviral action against HIV.

a. nucleoside reverse transcriptase inhibitors
b. protease inhibitors
c. entry inhibitors

(*) = Common theme throughout the course

Antiviral Agents

Since viruses lack the structures and metabolic processes that are altered by common antibiotics, antibiotics are virtually useless in treating viral infections. To date, relatively few antiviral chemotherapeutic agents are available and used to treat just a few limited viruses.

Most of the antiviral agents work by **inhibiting viral DNA synthesis**. These drugs chemically **resemble normal DNA nucleosides**, molecules containing deoxyribose and either adenine, guanine, cytosine, or thymine. Viral enzymes then add phosphate groups to these nucleoside analogs to form DNA nucleotide analogs. **The DNA nucleotide analogs are then inserted into the growing viral DNA strand in place of a normal nucleotide**. Once inserted, however, new nucleotides can't attach and DNA synthesis is stopped. They are selectively toxic because viral polymerases are more prone to incorporate nucleotide analogs into their nucleic acid than are host cell polymerases.

Antivirals used for viruses other than HIV

Antivirals used for viruses other than HIV include:

1. **amantadine** (*Symmetrel*): used prophylactically) against influenza A in high-risk individuals. It prevents influenza A viruses from the uncoating step necessary for viral replication.

2. **rimantidine** (*Flumadine*): used for treatment and prophylaxis of influenza A. It prevents influenza A viruses from the uncoating step necessary for viral replication.

3. **zanamivir** (*Relenza*): used to limit the duration of influenza A and B infections. It is an inhibitor of the influenza virus surface enzyme called neuraminidase that is needed for release of newly formed influenza viruses from the infected cell.

4. **oseltamivir** (*Tamiflu*): used limit the duration of influenza infections. It is an inhibitor of the influenza virus surface enzyme called neuraminidase that is needed for release of newly formed influenza viruses from the infected cell.

5. **acyclovir** (*Zovirax*): used against herpes simplex viruses (HSV) to treat genital herpes, mucocutaneous herpes in the immunosuppressed, HSV encephalitis, neonatal herpes, and to reduce the rate of recurrences of genital herpes. It is also used against varicella zoster viruses (VZV) to treat shingles). It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

6. **trifluridine** (*Viroptic*): used to treat eye infection (keratitis and conjunctivitis) caused by HSV. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

7. **famciclovir** (*Famvir*): used to treat HSV and VZV infections. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

8. **valacyclovir** (*Valtrex*): used to treat HSV and VZV infections. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

9. **penciclovir** (*Denavir*): used in treating HSV infections. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

10. **gancyclovir** (*Cytovene*; *Vitrasert*): used in treating severe cytomegalovirus (CMV) infections such as retinitis. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

11. **valganciclovir** (*Valcyte*): used in treating severe CMV infections such as retinitis). It chemically resembles a normal DNA nucleoside. Once inserted into the

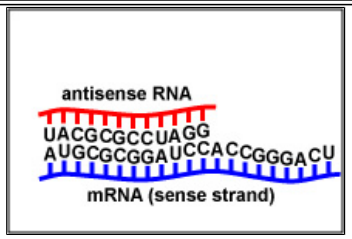
growing DNA chain it inhibits further viral DNA replication.

12. **foscarnet** (*Foscavir*): used in treating severe CMV infections such as retinitis. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

13. **cidofovir** (*Vistide*): used in treating CMV retinitis. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

14. **fomivirsen** (*Vitravene*): used in treating CMV retinitis. Fomivirsen inhibits cytomegalovirus (CMV) replication through an antisense RNA (microRNA or miRNA) mechanism. The nucleotide sequence of fomivirsen is complementary to a sequence in mRNA transcripts **(see Fig. 1)** that encodes several proteins responsible for regulation of viral gene expression that are essential for production of infectious CMV. Binding of fomivirsen to the target mRNA results in inhibition of protein synthesis, subsequently inhibiting virus replication.

Fig. 1: Antisense RNA (microRNA or miRNA)



When an antisense RNA (microRNA or miRNA) that is complementary to a mRNA coding for a particular protein or enzyme binds to the mRNA by complementary base pairing, that mRNA cannot be translated and the protein or enzyme is not made.

15. **ribavirin** (*Copegus; Rebetol; Virazole)*: used in treating severe acute respiratory syndrome (SARS). In combination with other drugs it is used to treat hepatitis C virus (HCV). It chemically resembles a normal RNA nucleoside. Once inserted into the growing RNA chain it inhibits further viral RNA replication.

16. **telaprevir** (*Incivek*) for the treatment of chronic hepatitis C (hepatitis C virus or HCV genotype 1). It is a protease inhibitor that binds to the active site of an HCV-encoded protease and prevent it from cleaving the long polyprotein from polycistronic HCV genes into proteins essential to the structure and function of HCV.

17. **boceprevir** (*Victrelis*) for the treatment of chronic hepatitis C (hepatitis C virus or HCV genotype 1) infection. It is used in combination with peginterferon alfa and ribavirin. Boceprevir is a protease inhibitor that binds to the active site of an HCV-encoded protease and prevent it from cleaving the long polyprotein from polycistronic HCV genes into proteins essential to the structure and function of HCV.

18. **simeprevir** (*Olysio*) for the treatment of chronic hepatitis C (hepatitis C virus or HCV genotype 1) infection. Used in combination with peginterferon alfa and ribavirin. Simeprevir is a protease inhibitor that binds to the active site of an HCV-encoded protease and prevent it from cleaving the long polyprotein from polycistronic HCV genes into proteins essential to the structure and function of HCV.

19. **sofosbuvir** (*Sovaldi*) for the treatment of chronic hepatitis C infection. Used in combination with ribavirin for hepatitis C virus or HCV genotypes 2 and 4; used in combination with peginterferon alfa and ribavirin for HCV genotypes 1 and 4. The second indication is the first approval of an interferon-free regimen for the treatment of chronic HCV infection. Sofosbuvir is a nucleotide polymerase inhibitor that binds to the active site of an HCV-encoded RNA polymerase preventing the synthesis of the viral RNA genome.

20. **lamivudine** (*Epivir-HBV*): used in treating chronic hepatitis B. It chemically resembles a normal DNA nucleoside. Once inserted into the growing DNA chain it inhibits further viral DNA replication.

21. **adefovir dipivoxil** (*Hepsera*): used in treating hepatitis B.

Quiz Group

Antiviral Agents Against HIV

Current anti-HIV drugs include the following (classified by their action):

1. HIV nucleoside-analog reverse transcriptase inhibitors

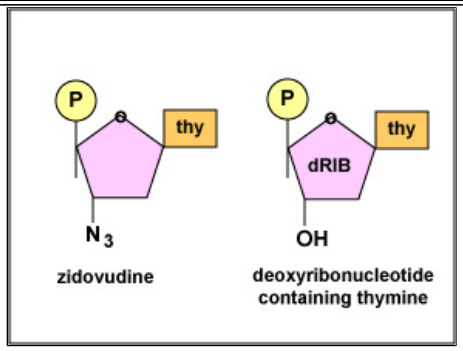
In order to replicate, HIV uses the enzyme reverse transcriptase to make a DNA copy of its RNA genome. A complementary copy of this DNA is then made to produce a double-stranded DNA intermediate which is able to insert into host cell chromosomes to form a provirus.

Most reverse transcriptase inhibitors are nucleoside analogs. A nucleoside is part of the building block of DNA, consisting of a nitrogenous base bound to the sugar deoxyribose but no phosphate group. A nucleoside analog chemically resembles a normal nucleoside.

Once phosphate groups are added by either viral or host cell enzymes, the drugs now **chemically resemble normal DNA nucleotides , the building block**

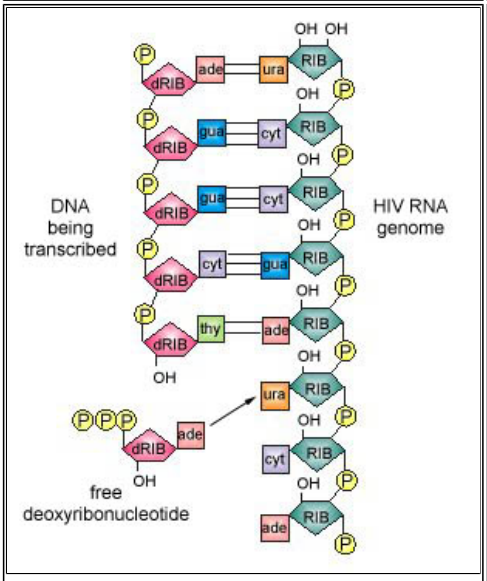
molecules for DNA synthesis. The nucleotide analog binds to the active site of the reverse transcriptase which, in turn, inserts it into the growing DNA strand in place of a normal nucleotide. Once inserted, however, new DNA nucleotides are unable to attach to the drug and DNA synthesis is stopped. This results in an incomplete provirus. For example, zidovudine (AZT, ZDV, *Retrovir*), as shown in **Fig. 1**, resembles the deoxyribonucleotide containing the base thymine. Once zidovudine is inserted into the growing DNA strand being transcribed from the viral RNA by reverse transcriptase, no further nucleotides can be attached **(see Fig. 2A, Fig. 2B , and Fig. 2C)**.

Fig. 1: Illustration of Zidovudine



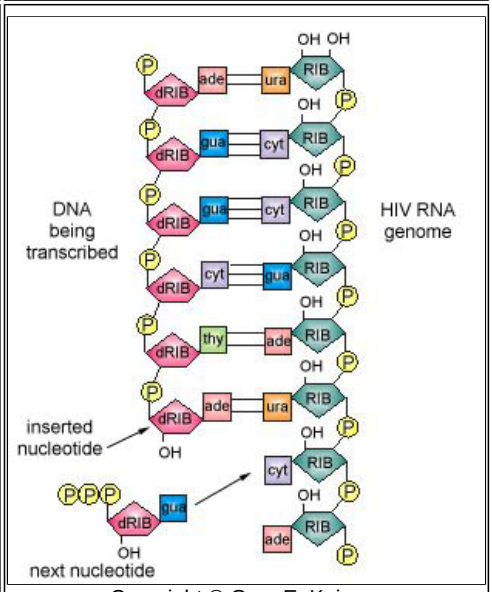
A comparison of zidovudine (AZT, ZDV) and the deoxyribonucleotide containing the base thymine.

Fig. 2A: Illustration of the Action of Zidovudine, Step-1



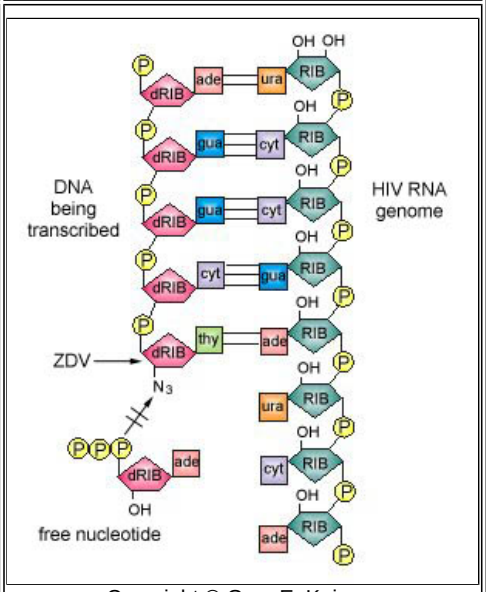
In order for a DNA strand to elongate, the phosphate group of a free deoxyribonucleotide bonds to the hydroxyl (OH) on the 3 prime carbon of the deoxyribose of the last deoxyribonucleotide in the strand.

Fig. 2B: Illustration of the Action of Zidovudine, Step-2



To see how zidovudine interferes with this process, see Step-3.

Fig. 2C: Illustration of the Action of Zidovudine, Step-3



Zidovudine (ZDV, AZT) has an azide (N_3) group instead of a hydroxyl (OH) group on its pentose sugar. Once the phosphate group of the zidovudine bonds to OH of the last deoxyribonucleotide in the strand, no further free deoxyribonucleotides can attach. (The phosphate groups of free deoxyribonucleotides can only bond to OH groups, they are unable to bond to N_3groups.) This results in an incomplete provirus.

For more information: Review of Deoxyribonucleic Acid DNA
For more information: Review of DNA Replication

Flash Animation showing normal transcription of DNA from the RNA genome of HIV
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing normal transcription of DNA from the RNA genome of HIV
In order for a DNA strand to elongate, the phosphate group of a free deoxyribonucleotide bonds to the hydroxyl (OH) on the 3' carbon of the deoxyribose of the last deoxyribonucleotide in the strand.

Flash Animation showing the action of ZDV in causing the formation of an incomplete provirus
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the action of ZDV in causing the formation of an incomplete provirus
Zidovudine (ZDV, AZT) has an azide (N_3) group instead of a hydroxyl (OH) group on its pentose sugar. Once the phosphate group of the zidovudine bonds to OH of the last deoxyribonucleotide in the strand, no further free deoxyribonucleotides can attach. (The phosphate groups of free deoxyribonucleotides can only bond to OH groups, they are unable to bond to N_3 groups.) This results in an incomplete provirus.

Examples of nucleoside reverse transcriptase inhibitors include:

a. **zidovudine** (AZT; ZDV; *Retrovir*)

b. **didanosine** (ddI; dideoxyinosine; *Videx*)

c. **stavudine** (d4T; *Zerit*)

d. **lamivudine** (3TC; *Epivir*)

e. **abacavir** (ABC; *Ziagen*)

f. **emtricitabine** (*FTC; Emtriva*, Coviracil)

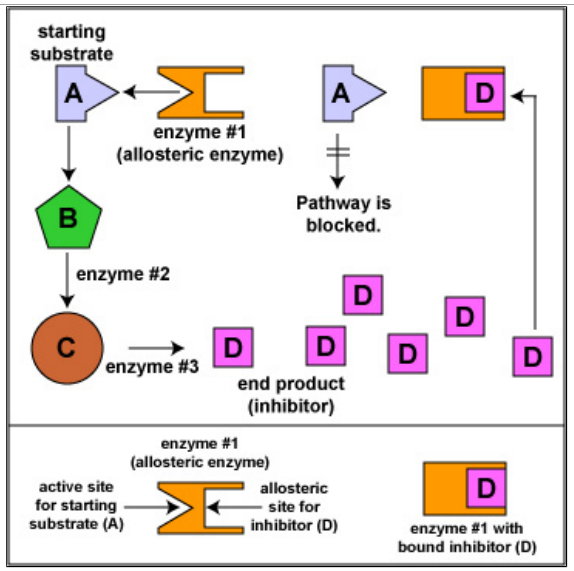
2. Nucleotide Reverse Transcriptase Inhibitors (NtRTIs)

A NtRTI inhibitor is a nucleotide analog. A nucleotide is the building block of DNA, consisting of a nitrogenous base bound to the sugar deoxyribose, and a phosphate group. A nucleotide analog chemically resembles a normal nucleotide. The nucleotide analog binds to the active site of the reverse transcriptase which, in turn, inserts it into the growing DNA strand in place of a normal nucleotide. Once inserted, however, new DNA nucleotides are unable to attach to the drug and DNA synthesis is stopped. This results in an incomplete provirus. An example of nucleoside reverse transcriptase inhibitor is tenofovir (TDF;*Viread*).

3. HIV Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

These drugs do not resemble regular DNA building blocks. They **bind to an allosteric site that regulates reverse transcriptase activity** rather than to the enzyme's active site itself as do the above nucleoside analogues **(see Fig. 3)**. This also prevents HIV provirus formation.

Fig. 3: Illustration of Noncompetitive Inhibition with Allosteric Enzymes



When the end product (inhibitor) of a pathway combines with the allosteric site of the enzyme, this alters the active site of the enzyme so it can no longer bind to the starting substrate of the pathway. This blocks production of the end product.

a. **nevirapine** (NVP*; Viramune*)

b. **delavirdine** (DLV;*Rescriptor*)

c. **efavirenz** (EFV; *Sustiva*)

d. **rilpivirine** (*Edurant*)

e. **etravirine** (ETR, TMC125; *Intelence*)

Flash animation showing the normal function of an allosteric enzyme
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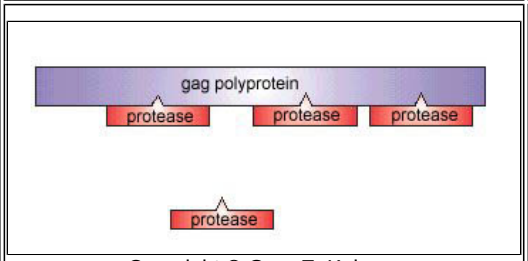
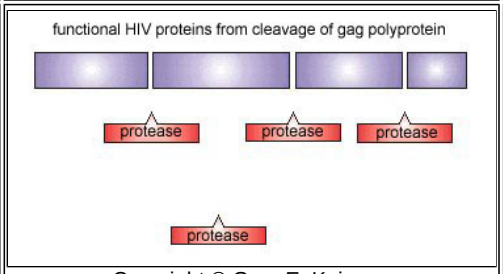
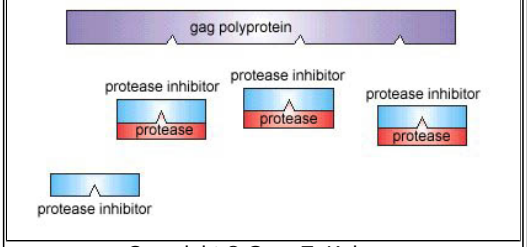
html5 version of animation for iPad showing the normal function of an allosteric enzyme.
An allosteric enzyme has both an active site for its substrate and an allosteric site for an inhibitor of the enzyme. In the absence of enzyme's inhibitor, the substrate is able to bind to the enzyme's active site and end products are produced. If the inhibitor is present, it will bind to the enzyme's allosteric site. This, in turn, alters the enzyme's active site so it can no longer bind its substrate and no end products are produced.

Flash animation showing the action of an inhibitor on an allosteric enzyme
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the action of an inhibitor on an allosteric enzyme
An allosteric enzyme has both an active site for its substrate and an allosteric site for an inhibitor of the enzyme. In the absence of enzyme's inhibitor, the substrate is able to bind to the enzyme's active site and end products are produced. If the inhibitor is present, it will bind to the enzyme's allosteric site. This, in turn, alters the enzyme's active site so it can no longer bind its substrate and no end products are produced.

3. HIV Protease Inhibitors (PIs)

In order for maturation of HIV to occur, a HIV enzyme termed a protease has to cleave a long HIV-encoded gag-pol polyprotein to produce reverse transcriptase and integrase (coded by the HIV *pol* gene) and gag polyprotein (coded by the HIV *gag* gene). The HIV protease then cleaves the gag polyprotein into capsid protein p17, matrix protein p24, and nucleocapsid protein p7, as well as proteins p6, p2, and p1 whose functions are not yet fully understood **(see Figs. 4A, 4B, and 4C)**. Proteases also cleave the env-polyprotein (coded by the HIV *env* gene) into the envelope glycoproteins gp120 and gp41 **(see Fig. 5)**. Protease inhibitors are drugs that bind to the active site of this HIV-encoded protease and prevent it from cleaving the long gag-pol polyprotein and the gag polyprotein into essential proteins essential to the structure of HIV and to RNA packaging within its nucleocapsid **(see 4C)**. As a result, viral maturation does not occur and noninfectious viral particles are produced.

Fig. 4A: Illustration of HIV Protease Activity, Step-1	Fig. 4B: Illustration of HIV Protease Activity, Step-2	Fig. 4C: Illustration of HIV Protease Activity, Step-3
 Copyright © Gary E. Kaiser	 Copyright © Gary E. Kaiser	 Copyright © Gary E. Kaiser
HIV must use a HIV encoded enzyme called protease in order to cleave a large Gag-Pol polyprotein (gp120), a Gag polyprotein (p55), and an Env polyprotein (gp160) into functional proteins essential to the structure of HIV and to its RNA packaging. The active site of the HIV protease binds to the polyproteins and cleaves them into functional proteins (see Step-2). The Gag polyproteins (p55) will eventually be cleaved by HIV proteases to become HIV matrix proteins (MA; p17), capsid proteins (CA; p24), and nucleocapsid proteins (NC, p7). The Gag-Pol polyproteins (p160) will eventually be cleaved to become HIV matrix proteins (MA; p17), capsid proteins (CA; p24), proteinase molecules (protease or PR; p10), reverse transcriptase molecules (RT; p66/p51), and integrase molecules (IN; p32). The Env polyproteins (gp160) will eventually be cleaved to become HIV envelope glycoproteins gp120 and gp41.	Once the active site of the HIV protease binds to the polyproteins, it cleaves them into functional proteins.	Protease inhibitors bind to the active site of the HIV protease and prevent the enzyme from attaching to its substrate and cleaving HIV polyproteins into functional proteins. As a result, HIV can not mature and noninfectious viruses are produced.

Flash animation showing the normal function of an HIV protease
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the normal function of an HIV protease
HIV must use a HIV encoded enzyme called protease in order to cleave a large *Gag-Pol* polyprotein (gp120), a *Gag* polyprotein (p55), and an *Env* polyprotein (gp160) into functional proteins essential to the structure of HIV and to its RNA packaging. The active site of the HIV protease binds to the polyproteins and cleaves them into the functional proteins needed for viral replication and maturation. The *Gag* polyproteins (p55) will eventually be cleaved by HIV proteases to become HIV matrix proteins (MA; p17), capsid proteins (CA; p24), nucleocapsid proteins (NC, p7), and protein p6. The *Gag-Pol* polyproteins (p160) will eventually be cleaved to become HIV matrix proteins (MA; p17), capsid proteins (CA; p24), proteinase molecules (protease or PR; p10), reverse transcriptase molecules (RT; p66/p51), and integrase molecules (IN; p32). The *Env* polyproteins (gp160) will eventually be cleaved to become HIV envelope glycoproteins gp120 and gp41.

Flash animation showing the action of protease inhibitors
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the action of protease inhibitors.

Protease inhibitors bind to the active site of the HIV proteases and prevent the enzymes from attaching to its substrate and cleaving the HIV *gag*, *gag-pol*, and *env* polyproteins into functional proteins. As a result, HIV can not mature and non-infectious viruses are produced.

Protease inhibitors include:

a. **saquinavir** (SQV; *Inverase*)

b. **ritonavir** (RTV; *Norvir*)

c. **idinavir** (IDV; *Crixivan*)

d. **nelfinavir** (NFV; *Viracept*)

e. **amprenavir** (APV; *Agenerase*)

f. **atazanavir** (ATV; *Reyataz*)

g. **fosamprenavir** (FPV; *Lexiva*)

h. **ritonavir** (RTV; *Norvir*)

i. **darunavir** (DRV; TMC114; *Prezista*)

j. **tipranavir** (TPV; *Aptivus*)

Animation of a protease inhibitor blocking maturation of HIV

Courtesy of HHMI's Biointeractive.

4. Entry Inhibitors (EIs)

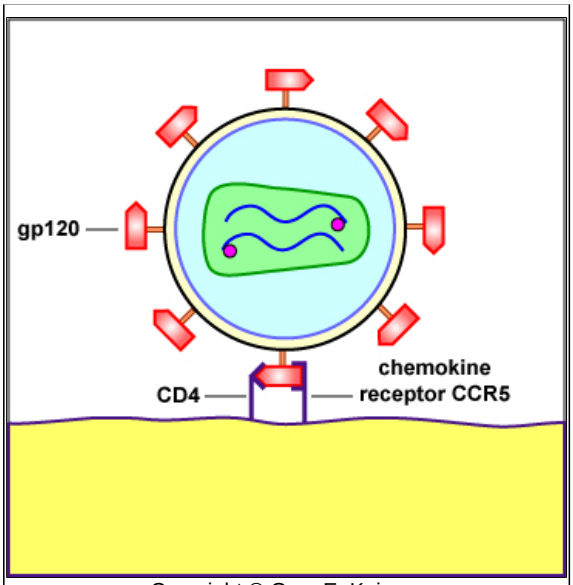
EIs are agents interfering with the entry of HIV-1 into cells. During the adsorption and penetration stages of the life cycle of HIV, a portion or domain of the HIV surface glycoprotein gp120 binds to a CD4 molecule on the host cell. This induces a change in shape that brings the chemokine receptor binding domains of the gp120 into proximity with the host cell chemokine receptor. This brings about another conformational change that exposes a previously buried portion of the transmembrane glycoprotein gp41 that enables the viral envelope to fuse with the host cell membrane. EIs interfere with various stages of this process.

a. **Agents that block the binding of gp120 to host chemokine receptor 5 (CCR5**).

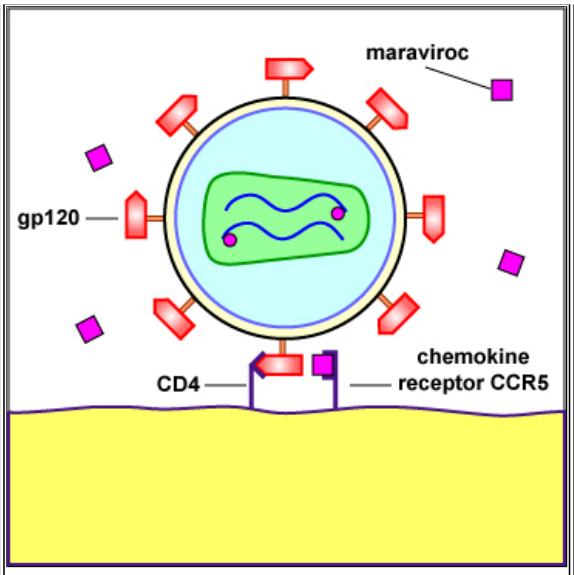
After the gp120 on the envelope of HIV binds to a CD4 molecule on the host cell, it must then also bind to a co-receptor - a chemokine receptor. CCR5-tropic strains of HIV bind to the chemokine receptor CCR5 **(see Fig. 6)**. (An estimated 50%-60% of people having previously received HIV medication have circulating CCR5-tropic HIV.)

maraviroc (MVC; *Selzentry*; *Celsentri*) is a chemokine receptor binding blocker that **binds to CCR5 and blocks the attachment of gp120 thus blocking HIV adsorption to the host cell**.

Fig. 6A: Illustration of HIV gp120 binding to a CD4 molecule and CCR5 co-receptor	Fig. 6B: Illustration of the Action of Maraviroc in Blocking CCR5



M-tropic strains of HIV bind to first to a CD4 molecule and then to a chemokine co-receptor called CCR5. This binding to dual receptors is necessary for the virus to enter the host cell.



The drug maraviroc (*Selzentry*) blocks the CCR5 co-receptor on host cells so that HIV cannot adsorb to the cell.

b. **Agents that block the fusion of the viral envelope with the cytoplasmic membrane of the host cell**.

enfuvirtide (ENF; T-20; *Fuzeon*) binds a gp41 subunit of the viral envelope glycoprotein and **prevents the conformational changes required for the fusion of the viral envelope with the cellular cytoplasmic membrane**.

5. Integrase Inhibitors

Integrase inhibitors **disable HIV integrase, the enzyme that inserts the HIV double-stranded DNA intermediate into host cell DNA**. It **prevents production of a provirus**.

raltegravir (*Isentress*)

6. Fixed-dose combinations

Tablets containing two or more anti-HIV medications:

1. abacivir + lamivudine (*Epzicom*)
2. abacivir + lamivudine + zidovudine (*Trizivir*)
3. efavirenz + emtricitabine + tenofovir DF (*Atripla*)
4. emtricitabine + tenofovir DF (*Truvada*)
5. lamivudine + zidovudine (*Combivir*)

Cytokines

Certain antiviral cytokines called type-1 interferons have been produced by recombinant DNA technology and several are used to treat certain severe viral infections. These include:

1. **recombinant interferon alfa-2a** (*Roferon-A*): a cytokine used to treat Kaposi's sarcoma, chronic myelogenous leukemia, and hairy cell leukemia.
2. **peginterferon alfa-2a** (*Pegasys*) : used to treat hepatitis C (HCV).
3. **recombinant interferon-alpha 2b** (*Intron A*): a cytokine produced by recombinant DNA technology and used to treat Hepatitis B; malignant melanoma, Kaposi's sarcoma, follicular lymphoma, hairy cell leukemia, warts, and Hepatitis C.
4. **peginterferon alfa-2b** (*PEG-Intron; PEG-Intron Redipen*): used to treat hepatitis C (HCV).

5. **recombinant Interferon alfa-2b plus the antiviral drug ribavirin** (*Rebetron*): used to treat hepatitis C (HCV).
6. **recombinant interferon-alpha n3** (*Alferon N*): used to treat warts.
7. **recombinant iInterferon alfacon-1** *(Infergen*) : used to treat hepatitis C (HCV).

Most of the current antiviral agents don't kill and eliminate the viruses, but rather inhibit their replication and decrease the severity of the disease. As with other microbes, **resistant virus strains can emerge** with treatment.

Since there are no antiviral drugs for the vast majority of viral infections and most of the drugs that are available are only partially effective against limited types of viruses, to control viruses, we must **rely on the body's immune responses**. As will be seen in detail in Units 5 and 6, the immune responses include innate immunity as well as adaptive immunity (antibody production and cell-mediated immunity). Adaptive immunity can be either naturally acquired or, in some cases, artificially acquired.

For a more detailed description of any specific antimicrobial agent, see the website of RxList - The Internet Drug Index.

Concept Map for Antiviral Agents

Self Quiz for Antiviral Agents

Quiz Group

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Overview of viral infections
GENERAL CATEGORIES OF VIRAL INFECTIONS

Viruses

General Categories of Viral Infections

Fundamental Statement for this Softchalk Lesson:

1. Acute infections are of relatively short duration with rapid recovery.
2. Persistent infections are where the viruses are continually present in the body.
3. In a latent viral infection the virus remains in equilibrium with the host for long periods of time before symptoms again appear, but the actual viruses cannot be detected until reactivation of the disease occurs.
4. In a chronic virus infection, the virus can be demonstrated in the body at all times and the disease may be present or absent for an extended period of time.
5. Slow infections are ones in which the infectious agents gradually increase in number over a very long period of time during which no significant symptoms are seen.

Detailed Learning Objectives

1. Describe and give an example of an acute viral infection, a late complication following an acute infection, a latent viral infection, a chronic viral infection, and a slow viral infection.

General Categories of Viral Infections

Most viruses that infect humans, such as those that cause routine respiratory infections (e.g., cold viruses, influenza viruses) and gastrointestinal infections (e.g., Rotaviruses, Noroviruses), cause acute infections. **Acute infections** are of relatively short duration with rapid recovery.

In **persistent infections**, the viruses are continually present in the body. Some persistent infections are **late complications following an acute infection** and include subacute sclerosing panencephalitis (SSPE) that can follow an acute measles infection and progressive encephalitis that can follow rubella. Other persistent infections are known as **latent viral infection**. In a latent viral infection the virus remains in equilibrium with the host for long periods of time before symptoms again appear, but the actual viruses cannot be detected until reactivation of the disease occurs. Examples include infections caused by HSV-1 (fever blisters), HSV-2 (genital herpes), and VZV (chickenpox-shingles). In the case of **chronic virus infections**, the virus can be demonstrated in the body at all times and the disease may be present or absent for an extended period of time. Examples include hepatitis B (caused by HBV) and hepatitis C (caused by HCV). **Slow infections** are ones in which the infectious agents gradually increase in number over a very long period of time during which no significant symptoms are seen. Examples include AIDS (caused by HIV-1 and HIV-2) and certain lentiviruses that cause tumors in animals. Although not viruses, prions also cause slow infections.

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- Adenoviruses

- Herpes Simplex
- Varicella-Zoster Virus
- Cytomegalovirus
- Hepatitis B
- Enteroviruses
- Rhinoviruses
- Rubella
- Hepatitis C
- Measles
- Influenza
- HIV Infection and AIDS

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