

Introduction to Viroids and Prions

Viroids – Viroids are plant pathogens made up of short, circular, single-stranded RNA molecules (usually around 246-375 bases in length) that are not surrounded by a protein coat. They have internal base-pairs that cause the formation of folded, three-dimensional, rod-like shapes. Viroids apparently do not code for any polypeptides (proteins), but do cause a variety of disease symptoms in plants. The mechanism for viroid replication is not thoroughly understood, but is apparently dependent on plant enzymes. Some evidence suggests they are related to introns, and that they may also infect animals. Disease processes may involve RNA-interference or activities similar to those involving mi-RNA.

Prions – Prions are proteinaceous infectious particles, associated with a number of disease conditions such as **Scrapie** in sheep, **Bovine Spongiform Encephalopathy (BSE)** or Mad Cow Disease in cattle, **Chronic Wasting Disease (CWD)** in wild ungulates such as muledeer and elk, and diseases in humans including **Creutzfeld-Jacob disease (CJD)**, **Gerstmann-Straussler-Scheinker syndrome (GSS)**, **Alpers syndrome** (in infants), **Fatal Familial Insomnia (FFI)** and **Kuru**. These diseases are characterized by loss of motor control, dementia, paralysis, wasting and eventually death.

Prions can be transmitted through ingestion, tissue transplantation, and through the use of contaminated surgical instruments, but can also be transmitted from one generation to the next genetically. This is because prion proteins are encoded by genes normally existing within the brain cells of various animals. Disease is caused by the conversion of normal cell proteins (glycoproteins) into prion proteins. Apparently the amino acid sequences of normal and infective proteins are the same, but prions have a unique three-dimensional configuration that is induced by the presence of other prions. Prions enter animal cells through endocytosis and accumulate within lysosomes, but are not digested. They cause the formation of amyloid plaques within brain tissues. The prion protein configuration is unusually stable, but not functional in the usual sense. By converting normal brain proteins into more prions, the infective prions ultimately destroy brain function.

Prions are potentially very dangerous because they remain stable within the environment for an extended period of time. They are resistant to denaturation by exposure to heat, radiation or toxic chemicals. Feed lots used to maintain stock animals have become contaminated by prions (through contaminated feed) and have remained infectious for years. Although infection with prion proteins is almost always fatal, researchers are working to develop therapeutic agents and a vaccine has been developed to prevent prion disease in mice. This may eventually lead to a similar vaccine for humans.

(See Internet links for more information)

Introduction to Viruses

The term **virus** is Latin for poison, and historically, the name was applied to any agent that caused illness in humans. Currently, the term virus applies to a specific type of microbial agent. A virus is a non-cellular entity made up of a **nucleic acid core** (DNA or RNA) surrounded by a protein coat. Viruses are obligate intracellular parasites (**hypotrophs**) and probably infect every type of living organism. Most viruses tend to be host specific, and are often categorized according to the type of organisms they infect. They are generally much smaller than cells, but they vary considerably in size. A complete virus particle as it exists outside of its host is called a **virion**, and is the infective form of a virus. The origin of viruses is not entirely clear, and it is possible that they came from cells. **Virology**, the science or study of viruses, is a relatively new science because electron microscopes were required to observe viruses.

A virus that infects bacteria is called a **bacteriophage** (meaning bacteria eater), and a virus that infects *E. coli* is called a **coliphage**.

Virion Structure in Greater Detail:

Though viruses vary considerably in size and structure, they typically have common components, e.g., a nucleic acid core and a protein coat or capsid. Some viruses have additional structures as described below.

- A. The **nucleic acid core** or **genome** of a virus can be either DNA or RNA (double or single-stranded), but viruses rarely contain both DNA and RNA (the Mimivirus of *Acanthamoeba polyphaga* and certain human herpesviruses carry m-RNA molecules). In viruses that contain double-stranded RNA the two strands are **complimentary** and **antiparallel** just as they are in DNA. The viral genome may be either a closed loop or linear, and may be made up of one or many segments. Viral genomes range in size from around 4 to over 400 genes.
- B. The protein coat surrounding the nucleic acid core of a virus is called a **capsid** and is made of units called **capsomers**. These are visible with an electron microscope and are often triangular in shape. Capsomers are made up of smaller units called **protomers** that are protein complexes. Many viral capsids are **polyhedrons** that are nearly spherical in shape, but some are shaped like long, slender rods.
- C. Some viruses have an additional layer called an **envelope** outside their protein capsid. This is made of protein and lipids like those found in cell membranes (lipid bilayer with integral and peripheral proteins). A viral envelope is formed from a section of cell membrane taken from the host cell as the virus leaves.
- D. A **tail assembly** is another viral structure that may or may not be present. Viruses known as T-even coliphages have very complex tail assemblies that attach to the **nucleocapsid** (capsid and nucleic acid core together). Just below the nucleocapsid is a **collar**, and below that the **tail core** surrounded by a **tail sheath**. At the base of the tail core is a structure called a **base plate**, equipped with **tail pins** (short, angular segments extending downward from the base plate) and **tail fibers** (long thin structures resembling spider legs).

Regardless of their structure, all viruses tend to have a similar strategy; that is:

1. Gain access to a host.
2. Reproduce within that host cell.
3. Get out.

If a virus gets into a cell, it will often disrupt the cell's activity, sometimes causing death. If the cell is a bacterium it may be lysed. If a virus gets into a multicellular organism such as a human, the damage it causes depends on what cells it infects and how many of these are killed. Host defense mechanisms influence the outcome. In some cases the entire organism dies.

Bacteriophages are often used as models for virus activity because they are readily available and not hazardous to humans. Bacteria are easy to grow in vitro, and the viruses that infect them are easily obtained. Bacteriophages can be divided into two categories based on how they affect their host, these are cytolitic and temperate phages.

- a. A **cytolitic bacteriophage** is one that causes cell lysis, so kills its host.
- b. A **temperate** (avirulent or asymptomatic) bacteriophage does not kill its host (at least not right away), and may become a **prophage**, i.e., may incorporate itself into the chromosome of its host.

Cytolytic Bacteriophage Life Cycle:

A group of viruses known as T-even coliphages (T stands for type, and the T-even phages include T2, T4 and T6) can be used to illustrate a typical virus life cycle. These viruses are cytolitic like the phages T2 and X174 viewed in the laboratory. The life cycle of a T-even phage can be divided into stages or phases as indicated below, and begins with a free **virion** outside the host cell.

1. **Adsorption** – The binding of a virus to the cell surface of its host is called **adsorption**. This step involves interaction between the tail fibers of the virus and receptors on the host cell surface (viruses are host specific due to this interaction). The virion can only attach to a specific type of cell, and can move around until each tail fiber is contacting a receptor site on the cell surface. When all tail fibers are attached, they flex and allow the tail core to snap down so that the tail pins are touching the cell surface. Once the tail pins attach, the virus is not likely to come off.
2. **Penetration** – During the penetration stage, the viral nucleic acid core enters the host cell. The tail sheath proteins undergo a molecular reconfiguration and the tail core penetrates the cell surface. The capsid and tail assembly are just packaging and a method of transmitting the virus to a new cell. During penetration, the nucleic acid core enters the cell leaving the other parts behind on the outside.
3. **Latent period** (or eclipse phase) – During the latent or eclipse period, the viral nucleic acid is transcribed and translated. The virus is dependent on the host cells ribosomes for the translation process because the virus does not have any. Transcription may involve viral enzymes after these have been made, but is initially dependent on host enzymes (unless viral enzymes have been packaged within the capsid). The viral genome is transcribed in segments or in a sequence as follows:

- a. **Immediate early phage genes** – These genes encode enzymes. Some of these preferentially transcribe viral genes, and others chop up the host cell's DNA. This provides a large quantity of nucleotides that are then available to make viral DNA strands. This also prevents the cell from carrying on its own DNA related activity.
 - b. **Delayed early phage genes** – These genes encode enzymes used to replicate the viral genome. The viral DNA may be reproduced 200 times or more.
 - c. **Late phage genes** – These genes encode the proteins needed to build the viral **capsid** and the **tail assembly**. Since during replication the viral DNA may experience mutation, the proteins encoded by the various genomes may not be identical. This can result in variation within the virus population.
4. **Assembly or maturation** – During the assembly phase, the viral components are assembled (put together) to form new virions. Some of the proteins come together by themselves and others require assistance from other proteins called **chaperonins** or chaperonin proteins.
 5. **Release or liberation** – During the liberation phase the cell is lysed and new virions are released into the environment. This process may involve viral enzymes that soften up the cell wall. The new virions are now free to infect other cells.

The length of time required for viral replication is variable, and is dependent on factors influencing metabolism within the host. For bacteria grown in a batch culture where fission can occur every 20 minutes, bacteriophage replication tends to be rapid. A single virus particle might cause the destruction of an entire population within a few hours. Restriction enzymes within the host cells provide the main line of defense for the bacteria.

Cytolytic bacteriophages are used clinically in some countries because they can kill bacteria that would otherwise be damaging to humans. In situations where antibiotics are not available, or are too expensive, bacteriophage cultures provide an alternative method for controlling gastrointestinal infections caused by *E. coli*, *Salmonella*, *Vibrio* and other infectious agents.

According to a recent report (October, 2006) the FDA has approved the use of a bacteriophage preparation from the Netherlands, sold as LISTEX, to control *Listeria monocytogenes* in cheese and poultry meat products. Bacteria in the genus *Listeria* are psychrophiles capable of growing under refrigerator conditions, and commonly associated with poultry, especially turkeys. They can cause potentially fatal listeriosis, a disease usually causing gastrointestinal symptoms (diarrhea, fever, malaise), but can also cause neurological symptoms (encephalitis and meningitis).

Temperate Phage (Bacteriophage lambda):

Temperate phages (also called **avirulent** or **asymptomatic** phages) are those that do not kill their host cell (at least not right away). These bacteriophages can reside within the host without causing damage, and may become incorporated into the host chromosome, i.e., can exist as **prophages**.

A temperate phage known as **Bacteriophage lambda** is one example of a bacteriophage that can become a prophage, but can also revert to a lytic life cycle. This virus has a

double-stranded DNA genome that may be represented as a loop being closed by overlapping cohesive termini. (These cohesive termini are sometimes referred to as cos-sites, and are the basis for the name **cosmid** applied to cloning vectors made from bacteriophage lambda as described earlier.) The cohesive termini allow the phage genome to enter into the chromosome of the host at a specific location (i.e., an endonuclease cut site that would have complimentary cohesive termini). The viral genome has numerous genes, and the regulation of these genes is somewhat complex. The short version of the regulation is as follows:

- a. When the bacteriophage lambda genome enters the host cell, it is initially not committed to either a lytic or a lysogenic pathway. Under certain circumstances, a portion of the viral genome known as the **cI gene** is expressed. This gene codes for a **repressor protein** that can block the transcription of the viral lytic genes. (Recall operon function - an active repressor protein will bind to the operator site of an operon and block the transcription of the structural genes in that operon.) As long as the repressor protein is present, the expression of lytic genes is repressed, and the virus will not be able to complete a lytic cycle. Other genes on the viral genome code for enzymes that allow the virus DNA to integrate itself into the host chromosome. When these are expressed, the viral genome can enter the chromosome and become a prophage.
- b. Whether the viral genome stays within the host chromosome or not is determined by a variety of factors, one being the activation of a host gene called **RecA**. The RecA gene of *E. coli* codes for a **proteolytic enzyme** that catalyses the cleavage and inactivation of the cI repressor protein. The RecA gene is activated when the *E. coli* cells are **stressed**, i.e., when they are exposed to stressful environmental conditions such as exposure to ultra violet light or toxic chemicals. Thus, host cells that are stressed produce a protein (coded for by the RecA gene) that ultimately causes the viral genome to exit the host chromosome and revert to a lytic cycle. In a sense, the dying cell releases the viral genome (genetic information) much as passengers in a sinking ship might release a message in a bottle.

Temperate phages are associated with a condition or phenomenon known as lysogeny. **Lysogeny** occurs when a temperate phage (the genome of a virus) establishes a stable, non-lytic relationship with its host cell. In most cases the viral genome enters into the chromosome of the host and is then referred to as a **prophage**. This viral DNA will be copied along with the host chromosome and maintained within the progeny population. In other instances, the viral genome is maintained outside the chromosome as a plasmid. The presence of viral genes within a bacterial population will sometimes result in **phenotypic changes**, i.e., the phenotype of the bacteria is influenced by the expression of viral genes. This phenomenon is referred to as **lysogenic conversion** (sometimes as **bacteriophage conversion**). A characteristic of bacteria that is often attributed to the expression of viral genes is **toxin production**. Bacteria such as *Clostridium tetani*, *Clostridium botulinum*, *Corynebacterium diphtheriae* and other bacteria that frequently produce potent toxins, are expressing viral genes. Thus the Latin meaning for the term virus (poison) is quite appropriate.

A bacterium that is carrying a prophage may be referred to as a **lysogenic cell**. If we consider the origin of this term, it had to do with bacteria populations that appeared to initiate self-destruction under certain circumstances, i.e., they generated their own lysis

(Lyso = lysis, and genic = generation). These lysogenic bacteria, when grown under laboratory conditions, would appear to generate their own lysis when subjected to stressful conditions (exposure to toxic substances, ultra violet light, etc.) As it turns out, the viral genome plays a significant role in this lytic process as explained above.

Viral Classification:

Viruses are non-cellular entities that reproduce only when within living host cells and most viruses are host specific; for this reason, viruses are often categorized according to the type of host they infect. Those infecting tomatoes, corn, beans, etc. can be called plant viruses and those infecting cats, horses and humans are called animal viruses. Viruses that infect bacteria are called bacteriophages (bacteria eaters), or sometimes just phages. Since the presence of viruses within their host often results in the development of disease symptoms, many viruses were initially named according to the diseases they caused. Plant viruses were named for the most common, first recognized or most important type of host plant they infected and for the primary symptoms they caused under natural conditions; for example, tobacco mosaic virus, barley yellow dwarf virus and apple chlorotic leafspot virus. Animal viruses were initially classified in a similar manner, but this was problematic because some are not host-specific. Thus although human wart viruses infect only humans, influenza viruses can infect humans, water fowl and swine, and the rabies virus infects a wide variety of mammals.

During the 1960s, virologists proposed that all viruses should be classified according to their own, shared properties rather than according to the organisms they infected, the symptoms they caused or other properties relating to their hosts. As a consequence, two classification systems were developed: The **Hierarchical virus classification system** and the **Baltimore Classification System**.

The Hierarchical virus classification system :

This system, proposed by A. Lwoff, R. W. Horne, and P. Tournier (1962) and modified by the International Committee on Taxonomy of Viruses (ICTV), bases viral classification on four main characteristics:

1. Nature of the nucleic acid: RNA or DNA
2. Symmetry of the capsid
3. Presence or absence of an envelope
4. Dimensions of the virion and capsid

Currently, this viral classification scheme is most important from the family-level down since higher taxa (phyla, classes, orders) are still being determined. In general, virus families are determined through genomics and proteomics, i.e., the elucidation of evolutionary relationships through the analysis of nucleic acid and amino acid sequence similarities. The names of virus families contain the suffix – viridae, e.g., Retroviridae, Rhabdoviridae, Reoviridae, and genera include the suffix – virus, e.g., enterovirus, cardiovirus, rhinovirus, etc. The definition of ‘species’ is difficult but usually includes a group of viruses sharing the same genetic information and host range, e.g., human immunodeficiency virus (HIV). Viral subspecies or strains are indicated by number, e.g., HIV-1 and HIV-2.

The Baltimore Classification system: (Not included on Exam 2)

The Baltimore classification system (David Baltimore - 1971) is based on the viral **genome**

composition and viral strategy for gene expression. By convention, m-RNA is designated as positive-sense or positive-strand RNA. DNA with the same sequence written in the 5' – 3' direction is positive-sense or positive-strand DNA. Complimentary sequences are designated as negative-sense or negative-strand RNA or DNA. All viral genomes, whether DNA or RNA, generate m-RNA molecules that allow for the production of viral proteins and facilitate viral replication. The precise mechanisms involved differ for each virus family. According to the Baltimore system of classification, all viruses can be classified into seven (arbitrary) groups as follows:

- I: Double-stranded DNA** (Adenoviruses; Herpesviruses; Poxviruses, etc)
These viruses function like cellular DNA in that the strands separate, and the negative-sense, DNA strand codes for m-RNA. Some of these replicate in the nucleus, e.g., adenoviruses, using cellular enzymes. Poxviruses replicate in the cytoplasm and make their own enzymes for nucleic acid replication.
- II: Single-stranded positive-sense DNA** (Parvoviruses)
These viruses replicate within the nucleus to form complimentary, negative-sense DNA strands. These then serve as templates for positive-strand m-RNA and DNA synthesis.
- III: Double-stranded RNA** (Reoviruses; Birnaviruses)
These viruses have segmented genomes. Each negative-strand, genome segment is transcribed separately to produce monocistronic m-RNA molecules.
- IV: Single-stranded positive-sense RNA** (Picornaviruses; Togaviruses, etc)
These viruses enter their host in a form that can be translated directly (genomic RNA is m-RNA), and occur in two forms:
 - a) Polycistronic m-RNA, e.g., Picornaviruses (Hepatitis A), where naked RNA is infectious and does not code for viral RNA-polymerase. Translation results in the formation of a polyprotein product that is later cleaved to form mature proteins.
 - b) Complex Transcription, e.g., Togaviruses, where two or more rounds of translation are necessary to produce genomic-RNA.
- V: Single-stranded negative-sense RNA** (Orthomyxoviruses, Rhabdoviruses, etc).
These viruses require RNA-polymerase enzymes directed toward viral RNA specifically, and occur in two forms:
 - a) Segmented, e.g., Orthomyxoviruses, where the first step in replication involves transcription of the negative-sense RNA genome by viral RNA-dependent RNA polymerase enzymes. This results in the formation of monocistronic m-RNA molecules that serve as the template for genome replication.
 - b) Non-segmented, e.g., Rhabdoviruses, where replication occurs as above and monocistronic m-RNAs are formed.
- VI: Single-stranded positive-sense RNA with DNA intermediate in life-cycle** (Retroviruses)
These viruses have a positive-sense genome, but are unique in that it is **diploid** (occurs as two copies), and does not serve as m-RNA. The viral genome (RNA) must be reverse transcribed to form DNA before new copies of viral genome can be generated.

VII: **Double-stranded DNA with RNA intermediate** (Hepadnaviruses)

These viruses also rely on reverse transcription, but unlike Retroviruses, form DNA inside the virus particle upon maturation. When the virion enters a host cell, the first thing it does is repair the gapped genome (DNA) which then serves as the template for transcription.

The discovery of new virus types has required some revision to this system, but it remains the most commonly used system for viral classification. In addition, this system provides students with an overview of variable strategies for gene expression. If you can imagine a new and unique method for storing and accessing information involving nucleic acids, there is probably some type of virus already using it. Aren't they amazing?

A Brief Look at Plant and Animal Viruses: (This is potential test material)

Viruses that infect plants (also those infecting algae and fungi) must cross a rather formidable barrier represented by the cell wall surrounding these cells. In the case of plant viruses, this barrier is passed with the aid of biting arthropods (mites, insects), nematodes, snails, etc. that feed on plant tissues. The same is probably true for viruses infecting fungi and algae.

Animal viruses have an easier access to their host cells because animal cells do not have cell walls. In most cases, animal viruses enter their hosts via **endocytosis**. Following **adsorption**, the host cell membrane invaginates (folds inward) taking the virus particle into the cytoplasm enclosed within a membranous bubble or vacuole. Enveloped viruses enter by means of a slightly different mechanism. The envelope of the virus (being composed of cell membrane materials) fuses with the membrane surface and allows the virus to enter the cell without being enclosed in a vacuole. (Note - the term **viropexis** is sometimes applied to the process involved when animal cells take in viruses, but may or may not involve invagination of the cell membrane.) In either case, the entire **virion** enters the host cell. Before the genome of an animal virus may be activated, the protein capsid must be removed. This process is referred to as **uncoating**.

HIV as a Representative Animal Virus:

The **Human Immunodeficiency Virus (HIV)** is a diploid, **single-stranded RNA** type virus known as a **retrovirus** because it has the ability to **reverse transcribe** the information of its viral genome from RNA into DNA. This virus has an envelope and enters its host as described above, i.e., receptor molecules (spike proteins) on the viral envelope bind with receptors on the host cell surface, and then the membranes fuse. Although this type of virus can infect a variety of host cells, one of its primary targets is **T4 lymphocytes** (helper-T cells or T-helper cells). Infection of a few host cells will leave some viral proteins on cell surfaces; however, this may not trigger an immune reaction (antibody production) sufficient for detection/diagnosis.

After the virus nucleocapsid enters the host, the capsid is removed (**uncoating occurs**) and the viral genome is released within the cytoplasm of the host cell. A type of enzyme called

reverse transcriptase (RNA dependent DNA polymerase), also contained within the viral capsid, then causes the viral RNA to be reverse transcribed into DNA.

The single-stranded DNA formed is then replicated to form a DNA duplex. Once the viral genome has been converted into a DNA format, it can become integrated into the chromosome of its host to form a **provirus**. (Note - a human virus cannot be called a prophage because it is not a bacteriophage.) The integration of the viral DNA into the host cell chromosome requires a second enzyme, also coded for by the viral genome, called **integrase**. This enzyme is contained within the viral capsid when HIV enters its host cell. The viral genome within the chromosome of the host cell will be replicated along with that chromosome, and new cells formed via mitosis will carry the virus. The length of time HIV can remain within the human body without causing disease symptoms is variable, but the entire disease syndrome (AIDS) does not usually develop until months or years after the initial infection.

Eventually the viral DNA will become active (possibly being triggered by other viral agents, stress, etc.) and will be transcribed. Viral genes coding for receptor proteins are transcribed into m-RNA that is then translated, and the viral proteins penetrate the cell membrane of the host cell. Viral genes coding for capsid proteins and enzymes are also transcribed into m-RNA that is translated into the appropriate proteins. Finally the entire viral genome is transcribed to yield a new copy of the original viral RNA. This RNA genome is packaged within the newly formed capsid proteins (along with enzymes) and the virus is ready to exit the cell.

The virus particles exit the host cell by means of a process called **budding**. Virus particles move to the cell surface and cause the cell membrane to bulge outward. The membrane eventually breaks, leaving a small hole, and the complete virion (wrapped in a membranous envelope) is released into the environment. Since the budding process causes the formation of holes in the cell membrane, the release of numerous virus particles will ultimately cause the host cell to die. It is the loss of the helper-T lymphocytes (along with associated imbalances within the immune system) that ultimately brings on the symptoms of **Acquired Immune Deficiency Syndrome** (AIDS). Loss of immune function leaves the host organism subject to infection by numerous opportunistic pathogens that can eventually kill the host.

Some Selected Viral Diseases and Agents:

A complete presentation of viral diseases and the viruses associated with these is beyond the scope of this course; however, a number of important human viruses are included in this section. For convenience, these are categorized according to the areas of the body or body systems involved.

Diseases of Skin and Oral/Genital regions:

- 1) **Herpesviruses (*Herpesviridae*)** – The herpesviruses are double-stranded DNA viruses with nucleocapsids and envelopes. All have a tendency to go into a latent stage following primary infection, and to reactivate at later intervals.

- a. **Human herpesvirus 1 and 2** (genus *Simplexvirus*) – **Herpes labialis and genitalis**. Most commonly associated with cold sores (fever blisters) and genital herpes (also neonatal herpes) respectively, but can cross infect.
 - b. **Human herpesvirus 3** (genus *Varicellovirus*) – **Varicella-zoster virus**. Human herpesvirus type 3 causes chickenpox (varicella), a mild highly contagious disease of children, and shingles (zoster) a sporadic, incapacitating disease of adults.
 - c. **Human herpesvirus 4** (genus *Lymphocryptovirus*) – **Epstein Bar herpesvirus** and **5** (*Cytomegalovirus*) cause infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinoma, and cytomegalic inclusions disease.
 - d. **Human herpesvirus 6** (genus *Roseolovirus*) Causes exanthema subitum or sixth disease, an acute, short-lived disease of infants and young children characterized by high fever (for 3-4 days) followed by a skin rash.
 - e. **Human herpesvirus 8 – Kaposi's sarcoma**. Causes tumor formation in tissues below the skin or in mucous membranes of the mouth, nose or anus. Lesions or abnormal tissue areas appear as red, purple or brown blotches or nodules that may be quite painful.
- 2) **Paramyxoviruses (*Paramyxoviridae*)** – The paramyxoviruses are single-stranded RNA viruses with nucleocapsids and envelopes. They tend to cause fusion of host cells with the resulting formation of giant cells.
- a. **Measles** (genus *Morbillivirus*) – **Rubeola virus**. Measles is an acute, highly infectious disease characterized by a maculopapular rash, fever, and respiratory symptoms. Rapid improvement usually occurs within three days.
 - b. **Mumps** (genus *Rubulavirus*) – Mumps is an acute, contagious disease characterized by enlargement of one or both parotid salivary glands. Entry may be via mouth or respiratory tract. Other glandular tissues may be involved.
- 3) **Togaviruses (*Togaviridae*)** – The togaviruses are single-stranded RNA viruses (positive-sense RNA) with envelopes. Many are transmitted by arthropod vectors, but some can be transmitted by the respiratory route.
- a. **German measles** (genus *Rubivirus*) – **Rubella virus**. German measles or three-day measles is an acute illness characterized by a rash, mild fever, and sore throat. It is not serious except in women in the first trimester of pregnancy when it can cause congenital rubella syndrome resulting in serious abnormalities of the eye, ear, heart, genitalia, and nervous system. Infection can lead to fatality.
- 4) **Poxviruses (*Poxviridae*)** – The poxviruses are double-stranded DNA viruses with envelopes. They tend to be large, complex and brick-shaped.
- a. **Smallpox** (genus *Orthopoxvirus*) – **Variola virus**. Smallpox is transmitted through the respiratory system, but infects various internal organs before entering the bloodstream and reaching the skin. Viral replication causes the formation of lesions

on the skin surface often resulting in scars. Smallpox was declared eradicated from the world in 1979, but remains a potential agent for bioterrorism.

- b. **Cowpox** (genus *Orthopoxvirus*) – Cowpox is a zoonosis commonly associated with rodents, but also transmitted to cats, cows and humans. Edward Jenner used the fluid from cowpox lesions to prevent smallpox.
 - c. **Monkeypox** (genus *Orthopoxvirus*) – Monkeypox is a disease with symptoms similar to smallpox. A recent outbreak in the United States involved transmission from rodents to prairie dogs and from prairie dogs to humans.
- 5) **Human Papovaviruses (*Papoviridae*)** – The name for this virus group is derived from three words, papilloma, polyoma, and vacuolating viruses. These are double-stranded DNA viruses with naked capsids.
- a. **Human wart virus** (genus *Papillomavirus*) – Human wart viruses induce the formation of warts on the skin surface and can be spread by scratching, direct or indirect contact. Their existence on the skin is usually self-limiting.

Diseases of the Gastrointestinal System:

- 1) **Picornaviruses (*Picornaviridae*)** – The Picornaviruses have single-stranded RNA genomes (positive-sense RNA) and are nonenveloped.
- a. **Hepatitis A virus (HAV)** – (Genus *Hepatitisvirus*) Hepatitis A, also known as infectious hepatitis, is caused by the RNA-type **hepatitis A virus**. Symptoms include tender abdomen, fever, nausea, loss of appetite, and eventually jaundice. Recovery is usually complete in 3 months. Transmission of (HAV) is typically via direct contact (orally or through sexual intercourse), but may involve the use of dirty needles.
- 2) **Hepadnaviruses (*Hepadnaviridae*)** – The Hepadnaviruses have double-stranded DNA genomes and are enveloped.
- a. **Hepatitis B virus (HBV)** – (genus *Hepadnavirus*) Hepatitis B, also known as serum hepatitis, is caused by the DNA-type hepatitis B virus (HBV), and is a severe form of hepatitis that is sometimes progressively fatal. Transmission of HBV is typically via contaminated needles or serum inoculations. HBV can also cause liver tumors.
- 3) **Flaviviruses (*Flaviviridae*)** – The Flaviviruses have single-stranded RNA genomes (positive-sense RNA) and are enveloped. Many are transmitted by arthropods.
- a. **Hepatitis C virus (HCV)** – (genus *Hepacivirus*) Hepatitis C is the etiological agent of the silent epidemic, a form of hepatitis that has killed more people in the United States than AIDS. These viruses are capable of rapid genetic variation and tend to invade the immune system. Their mode of transmission is not entirely clear.
- 4) **Deltaviruses (*Deltaviridae*)** – The Deltaviruses have one strand of negative-sense, single-stranded RNA as their genome.

- a. **Hepatitis D virus (HDV)** – Delta Hepatitis or Hepatitis D is viroid-like and related to HBV. It can cause both acute and chronic hepatitis. During coinfection with hepatitis B, HDV can cause progressively fatal damage to the liver.
- 5) **Caliciviruses (*Caliciviridae*)** – The Caliciviruses have single-stranded RNA (positive-sense RNA) genomes and are non-enveloped.
- a. **Norwalk-like virus (genus *Norovirus*)** – The genus *Norovirus* has recently been assigned to a group of viruses known as Norwalk-like viruses that are the most common cause of infectious gastroenteritis (often referred to as the 24-hour flu).
 - b. **Hepatitis E virus** – The Hepatitis E virus (HEV) causes symptoms indistinguishable from hepatitis A including malaise, anorexia, abdominal pain, joint pain and fever.

Diseases of the Respiratory System:

- 1) **Picornaviruses (*Picornaviridae*)** – The Picornaviruses have single-stranded RNA genomes, are nonenveloped, and carry spike-proteins on their virion surfaces.
- a. **Common cold or Acute Rhinitis (genus *Rhinovirus*)** – Between 90 and 95% of all upper respiratory tract infections are viral, the causative agent being one of at least 90-100 different kinds of Rhinovirus. Symptoms of the common cold are familiar to most people.
- 2) **Paramyxoviruses (*Paramyxoviridae*)** – The paramyxoviruses are single-stranded RNA viruses with nucleocapsids and envelopes. They tend to cause fusion of host cells with the resulting formation of giant cells.
- a. **Respiratory syncytial virus (genus *Pneumovirus*)** – Respiratory syncytial viruses are the most important cause of pneumonia in infants and children worldwide.
- 3) **Adenoviruses (*Adenoviridae*)** – The Adenoviruses have double-stranded DNA genomes (positive-sense RNA) and are nonenveloped.
- a. **Sore throat or pharyngitis** – Pharyngitis not due to streptococcus infection is often caused by adenoviruses.
 - b. **Viral pneumonia** – Viral pneumonia can be caused by adenoviruses as well as by orthomyxoviruses and respiratory syncytial viruses.
- 4) **Orthomyxoviruses (*Orthomyxoviridae*)** – The Orthomyxoviruses have single-stranded RNA genomes (negative-sense RNA) that occur as multiple strands. They are enveloped viruses and carry spike proteins.
- a. **Influenza (genus *Orthomyxovirus*)** – Influenza is a disease of the lower respiratory tract characterized by fever, chills, muscle ache, and respiratory symptoms. Secondary pneumonia, endocarditis, and/or central nervous system complications may occur. Influenza virus A, B, and C can all cause influenza, and since they often

cross infect humans, swine and waterfowl, tend to experience considerable genetic variation.

- 5) **Coronaviruses (*Coronaviridae*)** – The Coronaviruses have single-stranded RNA genomes (positive-sense RNA) and are enveloped. They can cause a variety of upper respiratory tract infections.
 - a. **Severe Acute Respiratory Syndrome (SARS)** – (genus *Coronavirus*) Severe Acute Respiratory Syndrome (SARS) is a newly identified disease responsible for killing over 800 people during an outbreak in China. Early symptoms resemble those of atypical pneumonia with low fever and are difficult to diagnose. Other symptoms include reduction in the number of circulating lymphocytes (CD4 and CD8), Monocytes and thrombocytes accompanied by electrolyte and enzyme imbalances.
- 6) **Bunyaviruses (*Bunyaviridae*)** – The Bunyaviruses are single-stranded RNA viruses (negative-sense RNA) with envelopes.
 - a. **Hantavirus Pulmonary Syndrome (genus *Hantavirus*)** – Hantaviruses can cause both hemorrhagic fever and Hantavirus pulmonary syndrome, an acute form of respiratory distress that can lead to rapid death. They are transmitted to humans through the excrement of rodents and rodents serve as the natural reservoir.

Diseases of the Nervous System:

- 1) **Picornaviruses (*Picornaviridae*)** – The Picornaviruses have single-stranded RNA genomes, are nonenveloped, and carry spike-proteins on their virion surfaces.
 - a. **Poliovirus (genus *Enterovirus*)** – Poliomyelitis is an acute infectious disease that in its serious form affects the CNS. The destruction of motor neurons in the spinal cord results in flaccid paralysis. Three types of enteroviruses are involved, and most cases result in subclinical infection.
- 2) **Togaviruses (*Togaviridae*)** – The togaviruses are single-stranded RNA viruses (positive-sense RNA) with envelopes. Many are transmitted by arthropod vectors, but some can be transmitted by the respiratory route.
 - a. **Encephalitis (genus *Alphavirus*)** – Encephalitis is caused by a number of viruses sometimes referred to as arboviruses. Arbovirus is an ecological name for a virus that can multiply within an arthropod and within a human host. Eastern, Western, and Venezuelan Equine encephalitis are caused by various types of arboviruses in the group called Togaviruses. All are zoonoses, the horse being the primary host. All involve vectors (mosquitoes) and cause permanent damage to nervous tissue involved.
- 3) **Rhabdoviruses (*Rhabdoviridae*)** – The Rhabdoviruses have single-stranded RNA genomes and spiked envelopes. They cause a variety of animal diseases.
 - a. **Rabies (genus *Rhabdovirus*)** – Rabies is an active viral disease resulting in the destruction of gray matter within the CNS and is almost always fatal.

Transmission is usually through a bite from an infected animal, but a bite is not required. Children that are licked in the face by infected animals may contract rabies.

- 4) **Flaviviruses (*Flaviviridae*)** – The Flaviviruses have single-stranded RNA genomes (positive-sense RNA) and are enveloped. Many are transmitted by arthropods.
 - a. **West Nile Fever** (genus *Flavivirus*) – West Nile fever is caused by a flavivirus common to Africa, West Asia and the Middle East. The virus is usually associated with birds, horses and other animals, but can be transmitted to humans. It generally causes flu-like symptoms, but can cause encephalitis and meningitis.
 - b. **Saint Louis Encephalitis** (genus *Flavivirus*) – Saint Louis encephalitis is similar to West Nile fever, but occurs in the United States.

Diseases of the Circulatory System:

- 1) **Flaviviruses (*Flaviviridae*)** – The Flaviviruses have single-stranded RNA genomes (positive-sense RNA) and are enveloped. Many are transmitted by arthropods and cause hemorrhagic fevers.
 - a. **Yellow Fever** (genus *Flavivirus*) – Yellow fever is caused by a flavivirus common to tropical regions. It is an acute, febrile, mosquito-borne illness. Severe cases are characterized by jaundice, proteinuria, and hemorrhage. Incubation is 3-6 days and is followed by fever, chills, headache, backache, nausea, and vomiting. Disease may be severe, resulting in death, or patient may recover completely.
 - b. **Dengue fever** (genus *Flavivirus*) – Dengue fever is caused by four different Dengue viruses, and is usually characterized by headache, fever, rash, muscle and bone pain and prostration. Dengue viruses can cause disease symptoms categorized as dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) and are a leading cause of death among children in South East Asia. Dengue fever has undergone a dramatic expansion in range and causes tens to hundreds of cases in humans each year.
- 2) **Filoviruses (*Filoviridae*)** – The Filoviruses are single-stranded RNA viruses (negative-sense RNA) with envelopes. Their virions are long and filamentous (hence the name) and they cause severe hemorrhagic fevers.
 - a. **Ebola** (genus *Filovirus*) – Ebola (Sudan, Zaire, Cote d'Ivoire, and Reston) is caused by a group of filoviruses called Ebola-like viruses. They cause severe hemorrhagic fever and are not well studied because they can only be handled under biosafety level 4 conditions.
 - b. **Marburg** (genus *Filovirus*) – The Marburg-like viruses are named for outbreaks of severe hemorrhagic fever that occurred in Marburg and Frankfurt, Germany in 1967. Although the virus was passed to humans from African Green Monkeys, the natural origin remains unknown.

3) **Retroviruses (*Retroviridae*)** – The Retroviruses are single-stranded RNA viruses (negative-sense RNA) with envelopes.

- a. **Acquired Immune Deficiency Syndrome (AIDS)** (genus *Retrovirus*) – The **Human Immunodeficiency Virus (HIV)**, formerly identified as LAV/HTLV III has a single-stranded RNA genome occurring in two segments. These viruses use reverse transcriptase enzymes to reverse transcribe their RNA into DNA and then use integrase enzymes to insert their viral DNA segments into the chromosomes of their host cells.

AIDS is a disease associated with, and transmitted through blood (blood products) and semen. It is caused by a human retrovirus that infects primarily T-4 lymphocytes (Helper/Inducer T-cells), but can also infect B-lymphocytes and monocytes. The destruction of these cells acts to cripple the immune system of the body, leaving the individual vulnerable to a variety of neoplasms (especially Kaposi's sarcoma), and severe opportunistic infections, the most common of which include: 1) Protozoa such as *Pneumocystis carinii* and *Toxoplasma gondii*, 2) Fungi such as *Candida albicans*, *Coccidioides immitis*, and *Histoplasma capsulatum*, 3) Bacteria such as *Listeria monocytogenes*, *Mycobacterium tuberculosis*, and *Salmonella*, and 4) viruses such as Cytomegalovirus, Herpes viruses, and Hepatitis B virus. The more serious complications are often preceded by symptoms such as fatigue, malaise, unexplained weight loss, fever, shortness of breath, chronic diarrhea, white patches on the tongue, and lymphadenopathy. The incubation period for the AIDS virus appears to be long, ranging from 6 months to more than two years. Individuals at highest risk of infection include homosexual males, intravenous drug users, female partners of bisexual males and/or intravenous drug users, and the infants of these females. There is currently no effective method for the prevention or cure of this devastating disease, and studies indicate that at least in most cases, the ultimate result of infection is death. HIV is also associated with brain disease, and several types of cancer.

Viruses and Cancer:

Cancer is recognized as a disease commonly associated with aging individuals and brought about by heritable changes in a cell's genetic material. Cancer cells give rise to more cancer cells through cell division. Although much remains unknown about the exact relationship between viruses and cancer, it seems now that several different types of viruses serve as cofactors or cocarcinogens in the development of **tumors** in humans. The seven families of viruses involved in tumor formation include hepadnaviruses, polyomaviruses, papillomaviruses, adenoviruses, herpesviruses, poxviruses and retroviruses. Of these, the first six are DNA viruses, and the seventh are RNA viruses.

Segments of DNA referred to as **oncogenes** are important players in cancer development. These genes serve as potential effectors, or components involved in the conversion of normal cells into cancer cells. These can be categorized as **cellular oncogenes** (being of cellular origin), **viral oncogenes** (being of viral origin) or **proto-oncogenes** (normal cellular genes that can give rise to cellular or retroviral oncogenes). Proto-oncogenes are highly conserved among various animal species. **Tumor suppressor genes**, genes able to prevent the conversion of normal cells into tumor cells also play an important role in

tumor development. Cellular oncogenes arise from normal cellular genes performing a variety of important cellular functions. This can occur through point mutations, sequence deletions, chromosome rearrangements, over expression and/or inappropriate expression of the genes. Factors such as mutagenic chemicals, high-energy radiation and/or viral infection can bring about these changes.

Viruses have been shown to alter cells through a process called **transformation**. Transformed cells display a number of abnormal characteristics including immortality, decreased dependence on anchorage and exogenous growth factors and a loss of contact inhibition of growth. Many tumor cells transformed by viruses carry virus specific antigens on their cell surfaces, i.e., **tumor-specific transplantation antigens (TSTA)**, or **T-antigens** in their nuclei. Viruses capable of inducing tumor formation in animals are called **oncogenic viruses**.

DNA Viruses and Cancer:

Hepadnaviruses – The Hepatitis B virus (HBV) is associated with hepatocellular carcinoma (liver cancer), but the effect is thought to be through chronic irritation and cirrhosis of the liver. No HBV genes have been specifically identified as oncogenes.

Papillomaviruses – The Human papilloma viruses (HPV) are recognized as the causative agents of common warts and have been linked to a variety of anogenital cancers. The epidemiology of Cervical carcinoma is strongly suggestive of an infectious sexually-transmitted disease, and HPV has been shown to cause neoplasia of the vagina, penis and anus. HPV genes have been shown to bind with and inactivate cellular tumor suppressor genes.

Herpesviruses – Human Herpesvirus 4, the Epstein-Barr virus (EBV), has been associated with Burkitt's lymphoma, nasopharyngeal carcinoma and Hodgkin's lymphoma. Viral DNA and sometimes entire viral genomes have been found in these cancer cells. Transformation initiated by EBV involves multiple genes, the products of which appear to bind with and inactivate tumor suppressor proteins.

Adenoviruses – Although the Adenoviruses have not been associated with cancer in humans, they have been shown to transform cells in vitro and cause tumors in rodents. Adenovirus gene products, like those of other DNA viruses, bind with and inactivate tumor suppressor gene products.

Poxviruses and Polyomaviruses – These viruses have been shown to cause tumors in laboratory animals. The Polyomavirus identified as human JC virus (a virus reported to infect most adults in the US) has been implicated as a possible cause of brain tumors, but no convincing evidence exists.

RNA Tumor Viruses:

Retroviruses transfer genes from one type of animal cell to another through transduction and induce oncogene formation through alteration of gene regulation. **Transducing**

retroviruses typically pick up cellular genes at the expense of their own, so are often defective i.e., unable to replicate without the assistance of other viruses. The human genes they carry are usually modified by point mutations or sequence deletions, and always have their introns removed. They are frequently fused with various viral genes. Transducing retroviruses induce cell transformation and tumor formation with very high frequency, often 100% under laboratory conditions. **Cis-activating Retroviruses** induce tumor formation by altering gene regulation; typically by inserting adjacent to proto-oncogenes and modifying their expression. **Trans-acting retroviruses** induce tumor formation through the action of their protein products and their efficiency of tumor induction is low (1%).

Retroviruses identified as **Human T-cell Leukemia viruses** (HTLV-1 and HTLV-2) are recognized as being the cause of adult T-cell leukemia and lymphoma in humans. **Feline leukemia virus** (FeLV) causes leukemia in cats. The sarcoma viruses of cats, chickens and rodents and the mouse mammary tumor viruses are also retroviruses.