Semester – V

15MBU502 **VIROLOGY** **5H – 5C**

Instruction Hours / week: L: 5 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

Virologist are highly demanded in the medical research companies, Pharmaceutical companies, governmental agencies, laboratory testing companies, or cancer treatment or research companies depending upon the specialization.

OBJECTIVES

To study general aspects of viral structure, classification, replication, interactions and immunity to viruses

UNIT – I

Early development of virology - Classification of viruses based on their genetic material - Virus cultivation – purification and assay of viruses.

UNIT – II

The structure of viruses - virion – general properties of viruses – viral symmetry (Helical capsid, icosahedral capsid and complex) - Nucleic acid – viral envelopes and enzymes, viral replications.

UNIT – III

Classification of Bacteriophage - Reproduction of double stranded DNA phage (T4- phage) – one step growth experiment - Reproduction of single stranded DNA phage (phage ΦX174)

UNIT – IV

Classification and reproduction of animal and plant viruses - Viruses and cancer - General properties of viroids and prions.

UNIT – V

Morphology, Replication, and Pathogenesis of plant viruses (Tobacco mosaic virus, Cauliflower mosaic virus, Gemini virus) - Morphology, Replication, and Pathogenesis of animal viruses (Human immuno deficiency virus, Rabies virus, Pox virus, Herpes viruses and Influenza viruses).

TEXTBOOKS

1. Ananthanarayanan, R. and C.K.J. Panicker, 2005. Text Book of Microbiology. 7th Edition. Orient Longman. New Delhi.
2. Chakraborty, P., 2003. A Text book of Microbiology. 2nd Edition. New Central Book Agency (P) Ltd., Calcutta.
3. Dubey, R. C. and D.K. Maheswari, 2004. A Text book of Microbiology 1st Edition, S.Chand and Company Ltd.
4. Pelczar Jr. M.J., E.C.S. Chan and N.R. Kreig, 2003. Microbiology. 5th Edition. Tata McGraw-Hill Publishing Company. New Delhi.

REFERENCES

1. Jawetz, E., J.L. Melnic and E.A. Adelberg, 2001. Review of Medical Microbiology. 22nd Edition. Lange Medical Publishers, New York.
2. Prescott, M., J.P. Harley and D.A. Klein, 2007. Microbiology. 7th Edition, McGraw-Hill Inc. New York.
3. Stanier R.Y., J.L. Ingraham, M.L. Wheelis and P. R. Painter, 2007. General Microbiology Macmillan Press Ltd. London.
4. Dimmock, N. J., A.J. Easton and K.N. Leppard, 2007. Introduction to Modern Virology. 6th Edition, Blackwell Scientific Publications, Oxford
5. White, D. O., Fenner, F. J. 1994. Medical Virology, 4th edn. Academic Press, New York.
6. Jay A. Levy, Heinz Fraenkel-Conrat, and Oliver S. Owens., 1994.Virology .3rd Edition Benjamin Cummings.
7. Edward,K. Wagner, Martinus .J. Hewllet, David. C. Bloom, David. Camerini, 2009. Basic Virology. Wiley Publishers
8. Alal. J. Cann, 2012. Principles of Molecular Virology. 5th Edition. Academic Press. US.

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III B. Sc Microbiology – Virology

Objective of the course

In addition to fulfilling the learning objectives provided by individual lecturers, the student should be able to do the following.

- Describe the structure and replication strategies of the individual viruses discussed, including the processes of entry into cells, control of gene transcription and where relevant translation and gene product stability, control of and mechanism(s) of genome replication from the cell.
- Define the process of virus latency and describe in molecular terms control of the process and activation of viral genomes during reactivation.
- Describe the growth behavior differences between normal cells and cells transformed by oncogenic DNA and RNA viruses and also about the bacteriophages.
- Describe the processes of senescence and apoptosis and discuss the impact of oncogenic viruses (and specific viral gene products or activities) on these processes.
- Describe the processes involved in the anti-tumor effects of "anti-tumor" viruses.
- Integrate experimental strategies learned in the context of individual viral systems into the design of experiments involving other systems.
- Describe the human viruses pathogenesis and other viruses

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III B. Sc Microbiology –Virology
Unit I

LECTURE PLAN

LECTURE PLAN - UNIT -1			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	Early development of virology	T1 1-4
2	1	Classification of Viruses	T1 4-8
3	1	Virus isolation and cultivation	T1 9-14
4	1	Purification of viruses	W1
5	1	Assay of viruses- Samples	T2 6.1-6.4
6	1	Assay of viruses- serology	W1
7	1	Revision of Unit I	J1
8	1	Unit I test	R1&J1
Textbooks :		T1-virology-P.saravanan-Mjp publishers T2-Microbiology- Dushyant Kumar Sharma – Narosa publishers T3-Textbook of Microbiology- Paniker- Orient longman publishers	
Reference books:		R1- Medical Microbiology –Jawertz – McGraw hill Publishers	
Website:		W1- www.virologyonline.com	
Journals:		J1 – the Viruses	

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Unit I

Introduction to Virology

Background/Discovery

The concept behind modern virology can be traced back to Adolf Mayer, Dimitri Ivanofsky and Martinus Beijerinck who, independently in the late 1880's, discovered what was later to be called tobacco mosaic virus (TMV). Their discoveries led to the descriptions of filterable agents, too small to be seen with the light microscope, that could be grown in living cells and cause disease. The first filterable agent from animals, foot and mouth disease virus, was described by Loeffler and Frosch in 1898 and the first human filterable agent discovered was yellow fever virus, discovered by Walter Reed in 1901. The term 'virus' derives from the Latin for slimy liquid or poison and was gradually introduced during this period to replace the term 'filterable agents'.

The first virus to be visualized by x-ray crystallography and electron microscopy was TMV, reported in 1941 and 1939, respectively. These advances introduced the notion that viruses were structurally composed of repeating subunits.

Frederick Twort and Felix d'Herelle, working independently, are credited with the discovery of viruses which could infect and lyse bacteria in 1915. D'Herelle introduced the term 'bacteriophages' for these agents and also described the concepts of virus adsorption to its target, cell lysis and release of infectious particles. Over the next 35-40 years, work with phages led to numerous discoveries including how the introduction of DNA into a target cell could reproduce itself and the regulation of cellular macromolecular synthesis directed by viruses. In essence, the field of molecular biology was opened up during this period.

Advances in animal virology were noted throughout the 20^{th} century but the major breakthrough came through the development of tissue culture systems that led, for example, to the isolation of poliovirus by Enders et al. in 1949. This markedly facilitated detailed study of this agent and, most importantly, the development of poliovirus vaccines. The ensuing 60 years have seen diagnostic virology mature as a field with the discovery of new agents and diseases and the parallel determination of the importance of viruses in our understanding of molecular biology and cancer.

History of virology

The history of virology – the scientific study of viruses and the infections they cause – began in the closing years of the 19th century. Although Louis Pasteur and Edward Jenner developed the first vaccines to protect against viral infections, they did not know that viruses existed. The first evidence of the existence of viruses came from experiments with filters that had pores small enough to retain bacteria. In 1892, Dmitry Ivanovsky used one of these filters to show that sap from a diseased tobacco plant remained infectious to healthy tobacco plants despite having been filtered. Martinus Beijerinck called the filtered, infectious substance a "virus" and this discovery is considered to be the beginning of virology. The subsequent discovery and partial characterization of bacteriophages by Felix d'Herelle further catalyzed the field, and by the early 20th century many viruses were discovered.

Pioneers

Despite his other successes, **Louis Pasteur** (1822–1895) was unable to find a causative agent for rabies and speculated about a pathogen too small to be detected using a microscope. In 1884, the French microbiologist **Charles Chamberland** (1851–1931) invented a filter – known today as the Chamberland filter – that had pores smaller than bacteria. Thus, he could pass a solution containing bacteria through the filter and completely remove them from the solution.

In 1892, the Russian biologist **Dmitry Ivanovsky** (1864–1920) used a Chamberland filter to study what is now known as the tobacco mosaic virus. His experiments showed that crushed leaf extracts from infected tobacco plants remain infectious after filtration. Ivanovsky suggested the infection might be caused by a toxin produced by bacteria, but did not pursue the idea.

In 1898, the Dutch microbiologist **Martinus Beijerinck** (1851–1931) repeated the experiments and became convinced that filtrate contained a new form of infectious agent. He observed that the agent multiplied only in cells that were dividing and he called it a *contagium vivum fluidum* (soluble living germ) and re-introduced the word *virus*. Beijerinck maintained that viruses were liquid in nature, a theory later discredited by the American biochemist and virologist **Wendell Meredith Stanley** (1904–1971), who proved that they were in fact, particles. In the same year **Friedrich Loeffler** (1852–1915) and **Paul Frosch** (1860–1928) passed the first animal virus through a similar filter and discovered the cause of foot-and-mouth disease.

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In 1881, **Carlos Finlay** (1833–1915), a Cuban physician, first suggested that mosquitoes were carrying the cause of yellow fever, a theory proved in 1900 by **Walter Reed** (1851–1902). During 1901 and 1902, **William Crawford Gorgas** (1854–1920) organised the destruction of the mosquitoes' breeding habitats in Cuba, which dramatically reduced the prevalence of the disease. Gorgas later organised the elimination of the mosquitoes from Panama, which allowed the Panama Canal to be opened in 1914. The virus was finally isolated by **Max Theiler** (1899–1972) in 1932 who went on to develop a successful vaccine.

By 1928 enough was known about viruses to enable the publication of *Filterable Viruses*, a collection of essays covering all known viruses edited by **Thomas Milton Rivers** (1888–1962). Rivers, a survivor of typhoid fever contracted at the age of twelve, went on to have a distinguished career in virology. In 1926, he was invited to speak at a meeting organised by the Society of American Bacteriology where he said for the first time, "Viruses appear to be obligate parasites in the sense that their reproduction is dependent on living cells."

The notion that viruses were particles was not considered unnatural and fitted in nicely with the germ theory. It is assumed that **Dr. J. Buist** of Edinburgh was the first person to see virus particles in 1886, when he reported seeing "micrococci" in vaccine lymph, though he had probably observed clumps of vaccinia. In the years that followed, as optical microscopes were improved "inclusion bodies" were seen in many virus-infected cells, but these aggregates of virus particles were still too small to reveal any detailed structure. It was not until the invention of the electron microscope in 1931 by the German engineers **Ernst Ruska** (1906–1988) and **Max Knoll** (1887–1969), that virus particles, especially bacteriophages, were shown to have complex structures. The sizes of viruses determined using this new microscope fitted in well with those estimated by filtration experiments. Viruses were expected to be small, but the range of sizes came as a surprise. Some were only a little smaller than the smallest known bacteria, and the smaller viruses were of similar sizes to complex organic molecules.

In 1935, **Wendell Stanley** examined the tobacco mosaic virus and found it was mostly made of protein. In 1939, **Stanley** and **Max Lauffer** (1914) separated the virus into protein and RNA parts. The discovery of RNA in the particles was important because in 1928, **Fred Griffith** (c.1879–1941) provided the first evidence that its "cousin", DNA, formed genes.

In Pasteur's day, and for many years after his death, the word "virus" was used to describe any cause of infectious disease. Many bacteriologists soon discovered the cause of numerous infections. However, some infections remained, many of them horrendous, for which no bacterial cause could be found. These agents were invisible and could only be grown in living animals. The discovery of viruses was the key that unlocked the door that withheld the secrets of the cause of these mysterious infections. And, although Koch's postulates could not be fulfilled for many of these infections, this did not stop the pioneer virologists from looking for viruses in infections for which no other cause could be found.[17]

Bacteriophages

Discovery

Bacteriophages are the viruses that infect and replicate in bacteria. They were discovered in the early 20th century, by the English bacteriologist **Frederick Twort** (1877–1950). But before this time, in 1896, the bacteriologist **Ernest Hanbury Hankin** (1865–1939) reported that something in the waters of the River Ganges could kill *Vibrio cholerae* – the cause of cholera. Whatever it was in the water could be passed through filters that remove bacteria but was destroyed by boiling. Twort discovered the action of bacteriophages on staphylococci bacteria. He noticed that when grown on nutrient agar some colonies of the bacteria became watery or "glassy". He collected some of these watery colonies and passed them through a Chamberland filter to remove the bacteria and discovered that when the filtrate was added to fresh cultures of bacteria, they in turn became watery. He proposed that the agent might be "an amoeba, an ultramicroscopic virus, living protoplasm, or an enzyme with the power of growth".

Félix d'Herelle (1873–1949) was a mainly self-taught French-Canadian microbiologist. In 1917 he discovered that "an invisible antagonist", when added to bacteria on agar, would produce areas of dead bacteria. The antagonist, now known to be a bacteriophage could pass through a Chamberland filter. He accurately diluted a suspension of these viruses and discovered that the highest dilutions (lowest virus concentrations), rather than killing all the bacteria, formed discrete areas of dead organisms. Counting these

areas and multiplying by the dilution factor allowed him to calculate the number of viruses in the original suspension. He realised that he had discovered a new form of virus and later coined the term "bacteriophage". Between 1918 and 1921 d'Herelle discovered different types of bacteriophages that could infect several other species of bacteria including *Vibrio cholerae*. Bacteriophages were heralded as a potential treatment for diseases such as typhoid and cholera, but their promise was forgotten with the development of penicillin. Since the early 1970s, bacteria have continued to develop resistance to antibiotics such as penicillin, and this has led to a renewed interest in the use of bacteriophages to treat serious infections.

Early research 1920–1940

D'Herelle travelled widely to promote the use of bacteriophages in the treatment of bacterial infections. In 1928, he became professor of biology at Yale and founded several research institutes. He was convinced that bacteriophages were viruses despite opposition from established bacteriologists such as the Nobel Prize winner **Jules Bordet (**1870–1961). Bordet argued that bacteriophages were not viruses but just enzymes released from "lysogenic" bacteria. He said "the invisible world of d'Herelle does not exist". But in the 1930s, the proof that bacteriophages were viruses was provided by **Christopher Andrewes** (1896–1988) and others. They showed that these viruses differed in size and in their chemical and serological properties. In 1940, the first electron micrograph of a bacteriophage was published and this silenced sceptics who had argued that bacteriophages were relatively simple enzymes and not viruses. Numerous other types of bacteriophages were quickly discovered and were shown to infect bacteria wherever they are found. But this early research was interrupted by World War II. Even d'Herelle was silenced. Despite his Canadian citizenship, he was interned by the Vichy Government until the end of the war.

Modern era

Knowledge of bacteriophages increased in the 1940s following the formation of the Phage Group by scientists throughout the US. Among the members were **Max Delbrück**(1906–1981) who founded a course on bacteriophages at Cold Spring Harbor Laboratory. Other key members of the Phage Group included Salvador Luria (1912–1991) and **Alfred Hershey** (1908–1997). During the 1950s, Hershey and Chase made important discoveries on the replication of DNA during their studies on a bacteriophage called T2. Together with Delbruck they were jointly awarded the 1969 Nobel Prize in Physiology or Medicine "for their discoveries concerning the replication mechanism and the genetic structure of viruses".[29] Since then, the study of bacteriophages has provided insights into the switching on and off of genes, and a useful mechanism for introducing foreign genes into bacteria and many other fundamental mechanisms of molecular biology

Plant viruses

In 1882, **Adolf Mayer** (1843–1942) described a condition of tobacco plants, which he called "mosaic disease" ("mozaïkziekte"). The diseased plants had variegated leaves that were mottled. He excluded the possibility of a fungal infection and could not detect any bacterium and speculated that a "soluble, enzyme-like infectious principle was involved". He did not pursue his idea any further, and it was the filtration experiments of Ivanovsky and Beijerinck that suggested the cause was a previously unrecognised infectious agent. After tobacco mosaic was recognized as a virus disease, virus infections of many other plants were discovered.

The importance of tobacco mosaic virus in the history of viruses cannot be overstated. It was the first virus to be discovered, and the first to be crystallised and its structure shown in detail. The first X-ray diffraction pictures of the crystallised virus were obtained by **Bernal and Fankuchen** in 1941. On the basis of her pictures, **Rosalind Franklin** discovered the full structure of the virus in 1955. In the same year, **Heinz Fraenkel-Conrat and Robley Williams** showed that purified tobacco mosaic virus RNA and its coat protein can assemble by themselves to form functional viruses, suggesting that this simple mechanism was probably the means through which viruses were created within their host cells.

By 1935 many plant diseases were thought to be caused by viruses. In 1922, **John Kunkel** Small (1869–1938) discovered that insects could act as vectors and transmit virus to plants. In the following decade many diseases of plants were shown to be caused by viruses that were carried by insects and in 1939, **Francis Holmes,** a pioneer in plant virology described 129 viruses that caused disease of plants. Modern, intensive agriculture provides a rich environment for many plant viruses. In 1948, in Kansas, US, 7%

of the wheat crop was destroyed by wheat streak mosaic virus. The virus was spread by mites called *Aceria tulipae*.

In 1970, the Russian plant virologist **Joseph Atabekov** discovered that many plant viruses only infect a single species of host plant. The International Committee on Taxonomy of Viruses now recognises over 900 plant viruses.

20th century

By the end of the 19th century, viruses were defined in terms of their infectivity, their ability to be filtered, and their requirement for living hosts. Up until this time, viruses had only been grown in plants and animals, but in 1906, **Ross Granville Harrison** (1870–1959) invented a method for growing tissue in lymph and, in 1913, **E Steinhardt**, **C Israeli**, and **RA Lambert** used this method to grow vaccinia virus in fragments of guinea pig corneal tissue. In 1928, **HB** and **MC Maitland** grew vaccinia virus in suspensions of minced hens' kidneys. Their method was not widely adopted until the 1950s, when poliovirus was grown on a large scale for vaccine production. In 1941–42, **George Hirst** (1909–94) developed assays based on haemagglutination to quantify a wide range of viruses as well as virus-specific antibodies in serum.

Late 20th century

The second half of the 20th century was the golden age of virus discovery and most of the 2,000 recognized species of animal, plant, and bacterial viruses were discovered during these years. In 1946, Bovine virus diarrhea was discovered, which is still possibly the commonest pathogen of cattle throughout the world and in 1957, equine arteri virus was discovered. In the 1950s, improvements in virus isolation and detection methods resulted in the discovery of several important human viruses including Varicella zoster virus, the paramyxoviruses,– which include measles virus and respiratory syncytial virus and the rhinoviruses that cause the common cold. In the 1960s more viruses were discovered. In 1963, the hepatitis B virus was discovered by Baruch Blumberg (b. 1925) and in 1965, Howard Temin (1934–1994) described the first retrovirus. Reverse transcriptase, the key enzyme that retroviruses use to translate their RNA into DNA, was first described in 1970, independently by Howard Temin and David Baltimore (b. 1938). This was important to the development of antiviral drugs – a key turning-point in the history of viral infections. In 1983 **Luc Montagnier** (b. 1932) and his team at the Pasteur Institute in France, first isolated the retrovirus now called HIV. New viruses and strains of viruses were discovered in every decade of the second half of the 20th century. These discoveries have continued in the 21st century as new viral diseases such as SARS and nipah virus have emerged. Despite scientists' achievements over the past one hundred years, viruses continue to pose new threats and challenges.

Classification

Viral classification has been confusing and oft-changing over the years. In the past, viruses were often classified by host, target organ or vector and these are still used vernacularly (e.g., the hepatitis viruses). Modern classification is based on the following three characteristics:

Type of viral nucleic acid (RNA or DNA, single-stranded or double-stranded) and its replication strategy.

Capsid symmetry (icosahedral or helical).

Presence and absence of envelope

Pathogenesis of Viral Diseases

As with other infectious agents which cause human disease, the outcome of the interaction of a particular virus with the human host is dependent on both pathogen and host factors. Viral strains within a genus may have differential cell tropisms, replication capacities and cytopathogenic effects. As an example, strains of HIV may preferentially target monocyte/macrophages or T-lymphocytes, may use different co-receptors (e.g., the chemokine receptors, CCR5 or CXCR4) on the cell surface, may replicate to different levels and may induce different degrees of cell killing. These traits have direct clinical correlates for HIV infected persons with respect to the rates of CD4 cell decline and progression to clinical AIDS. On the host side, the nature of the exposure and the host immune status are probably the two most important determinants of outcome. Thus, the key elements of the virus-host interaction are:

1. Viral strain.
2. Inoculum size.
3. Route of exposure.
4. Susceptibility of host (i.e., is there pre-existent immunity from past exposure or vaccination?).

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5. Immune status and age of host.

The net result of this interaction may be:

1. No infection.
2. Abortive infection with limited viral replication.
2. Asymptomatic infection.
3. Symptomatic infection.
4. Depending upon the agent and the immune status of the host, persistent/latent or self-limited infection.

Pathogenetic Steps in Human Infection

A generalized schema of viral infection leading to disease in the human host is as follows:

Depending upon the agent, the virus enters through the skin, mucous membranes, respiratory tract, gastrointestinal tract, via a transfusion or transplanted organ or via maternal-fetal transmission.

There is local replication at the site of the inoculation. Certain agents exhibit pathology at the skin or mucous membrane surface – e.g., herpes simplex virus, human papillomavirus.

For some neurotropic viruses there may be spread along peripheral nerve routes to ganglia (e.g., herpes simplex virus) or the central nervous system (e.g., rabies virus). For other neurotropic agents, the central nervous system is seeded following viremia.

For many agents, there is replication in regional lymph nodes with subsequent viremia and spread to target organs. Some viruses travel in the bloodstream free in plasma (e.g., picornaviruses); others are cell associated (e.g., cytomegalovirus).

Replication in target organs may lead to local damage and further rounds of viremia..

Non-specific and specific host immune responses come into play to try to control and downregulate the viral replicative process.

Immune Responses to Viral Infections

Innate (non-specific) immunity refers to those elements of the immune system that can clear virus or virus infected cells immediately upon or shortly after viral exposure and which are not dependent upon immunologic memory. Non-specific immunity may include:

a. Phagocytic cells (neutrophils and monocyte/macrophages).
b. Cytokines (e.g., interferons) and chemokines.
c. Natural killer cells.
d. Poorly defined antiviral factors that may exist in blood or body fluids.

Adaptive (specific) immunity refers to antigen specific B and T cell responses that lead to the development of antibodies, cytotoxic T cells and antibody dependent cellular cytotoxicity.

In some instances, an intense immunologic reaction to a viral agent can result in immunopathology and a serious clinical syndrome. A prime example is dengue hemorrhagic fever which is likely due to antibody dependent enhancement and T cell activation on re-exposure to dengue virus.

Mechanisms of Viral Persistence

Viruses may cause chronic, persistent infection with continuous viral replication in the face of an immune response. Examples include HIV, hepatitis B virus and hepatitis C virus. Some viruses may demonstrate persistent infection in immune compromised hosts. These include the herpesviruses, human papillomavirus and rubella virus, among others.

Some viruses are able to cause latent infection. Latency is characterized by a quiescent or minimally transcriptionally active viral genome with periods of reactivation. Latent viruses include the herpesviruses (cytomegalovirus, Epstein-Barr virus, herpes simplex virus, varicella-zoster virus), human papillomavirus, human retroviruses. Recurrent herpes labialis or genital herpes due to HSV or herpes zoster due to varicella zoster virus are classic examples of latency and reactivation. Viruses which exhibit latency may also exhibit chronic, persistent replication in the setting of immune compromise of the host. Mechanisms of persistence of viruses which produce chronic infections include antigenic variation to escape antibody or cytotoxic T cell responses, downregulation of class I major histocompatibility antigens resulting in diminished recognition by cytotoxic T cells and modulation of apoptosis. Viruses which establish latent infection escape recognition by the immune system through decreased viral antigen expression and presentation.Sites of persistence include the nervous system (herpes simplex virus, varicella zoster virus, measles virus, poliovirus, JC virus), the liver (hepatitis B virus, hepatitis C virus), and leukocytes (HIV, cytomegalovirus, Epstein-Barr virus).

Oncogenesis

Several viruses are associated with human cancers. These include: Epstein-Barr virus with lymphoma, nasopharyngeal carcinoma and leiomyosarcoma; herpesvirus 8 with Kaposi's sarcoma and body cavity B-cell

lymphoma; hepatitis B and C viruses with hepatocellular carcinoma; and human papillomavirus with cervical cancer and anogenital carcinoma. Mechanisms of oncogenesis can include transformation (Epstein-Barr virus and herpesvirus 8) and binding of tumor suppressor proteins (human papillomavirus), among others

Diagnosis of Viral Infections

The diagnosis of viral infections relies first on the recognition of a distinct clinical syndrome (e.g., herpes zoster infection) or a consideration of the viral infection in the differential diagnosis of a presenting syndrome (e.g., aseptic meningitis). The second consideration is the knowledge of the appropriate specimens to send to the laboratory (blood, body fluids, lesion scraping, tissue) to diagnose a particular infection. One general point to remember is that the isolation of viruses relies on the use of proper viral transport medium and quick delivery to the laboratory. A variety of methods exist to diagnose viral infections with the recent trend being toward molecular diagnostics. These methods include: Isolation of virus in tissue culture, animals, embryonated eggs. Most diagnostic laboratories only use tissue culture for virus isolation. A specific cytopathic effect or induction of a characteristic function (e.g., hemagglutination) can indicate the growth of viruses in tissue culture. This can be confirmed with virus specific antisera applied to the tissue monolayer to neutralize the cytopathic effect or the hemagglutination reaction. Antigen detection in body fluids (e.g., respiratory tract for respiratory viruses) or blood (e.g., cytomegalovirus) or lesion scrapings (e.g, for herpes simplex virus or varicella-zoster virus) with specific immune sera linked to fluorescence or enzyme immunoassay detection.

PCR amplification and/or nucleic acid probes to detect viral nucleic acid in body fluids or tissues.

Antibody detection. IgM antibody detection can assist with acute diagnosis. Four-fold rises in IgG specific antibody or conversion from seronegative status to seropositive status can secure a diagnosis but this may not be helpful in the acute setting.Examination of tissue samples by light microscopy for viral induced cytopathology and antigen detection by immunohistochemical staining. Examination of body fluids or tissues by electron microscopy. This is not an efficient method and is dependent upon sufficient numbers of virions being present to permit detection.

Prevention and Therapy

Vaccines for the prevention of life threatening viral infections are one of the most significant advances in human health. The eradication of smallpox is the hallmark example of the effectiveness of a viral vaccine.

Effective vaccines exist for polio, mumps, measles, rubella, influenza, hepatitis A, hepatitis B, varicella-zoster, rabies, adenovirus, Japanese B encephalitis and yellow fever.

Immune globulin can prevent or ameliorate clinical disease due to certain viral agents. Examples include varicella-zoster immune globulin for exposure in immune compromised hosts, rabies immune globulin (administered with rabies vaccine) following an exposure, cytomegalovirus immune globulin for transplant recipients, respiratory syncytial virus immune globulin and immune serum globulin for hepatitis A.

Screening of blood for prevention of transmission of HIV, hepatitis B, hepatitis C and in certain transplant situations, cytomegalovirus.

Safe sexual practices for the prevention of HIV, hepatitis B and human papillomavirus infections.

Advances in specific antiviral therapy over the past 30 years have been marked. Effective therapy exists for herpes simplex virus, varicella-zoster virus, cytomegalovirus, HIV, influenza virus, respiratory syncytial virus, hepatitis B and hepatitis C.

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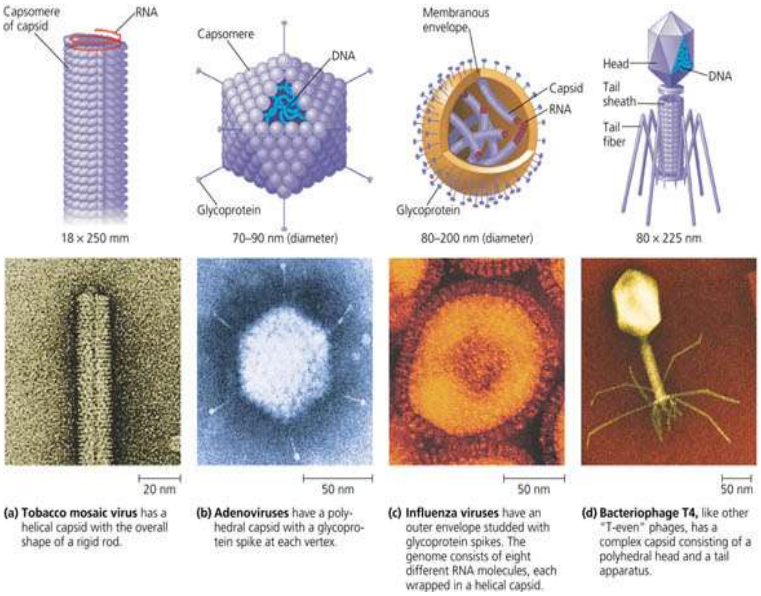

(a) Tobacco mosaic virus has a helical capsid with the overall shape of a rigid rod.

(b) Adenoviruses have a polyhedral capsid with a glycoprotein spike at each vertex.

(c) Influenza viruses have an outer envelope studded with glycoprotein spikes. The genome consists of eight different RNA molecules, each wrapped in a helical capsid.

(d) Bacteriophage T4, like other "T-even" phages, has a complex capsid consisting of a polyhedral head and a tail apparatus.

Viruses contribute significantly to the global burden of infectious diseases. Most of the diseases are mild, but viruses may cause severe diseases in susceptible individuals, such as the mal-nourished, immuno-compromised, the very old and the very young.

What is a virus? Very simple structures consisting essentially of a nucleic acid genome, protected by a shell of protein. May or may not have a lipoprotein envelope. Has no organelle. Very small, sizes range 20 - 200 nm, beyond the resolving power of the light microscope. Metabolically inert and can only replicate inside a host cell. Genome consists of ONLY one type of nucleic acid; either RNA or DNA.

Viral genome codes for the few proteins necessary for replication: some proteins are non-structural e.g. polymerase, and some are structural, i.e. form part of the virion structure.

Type of infection Virus replicates initially at the site of entry, but then enters the blood (viraemia) or lymphatics and spreads throughout the body .Other viruses may replicate locally initially, and then enter nerve endings and travel up the axon to infect the central nervous system.

Time from exposure to an organism to the onset of clinical disease. Viruses that cause localized infections have short incubation periods **Incubation period** (<7 days), while in disseminated infections, the incubation period tends to be longer.

Immune response

Viruses replicate intracellularly, so recovery from a viral infection requires the action of specific cytotoxic T lymphocytes. Virus-specific antibody levels rise during the course of the infection, but antibody plays only a limited role in recovery. Specific antibodies play a very important role in preventing reinfection of the host with the same virus.

Viral cell interactions

When an intact of infectious virus particles makes contacts with a susceptible host cell may develop a number of reactions at the cell surface lead to release of the genetic material at the virus within the cell. This is immediately followed by a series of biosynthetic processes lead to formation of new virus like e.g.

1. Defective virus:- viruses that have lost ability to perform any one of the essential steps required for successful replication.
2. Incomplete virus:- abnormal viruses produced due to inoculation of high titer virus solution in limited number of host susceptible cells like inoculums containing a high rating of infective units to cells this called Von Magnus phenomena. That the produced viruses without nucleic acid e.g. influenza virus.

Interferon

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Soluble substance produced by living cells of many different types in cell cultures, embryonated eggs, in lab. Animals when infected by some animal viruses either DNA or RNA and can inhibit multiplication of active virus e.g. influenza virus.

Characteristics of interferon molecules:-

1. It is small protein without nucleic acid.
2. Low molecular weight of about 25- 45000 Dalton.
3. Thermo stable at 4 C° and resist heating at 50 C° for I hour.
4. Interferon is active through a wide range of pH values (2-12).
5. It is relatively non-toxic, weakly antigenic and cannot neutralized by the specific antiserum.
6. Inactivated by protolytic enzymes such as trypsin.
7. Not affected with RNase & DNase.
8. Interferon specific to animal species but not to viruses species i.e.: it act against wide variety of viruses.

Viral culture

Viral culture is a laboratory test in which samples are placed with a cell type that the virus being tested for is able to infect. If the cells show changes, known as cytopathic effects, then the culture is positive.[1]

Traditional viral culture has been generally superseded by shell vial culture, in which the sample is centrifuged onto a single layer of cells and viral growth is measured by antigen detection methods. This greatly reduces the time to detection for slow growing viruses such as cytomegalovirus, for which the method was developed.[2] In addition, the centrifugation step in shell viral culture enhances the sensitivity of this method because after centrifugation, the viral particles of the sample are in close proximity to the cells.

Human and monkey cells are used in both traditional viral culture and shell vial culture. Human virus types that can be identified by viral culture include adenovirus, cytomegalovirus, entero viruses, herpes simplex virus, influenza virus, para-influenza virus, rhinovirus, respiratory syncytial virus, varicella zoster virus, measles and mumps.[3] For these, the final identification method is generally by immunofluorescence, with exception of cytomegalovirus and rhinoviruses, whose identification in a viral culture are determined by cytopathic effects.

General Characteristics of Viruses

Definition: Obligate intracellular parasite composed of:
Nucleic acid - either DNA or RNA
Protein coat

Characteristics

Single type of nucleic acid - DNA or RNA
Protein coat, or capsid, some has envelopes
Multiply inside of living cells using the host cell machinery
Direct the synthesis of structures to transfer viral nucleic acid to other cells

TABLE 13.1 Viruses and Bacteria Compared

	Bacteria		Viruses
	Typical Bacteria	Rickettsias/ Chlamydias	
Intracellular parasite	No	Yes	Yes
Plasma membrane	Yes	Yes	No
Binary fission	Yes	Yes	No
Pass through bacteriological filters	No	No/Yes	Yes
Possess both DNA and RNA	Yes	Yes	No
ATP-generating metabolism	Yes	Yes/No	No
Ribosomes	Yes	Yes	No
Sensitive to antibiotics	Yes	Yes	No
Sensitive to interferon	No	No	Yes

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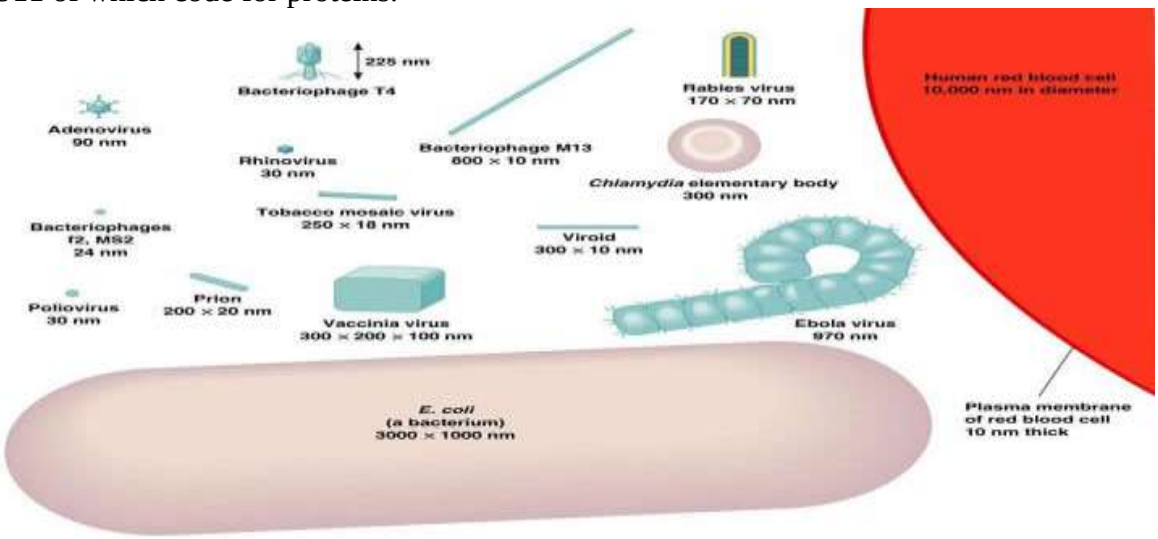
Host Range

The specific types of cells a virus can infect in its host species represent the host range of the virus.
Usually species specific Classification:
Animal virus
Plant virus
Bacterial virus (bacteriophage)
Host range is determined by attachment sites (receptors).
Anti-bacterial therapy - phage therapy
Anti-tumor therapy - oncolytic viruses

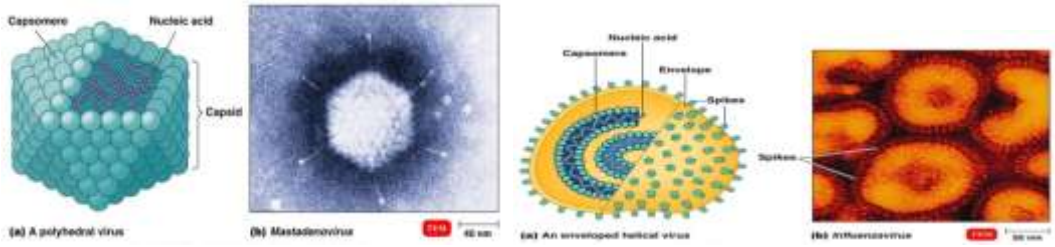
Viral Size

Determined by electron microscopy.
Ranges from 20 to 14,000 nm in length.
There is also a group of giant viruses, including the giant mimi virus, which is something like 800 nm in diameter and has a genome with 1.2Mbp base pairs carrying somewhere in the neighborhood of 1000 genes, 911 of which code for proteins.



Viral Structure

Virions are complete, fully developed viral particles composed of nucleic acid surrounded by a protein coat. Some viruses have an envelope composed of a phospholipid bilayer with viral glycoproteins.
1. Nucleic acid
Viral genomes are either DNA or RNA (not both).
Nucleic acid may be single- or double-stranded
Nucleic acid may be circular or linear or separate molecules.
Nucleic acid:protein ranges from about 1% - 50%.
2. Capsid
Capsid - protein coat
Capsomeres are subunits of the capsid
Protomeres are capsomere subunits.



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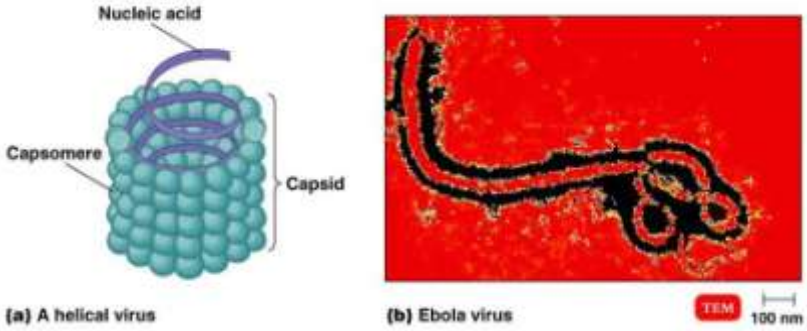
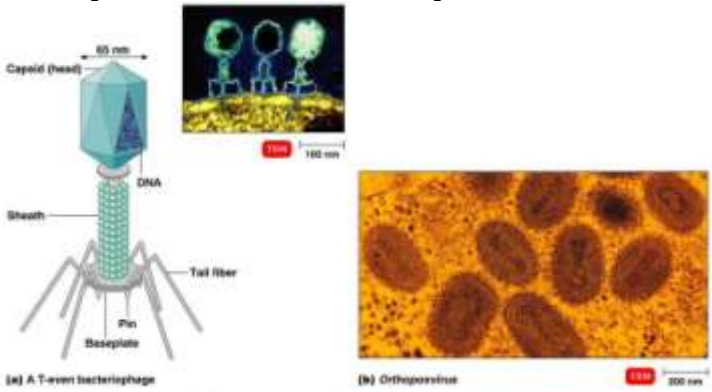
3. Envelope – the outer covering of some viruses, the envelope is derived from the host cell plasma membrane when the virus buds out. Some enveloped viruses have spikes, which are viral glycoproteins that project from the envelope.
Influenzavirus has two kinds of spikes, H (hemagglutinin) and N (neuraminidase). The H spike allows the virus to attach to host cells (and red blood cells), the N spike is an enzyme that allows the mature viral particles to escape from the host cell
Non-enveloped or naked viruses are protected by their capsid alone.

General Morphology

Based on capsid architecture, although enveloped viruses end up being approximately spherical.
1. Helical, non-enveloped
2. Helical, enveloped



3. Polyhedral, non-enveloped
4. Polyhedral, enveloped
Polyhedral means many sides (most are icosahedral - 20 triangular faces and 12 corners)
5. Complex viruses are, well, complex.



Taxonomy of Viruses

Classification of viruses is based on type of nucleic acid, strategy for replication, and morphology.
Virus family names end in *–viridae*; genus names end in *–virus*, order names end in *-ales*.
A viral species is a group of viruses sharing the same genetic information and ecological niche. There is no specific epithet used, common names that are descriptive are used; subspecies are designated with a number.
Families of viruses that affect humans:
DNA viruses RNA viruses

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TABLE 13.2 Families of Viruses That Affect Humans

Characteristics/ Dimensions	Viral Family	Important Genera	Clinical or Special Features
Single-stranded DNA nonenveloped 18–25 nm	Parvoviridae	Human parvovirus B19	Fifth disease; anemia in immunocompromised patients. Refer to Chapter 21.
Double-stranded DNA nonenveloped 70–90 nm	Adenoviridae	*Mastadenovirus*	Medium-sized viruses that cause various respiratory infections in humans; some cause tumors in animals.
40–57 nm	Papovaviridae	*Papillomavirus* (human wart virus) *Polyomavirus*	Small viruses that induce tumors; the human wart virus (papilloma) and certain viruses that produce cancer in animals (polyoma and simian) belong to this family. Refer to Chapters 21 and 26.
Double-stranded DNA enveloped 200–350 nm	Poxviridae	*Orthopoxvirus* (vaccinia and smallpox viruses) *Molluscipoxvirus*	Very large, complex, brick-shaped viruses that cause diseases such as smallpox (variola), molluscum contagiosum (wartlike skin lesion), and cowpox. Refer to Chapter 21.
150–200 nm	Herpesviridae	*Simplexvirus* (HHV-1 and 2) *Varicellovirus* (HHV-3) *lymphocryptovirus* (HHV-4) *Cytomegalovirus* (HHV-5) *Roseolovirus* (HHV-6) HHV-7 Kaposi's sarcoma (HHV-8)	Medium-sized viruses that cause various human diseases, such as fever blisters, chickenpox, shingles, and infectious mononucleosis; causes a type of human cancer called Burkitt's lymphoma. Refer to Chapters 21, 23, and 26.
Double-stranded DNA enveloped 42 nm	Hepadnaviridae	*Hepadnavirus* (hepatitis B virus)	After protein synthesis, hepatitis B virus uses reverse transcriptase to produce its DNA from mRNA; causes hepatitis B and liver tumors. Refer to Chapter 25.
Single-stranded RNA, + strand nonenveloped 28–30 nm	Picornaviridae	*Enterovirus* *Rhinovirus* (common cold virus) Hepatitis A virus	At least 70 human enteroviruses are known, including the polio-, coxsackie-, and echoviruses; more than 100 rhinoviruses exist and are the most common cause of colds. Refer to Chapters 22, 24, and 25.
35–40 nm	Caliciviridae	Hepatitis E virus *Norovirus*	Includes causes of gastroenteritis and one cause of human hepatitis. Refer to Chapter 25.
Single-stranded RNA, + strand enveloped 60–70 nm	Togaviridae	*Alphavirus* *Rubivirus* (rubella virus)	Included are many viruses transmitted by arthropods (*Alphavirus*); diseases include eastern equine encephalitis (EEE) and western equine encephalitis (WEE). Rubella virus is transmitted by the respiratory route. Refer to Chapters 21, 22, and 23.
40–50 nm	Flaviviridae	*Flavivirus* *Pestivirus* Hepatitis C virus	Can replicate in arthropods that transmit them; diseases include yellow fever, dengue and St. Louis and West Nile encephalitis. Refer to Chapters 22, 23, and 25.
Nidovirales 80–160 nm	Coronaviridae	Coronavirus	Associated with upper respiratory tract infections and the common cold; SARS virus. Refer to Chapter 24.
Mononegavirales – strand, one strand of RNA 70–180 nm	Rhabdoviridae	*Vesiculovirus* (vesicular stomatitis virus) *Lyssavirus* (rabies virus)	Bullet-shaped viruses with a spiked envelope; cause rabies and numerous animal diseases. Refer to Chapter 22.
80–14,000 nm	Filoviridae	*Filovirus*	Enveloped, helical viruses; Ebola and Marburg viruses are filoviruses. Refer to Chapter 23.
150–300 nm	Paramyxoviridae	*Paramyxovirus* *Morbillivirus* (measleslike virus)	Paramyxoviruses cause parainfluenza, mumps, and Newcastle disease in chickens. Refer to Chapters 21, 24, and 25.
– strand, one strand of RNA 32 nm	Deltaviridae	Hepatitis D	Depend on coinfection with hepadnavirus. Refer to Chapter 25.
– strand, multiple strands of RNA 80–200 nm	Orthomyxoviridae	Influenza virus A, B, and C	Envelope spikes can agglutinate red blood cells. Refer to Chapter 24.
90–120 nm	Bunyaviridae	*Bunyavirus* (California encephalitis virus) *Hantavirus*	Hantaviruses cause hemorrhagic fevers such as Korean hemorrhagic fever and Hantavirus pulmonary syndrome; associated with rodents. Refer to Chapters 22, 23.
110–130 nm	Arenaviridae	*Arenavirus*	Helical capsids contain RNA-containing granules; cause lymphocytic choriomeningitis, Venezuelan hemorrhagic fever, and Lassa fever. Refer to Chapter 23.
Produce DNA 100–120 nm	Retroviridae	*Oncovirus* *Lentivirus* (HIV)	Includes all RNA tumor viruses. Oncoviruses cause leukemia and tumors in animals; the *Lentivirus* HIV causes AIDS. Refer to Chapter 19.
Double-stranded RNA nonenveloped 60–80 nm	Reoviridae	*Reovirus* *Rotavirus*	Involved in mild respiratory infections and gastroenteritis; an unclassified species causes Colorado tick fever. Refer to Chapter 25.

The Isolation, Cultivation and Identification of Viruses

Viruses must be grown in living cells. They can't be grown in culture media or on agar plates alone, they must have living cells to support their replication.

The easiest viruses to grow are bacteriophages (because the easiest cells to grow in the lab are bacteria).

Growing Bacteriophages In The Laboratory

Once viruses have replicated and been harvested the concentration of viral particles (virions) in the viral stock solution must be determined. One of the easiest ways to determine the concentration of a stock solution of bacteriophages is to use the plaque method.

The plaque method:

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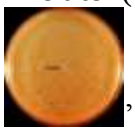
Virus, bacteria, and agar mixed, plated and incubated.
After replication the virus lyses the bacteria, forming plaques, or clear zones.
Each plaque is assumed to come from a single viral particle.
The titer (concentration of the stock solution) of the virus is given in plaque forming units.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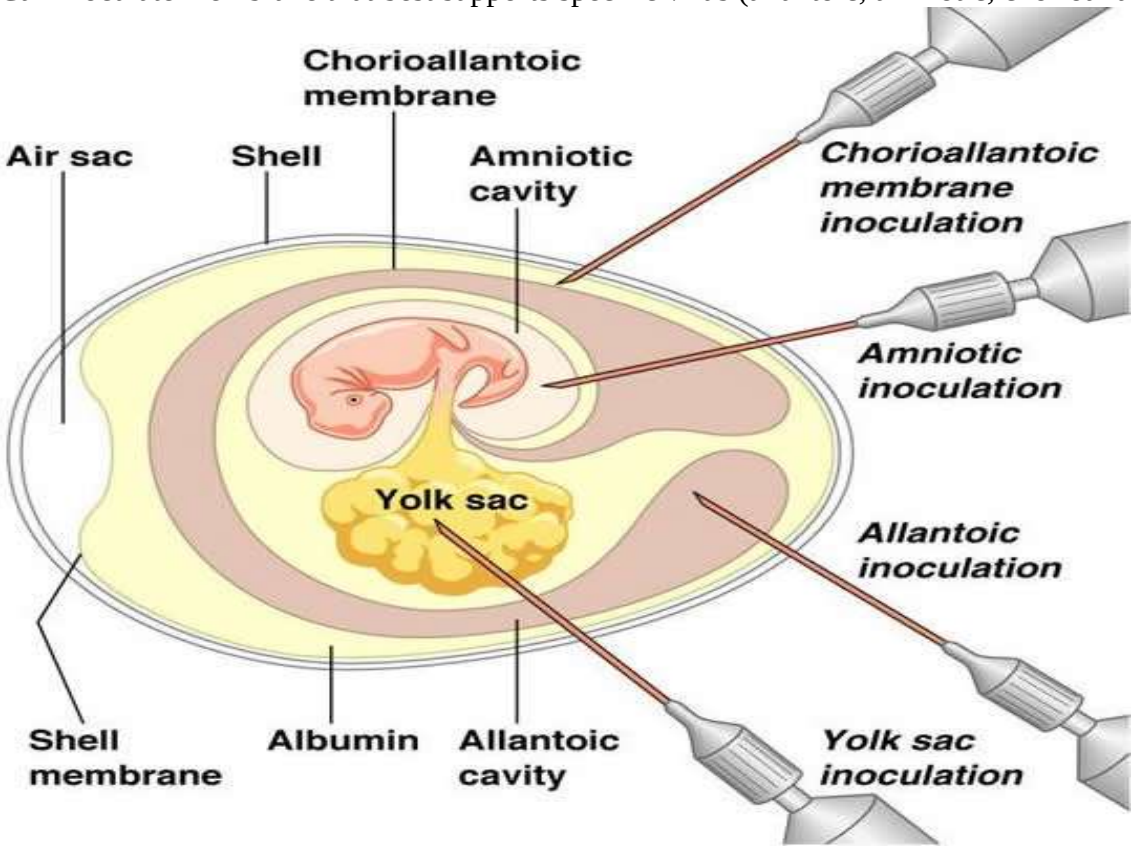
Growing Animal Viruses In The Laboratory

1. Live animal cultures have to be used for some animal viruses.
Simian AIDS and feline AIDS provide models for studying human AIDS.
2. Embryonated eggs can serve as substitutes for some viruses.
Can inoculate membrane that best supports specific virus (allantoic, amniotic, chorioallantoic, or yolk sac).



3. Cell culture is a lot cheaper and easier to work with (contamination can be a problem however).
Primary cell lines have a short lifespan in culture – a few generations before reaching senescence.
Diploid cell lines are derived from embryos and can grow for up to 100 population doublings before senescence.
Continuous cell lines are derived from ***transformed*** cells and grow indefinitely in culture.
Hela cells – 1st continuous cell line, derived from Helen Lane (fictional name - actually named Henrietta Lacks), a cervical cancer patient who died in 1951. This is the oldest continuous cell line and was first used to culture and identify polio virus.

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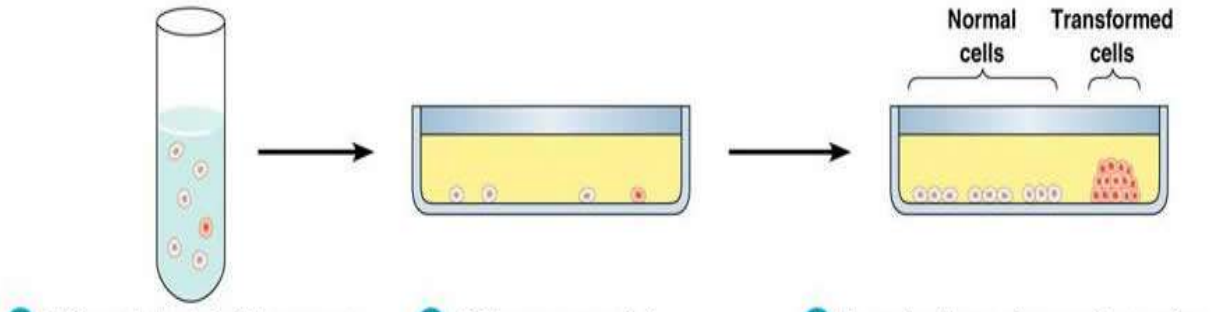
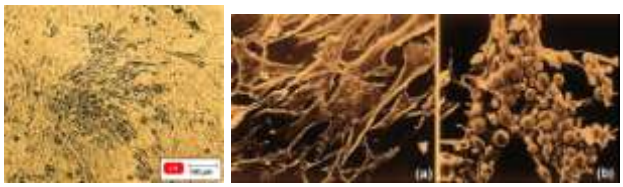


Transformed Cells in Culture



Viral growth can cause cytopathic effects in the cell culture.
Cytopathic effects can appear early or late in the course of the viral infection.
Cytopathic effects may be cytocidal (cell death) or non-cytocidal.
Non-cytocidal effects include acidophilic or basophilic inclusion bodies in the nucleus, cytoplasm, or both; cell fusion, and transformation.
Cytopathic effects can be so characteristic of individual viruses that they can often be used to identify viruses.

Viral Identification
Serological methods
Western blotting
Cytopathic effects
Diagnostic inclusion bodies are associated with rabies virus, measles virus, vaccinia virus, smallpox virus, herpesvirus, and adenoviruses.
Molecular methods include PCR and RFLPs.
PCR was used to identify the West Nile virus and the SARS-associated coronavirus

Study of Viruses
The study of viruses is known as virology. Viruses can be studied in two ways. The first way is through isolation and cultivation, and the second way through detection, identification and diagnosis. For isolation and cultivation, animals, plants, chick embryo and tissue culture are used. For detection, identification and diagnosis, there are several methods. These methods include tissue culture methods, physical methods, serological methods, immunological methods, and others and molecular biology.

Techniques of Virus Cultivation
Viruses are obligate intracellular parasites so they depend on host for their survival. They cannot be grown in non-living culture media or on agar plates alone, they must require living cells to support their replication.
The primary purpose of virus cultivation is:

1. To isolate and identify viruses in clinical samples.
2. To do research on viral structure, replication, genetics and effects on host cell.
3. To prepare viruses for vaccine production.

Cultivation of viruses can be discussed under following headings:

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1. Animal Inoculation
2. Inoculation into embryonated egg
3. Cell Culture

1. Animal Inoculation

- Viruses which are not cultivated in embryonated egg and tissue culture are cultivated in laboratory animals such as mice, guinea pig, hamster, rabbits and primates are used.
- The selected animals should be healthy and free from any communicable diseases.
- Suckling mice(less than 48 hours old) are most commonly used.
- Suckling mice are susceptible to togavirus and coxsackie virues, which are inoculated by intracerebral and intranasal route.
- Viruses can also be inoculated by intraperitoneal and subcutaneous route.
- After inoculation, virus multiply in host and develops disease. The animals are observed for symptoms of disease and death.
- Then the virus is isolated and purified from the tissue of these animals.
- Live inoculation was first used on human volunteers for the study of yellow fever virus.

Advantages of Animal Inoculation

1. Diagnosis, Pathogenesis and clinical symptoms are determined.
2. Production of antibodies can be identified.
3. Primary isolation of certain viruses.
4. Mice provide a reliable model for studying viral replication.
5. Used for the study of immune responses, epidemiology and oncogenesis.

Disadvantages of Animal Inoculation

1. Expensive and difficulties in maintenance of animals.
2. Difficulty in choosing of animals for particular virus
3. Some human viruses cannot be grown in animals or can be grown but do not cause disease.
4. Mice do not provide models for vaccine development.
5. It will lead to generation of escape mutants
6. Issues related to animal welfare systems

2. Inoculation into embryonated egg

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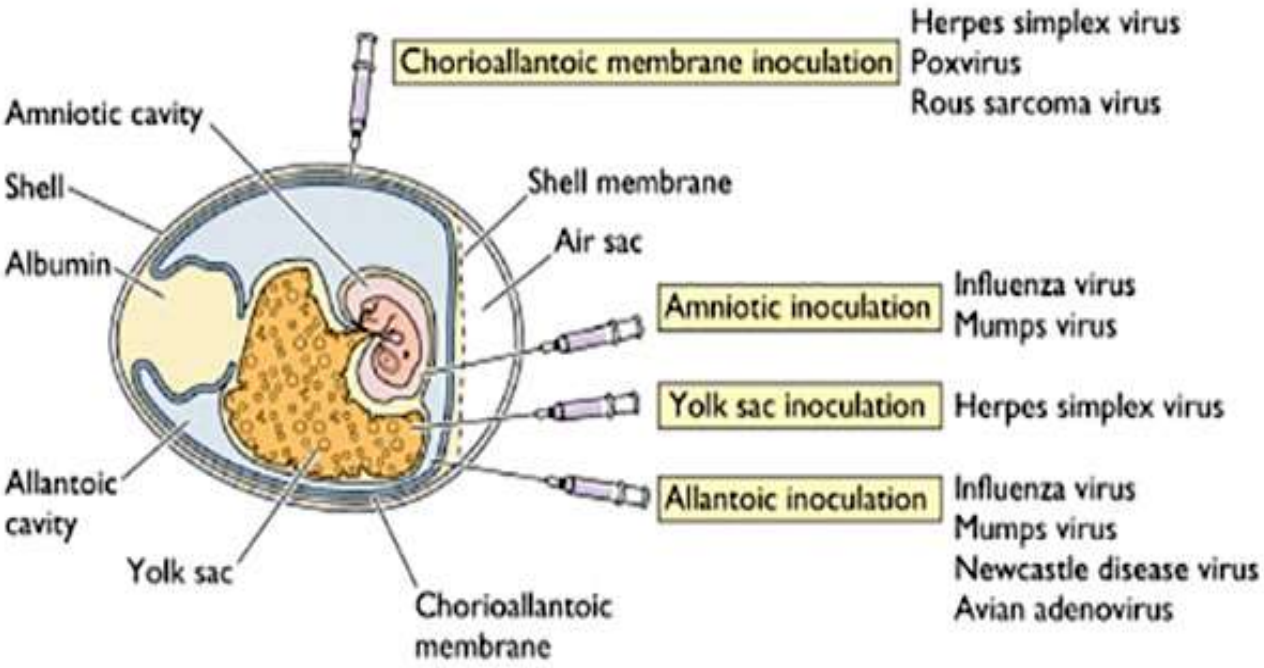

- Good pasture in 1931 first used the embryonated hen's egg for the cultivation of virus.
- The process of cultivation of viruses in embryonated eggs depends on the type of egg which is used.
- Viruses are inoculated into chick embryo of 7-12 days old.
- For inoculation, eggs are first prepared for cultivation, the shell surface is first disinfected with iodine and penetrated with a small sterile drill.
- After inoculation, the opening is sealed with gelatin or paraffin and incubated at 36°c for 2-3 days.
- After incubation, the egg is broken and virus is isolated from tissue of egg.
- Viral growth and multiplication in the egg embryo is indicated by the death of the embryo, by embryo cell damage, or by the formation of typical pocks or lesions on the egg membranes
- Viruses can be cultivated in various parts of egg like chorioallantoic membrane, allantoic cavity, amniotic sac and yolk sac.

1. **Chorioallantoic Membrane (CAM):**
   - Inoculation is mainly for growing poxvirus.
   - After incubation and incubation, visible lesions called pocks are observed, which is grey white area in transparent CAM.
   - Herpes simplex virus is also grown.
   - Single virus gives single pocks
   - This method is suitable for plaque studies.

2. **Allantoic cavity:**
   - Inoculation is mainly done for production of vaccine of influenza virus, yellow fever, rabies.
   - Most of avian viruses can be isolated using this method.

3. **Amniotic sac:**
   - Inoculation is mainly done for primary isolation of influenza virus and the mumps virus.
   - Growth and replication of virus in egg embryo can be detected by haemagglutination assay.

4. **Yolk sac inoculation:**
   - It is also a simplest method for growth and multiplication of virus.
   - It is inoculated for cultivation of some viruses and some bacteria (Chlamydia, Rickettsiae)
   - Immune interference mechanism can be detected in most of avian viruses.

Advantages of Inoculation into embryonated egg

1. Widely used method for the isolation of virus and growth.

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2. Ideal substrate for the viral growth and replication.
3. Isolation and cultivation of many avian and few mammalian viruses.
4. Cost effective and maintenance is much easier.
5. Less labor is needed.
6. The embryonated eggs are readily available.
7. Sterile and wide range of tissues and fluids
8. They are free from contaminating bacteria and many latent viruses.
9. Specific and non specific factors of defense are not involved in embryonated eggs.
10. Widely used method to grow virus for some vaccine production.

Disadvantages of Inoculation into embryonated egg

1. The site of inoculation for varies with different virus. That is, each virus have different sites for their growth and replication.

3. Cell Culture (Tissue Culture)

There are three types of tissue culture; organ culture, explant culture and cell culture.

Organ cultures are mainly done for highly specialized parasites of certain organs e.g. tracheal ring culture is done for isolation of coronavirus.

Explant culture is rarely done.

Cell culture is mostly used for identification and cultivation of viruses.

- Cell culture is the process by which cells are grown under controlled conditions.
- Cells are grown in vitro on glass or a treated plastic surface in a suitable growth medium.
- At first growth medium, usually balanced salt solution containing 13 amino acids, sugar, proteins, salts, calf serum, buffer, antibiotics and phenol red are taken and the host tissue or cell is inoculated.
- On incubation the cell divide and spread out on the glass surface to form a confluent monolayer.

Types of cell culture

1. Primary cell culture:

- These are normal cells derived from animal or human cells.
- They are able to grow only for limited time and cannot be maintained in serial culture.
- They are used for the primary isolation of viruses and production of vaccine.
- Examples: Monkey kidney cell culture, Human amnion cell culture

2. Diploid cell culture (Semi-continuous cell lines):

- They are diploid and contain the same number of chromosomes as the parent cells.
- They can be sub-cultured up to 50 times by serial transfer following senescence and the cell strain is lost.
- They are used for the isolation of some fastidious viruses and production of viral vaccines.
- Examples: Human embryonic lung strain, Rhesus embryo cell strain

3. Heteroploid cultures (Continuous cell lines):

- They are derived from cancer cells.
- They can be serially cultured indefinitely so named as continuous cell lines
- They can be maintained either by serial subculture or by storing in deep freeze at -70°c.
- Due to derivation from cancer cells they are not useful for vaccine production.
- Examples: HeLa (Human Carcinoma of cervix cell line), HEP-2 (Humman Epithelioma of larynx cell line), Vero (Vervet monkey) kidney cell lines, BHK-21 (Baby Hamster Kidney cell line).

Susceptible Cell Lines

1. **Herpes Simplex** Vero Hep-2, human diploid (HEK and HEL),human amnion
2. **VZV** human diploid (HEL, HEK)
3. **CMV** human diploid fibroblasts
4. **Adenovirus** Hep2, HEK,
5. **Poliovirus** MK, BGM, LLC-MK2, human diploid, Vero, Hep-2,Rhadomyosarcoma
6. **Coxsackie B** MK, BGM, LLC-MK2, vero, hep-2

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7. **Echo** MK, BGM, LLC-MK2, human diploid, Rd
8. **Influenza A** MK, LLC-MK2, MDCK
9. **Influenza B** MK, LLC-MK2, MDCK
10. **Parainfluenza** MK, LLC-MK2
11. **Mumps** MK, LLC-MK2, HEK, Vero
12. **RSV** Hep-2, Vero
13. **Rhinovirus** human diploid (HEK, HEL)
14. **Measles** MK, HEK
15. **Rubella** Vero, RK13

Advantages of cell culture

1. Relative ease, broad spectrum, cheaper and sensitivity

Disadvantage of cell culture

1. The process requires trained technicians with experience in working on a full time basis.
2. State health laboratories and hospital laboratories do not isolate and identify viruses in clinical work.
3. Tissue or serum for analysis is sent to central laboratories to identify virus.

Cultivation of plant viruses and bacteriophages

Cultivation of plant viruses

There are some methods of Cultivation of plant viruses such as plant tissue cultures, cultures of separated cells, or cultures of protoplasts, etc. viruses can be grown in whole plants.

Leaves are mechanically inoculated by rubbing with a mixture of viruses and an abrasive. When the cell wall is broken by the abrasive, the viruses directly contact the plasma membrane and infect the exposed host cells. A localized necrotic lesion often develops due to the rapid death of cells in the infected area. Some plant viruses can be transmitted only if a diseased part is grafted onto a healthy plant.

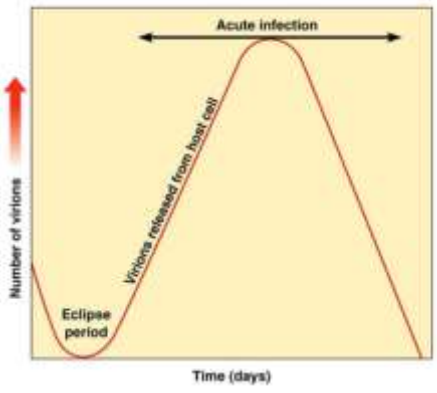
Cultivation of bacteriophages

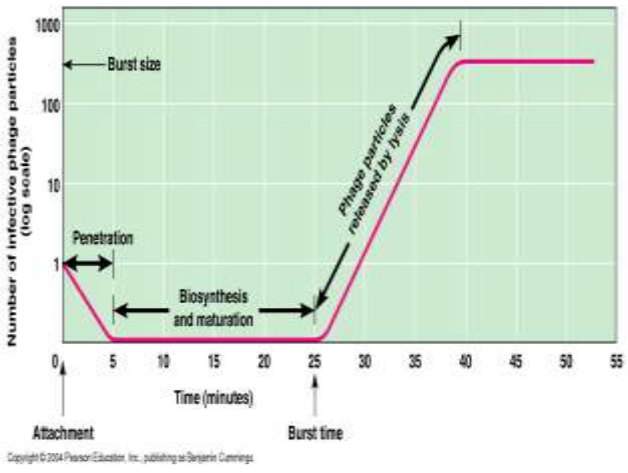
Bacteriophages are cultivated in either broth or agar cultures of young, actively growing bacterial cells.

Viral Multiplication

Viruses do not contain enzymes for energy production or protein synthesis.

For a virus to multiply, it must invade a host cell and direct the host's metabolic machinery to produce viral enzymes, viral proteins, and copies of its nucleic acid, using the host cell's ATP to power the reactions.





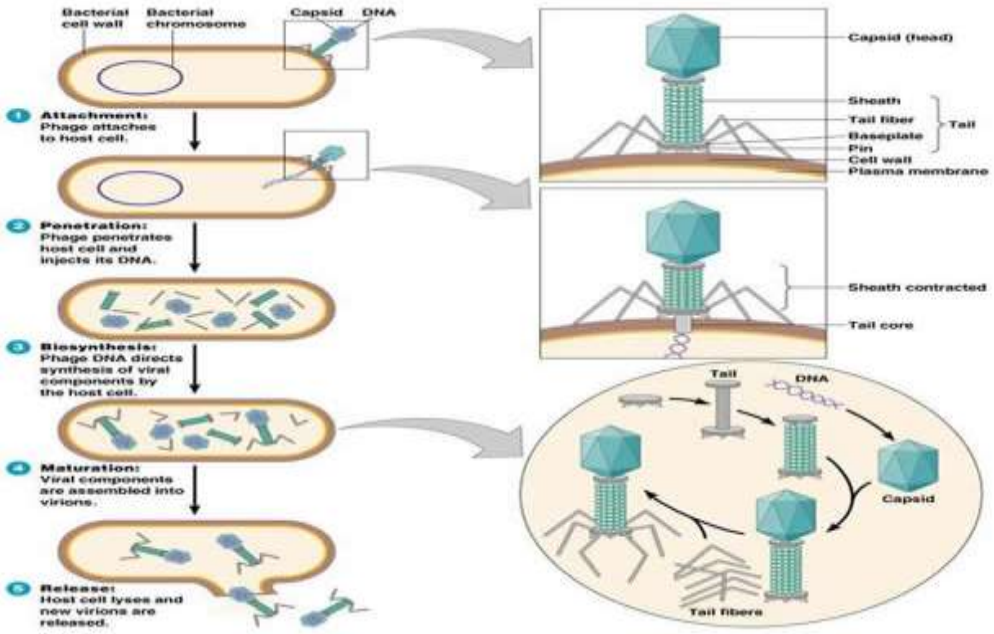
Viral particles disappear upon penetration, none are seen during biosynthesis and assembly, and eventually all cells die so no new virions can be produced.

The **eclipse period** is the period when all viral particles are present but before they are assembled.

Burst time is the time from phage adsorption to release.
Burst size is the number of newly synthesized phages produced from one infected cell.

Multiplication of Bacteriophages

The virus may cause lysis or lysogeny.
Events of the **lytic cycle**:
Attachment or adsorption
Requires a receptor
Penetration
T-evens release lysozyme to break down a portion of the cell wall.
The tail sheath contracts and the tail core is driven through the hole in the wall to the plasma membrane.
The viral genome is then injected into the bacterium.
Biosynthesis
Viral DNA and proteins are synthesized.
Host protein synthesis is stopped by degradation of host DNA, interference with transcription, or repression of translation.
Maturation
During maturation or assembly phage DNA and capsids are assembled into complete viruses.
Release
Release occurs when phage lysozyme breaks down the cell wall and newly synthesized phage particles are released.



Lysogeny is a cycle in which the phage DNA recombines with the bacterial chromosome.
The incorporated viral DNA is now a prophage.
The prophage genes are regulated by a repressor coded for by the prophage, the prophage is replicated each time the host DNA is replicated.
Exposure to mutagens can lead to excision of the prophage and initiation of the lytic cycle.

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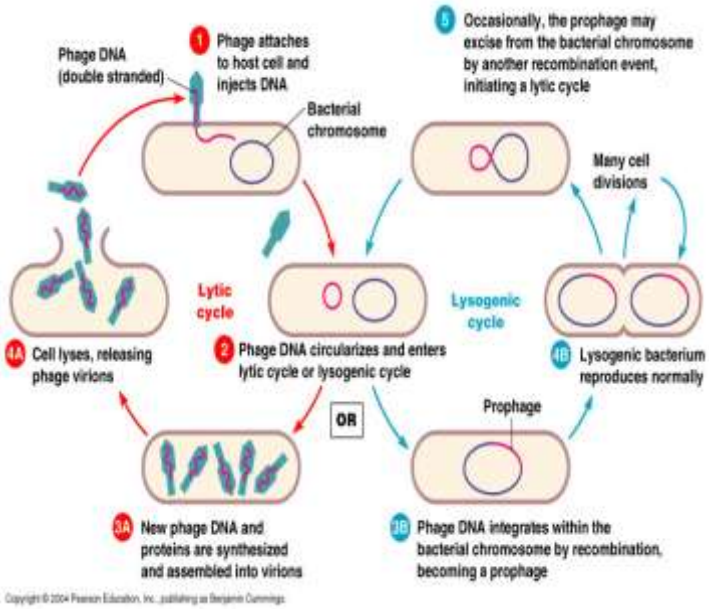
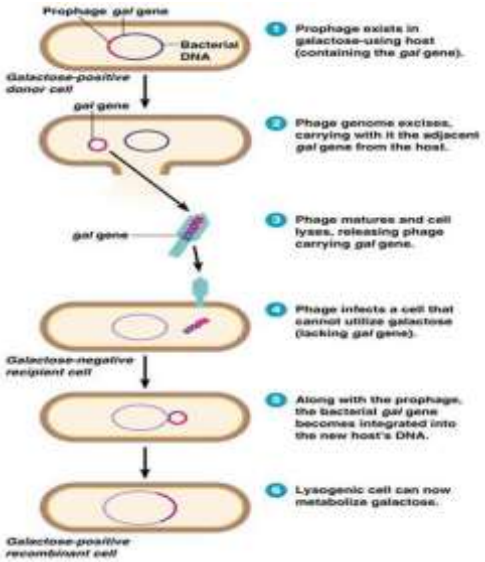


Outcomes of lysogeny

Bacterium can't be reinfected by the same kind of phage.

Host cell may exhibit new properties due to viral genes carried on the prophage

Specialized transduction - host cell may gain new bacterial genes packaged with the phage



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Multiplication of Animal Viruses

TABLE 13.3 Bacteriophage and Viral Multiplication Compared

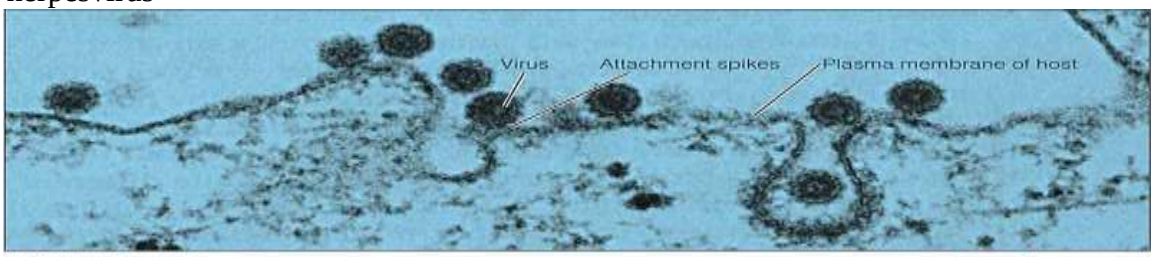
Stage	Bacteriophages	Animal Viruses
Attachment	Tail fibers attach to cell wall proteins	Attachment sites are plasma membrane proteins and glycoproteins
Entry	Viral DNA injected into host cell	Capsid enters by endocytosis or fusion
Uncoating	Not required	Enzymatic removal of capsid proteins
Biosynthesis	In cytoplasm	In nucleus (DNA viruses) or cytoplasm (RNA viruses)
Chronic infection	Lysogeny	Latency; slow viral infections; cancer
Release	Host cell lysed	Enveloped viruses bud out; nonenveloped viruses rupture plasma membrane

Attachment or adsorption

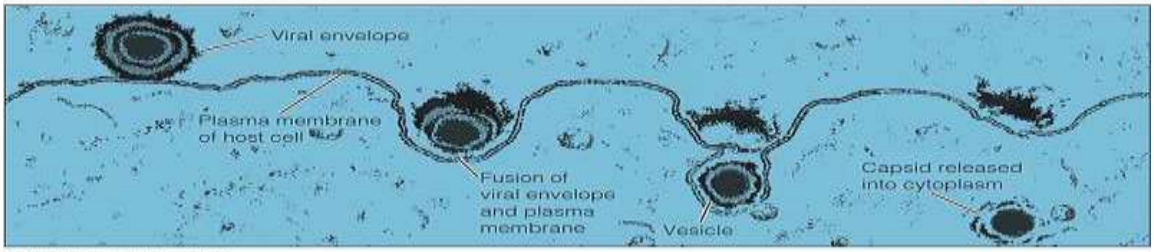
Penetration

a) Endocytosis (pinocytosis) - togavirus

b) Fusion - herpesvirus



(a) Entry of togavirus



(b) Entry of herpesvirus

Uncoating

Uncoating of viral nucleic acid may be accomplished by host or viral enzymes. Bacteriophages don't require uncoating because their nucleic acid is injected into the host cell.

Biosynthesis

Biosynthesis of DNA viruses

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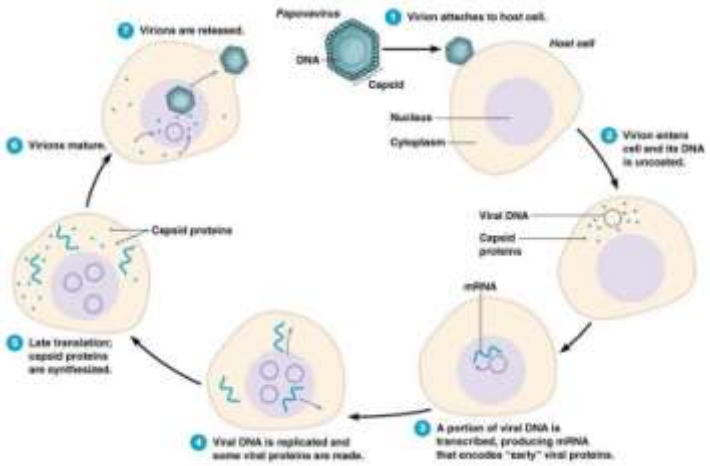
TABLE 13.4 The Biosynthesis of DNA and RNA Viruses Compared

Viral Nucleic Acid	Virus Family	Special Features of Biosynthesis
DNA, single-stranded	Parvoviridae	Cellular enzyme transcribes viral DNA in nucleus
DNA, double-stranded	Herpesviridae Papovaviridae Poxviridae	Cellular enzyme transcribes viral DNA in nucleus Viral enzyme transcribes viral DNA in virion, in cytoplasm
DNA, reverse transcriptase	Hepadnaviridae	Cellular enzyme transcribes viral DNA in nucleus; reverse transcriptase copies mRNA to make viral DNA
RNA, + strand	Picornaviridae Togaviridae	Viral RNA functions as a template for synthesis of RNA polymerase which copies − strand RNA to make mRNA in cytoplasm
RNA, − strand	Rhabdoviridae	Viral enzyme copies viral RNA to make mRNA in cytoplasm
RNA, double-stranded	Reoviridae	Viral enzyme copies − strand RNA to make mRNA in cytoplasm
RNA, reverse transcriptase	Retroviridae	Viral enzyme copies viral RNA to make DNA in cytoplasm; DNA moves to nucleus

DNA of most DNA viruses is released into the nucleus of the host cell.
Transcription and translation of early genes produces enzymes to reproduce viral DNA
Transcription and translation of late genes produces capsid proteins in the cytoplasm.

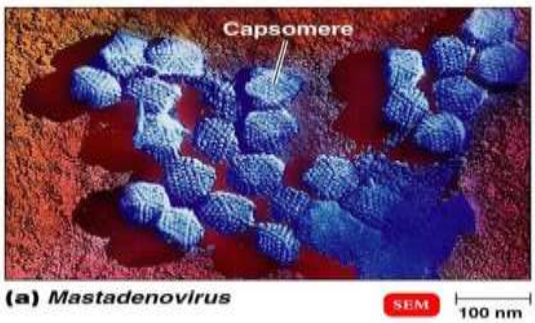


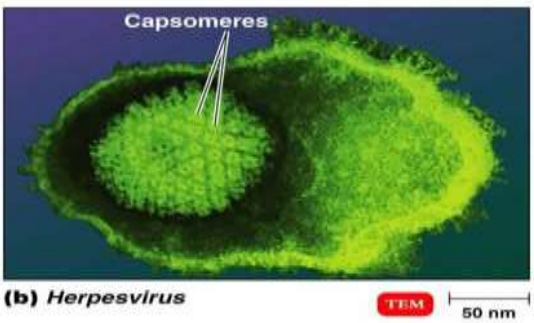
Advantages and Limits

Lytic cycle
- Replication of new viruses is fast
- However, the host is also immediately killed preventing the viral genome from passing onto the next generation of host cells

Lysogenic cycle
- Many more viruses can be made because the viral genome is passed onto future generation of host cells
- However, replication is takes longer because it is dependent on the host cell's replication

DNA-containing animal viruses





Some examples of DNA viruses:

Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Adenoviridae - from adenoids, cause respiratory diseases.
Poxviridae - pox refers to the pus-filled lesions that accompanies the diseases caused by these viruses
Herpesviridae - named after spreading (herpetic) appearance of cold sores
Papoviridae - named for ***pa***pillomas (warts), ***po***lyomas (tumors), and ***va***cuolation (cytoplasmic vacuoles)
Hepadnaviridae - name comes from the fact that they cause ***hepa***titis and contain ***DNA***.

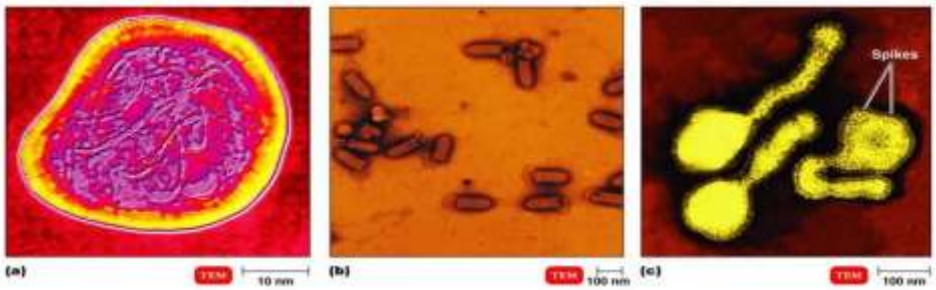
Biosynthesis of RNA Viruses
RNA viruses multiply in the cytoplasm. RNA-dependent RNA polymerase synthesizes a double-stranded RNA. Sense strand (+ strand) can act as mRNA directly and as a template for antisense strand (- strand) synthesis.

ss + strand RNA viral replication: The viral genome, a single stranded sense strand, is transcribed to make antisense (-) strands. The antisense strands serve as the template for making mRNA (sense, or + strands), which code for viral proteins and serve as the viral genome that is packaged inside the capsid during assembly.

ss - strand RNA viral replication: The viral genome, a single stranded antisense strand, is transcribed to make sense (+) strands, which serve as mRNA to code for viral proteins and also as a template to make more copies of the viral genome, single stranded antisense (-) strands, which will be packaged inside the capsid during assembly.

ds +/- RNA viral replication: transcription of - strand makes more copies of the + strand, which serves as mRNA. Transcription of the + strand provides viral protiens (including RNA-directed RNA polymerase) and more copies of - strand, which is packaged along with the complementary + strands in the capsid during assembly.

RNA-containing animal viruses



Picornaviridae - some of the smallest viruses (pico-); contain RNA, name comes from pico + RNA. Single stranded + strand viruses.
Example: poliovirus
Togaviridae - enveloped, name comes from toga (covering). Single stranded + strand viruses - transcription of a - strand serves as a template, the + strands transcribed from the - strand template are produced as a short strand mRNA that codes for envelope proteins and a long strand mRNA that codes for capsid proteins.
Examples: *Art*hropod-*bo*rne *arbo*viruses or alphaviruses which cause viral encephalitis.
Rhabdoviridae - Rhabdo- is from the Greek for rod (they're really more bullet shaped). Single stranded - strand viruses.
Example: *Lyssavirus* (rabiesvirus)
Reoviridae - named for habitat, respiratory and enteric tract. Before they were associated with disease they were considered orphan viruses, name comes from ***r***espiratory, ***e***nteric, and ***o***rphan. Double stranded RNA viruses.
Example: Rotavirus
Retroviridae - *Lentivirus* (HIV-1, HIV-2, HTLV-1, HTLV-2)

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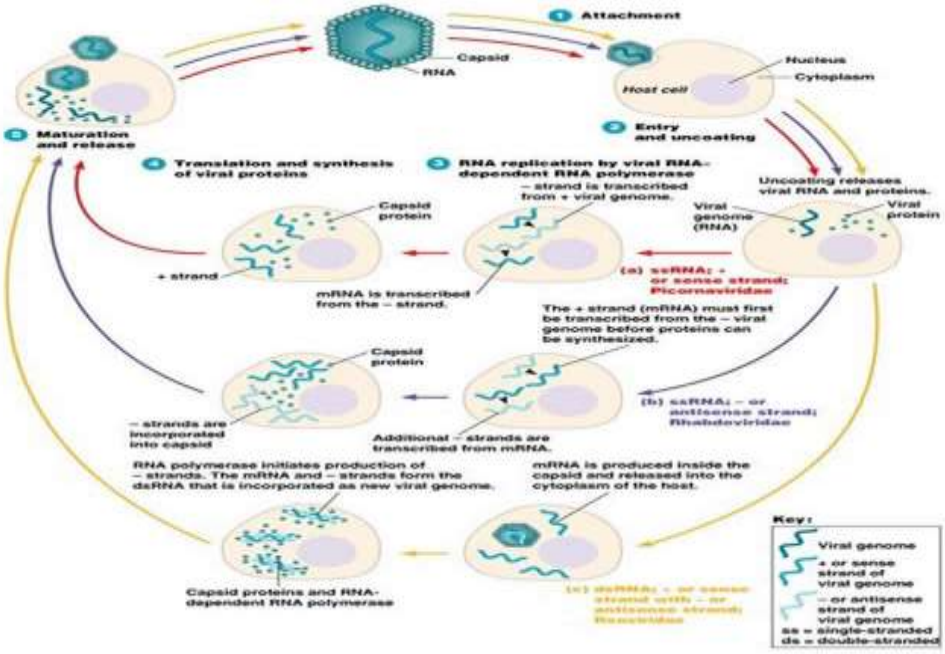
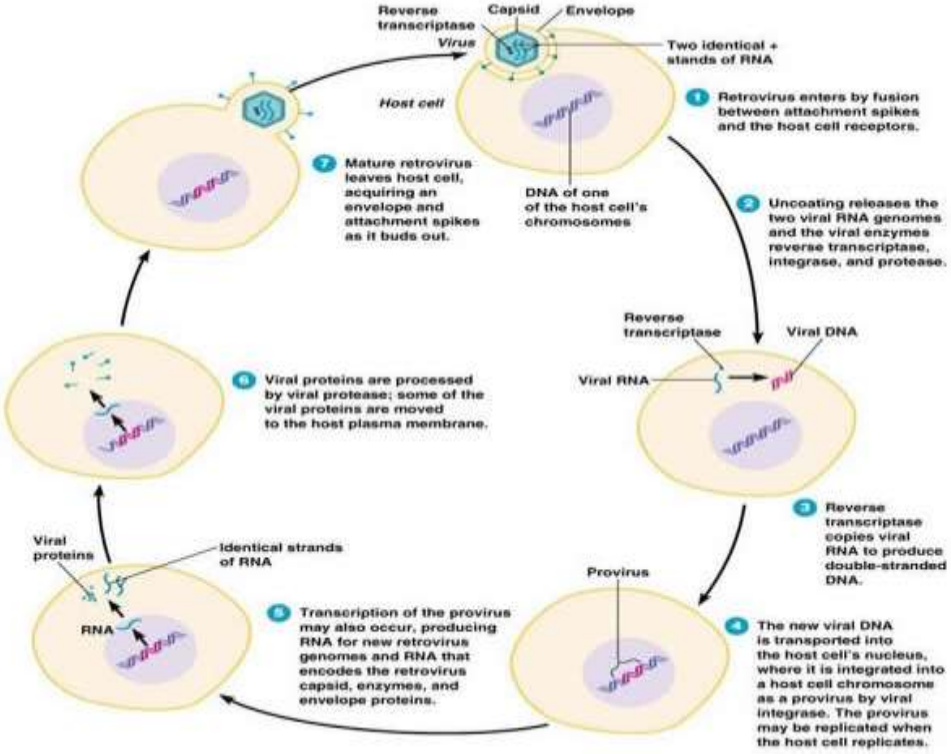


Multiplication of Retroviruses

Retroviruses use reverse transcriptase (RNA-dependent DNA polymerase) to transcribe DNA from RNA. Both viral RNA strands are + strands (making the virus <u>diploid</u>, how about that?) which are transcribed by reverse transcriptase to make complementary DNA strands.

The original viral RNA is degraded and the DNA copies integrate into the host cell's genome.



Maturation or Assembly

Release

Rupture – naked viruses

Budding – enveloped viruses

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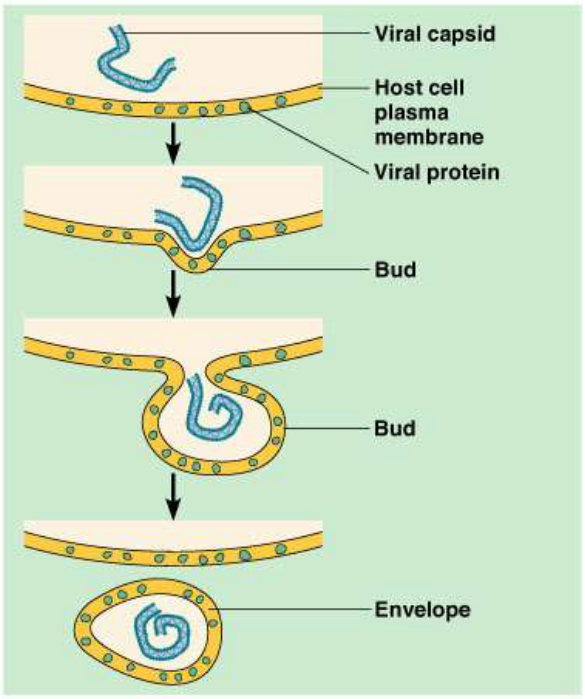
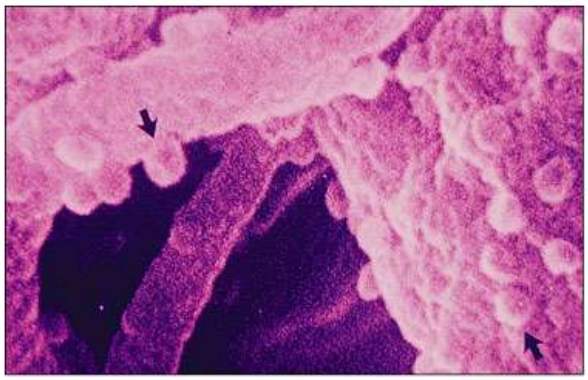


(a) Release by budding



(b) *Alphavirus*

Methods of Study of Viruses

Virus purification

The purification protocol utilizes *gradient ultracentrifugation* to isolate the virus particle on the basis of its size and density. When macromolecules are exposed to a centrifugal force, they will migrate away from the centrifugal axis at a speed roughly proportional to their size. Since virus particles have a unique size compared to cellular macromolecules and organelles, the rate of migration can be used as a tool for purification. This type of purification is called *velocity sedimentation*. The farther away from the centrifugal axis that the macromolecule migrates, the greater the centrifugal force becomes and in turn the rate of migration becomes more rapid. To counterbalance this increase in migration rate, centrifugation is done through a gradient of viscous liquid, such as sucrose or glycerol, so that migration will occur at a relatively constant rate irregardless of the distance from the centrifugal axis. Experimentally, centrifugation is carried out for a period of time such that the virus migrates roughly halfway through the gradient.

The second way in which ultracentrifugation can be used in virus purification is on the basis of density, a technique known as *isopycnic density centrifugation*. In this technique, the virus preparation is placed on a gradient of viscous material, the bottom part of which has a greater density than does the virus. When centrifuged, the virus particles will migration until they reach their density, after which point their migration will cease.

The most powerful purification procedure is a two step procedure employing both velocity sedimentation and isopycnic density centrifugation steps. However, because of time constraints in this laboratory only isopycnic density centrifugation will be done.

Purification of virus and components:

Ultracentrifugation:

The viruses are usually purified with the help of ultracentrifugation. The machine is capable of rotating the samples at 20,000-100,000 rpm under the density gradient of CsCl2 or sucrose. Density at which viruses neither sink nor float when suspended in a density gradient is called as **buoyant density** . The rate at which viral particles sediment under a defined gravitational force is called as**sedimentation coefficient** . The basic unit is the Svedberg (S) which is 10 -13 sec. The S value of a virus is used to estimate its molecular weight.

Types of sedimentation medium:

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A. Sucrose cushions or gradient - A fixed concentration or a linear gradient of sucrose is used. Increasing the density and viscosity of the medium decreases the rate at which virus sediments through them. In g eneral a "cushion" of sucrose is prepared at the bottom of the centrifuge tube and the sample containing virus is overlaid over the cushion. Since most viruses have greater densities than sucrose, separation is based on S values. This method can be used to separate molecules with relatively close S values. Sometime glycerol is also used in place of sucrose.

B. CsCl 2 gradient centrifugation - A linear gradient of CsCl 2 in buffer is prepared in the ultracentrifuge tube. As the concentration of the CsCl 2 is increased the density of the medium increases in the tube so that density is low at the top and high at the bottom. Viral particle centrifuged through this medium will form a band at a position equal to their buoyant density. These are useful to separate viruses of different densities. Limitation of this method is that CsCl 2 can permanently inactivate some viruses.

Other techniques for separation:

Viruses can also be separated by electrophoresis and column chromatography but these are not the preferred way to separate virus while sometimes they are used to separate viral nucleic acids or proteins. Both the methods separate the virus on the basis of charge and/or size. Virus contains a variety of charged macromolecule on its surface which contributes to its electrophoretic mobility or ion-exchange characteristics. Viruses are sometimes ligated with the charged group to be separated by ion exchange chromatography. Molecular sieve chromatography can also be used to purify the viruses where large pores are formed with the help of special agarose through which virus particles can enter.

Purity of viruses:

Many methods are used to assess the purity of virus. The ratio of UV absorption at 260 and 280 nm during a spectrophotometric analysis (260/280) is a characteristic feature to measure the purity of a virus sample and is dependent on the amount of nucleic acid and protein present in the virion. Serological methods such as enzyme-linked immunosorbent assay (ELISA), radioimmuno precipitation assay (RIPA), western blot, virus neutralization test (VNT), and complement fixation are also used to check the puirity of a virus sample. These methods require antibodies specific to viral proteins that may be monoclonal (single type of antibody specific to a single viral protein) or polyclonal (several different antibodies that may recognize several viral proteins or epitopes). Plaque assay is also performed in order to isolate the single colony from a pool of quasispecies viruses.

15MBU502
III BSC MICROBIOLOGY
VIROLOGY

Unit I Qu	Opt 1	Opt 2	Opt 3	Opt 4	Opt 5	Opt 6	Answer
How viru	centrifuga	sedimenta	concentra	Dilution			centrifugation
Which as	Transforn	Endpoint	MAGI as	ELISA			MAGI assay
What RB	human	monkey	sheep	Dog			sheep
Which m	Cs	Mg	Cu	Ag			Cu
_____	western	southern	northern	eastern			northern
Which en	ligase	protease	polymera	helicase			polymerase
Real time	RIA	HIA	FRET	SRID			FRET
The upco	Biotechno	molecular	Microarra	immuno technology			Microarray technolo
Plaque fo	5 to 10 da	3 to 10 da	3 to 5 day	3 to 14 days			3 to 14 days
_____	cellulose	nitrocellu	sulfocellu	ferricellulose			nitrocellulose
qPCR me	quantifica	qualifying	quantitati	quality PCR			quantitation pCR
_____	lung	liver	trachea	Kidney			trachea
At which	attachmer	biosynthes	uncoating	release			release
Advantag	replicatio	replicatio	host rema	Generation passes on			replication is fast
_____	human ce	kidney ce	human cel	Plant cells			human cells & kidn
Viruses ar	Obligate	aerobic	anaerobic	Facultative			Obligate
Who disc	Ivanosky	Twort and	Edward J	Louis Pasteur			Twort and Felix
Which ye	1985	1965	1997	1983			1965
Virus mea	pellet	poison	protein	incomplete			poison
The comp	capsid	protein co	nucleocap	nucleic acid			nucleocapsid
Virus whi	defective	direct viru	temperate	Provirus			defective virus
Infectious	virion	viriod	prion	capsid			virion
Virus is cl	DNA	RNA	DNA & R	Host			DNA & RNA
_____	David Ba	Edward je	Montangi	Felix			David Baltimore
Virus rep	host	own	cell	Direct			host
Virus is s	Interleuki	Interferon	antivirals	Antitumours			Interferons
_____	young	trickling	suckling	Old			suckling
_____	CFE	CPE	CDE	CHE			CPE
Yolk sac	HIV	Pox	HSV	Influenza			Pox
TCID 50	time cons	Tissue cu	Tissue cu	time consumed infectious dose			Tissue culture infect
For propa	Host	other	own	neighbour			Host
Monopart	one nucle	two nucle	multiple r	no nucleic acid			one nucleic acid
Proteins	Proteins	Nucleous	Nucleopr	Capsid			Nucleoproteins
Envelope	virus	host	protein	nucleic acid			host
Single typ	capsid	protein co	nucleocap	nucleic acid			capsid
Virus whi	defective	helical str	complex	Provirus			defective virus
Size of Fi	80 & 400	18 & 40 r	80 & 40 r	18 & 400 nm			80 & 400 nm
Protectio	Viral end	binding o	Synthesis	Uncoating of nucleic acid			Viral endonuclease a
Which on	Diphtheri	Haemoph	Hepatitis	HIV Vaccine			Diphtheria-pertusis-t

Which on	Blood tra	Fetal cont	Ingestion	Transmission of the virus from				Fetal contact with in
The findi	Bordetella	Epstein-B	Mycoplas	Respiratory syncytial virus (RS				Respiratory syncytial
All of the	Coxsackie	Coxsackie	Echo viru	Rhinovirus				Echo virus
A divorce	Treatmen	All the pa	A western	The patient should be reassured				Treatment with zido
In a chron	Hepatitis	Hepatitis	Hepatitis	Anti-HBs Ag				Hepatitis B Surface
A retrovir	gag	pol	env	onc				gag
A sexuall	A prolong	A second	failure of	reactivation of a latent infection				A prolonged period o
Viruses ra	1-100 nm	25-300 nr	10-100 µr	400-1000 nm				25-300 nm
Virus is c	DNA	RNA	DNA & R	Host				DNA & RNA
_____	Helical	Icosahedr	radical	spiral				Icosahedral
Capsomer	tetrads	trions	hexons	pentons				hexons
Minimum	15	5	12	10				12
Which vir	Mega	Mimi	Pandora	Alien				Pandora
_____	4 - 6 kb	1.7 - 2.3 k	5.1 - 7.8 k	6 - 7.8 kb				4 - 6 kb
How man	5-8	5-6	5-9	5-7				5-6
How man	3 or 2	3 or 1	3 or 4	3 or 5				3 or 2
What is a	conjuctio	Assortme	recombin	reassortment				reassortment
What type	reverse	frame shi	point	active				point
Viruses ca	penetratio	adsoption	Entry	absorption				adsoption
During U	lytic	lipolytic	proteolyti	digestive				proteolytic
_____	Prions	Virions	Pseudovir	viriods				Pseudovirions
What is th	Entero vir	Herpes vi	Arbo viru	Retroviruses				Herpes viruses

›gy

ıey cells

tious dose

ıctivity
:etanus (DPT) vaccine

fected blood during childbirth
l virus (RSV)

ovudine (azidothymidine, AZT)
Antigen (Hbs Ag)

of viremia following the initial infection

2017

III B. Sc Microbiology –Virology
Unit II

LECTURE PLAN

LECTURE PLAN - UNIT -11			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	The structure of virus	T2 30 -48
2	1	virus and prion	T2 401 - 414
3	1	General properties of viruses	T3 -417 - 429
4	1	viral symmetry	W1
5	1	Viral Capsids	
6	1	viral nucleic acid	R1 367- 395
7	1	viral enzymes	R1 367 -395
8	1	viral envelopes	R1 367 -395
9	1	Host cell-viral interaction	T3 436-447
10	1	viral replication	T1 31-41
11	1	Lytic & Lysogenic cycles	T1 41-52
12	1	Revision of UNIT II	
13	1	UNIT II Test	
Textbooks :		T1-medical virology -White & fenner, Academic press publishers T2-Modern virology -dimmock-Black well publishing T3-Textbook of Microbiology- Paniker- Orient longman publishers	
Reference books:		R1- Medical Microbiology –Jawertz – McGraw hill Publishers	
Website:		W1- www.microbesonline.com W2 - www.virologyonline.com	
Journals:			

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Opinions differ on whether viruses are a form of life, or organic structures that interact with living organisms. They have been described as "organisms at the edge of life", since they resemble organisms in that they possess genes, evolve by natural selection and reproduce by creating multiple copies of themselves through self-assembly. Although they have genes, they do not have a cellular structure, which is often seen as the basic unit of life. Viruses do not have their own metabolism, and require a host cell to make new products. They therefore cannot naturally reproduce outside a host cell – although bacterial species such as rickettsia and chlamydia are considered living organisms despite the same limitation. Accepted forms of life use cell division to reproduce, whereas viruses spontaneously assemble within cells. They differ from autonomous growth of crystals as they inherit genetic mutations while being subject to natural selection. Virus self-assembly within host cells has implications for the study of the origin of life, as it lends further credence to the hypothesis that life could have started as self-assembling organic molecules

Structure and Classification of Viruses

General Concepts

Structure and Function

Viruses are small obligate intracellular parasites, which by definition contain either a RNA or DNA genome surrounded by a protective, virus-coded protein coat. Viruses may be viewed as mobile genetic elements, most probably of cellular origin and characterized by a long co-evolution of virus and host. For propagation, viruses depend on specialized host cells supplying the complex metabolic and biosynthetic machinery of eukaryotic or prokaryotic cells. A complete virus particle is called a virion. The main function of the virion is to deliver its DNA or RNA genome into the host cell so that the genome can be expressed (transcribed and translated) by the host cell. The viral genome, often with associated basic proteins, is packaged inside a symmetric protein capsid. The nucleic acid-associated protein, called nucleoprotein, together with the genome, forms the nucleocapsid. In enveloped viruses, the nucleocapsid is surrounded by a lipid bilayer derived from the modified host cell membrane and studded with an outer layer of virus envelope glycoproteins.

Classification of Viruses

Morphology: Viruses are grouped on the basis of size and shape, chemical composition and structure of the genome and mode of replication. Helical morphology is seen in nucleocapsids of many filamentous and pleomorphic viruses. Helical nucleocapsids consist of a helical array of capsid proteins (protomers) wrapped around a helical filament of nucleic acid. Icosahedral morphology is characteristic of the nucleocapsids of many “spherical” viruses. The number and arrangement of the capsomeres (morphologic subunits of the icosahedron) are useful in identification and classification. Many viruses also have an outer envelope.

Chemical Composition and Mode of Replication: The genome of a virus may consist of DNA or RNA, which may be single stranded (ss) or double stranded (ds), linear or circular. The entire genome may occupy either one nucleic acid molecule (monopartite genome) or several nucleic acid segments (multipartite genome). The different types of genome necessitate different replication strategies.

Nomenclature

Aside from physical data, genome structure and mode of replication are criteria applied in the classification and nomenclature of viruses, including the chemical composition and configuration of the nucleic acid, whether the genome is monopartite or multipartite. The genomic RNA strand of single-stranded RNA viruses is called sense (positive sense, plus sense) in orientation if it can serve as mRNA, and antisense (negative sense, minus sense) if a complementary strand synthesized by a viral RNA transcriptase serves as mRNA. Also considered in viral classification is the site of capsid assembly and, in enveloped viruses, the site of envelopment.

Structure and Function

Viruses are inert outside the host cell. Small viruses, e.g., polio and tobacco mosaic virus, can even be crystallized. Viruses are unable to generate energy. As obligate intracellular parasites, during replication, they fully depend on the complicated biochemical machinery of eukaryotic or prokaryotic cells. The main purpose of a virus is to deliver its genome into the host cell to allow its expression (transcription and translation) by the host cell.

A fully assembled infectious virus is called a virion. The simplest virions consist of two basic components: nucleic acid (single- or double-stranded RNA or DNA) and a protein coat, the capsid, which functions as a shell

to protect the viral genome from nucleases and which during infection attaches the virion to specific receptors exposed on the prospective host cell. Capsid proteins are coded for by the virus genome. Because of its limited size (Table 41-1) the genome codes for only a few structural proteins (besides non-structural regulatory proteins involved in virus replication). Capsids are formed as single or double protein shells and consist of only one or a few structural protein species. Therefore, multiple protein copies must self assemble to form the continuous three-dimensional capsid structure. Self assembly of virus capsids follows two basic patterns: helical symmetry, in which the protein subunits and the nucleic acid are arranged in a helix, and icosahedral symmetry, in which the protein subunits assemble into a symmetric shell that covers the nucleic acid-containingcore.

Some virus families have an additional covering, called the envelope, which is usually derived in part from modified host cell membranes. Viral envelopes consist of a lipid bilayer that closely surrounds a shell of virus-encoded membrane-associated proteins. The exterior of the bilayer is studded with virus-coded, glycosylated (trans-) membrane proteins. Therefore, enveloped viruses often exhibit a fringe of glycoprotein spikes or knobs, also called peplomers. In viruses that acquire their envelope by budding through the plasma or another intracellular cell membrane, the lipid composition of the viral envelope closely reflects that of the particular host membrane. The outer capsid and the envelope proteins of viruses are glycosylated and important in determining the host range and antigenic composition of the virion. In addition to virus-specified envelope proteins, budding viruses carry also certain host cell proteins as integral constituents of the viral envelope. Virus envelopes can be considered an additional protective coat. Larger viruses often have a complex architecture consisting of both helical and isometric symmetries confined to different structural components. Small viruses, e.g., hepatitis B virus or the members of the picornavirus or parvovirus family are orders of magnitude more resistant than are the larger complex viruses, e.g. members of the herpes or retrovirus families.

TABLE 41-1 Chemical and Morphologic Properties of Animal Virus Families Relevant to Human Disease

	Virion							
Family	Viral Genome: Type, Configuration[a] and Number of Bases per strand (x 10^3)	Shape[b]	Diameter (nm)	Enveloped[c]	Capsid Symmetry	Number of Capsomeres[d]	Site of Capsid Assembly	Enzymes, e.g. Transcriptase present in Virion
Circoviridae	ssDNA, circular; 0.6-1.2	s	17-22	0	Icosahedral	32?	Nucleus	None
Parvoviridae	ssDNA, linear, sense or antisense; 4-6	s	18-26	0	Icosahedral	32	Nucleus	None
Papovaviridae	dsDNA, circular; 5.1 / 7.9	s	45 / 55	0	Icosahedral	72	Nucleus	None
Adenoviridae	dsDNA, linear; 35-40	s	75-80	0	Icosahedral	252	Nucleus	None
Herpesviridae	dsDNA, linear; 124-235	s	120-200	+	Icosahedral	162	Nucleus	Thymidine kinase
Iridoviridae	dsDNA, linear; 170-200	s	125-300	+	Icosahedral	ca. 1,500	Cytoplasm	DNA-dependent RNA polymerase
Poxviridae	dsDNA, linear, covalently closed; 130-370	x	240x300	+	Complex	–	Cytoplasm	DNA-dependent RNA polymerase Protein kinase
Hepadnaviridae	dsDNA, circular, 1 ss-region; 3.0-3.3/2.0	s	40-48	+	Icosahedral	180	Nucleus	DNA-dependent DNA polymerase

TABLE 41-1 Chemical and Morphologic Properties of Animal Virus Families Relevant to Human Disease (continued)

Family	Viral Genome: Type, Configuration[a] and Number of Bases per strand (X 10^3)	Shape[b]	Diameter (nm)	Enveloped[c]	Capsid Symmetry	Number of Capsomeres[d]	Site of Capsid Assembly	Enzymes, e.g. Transcriptase present in Virion
Picornaviridae	ssRNA, linear; 7-8.5	s	22-30	0	Icosahedral	32	Cytoplasm	None
Astroviridae	ssRNA, linear, sense 6.8-7.9	s	28-30	0	Icosahedral	32?	Cytoplasm	None
Caliciviridae	ssRNA, linear, sense 7.4-7.7	s	35-39	0	Icosahedral	90	Cytoplasm	None
Togaviridae	ssRNA, linear, sense 9.7-11.8	s	70	+	Icosahedral	?	Cytoplasm	None
Flaviviridae	dsRNA, linear, sense 10-12	s	45-50	+	Icosahedral	unknown	Cytoplasm	None
Reoviridae	dsRNA, linear, 10-12 segments; 18-23	s	60-80	0	Icosahedral	32 or 92	Cytoplasm	RNA-dependent RNA polymerase
Orthomyxoviridae	dsRNA, linear, 8 molecules, antisense; 10-13.6	s-pleom[e]	80-120	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Paramyxoviridae	dsRNA, linear, antisense; 15	s-pleom	150-300	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Rhabdoviridae	ssRNA, linear, antisense;11-15	u	60x180	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Bunyaviridae	ssRNA, linear, 3 molecules, antisense; 11-20	s-pleom	90-120	+	Helical	–	Cytoplasm	RNA dependent RNA polymerase
Coronaviridae	ssRNA, linear, sense; 30	s-pleom	120-160	+	Helical	–	Cytoplasm	None
Arenaviridae	ssRNA, linear, 2 species + ribosomal RNA; 3.4	s-pleom	110-130	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Retroviridae	ssRNA, linear, inverted dimer of sense strand; 7-11	s-pleom	90-120	+	Icosahedral (type C)	–	Cytoplasm	RNA-dependent DNA polymerase Protease, Integrase
Filoviridae	ssRNA, linear, antisense; 19.1	Bacilli-form[e]	80x800-2,500	+	Helical	–	Cytoplasm	RNA-transcrip-tase/poly merase

[a]ss = single stranded; ds = double stranded. [b]S = spherical; X = brickshaped or ovoid; U = elongated with parallel sides and a round end; pleom = pleomorphic. [c]Most enveloped viruses are sensitive to lipid solvents. [d]Applicable to viruses with icosahedral symmetry. [e]Filamentous forms also occur.

Table 41-1 Chemical and Morphologic Properties of Animal Virus Families Relevant to Human Disease.

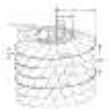
Classification of Viruses

Viruses are classified on the basis of morphology, chemical composition, and mode of replication. The viruses that infect humans are currently grouped into 21 families, reflecting only a small part of the spectrum of the multitude of different viruses whose host ranges extend from vertebrates to protozoa and from plants and fungi to bacteria.

Morphology

Helical Symmetry

In the replication of viruses with helical symmetry, identical protein subunits (protomers) self-assemble into a helical array surrounding the nucleic acid, which follows a similar spiral path. Such nucleocapsids form rigid, highly elongated rods or flexible filaments; in either case, details of the capsid structure are often discernible by electron microscopy. In addition to classification as flexible or rigid and as naked or enveloped, helical nucleocapsids are characterized by length, width, pitch of the helix, and number of protomers per helical turn. The most extensively studied helical virus is tobacco mosaic virus (Fig. 41-1). Many important structural features of this plant virus have been detected by x-ray diffraction studies. Figure 41-2 shows Sendai virus, an enveloped virus with helical nucleocapsid symmetry, a member of the paramyxovirus family.



The helical structure of the rigid tobacco mosaic virus rod. About 5 percent of the length of the virion is depicted. Individual 17,400-Da protein subunits (protomers) assemble in a helix with an axial repeat of 6.9 nm (49 subunits per three turns). About 5 percent of the length of the virion is depicted. Individual 17,400-Da protein subunits (protomers) assemble in a helix with an axial repeat of 6.9 nm (49 subunits per three turns). Each turn contains a nonintegral number of subunits (16-1/3), producing a pitch of 2.3 nm. The RNA ($2\times1O^6$ Da) is sandwiched internally between adjacent turns of capsid protein, forming a RNA helix of the same pitch, 8 nm in diameter, that extends the length of virus, with three nucleotide bases in contact with each subunit. Some 2,130 protomers per virion cover and protect the RNA. The complete virus is 300 nm long and 18 nm in diameter with a hollow cylindrical core 4 nm in diameter.

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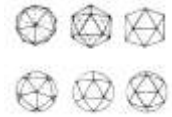
Fragments of flexible helical nucleocapsids (NC) of Sendai virus, a paramyxovirus, are seen either within the protective envelope (E) or free, after rupture of the envelope. The intact nucleocapsid is about 1,000 nm long and 17 nm in diameter; its pitch

Icosahedral Symmetry

An icosahedron is a polyhedron having 20 equilateral triangular faces and 12 vertices (Fig. 41-3). Lines through opposite vertices define axes of fivefold rotational symmetry: all structural features of the polyhedron repeat five times within each 360° of rotation about any of the fivefold axes. Lines through the centers of opposite triangular faces form axes of threefold rotational symmetry; twofold rotational symmetry axes are formed by lines through midpoints of opposite edges. An icosaheron (polyhedral or spherical) with fivefold, threefold, and twofold axes of rotational symmetry (Fig. 41-3) is defined as having 532 symmetry (read as 5,3,2).



Icosahedral models seen, left to right, on fivefold, threefold, and twofold axes of rotational symmetry. These axes are perpendicular to the plane of the page and pass through the centers of each figure. Both polyhedral (upper) and spherical (lower) forms.

Viruses were first found to have 532 symmetry by x-ray diffraction studies and subsequently by electron microscopy with negative-staining techniques. In most icosahedral viruses, the protomers, i.e. the structural polypeptide chains, are arranged in oligomeric clusters called capsomeres, which are readily delineated by negative staining electron microscopy and form the closed capsid shell (Fig. 41-4 a/b). The arrangement of capsomeres into an icosahedral shell (compare Fig. 41-4 with the upper right model in Fig. 41-3) permits the classification of such viruses by capsomere number and pattern. This requires the identification of the nearest pair of vertex capsomeres (called penton: those through which the fivefold symmetry axes pass) and the distribution of capsomeres between them.

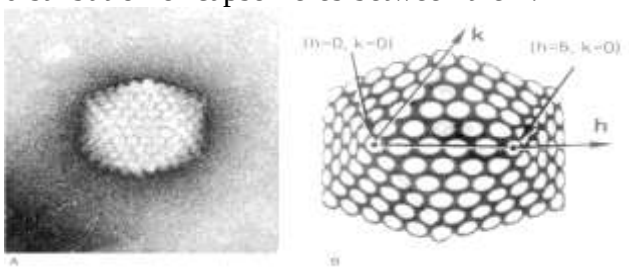


Figure 41-4

Adenovirus after negative stain electron microscopy. (A) The capsid reveals the typical isometric shell made up from 20 equilateral triangular faces. The 252 capsomeres, 12 pentons and the 240 hollow hexon capsomeres are arranged in a T = 25 symmetry

In the adenovirus model in Figure 41-4, one of the penton capsomeres is arbitrarily assigned the indices h = 0, k = 0 (origin), where h and k are the indicated axes of the inclined (60°) net of capsomeres. The net axes are formed by lines of the closest-packed neighboring capsomeres. In adenoviruses, the h and k axes also coincide with the edges of the triangular faces. Any second neighboring vertex capsomere has indices h = 5, k = 0 (or h = 0, k = 5). The capsomere number (C) can be determined to be 252 from the h and k indices and the equation: $C = 10(h^2 + hk + k^2) + 2$. This symmetry and number of capsomeres is typical of all members of the adenovirus family.

Structure

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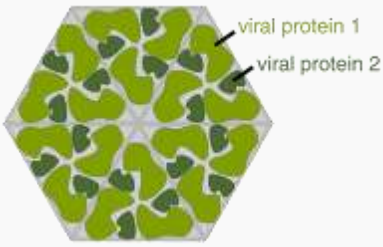
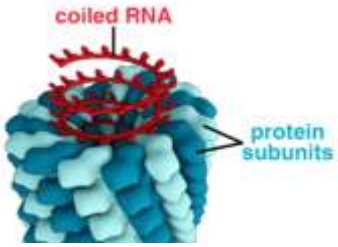
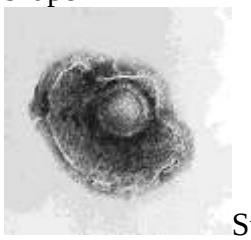


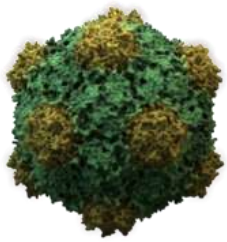
Diagram of how a virus capsid can be constructed using multiple copies of just two protein molecules



Structure of tobacco mosaic virus: RNA coiled in a helix of repeating protein sub-units


Structure of icosahedraladenovirus. Electron micrograph of with a cartoon to show shape


Structure of chickenpox virus. They have a lipid envelope


Structure of an icosahedralcowpea mosaic virus

Viruses display a wide diversity of shapes and sizes, called *morphologies*. In general, viruses are much smaller than bacteria. Most viruses that have been studied have a diameter between 20 and 300 nanometres. Some filoviruses have a total length of up to 1400 nm; their diameters are only about 80 nm. Most viruses cannot be seen with an optical microscope so scanning and transmission electron microscopes are used to visualise virions. To increase the contrast between viruses and the background, electron-dense "stains" are used. These are solutions of salts of heavy metals, such as tungsten, that scatter the electrons from regions covered with the stain. When virions are coated with stain (positive staining), fine detail is obscured. Negative staining overcomes this problem by staining the background only.

A complete virus particle, known as a virion, consists of nucleic acid surrounded by a protective coat of protein called a capsid. These are formed from identical protein subunits called capsomeres. Viruses can have a lipid "envelope" derived from the host cell membrane. The capsid is made from proteins encoded by the viral genome and its shape serves as the basis for morphological distinction. Virally coded protein subunits will self-assemble to form a capsid, in general requiring the presence of the virus genome. Complex viruses code for proteins that assist in the construction of their capsid. Proteins associated with nucleic acid are known as nucleoproteins, and the association of viral capsid proteins with viral nucleic acid is called a nucleocapsid.

Prepared by :Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

The capsid and entire virus structure can be mechanically (physically) probed through atomic force microscopy. In general, there are four main morphological virus types:

Helical

These viruses are composed of a single type of capsomere stacked around a central axis to form a helical structure, which may have a central cavity, or tube. This arrangement results in rod-shaped or filamentous virions: These can be short and highly rigid, or long and very flexible. The genetic material, in general, single-stranded RNA, but ssDNA in some cases, is bound into the protein helix by interactions between the negatively charged nucleic acid and positive charges on the protein. Overall, the length of a helical capsid is related to the length of the nucleic acid contained within it and the diameter is dependent on the size and arrangement of capsomeres. The well-studied tobacco mosaic virus is an example of a helical virus.

Icosahedral

Most animal viruses are icosahedral or near-spherical with chiral icosahedral symmetry. A regular icosahedron is the optimum way of forming a closed shell from identical sub-units. The minimum number of identical capsomeres required is twelve, each composed of five identical sub-units. Many viruses, such as rotavirus, have more than twelve capsomers and appear spherical but they retain this symmetry. Capsomeres at the apices are surrounded by five other capsomeres and are called pentons. Capsomeres on the triangular faces are surrounded by six others and are called hexons. Hexons are in essence flat and pentons, which form the 12 vertices, are curved. The same protein may act as the subunit of both the pentamers and hexamers or they may be composed of different proteins.

Prolate

This is an icosahedron elongated along the fivefold axis and is a common arrangement of the heads of bacteriophages. This structure is composed of a cylinder with a cap at either end.

Envelope

Some species of virus envelop themselves in a modified form of one of the cell membranes, either the outer membrane surrounding an infected host cell or internal membranes such as nuclear membrane or endoplasmic reticulum, thus gaining an outer lipid bilayer known as aviral envelope. This membrane is studded with proteins coded for by the viral genome and host genome; the lipid membrane itself and any carbohydrates present originate entirely from the host. The influenza virus and HIV use this strategy. Most enveloped viruses are dependent on the envelope for their infectivity.

Complex

These viruses possess a capsid that is neither purely helical nor purely icosahedral, and that may possess extra structures such as protein tails or a complex outer wall. Some bacteriophages, such as Enterobacteria phage T4, have a complex structure consisting of an icosahedral head bound to a helical tail, which may have a hexagonal base plate with protruding protein tail fibres. This tail structure acts like a molecular syringe, attaching to the bacterial host and then injecting the viral genome into the cell.

The poxviruses are large, complex viruses that have an unusual morphology. The viral genome is associated with proteins within a central disc structure known as a nucleoid. The nucleoid is surrounded by a membrane and two lateral bodies of unknown function. The virus has an outer envelope with a thick layer of protein studded over its surface. The whole virion is slightly pleiomorphic, ranging from ovoid to brick shape. Mimivirus is one of the largest characterised viruses, with a capsid diameter of 400 nm. Protein filaments measuring 100 nm project from the surface. The capsid appears hexagonal under an electron microscope, therefore the capsid is probably icosahedral. In 2011, researchers discovered the largest then known virus in samples of water collected from the ocean floor off the coast of Las Cruces, Chile. Provisionally named *Megavirus chilensis*, it can be seen with a basic optical microscope. In 2013, thePandoravirus genus was discovered in Chile and Australia, and has genomes about twice as large as Megavirus and Mimivirus.[85]

Some viruses that infect Archaea have complex structures that are unrelated to any other form of virus, with a wide variety of unusual shapes, ranging from spindle-shaped structures, to viruses that resemble hooked rods, teardrops or even bottles. Other archaeal viruses resemble the tailed bacteriophages, and can have multiple tail structures.

Genome

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Genomic diversity among viruses

Property	Parameters
Nucleic acid	• DNA • RNA • Both DNA and RNA (at different stages in the life cycle)
Shape	• Linear • Circular • Segmented
Strandedness	• Single-stranded • Double-stranded • Double-stranded with regions of single-strandedness
Sense	• Positive sense (+) • Negative sense (−) • Ambisense (+/−)

An enormous variety of genomic structures can be seen among viral species; as a group, they contain more structural genomic diversity than plants, animals, archaea, or bacteria. There are millions of different types of viruses, although only about 5,000 types have been described in detail. As of September 2015, the NCBI Virus genome database has more than 75,000 complete genome sequences. but there are doubtlessly many more to be discovered.

A virus has either a **DNA** or an **RNA** genome and is called a DNA virus or an RNA virus, respectively. The vast majority of viruses have RNA genomes. Plant viruses tend to have single-stranded RNA genomes and bacteriophages tend to have double-stranded DNA genomes.

Viral genomes are *circular*, as in the polyomaviruses, or *linear*, as in the adenoviruses. The type of nucleic acid is irrelevant to the shape of the genome. Among RNA viruses and certain DNA viruses, the genome is often divided up into separate parts, in which case it is called segmented. For RNA viruses, each segment often codes for only one protein and they are usually found together in one capsid. However, all segments are not required to be in the same virion for the virus to be infectious, as demonstrated by brome mosaic virusand several other plant viruses.

A viral genome, irrespective of nucleic acid type, is almost always either *single-stranded* or *double-stranded*. Single-stranded genomes consist of an unpaired nucleic acid, analogous to one-half of a ladder split down the middle. Double-stranded genomes consist of two complementary paired nucleic acids, analogous to a ladder. The virus particles of some virus families, such as those belonging to the*Hepadnaviridae*, contain a genome that is partially double-stranded and partially single-stranded.

For most viruses with RNA genomes and some with single-stranded DNA genomes, the single strands are said to be either positive-sense (called the *plus-strand*) or negative-sense (called the *minus-strand*), depending on if they are complementary to the viral messenger RNA (mRNA). Positive-sense viral RNA is in the same sense as viral mRNA and thus at least a part of it can be immediately translated by the host cell. Negative-sense viral RNA is complementary to mRNA and thus must be converted to positive-sense RNA by an RNA-dependent RNA polymerase before translation. DNA nomenclature for viruses with single-sense genomic ssDNA is similar to RNA nomenclature, in that the *template strand* for the viral mRNA is complementary to it (−), and the *coding strand* is a copy of it (+). However, several types of ssDNA and ssRNA viruses have genomes that are ambisense in that transcription can occur off both strands in a double-stranded replicative intermediate. Examples include geminiviruses, which are ssDNA plant viruses and arenaviruses, which are ssRNA viruses of animals.

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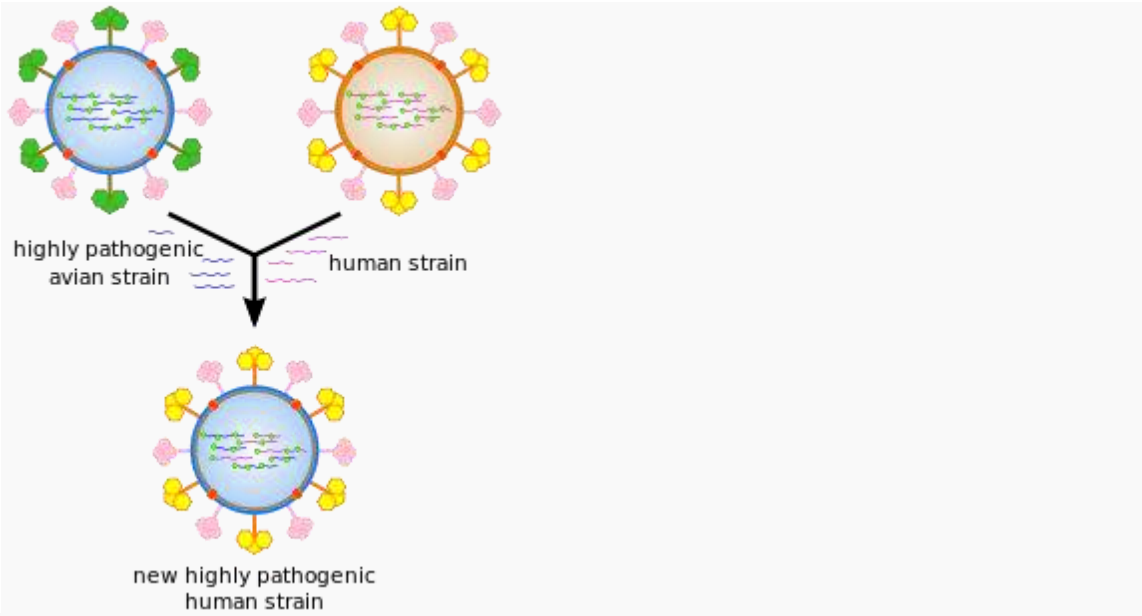
Genome size varies greatly between species. The smallest viral genomes – the ssDNA circoviruses, family *Circoviridae* – code for only two proteins and have a genome size of only two kilobases; the largest– the pandoraviruses–have genome sizes of around two megabases which code for about 2500 proteins.
In general, RNA viruses have smaller genome sizes than DNA viruses because of a higher error-rate when replicating, and have a maximum upper size limit. Beyond this limit, errors in the genome when replicating render the virus useless or uncompetitive. To compensate for this, RNA viruses often have segmented genomes – the genome is split into smaller molecules – thus reducing the chance that an error in a single-component genome will incapacitate the entire genome. In contrast, DNA viruses generally have larger genomes because of the high fidelity of their replication enzymes.[93] Single-strand DNA viruses are an exception to this rule, however, as mutation rates for these genomes can approach the extreme of the ssRNA virus case.

Genetic mutation



How antigenic shift, or reassortment, can result in novel and highly pathogenic strains of human flu
Viruses undergo genetic change by several mechanisms. These include a process called antigenic drift where individual bases in the DNA or RNA mutate to other bases. Most of these point mutations are "silent" – they do not change the protein that the gene encodes – but others can confer evolutionary advantages such as resistance to antiviral drugs. Antigenic shift occurs when there is a major change in the genome of the virus. This can be a result of recombination or reassortment. When this happens with influenza viruses, pandemics might result. RNA viruses often exist as quasispecies or swarms of viruses of the same species but with slightly different genome nucleoside sequences. Such quasispecies are a prime target for natural selection.
Segmented genomes confer evolutionary advantages; different strains of a virus with a segmented genome can shuffle and combine genes and produce progeny viruses or (offspring) that have unique characteristics. This is called reassortment or *viral sex*.
Genetic recombination is the process by which a strand of DNA is broken and then joined to the end of a different DNA molecule. This can occur when viruses infect cells simultaneously and studies of viral evolution have shown that recombination has been rampant in the species studied.[100] Recombination is common to both RNA and DNA viruses.

Virus Core Structure

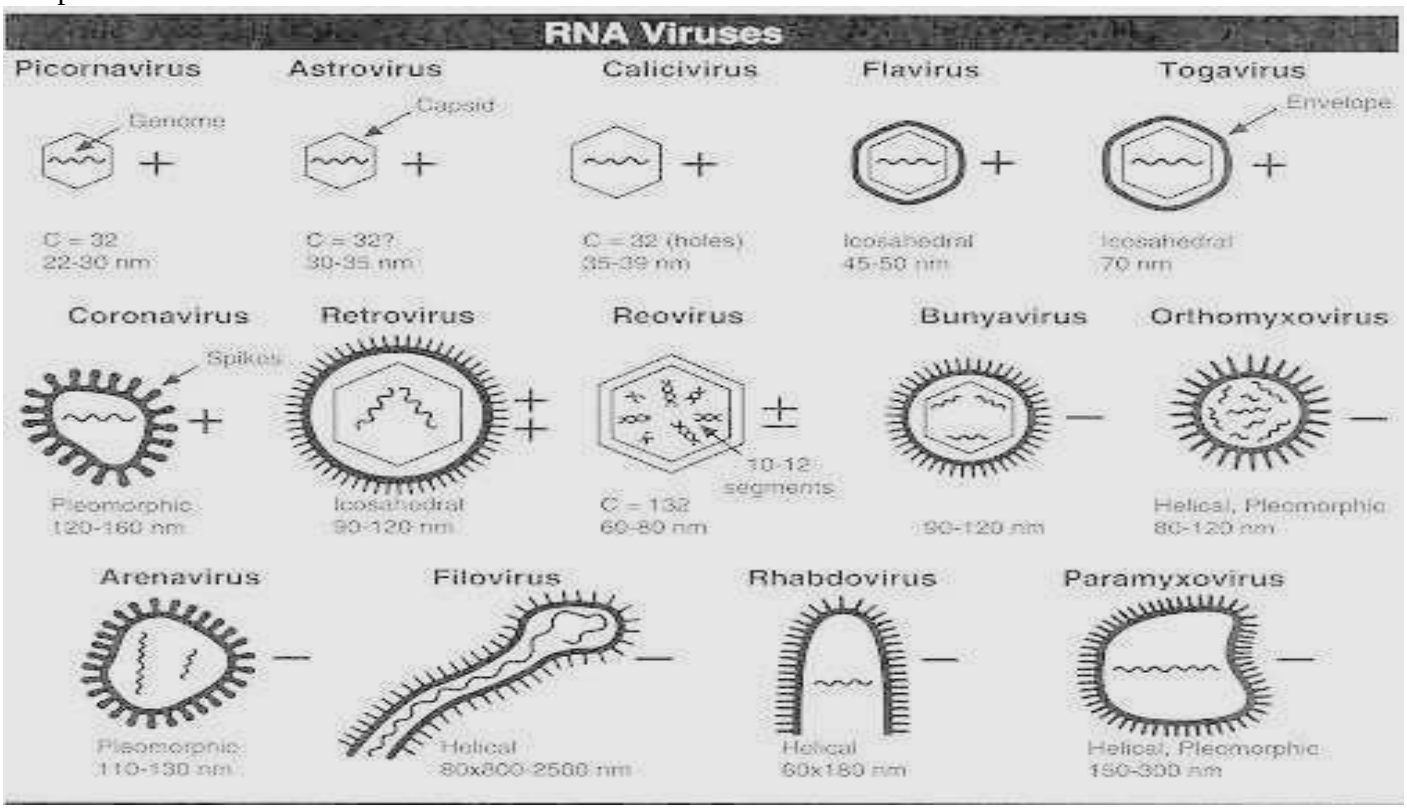
Except in helical nucleocapsids, little is known about the packaging or organization of the viral genome within the core. Small virions are simple nucleocapsids containing 1 to 2 protein species. The larger viruses contain in a core the nucleic acid genome complexed with basic protein(s) and protected by a single- or double layered capsid (consisting of more than one species of protein) or by an envelope (Fig. 41-5).

Figure 41-5 -Two-dimensional diagram of HIV-1 correlating (immuno-) electron microscopic findings with the recent nomenclature for the structural components in a 2-letter code and with the molecular weights of the virus structural (glyco-) proteins. SU stands for outer surface glycoprotein, TM for transmembrane gp, MA for membrane associated or matrix protein, LI for core-envelope-link, CA for major capsid, NC for nucleocapsid protein, respectively. PR, RT and IN represent the virus-coded enzymes protease, reverse transcriptase and integrase that are functional during the life cycle of a retrovirus

Chemical Composition and Mode of Replication

RNA Virus Genomes

RNA viruses, comprising 70% of all viruses, vary remarkably in genome structure (Fig. 41-6). Because of the error rate of the enzymes involved in RNA replication, these viruses usually show much higher mutation rates than do the DNA viruses. Mutation rates of 10^{-4} lead to the continuous generation of virus variants which show great adaptability to new hosts. The viral RNA may be single-stranded (ss) or double-stranded (ds), and the genome may occupy a single RNA segment or be distributed on two or more separate segments (segmented genomes). In addition, the RNA strand of a single-stranded genome may be either a sense strand (plus strand), which can function as messenger RNA (mRNA), or an antisense strand (minus strand), which is complementary to the sense strand and cannot function as mRNA protein translation. Sense viral RNA alone can replicate if injected into cells, since it can function as mRNA and initiate translation of virus-encoded proteins. Antisense RNA, on the other hand, has no translational function and cannot per se produce viral components.



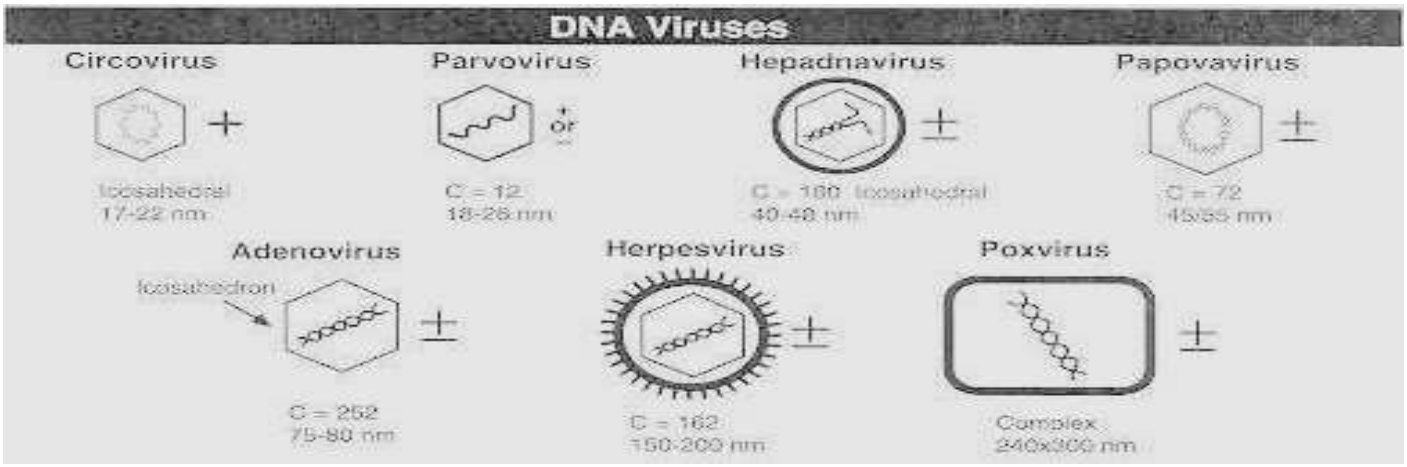


Figure 41-6

Schemes of 21 virus families infecting humans showing a number of distinctive criteria: presence of an envelope or (double-) capsid and internal nucleic acid genome. +, Sense strand; -, antisense strand; ±, dsRNA or DNA; 0, circular DNA; C, number +, Sense strand; -, antisense strand; ±, dsRNA or DNA; 0, circular DNA; C, number of capsomeres or holes, where known; nm, dimensions of capsid, or envelope when present; the hexagon designates the presence of an isometric or icosahedral outline.

DsRNA viruses, e.g., members of the reovirus family, contain 10, 11 or 12 separate genome segments coding for 3 enzymes involved in RNA replication, 3 major capsid proteins and a number of smaller structural proteins. Each segment consists of a complementary sense and antisense strand that is hydrogen bonded into a linear ds molecule. The replication of these viruses is complex; only the sense RNA strands are released from the infecting virion to initiate replication.

The retrovirus genome comprises two identical, plus-sense ssRNA molecules, each monomer 7–11 kb in size, that are noncovalently linked over a short terminal region. Retroviruses contain 2 envelope proteins encoded by the env-gene, 4–6 nonglycosylated core proteins and 3 non-structural functional proteins (reverse transcriptase, integrase, protease: RT, IN, PR) specified by the gag-gene (Fig. 41-5). The RT transcribes the viral ssRNA into double-stranded, circular proviral DNA. This DNA, mediated by the viral integrase, becomes covalently bonded into the DNA of the host cell to make possible the subsequent transcription of the sense strands that eventually give rise to retrovirus progeny. After assembly and budding, retroviruses show structural and functional maturation. In immature virions the structural proteins of the core are present as a large precursor protein shell. After proteolytic processing by the viral protease the proteins of the mature virion are rearranged and form the dense isometric or cone-shaped core typical of the mature virion, and the particle becomes infectious.

DNA Virus Genomes

Most DNA viruses (Fig. 41-6) contain a single genome of linear dsDNA. The papovaviruses, comprising the polyoma- and papillomaviruses, however, have circular DNA genomes, about 5.1 and 7.8 kb pairs in size. DsDNA serves as a template both for mRNA and for self-transcription. Three or 2 structural proteins make up the papovavirus capsid: in addition, 5-6 non-structural proteins are encoded that are functional in virus transcription, DNA replication and cell transformation.

Single-stranded linear DNA, 4–6 kb in size, is found with the members of the Parvovirus family that comprises the parvo-, the erythro- and the dependoviruses. The virion contains 2–4 structural protein species which are differently derived from the same gene product. The adeno-associated virus (AAV, a dependovirus) is incapable of producing progeny virions except in the presence of helper viruses (adenovirus or herpesvirus). It is therefore said to be replication defective.

Circular single-stranded DNA of only 1.7 to 2.3 kb is found in members of the Circovirus family which comprise the smallest autonomously propagated viruses. The isometric capsid measures 17 nm and is composed of 2 protein species only.

Virus Classification

2017

On the basis of shared properties viruses are grouped at different hierarchical levels of order, family, subfamily, genus and species. More than 30,000 different virus isolates are known today and grouped in more than 3,600 species, in 164 genera and 71 families. Viral morphology provides the basis for grouping viruses into families. A virus family may consist of members that replicate only in vertebrates, only in invertebrates, only in plants, or only in bacteria. Certain families contain viruses that replicate in more than one of these hosts. This section concerns only the 21 families and genera of medical importance.

Besides physical properties, several factors pertaining to the mode of replication play a role in classification: the configuration of the nucleic acid (ss or ds, linear or circular), whether the genome consists of one molecule of nucleic acid or is segmented, and whether the strand of ss RNA is sense or antisense. Also considered in classification is the site of viral capsid assembly and, in enveloped viruses, the site of nucleocapsid envelopment. Table 41-1 lists the major chemical and morphologic properties of the families of viruses that cause disease in humans.

The use of Latinized names ending in -viridae for virus families and ending in -virus for viral genera has gained wide acceptance. The names of subfamilies end in -virinae. Vernacular names continue to be used to describe the viruses within a genus. In this text, Latinized endings for families and subfamilies usually are not used. Table 41-2 shows the current classification of medically significant viruses.

TABLE 41-2 Current Classification of Major Groups of Viruses of Medical Significance

Family	Genera (or Subfamilies)	Vernacular Name of Type Species or Typical Member	Viruses Shown to Produce Infection in Humans
DNA Viruses			
Parvoviridae	*Erythrovirus*	B19 virus	B19 virus associated with erythema infectiosum and aplastic crisis of sickle cell anemia
	Dependovirus	Adeno-associated virus (AAV) 2	Defective viruses (infect humans in presence of a helper adenovirus)
Papovaviridae	*Papillomavirus*	Human papilloma virus (HPV) 1	More than 60 HPV types
	Polyomavirus	Polyomavirus (simian, human, mouse)	JC and BK viruses, simian virus 40 (SV40)
Adenoviridae	*Mastadenovirus*	Human adenovirus 2	Human adenovirus serotypes 1-47
Herpesviridae	Alphaherpesvirinae	Human herpesvirus 1 Human herpesvirus 2	Herpes simplex virus 1 Herpes simplex virus 2
	Varicellovirus	Human herpesvirus 3	Varicella-zoster virus
	Gammaherpesvirinae	Human herpesvirus 4	Epstein-Barr virus
	Betaherpesvirinae	Human herpesvirus 5	Human cytomegalovirus
	Roseolovirus	Human herpesvirus 6	HHV-6; Roseola infantum
	Unclassified	Human herpesvirus 7	?
Poxviridae	Orthopoxvirus	Vaccinia virus	Vaccinia, Variola (eradicated), cowpox, monkeypox viruses
	Parapoxvirus	Orf virus	Orf, bovine pustular stomatitis, milker's node viruses
	Molluscipoxvirus	Molluscum contagiosum virus	Molluscum contagiosum
Hepadnaviridae	*Orthohepadna viruses*	Hepatitis B virus	Hepatitis B virus

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TABLE 41-2 continued

Family	Genera (or Subfamilies)	Vernacular Name of Type Species or Typical Member	Viruses Shown to Produce Infection in Humans
Rhabdoviridae	*Vesiculovirus*	Vesicular stomatitis virus (VSV)	VSV and Chandipura virus
	Lyssavirus	Rabies virus	Rabies, Mokola, European bat, Duvenhage viruses
Bunyaviridae	*Bunyavirus*	Bunyamwera virus, La Crosse virus	various arthropod-transmitted viruses
	Hantavirus	Hantaan, Puumula, Seoul virus	Hemorrhagic fever with renal syndrome
	Nairovirus	Crimean-Congo hemorrhagic fever virus	Crimean-Congo hemorrhagic fever virus, Sakhalin virus group
	Phlebovirus	Sandfly fever (Sicilian) virus, Rift Valley fever virus, Uukuniemi virus	Sandfly fever virus, Rift Valley fever virus, Uukuniemi virus
Coronaviridae	*Coronavirus*	Avian infectious bronchitis virus	Human coronaviruses: several types
Arenaviridae	*Arenavirus*	Lymphocytic choriomeningitis virus	Lymphocytic choriomeningitis virus, Lassa viruses; Viruses of the Tacaribe complex
Retroviridae	BLV-HTLV-Retroviruses	Human T-lymphotropic virus I	Human T-cell leukemia viruses, Tropical spastic paresis
	Lentivirinae	Human immunodeficiency virus 1	HIV 1, HIV 2; acquired immunodeficiency syndrome
	Spumavirinae	Human spuma retrovirus	Human foamy virus; (in search of a disease)
Filoviridae	*Filovirus*	Marburg virus	Marburg, Ebola virus; hemorrhagic fever

Current Classification of Major Groups Of viruses of Medical Significance

In the early days of virology, viruses were named according to common pathogenic properties, e.g. organ tropism and/or modes of transmission, and often also after their discoverers. From the early 1950s until the mid-1960s, when many new viruses were being discovered, it was popular to compose virus names by using sigla (abbreviations derived from a few or initial letters). Thus the name Picornaviridae is derived from pico (small) and RNA; the name Reoviridae is derived from respiratory, enteric, and orphan viruses because the agents were found in both respiratory and enteric specimens and were not related to other classified viruses; Papovaviridae is from papilloma, polyoma, and vacuolating agent (simian virus 40 [SV40]); retrovirus is from reverse transcriptase; Hepadnaviridae is from the replication of the virus in hepatocytes and their DNA genomes, as seen in hepatitis B virus. Hepatitis A virus is classified now in the family Picornaviridae, genus Hepatovirus. Although the current rules for nomenclature do not prohibit the introduction of new sigla, they require that the siglum be meaningful to workers in the field and be recognized by international study groups.

The names of the other families that contain viruses pathogenic for humans are derived as follows: Adenoviridae (adeno, "gland"; refers to the adenoid tissue from which the viruses were first isolated); Astroviridae (astron means star); Arenaviridae (arena "sand") describes the sandy appearance of the virion. Bunyaviridae (from Bunyamwera, the place in Africa where the type strain was isolated); Calicivirus (calix, "cup" or "goblet" from the cup-shaped depressions on the viral surfaces); Coronaviridae (corona, "crown") describes the appearance of the peplomers protruding from the viral surface; Filoviridae (from the Latin filum, "thread" or "filament") describes the morphology of these viruses. Herpesviridae (herpes, "creeping") describes the nature of the lesions; Orthomyxoviridae (ortho, "true," plus myxo "mucus," a substance for which the viruses have an affinity; Paramyxoviridae derived from para, "closely resembling" and myxo; Parvoviridae (parvus means, "small"); Poxviridae (pock means, "pustule"); Rhabdoviridae (rhabdo, "rod" describes the shape of the viruses and Togaviridae (toga, "cloak") refers to the tight viral envelope.

Several viruses of medical importance still remain unclassified. Some are difficult or impossible to propagate in standard laboratory host systems and thus cannot be obtained in sufficient quantity to permit more precise characterization. Hepatitis E virus, the Norwalk virus and similar agents that cause nonbacterial gastroenteritis in humans are now assigned to the calicivirus family.

The fatal transmissible dementias in humans and other animals (scrapie in sheep and goat; bovine spongiform encephalopathy in cattle, transmissible mink encephalopathy; Kuru, Creutzfeldt-Jakob disease, and Gerstmann-

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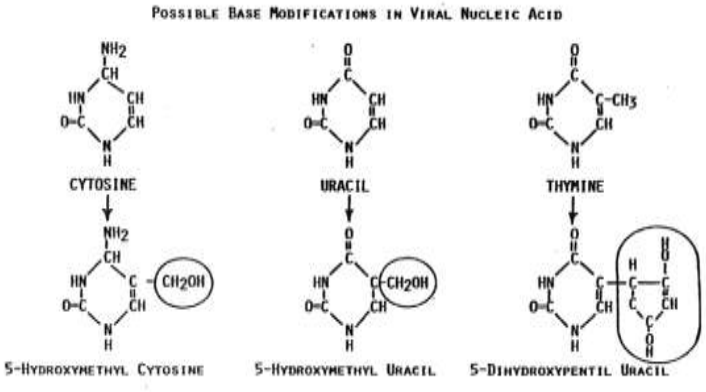
Straussler-Scheinker syndrome in humans are caused by the accumulation of non-soluble amyloid fibrils in the central nervous systems. The agents causing transmissible subacute spongiform encephalopathies have been linked to viroids or virions (i.e. plant pathogens consisting of naked, but very stable circular RNA molecules of about 3-400 bases in size, or infectious genomes enwrapped into a host cell coat) because of their resistance to chemical and physical agents. According to an alternative theory, the term "prion" has been coined to point to an essential nonviral infectious cause for these fatal encephalopathies—prion standing for self-replicating proteinaceous agent devoid of demonstrable nucleic acid. Some of the transmissible amyloidoses show a familiar pattern and can be explained by defined mutations which render a primary soluble glycoprotein insoluble, which in turn leads to the pathogenomonic accumulation of amyloid fibers and plaques. The pathogenesis of the sporadic amyloidoses, however, is still a matter of highly ambitious research

General Properties of Viruses

Structure



1. Nucleic acid -contains 3-400 genes

Deoxyribonucleic Acid (DNA) -unique features

- Single and/or double stranded
- Glycosylated and/or methylated
- Gaps present in double stranded molecule
- Circular or linear
- Bound protein molecules
- Unique purine and/or pyrimidine bases present
- Ribonucleotides present

Ribonucleic Acid (RNA) - Unique features

- Single or double stranded
- Segmented or unsegmented
- Bound protein molecules
- Unique purine and/or pyrimidine bases present
- Folding pattern

2. Capsid -The capsid accounts for most of the virion mass. It is the protein coat of the virus. It is a complex and highly organized entity which gives form to the virus. Subunits called protomeres aggregate to form capsomeres which in turn aggregate to form the capsid.

3. Envelope -this is an amorphous structure composed of lipid, protein and carbohydrate which lies to the outside of the capsid. It contains a mosaic of antigens from the host and the virus. A naked virus is one without an envelope.

4. Spikes. These are glycoprotein projections which have enzymatic and/or adsorption and/or hemagglutinating activity. They arise from the envelope and are highly antigenic.

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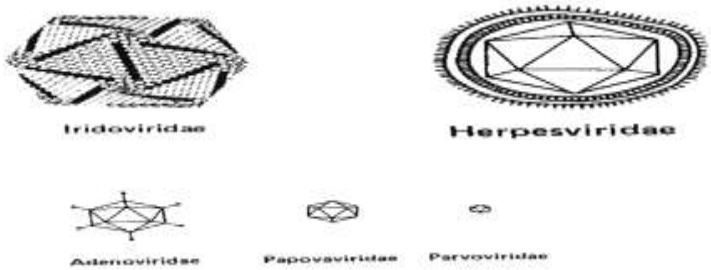
Morphology (Symmetry)

1. Icosahedral -The protomeres aggregate in groups of five or six to form the capsomere. In electron micrographs,
capsomeres are recognized as regularly spaced rings with a central hole. The shape and dimensions of the icosahedron depends on characteristics of its protomeres. All icosahedral capsids have 12 corners each occupied by a penton
capsomere and 20 triangular faces, each containing the same number of hexon capsomeres. Icosahedral symmetry is
identical to cubic symmetry.



2. Helical -The protomeres are not grouped in capsomeres, but are bound to each other so as to form a ribbon-like structure.
This structure folds into a helix because the protomeres are thicker at one end than at the other. The diameter of the helical
capsid is determined by characteristics of its protomeres, while its length is determined by the length of the nucleic acid it
encloses.

3. Complex -e.g., that exhibited by poxvirus and rhabdovirus. This group comprises all those viruses which do not fit into either
of the above two groups.

Viral Replication Cycle

1. **Adsorption -**Viruses can enter cells via phagocytosis, viropexis or adsorption. Adsorption is the most common process and
the most highly specific process. It requires the interaction of a unique protein on the surface of the virus with a
highly specific receptor site on the surface of the cell.

2. **Penetration** -This occurs by one or more processes.

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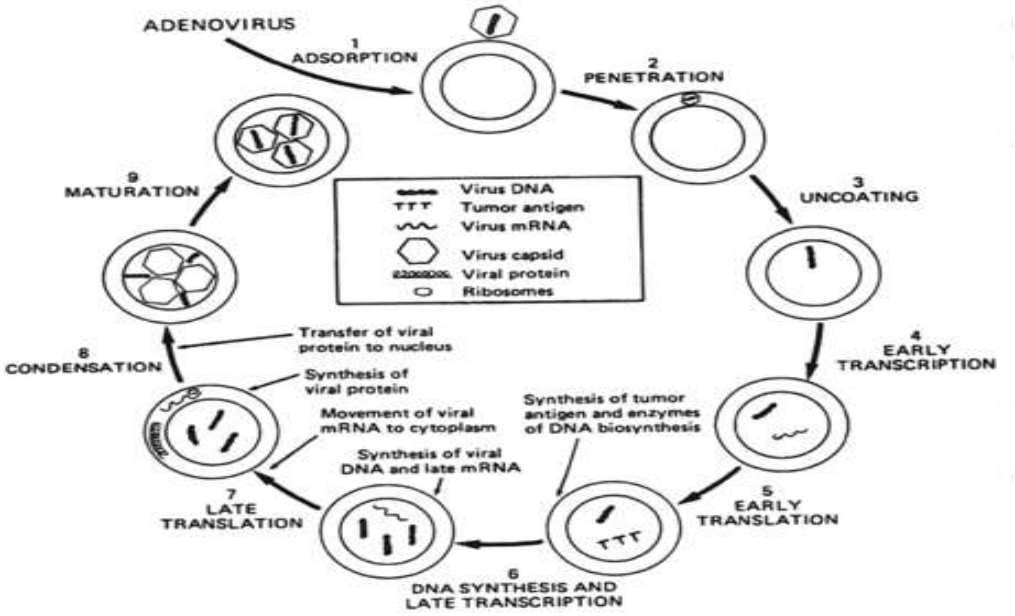
- Enveloped viruses fuse their envelope with the membrane of the host cell. This involves local digestion of the viral and cellular membranes, fusion of the membranes and concomitant release of the nucleocapsid into the cytoplasm.
- Naked viruses bind to receptor sites on the cellular membrane, digest the membrane and enter into the cytoplasm intact.
- Both naked and enveloped viruses can be ingested by phagocytic cells. However, in this process they enter the cytoplasm enclosed in a cytoplasmic membrane derived from the phagocytic cell.

3. **Uncoating** -During this stage cellular proteolytic enzymes digest the capsid away from the nucleic acid. This always occurs in the cytoplasm of the host cell. The period of the replication cycle between the end of the uncoating stage and maturation of new viral particles is termed the eclipse. Thus during the eclipse stage, no complete viral particles can be viewed within the cell.

4. **Biosynthesis** - Replication of nucleic acid. Replication of viral nucleic acid is a complex and variable process. The specific process depends on the nucleic acid type.



Steps in the replication of adenovirus, which contains DNA in its genome. (See text.)

NOTE: Symmetrical transcription of DNA gives rise to double-stranded RNA.

DNA virus replication -<u>with the exception of the poxviruses, all DNA viruses replicate in the nucleus</u>. In some cases one of the DNA strands is transcribed (in others both strands of a small part of the DNA may be transcribed) (step 4) into specific mRNA, which in turn is translated (step 5) to synthesize virus-specific proteins such as tumor antigen and enzymes necessary for biosynthesis of virus DNA. This period encompasses the early virus functions. Host cell DNA synthesis is temporarily elevated and is then suppressed as the cell shifts over to the manufacture of viral DNA (step 6). As the viral DNA continues to be transcribed, late virus functions become apparent. Messenger RNA transcribed during the later phase of infection (step 6) migrates to the cytoplasm and is translated (step 7). Proteins for virus capsids are synthesized and are transported to the nucleus to be incorporated into the complete virion (step 8).

Assembly of the protein subunits around the viral DNA results in the formation of complete virions (step 9), which are released after cell lysis.

The single-stranded DNA viruses first form a double stranded DNA, utilizing a host DNA-dependent DNA polymerase. They then undergo a typical replication cycle.

RNA virus replication -with the exception of the orthomyxoviruses and retroviruses, all RNA viruses replicate in the cytoplasm of the host cell. The exact process varies with the species of virus. The single-stranded RNA that is released after uncoating will act as either: (a) the mRNA to synthesize viral-coded proteins; or (b) a template to synthesize mRNA; or (c) a template to synthesize double stranded RNA, which is then used as a template to synthesize mRNA; or (d) a template to synthesize double-stranded DNA, which is then utilized as a template to synthesize mRNA. This latter process occurs only with the retroviruses (oncornaviruses).

The replication of poliovirus, which contains a single-stranded RNA as its genome, provides a useful example. All of the steps are independent of host DNA and occur in the cell cytoplasm. Polioviruses absorb to cells at specific cell receptor sites (step 1), losing in the process one virus polypeptide. The sites are specific for virus coat-cell interactions. After attachment, the virus particles are taken into the cell by viropexis (similar to pinocytosis) (step 2), and the viral RNA is uncoated (step 3). The single-stranded RNA then serves as its own messenger RNA. This messenger RNA is translated (step 4), resulting in the formation of an RNA-dependent RNA polymerase that catalyzes the production of a replication intermediate (RI), a partially double-stranded molecule consisting of a complete RNA strand and numerous partially completed strands (step 5). At the same time, inhibitors of cellular RNA and protein synthesis are produced. Synthesis of (+) and (-) strands of RNA occurs by similar mechanisms. The RI consists of one complete (-) strand and many small pieces of newly synthesized (+) strand RNA (step 6). The replicative form (RF) consists of two complete RNA strands, one (+) and one (-).

The single (+) strand RNA is made in large amounts and may perform any one of three functions: (a) serve as messenger RNA for synthesis of structural proteins; b) serve as template for continued RNA replication; or (c) become encapsulated, resulting in mature progeny virions. The synthesis of viral capsid proteins (step 7) is initiated at about the same time as RNA synthesis.

The entire poliovirus genome acts as its own mRNA, forming a polysome of approximately 350S, and is translated to form a single large polypeptide that is subsequently cleaved to produce the various viral capsid polypeptides. Thus, the poliovirus genome serves as a polycistronic messenger molecule. Poliovirus contains four polypeptides.

5. **Maturation and Release**

- Naked viruses -Maturation consists of two main processes: the assembly of the capsid, and its association with the nucleic acid. Maturation occurs at the site of nucleic acid replication. After they are assembled into mature viruses, naked virions may become concentrated in large numbers at the site of maturation, forming inclusion bodies. Naked virions are released in different ways, which depend on the virus and the cell type. Generally, RNA-containing naked viruses are released rapidly after maturation and there is little intracellular accumulation; therefore, these viruses do not form predominant inclusion bodies. On the other hand, DNA-containing naked icosahedral viruses that mature in the nucleus do not reach the cell surface as rapidly, and are released when the cells undergo autolysis or in some cases are extruded without lysis. In either case they tend to accumulate within the infected cells over a long period of time. Thus, they generally produce highly visible inclusion bodies.
- Enveloped viruses -In the maturation of enveloped viruses, a capsid must first be assembled around the nucleic acid to form the nucleocapsid, which is then surrounded by the envelope. During the assembly of the nucleocapsid, virus-coded envelope proteins are also synthesized. These migrate to the plasma membrane (if assembly occurs in the cytoplasm) or to the nuclear membrane (if assembly occurs in the nucleus) and become incorporated into that membrane. Envelopes are formed around the nucleocapsids by budding of cellular membranes. NOTE: Enveloped viruses will have an antigenic mosaicism characteristic of the virus and the host cell. Viruses are slowly and continuously released by the budding process with the results that: (a) the cell is not lysed; and (b) little intracellular accumulation of virus occurs; and (c) inclusion bodies are not as evident as with naked viruses.
- Complex viruses -These viruses, of which the poxvirus is a good example, begin the maturation process by forming multilayered membranes around the DNA. These layers differentiate into two membranes:

The inner one contains the characteristic nucleoid, while the external one acquires the characteristic pattern of the surface of the virion.

These form very characteristic cytoplasmic inclusion bodies. The viruses are generally released from the cell via cell lysis.

Five Basic Structural Forms

Based upon basic morphology, as indicated above, there are five different basic structural forms of viruses. These forms are listed below with examples:

- Naked icosahedral - adenoviruses and picornaviruses.
- Naked helical - tobacco mosaic virus; no known human or animal viruses have this structure.
- Enveloped icosahedral - togaviruses and flaviviruses.
- Enveloped helical - rhabdoviruses and paramyxoviruses.
- Complex - bacteriophages and poxviruses.

Viral Envelopes

The viral envelope, characteristic of some virus families, is derived from membranes of the host cell by budding, which occurs during the release of the virions from the cell. This membrane is mainly a piece of the plasma membrane; however, it may be part of the Golgi Apparatus, endoplasmic reticulum or the nuclear membrane, depending upon the virus and the cellular compartment where the replication takes place. Regardless of origin, the envelope is composed by a lipid bilayer – of cellular origin - and associated proteins. The proteins associated with the lipid bilayer are largely of viral origin (virus encoded) and are mainly glycoproteins. The number of viral proteins in the envelope may vary from one up to more than ten, depending on the virus. Virus envelope glycoproteins perform several functions, including the initial attachment of the virion to the target cell, penetration, fusion, and cell-to-cell spread, amongst others. The attachment of a virion to the cellular surface requires the envelope to be intact and the glycoproteins in their native conformation. Antiviral drugs that are directed against the envelope proteins can decrease the ability of the virus to attach and initiate infection, thereby decreasing infectivity.

The process of budding, and thus acquisition of the envelope by the newly formed virions, may or may not result in death of the host cell. If many virions are released simultaneously, the integrity of the host cell membrane may be compromised enough to lead to death of the cell. Alternatively, the release of virions may be slow and consistent resulting in chronic shedding and persistent infections. Indeed, unlike the non-enveloped viruses, which are released from the cell mainly through cell lysis and consequently death, egress of enveloped viruses is often compatible with cell survival. Therefore, budding provides a means of viral egress without leading to cell death.

Viral Proteins

There are two basic types of virus-encoded proteins: structural and non-structural. The structural proteins are those that are part of the physical structure of the virion (capsid, envelope), while nonstructural proteins are produced inside infected cells and play roles in different steps of viral replication. The number of proteins encoded by viral genomes varies greatly, from as few as two proteins to over hundreds. Structural proteins are typically those that compose the capsid and package the nucleic acid genome.

In some enveloped viruses, there is a protein layer between the capsid and the envelope (the tegument). The proteins that make up the tegument are also structural. External structural proteins of the capsid or envelope are ligands, which interact with receptors on the surface of target cells. Some of these proteins (glycoproteins) are processed in the lumen of the rough endoplasmic reticulum, where oligosaccharides are attached to the polypeptide chain. They are then sent to the Golgi apparatus, to secretory vesicles, and ultimately fuse with the plasma membrane where they are present on the surface of the infected cell. This is especially important for enveloped viruses. Envelope glycoproteins play roles in mediating interactions

between the virions and cells (attachment, penetration, fusion, cell-to-cell spread) and are major targets for neutralizing antibodies.

Nonstructural proteins are primarily, but not exclusively, enzymes, such as those associated with the processes of genome transcription, replication and protein processing. An example of a nonstructural protein is reverse transcriptase of retroviruses, which makes a DNA copy of a RNA template. This step is an important feature of retroviruses whose RNA needs to be converted to DNA in order to be incorporated into the host chromosome. Some viruses encode several non-structural proteins that play diverse accessory roles in the regulation of viral and cellular gene expression, regulation of different steps of the viral cycle, counteraction of host defenses, cell transformation, *et cetera*.

Other Viral Components

Lipids - The lipids of viruses are derived from the cellular membranes of the host cell. These are composed mainly of phospholipids (50 - 60%) and the remainder is cholesterol. As a result of being derived from host cell membranes, the composition of lipids varies. The lipid bilayer of the host membranes surrounding the virion of enveloped viruses also possesses viral proteins and glycoproteins, such as the characteristic spikes of some enveloped viruses. The overall lipid composition of enveloped viruses is approximately 20 - 35% dry weight. The remainder is divided between the nucleic acid and protein portions.

Carbohydrates - The carbohydrates of viruses occur as oligosaccharide side chains of glycoproteins, glycolipids, and mucopolysaccharides. The composition of the carbohydrates corresponds to that of the host cell. However, the glycoproteins typically have an *N*– or *O*- glycosidic linkage. Viral carbohydrates are mainly found in the envelope. Some of the larger, more complex viruses contain internal glycoproteins or glycosylated capsid proteins.

Viral Taxonomy - Viruses constitute a large and heterogeneous group. They are classified in hierarchical taxonomic categories based on many features. The classification is dynamic in that new viruses are continuously being discovered and more information is accumulating about viruses already known
The basic viral hierarchical classification scheme is: Order - Family - Subfamily - Genus - Species - Strain / Type. A number of viral characteristics, referred to below, define each of these taxonomic categories. Orders have the suffix *-virales*, families contain the suffix *-viridae*, while genera contain the suffix *-virus*. A virus species constitutes a replicating lineage that occupies an ecological niche, for example, a particular disease.

Viruses are placed in families on the basis of many features.

A basic characteristic is nucleic acid type (DNA or RNA) and morphology, that is, the virion size, shape, and the presence or absence of an envelope. The host range and immunological properties (serotypes) of the virus are also used. Physical and physicochemical properties such as molecular mass, buoyant density, thermal inactivation, pH stability, and sensitivity to various solvents are used in classification. Whether the RNA or DNA is single or double stranded, the organization of the genome and the presence of particular genes comprise important aspects of the current taxonomy of viruses. All of the former are used to place a virus into a particular order or family. For example, the order Mononegavirales encompasses those viruses possessing a negative sense, single stranded RNA genome. Lastly, classification is based upon macromolecules produced (structural proteins and enzymes), antigenic properties and biological properties (e.g., accumulation of virions in cells, infectivity, hemagglutination).

Atypical Particles Associated with Infections

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Defective Viruses - Defective viruses are those virus particles whose genome lacks a specific gene or genes due to either mutation or deletion. As a result, defective viruses are not capable of undergoing a productive life cycle in cells. However, if the cell infected with the defective virus is co-infected with a "helper virus", the gene product lacking in the defective one is complemented by the helper and defective virus can replicate. Interestingly, for some viruses, during infection a greater quantity of defective virions is produced than infectious virions (as much as 100:1). The production of defective particles is a characteristic of some virus species and is believed to moderate the severity of the infection/disease in *vivo*.

Pseudovirions - Pseudovirions may be produced during viral replication when the host genome is fragmented. As a result of this process, host DNA fragments are incorporated into the capsid instead of viral DNA. Thus, pseudovirions possess the viral capsid to which antibodies may bind and facilitate attachment and penetration into a host cell, but they cannot replicate once they have gained access to a host cell, as they have none of the essential viral genes for the process.

Prions - Although not viral, prions are proteinaceous infectious particles associated with transmissible spongiform encephalopathies (TSE) of humans and animals. TSEs include the Creutzfeldt-Jacob disease of humans, scrapie of sheep and bovine spongiform encephalopathy. At postmortem, the brain has large vacuoles in the cortex and cerebellum regions and thus prion diseases are called "spongiform encephalopathies". Closer examination of brain tissue reveals the accumulation of prion-protein associated fibrils and amyloid plaques. These diseases are characterized by loss of motor control, dementia, paralysis, wasting and eventually death. Details of pathogenesis are largely unknown.

Viroids - Viroids are naked, low-molecular weight nucleic acids that are extremely resistant to heat, ultraviolet, and ionizing radiation. These particles are composed exclusively of a single piece of circular, single stranded RNA that has some double stranded regions. Viroids mainly cause plant diseases, such as potato spindle tuber disease.

Virusoids - Virusoids (also called satellite RNAs) are similar to viroids in that they are naked, low-molecular weight nucleic acids that are extremely resistant to heat and ultraviolet and ionizing radiation. However, they depend on a helper virus for replication. Virusoids replicate in cytoplasm via a RNA dependent RNA polymerase virus this process; host DNA fragments are incorporated into the capsid instead of viral DNA. Thus, pseudovirions possess the viral capsid to which antibodies may bind and facilitate attachment and penetration into a host cell, but they cannot replicate once they have gained access to a host cell, as they have none of the essential viral genes for the process.

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15MBU502
III BSC MICROBIOLOGY
VIROLOGY

Unit II Q	Opt 1	Opt 2	Opt 3	Opt 4	Opt 5	Opt 6	Answer
A structur	The envel	DNA	Capsid	Tail fibers			Capsid
A chemic	Protein	Lipid	DNA	RNA			Protein
A commo	Pentagon	Cube	Icosahedr	Pyramid			Icosahedron
Which of	Hepatitis	T cell lym	Epstein-B	CAMV			T cell lymphotronic
Which of	Denaturat	Enzyme t	Pressure	Sedimentation			Denaturation
The viral	lysogeny	spontaneo	lytic phas	Induced induction			lysogeny
Which of	Viruses h	All viruse	All viruse	Viruses probably arose from sn			Viruses have been s
Which of	Hepatitis	Hepatitis	Hepatitis	Hepatitis A virus			Hepatitis E virus
In the sim	penton	polyhedra	icosahedr	helical			penton
The size o	centimete	micromet	nanomete	millimeters			nanometers
The temp	λ phage e	λ DNA	Phage Mu	Phage Mn			Phage Mn
Enzyme n	Human in	Epstein-B	Influenza	Adenovirus			Influenza virus
Lysozyme	immediat	late genes	delayed e	Early genes			late genes
Which of	HeLa	HEp-2	KB	All of these			HeLa
The repre	immunity	immunity	operon re	Operon deppressor			immunity repressor
Which of	Herpes	Influenza	Measles	HIV			Measles
Group E p	single stra	double str	single stra	double stranded RNA			double stranded DN
The temp	phage dest	lytic infec	Both (a) a	lysogenic			lytic infection by oth
The bacte	A	B	C	D			D
The proce	infection	integratio	repressior	induction			infection
The onco	how chen	how virus	how virus	no change			how viruses transfor
In cell cul	nuclear py	transform	syncytium	rounding and aggregation of ce			syncytium formatior
A change	ultraviole	chemicals	irradiatior	alcohol			alcohol
The viral	spontaneo	inductive	resultant i	spontaneous infection			spontaneous infectio
The lysog	immunity	immunity	operon re	Lac operon			immunity repressor
The capso	protomers	caproprot	bprocapsi	capsomers			caproprotein
In order f	the capsid	the host c	the genon	the host cell must lack a cell me			the genome must be
Which of	Rubella v	Yellow fe	Hepatitis	Dengue			Dengue
Enteroviru	Type of n	Size	Capsid sh	Ability to survive acidic condit			Capsid shape
Viruses th	. Toga vir	Herpes vi	Entero vir	Rhinoviruses			Herpes viruses
What type	Bacteriop	Animal V	Plant Viru	Fungal Viruses			Animal Viruses
Bacteriop	Immunoa	ELISA	Plaque as	Tissue cell culture			Plaque assays
A type of	Primary c	Continuo	Cell strain	Diploid fibroblast			Continuous cell line
Which of	Retroviru	Entero vir	Rhabado	Adenovirus			Entero virus
Which of	Hydrogen	Hypochlo	Formalde	chlorine			Formaldehyde
Viruses la	protein	carbohydr	alcohol	lipids			protein
Viruses re	bacteria	plants	animals	living cells			living cells
Reverse t	an RNA v	there are n	nutrients a	spikes are forming in the new v			an RNA virus conve
The seque	among di	among di	among di	between viruses and their hosts			among different viru

Area of ly	pock	plaque	pox	Colony			plaque
The proca	ladder	framing	scaffoldin	form			ladder
Which of	Phage stru	Proteins t	Proteins i	Complex protein			Proteins involved in
Which ca	Helical	Icosahedr	Complex	Cylinder			Icosahedral
Contracti	T3	T2	P22	P322			T2
One of th	RNA tran	RNA poly	RNA liga	RNA replicase			RNA polymerase
In viruses	the envelo	the envelo	the envelo	the envelope and its imbedded			the envelope is code
Which of	Staphyloc	Salmonel	Vibrio ch	E coli			Salmonella typhi
When a v	lysogeny	fermentat	symbiosis	synergism			lysogeny
Viruses a	nature of	nucleic ac	capsid syn	diameter of the viroin or nucleo			nature of the host
The first s	adsorption	absorption	penetratio	replication			adsorption
Which of	Hepatitis	Hepatitis	Varicella-	Herpes simplex virus type 2			Varicella-Zoster viru
The viral	genome a	capsid and	envelope	capsomere and genome			genome and Capsid
Edward J	Smallpox	Avianpox	Cowpox	Chickenpox			Cowpox
The virus	have no g	continue t	are usuall	is altered with chemicals			have no genome
Envelope	icosahedr	helical	roughly sp	complex			complex
The envel	Paramyxo	Retro viru	Orthomyx	Herpes viruses			Retro viruses
Which of	HeLa	HEp-2	WI-38	KB			HeLa
Plant viru	tissue cult	cultures o	whole pla	human cells			tissue culture
_____	Operator	Promotor	Repressor	Enhancer			Promotor

virus type I

uccessfully grown in pure cultures in test tubes

A
er viruses

m normal cells into tumor cells
n

n

released in the cytoplasm

rts its RNA to DNA
ses than between viruses and their hosts

ı cell lysis and phage release

d by the viral nucleic acids, but the proteins come from the host's membrane proteins

ıs

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III B. Sc Microbiology –Virology
Unit III

LECTURE PLAN

LECTURE PLAN - UNIT -111			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	Classification of bacteriophages	T1 448 - 452
2	1	Double Stranded DNA Phage	W1
3	1	one step growth experiment	J1
4	1	single stranded DNA phage	W1
5	1	Laboratory diagnostic of phages	T1 54-57
6	1	Revision of Unit III	
7	1	Unit III test	
Textbooks :		T1-medical virology -White & fenner, Academic press publishers T2-Modern virology -dimmock-Black well publishing T3-Textbook of Microbiology- Paniker- Orient longman publishers	
Reference books:		R1- Medical Microbiology –Jawertz – McGraw hill Publishers	
Website:		W1- www.microbesonline.com W2 - www.virologyonline.com	
Journals:			

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Bacteriophage

A **bacteriophage** /□ bæk□ t ər.i.oʊ □ feɪ dʒ / (informally, *phage* /□ feɪ dʒ /) is a virus that infects and replicates within a bacterium. The term is derived from "bacteria" and the Greek: φαγεῖν (*phagein*), "to devour". Bacteriophages are composed of proteins that encapsulate a DNA or RNA genome, and may have relatively simple or elaborate structures. Their genomes may encode as few as four genes, and as many as hundreds of genes. Phages replicate within the bacterium following the injection of their genome into its cytoplasm. Bacteriophages are among the most common and diverse entities in the biosphere.

Phages are widely distributed in locations populated by bacterial hosts, such as soil or the intestines of animals. One of the densest natural sources for phages and other viruses is sea water, where up to 9×10^8 virions per milliliter have been found in microbial mats at the surface, and up to 70% of marine bacteria may be infected by phages. They have been used for over 90 years as an alternative to antibiotics in the former Soviet Union and Central Europe, as well as in France. They are seen as a possible therapy against multi-drug-resistant strains of many bacteria. Nevertheless, phages of Inoviridae have been shown to complicate biofilms involved in pneumonia and cystic fibrosis, shelter the bacteria from drugs meant to eradicate disease and promote persistent infection.

Classification

Bacteriophages occur abundantly in the biosphere, with different virions, genomes, and lifestyles. Phages are classified by the International Committee on Taxonomy of Viruses (ICTV) according to morphology and nucleic acid.

Nineteen families are currently recognized by the ICTV that infect bacteria and archaea. Of these, only two families have RNA genomes, and only five families are enveloped. Of the viral families with DNA genomes, only two have single-stranded genomes. Eight of the viral families with DNA genomes have circular genomes while nine have linear genomes. Nine families infect bacteria only, nine infect archaea only, and one (*Tectiviridae*) infects both bacteria and archaea.

Bacteriophage P22, a member of the *Podoviridae* by morphology due to its short, non-contractile tail.

ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]

Order	Family	Morphology	Nucleic acid	Examples
Caudovirales	*Myoviridae*	Nonenveloped, contractile tail	Linear dsDNA	T4 phage, Mu, PBSX, P1Puna-like, P2, I3, Bcep 1, Bcep 43, Bcep 78
	Siphoviridae	Nonenveloped, noncontractile tail (long)	Linear dsDNA	λ phage, T5 phage, phi, C2, L5, HK97, N15
	Podoviridae	Nonenveloped, noncontractile tail (short)	Linear dsDNA	T7 phage, T3 phage, Φ29, P22, P37
Ligamenvirales	*Lipothrixviridae*	Enveloped, rod-shaped	Linear dsDNA	Acidianus filamentous virus 1
	Rudiviridae	Nonenveloped, rod-shaped	Linear dsDNA	Sulfolobus islandicus rod-shaped virus 1
Unassigned	*Ampullaviridae*	Enveloped, bottle-shaped	Linear dsDNA	
	Bicaudaviridae	Nonenveloped, lemon-shaped	Circular dsDNA	
	Clavaviridae	Nonenveloped, rod-	Circular	

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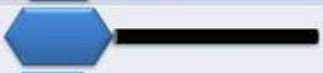
ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]

Order	Family	Morphology	Nucleic acid	Examples
		shaped	dsDNA	
	Corticoviridae	Nonenveloped, isometric	Circular dsDNA	
	Cystoviridae	Enveloped, spherical	Segmented dsRNA	
	Fuselloviridae	Nonenveloped, lemon-shaped	Circular dsDNA	
	Globuloviridae	Enveloped, isometric	Linear dsDNA	
	Guttaviridae	Nonenveloped, ovoid	Circular dsDNA	
	Inoviridae	Nonenveloped, filamentous	Circular ssDNA	M13
	Leviviridae	Nonenveloped, isometric	Linear ssRNA	MS2, Qβ
	Microviridae	Nonenveloped, isometric	Circular ssDNA	ΦX174
	Plasmaviridae	Enveloped, pleomorphic	Circular dsDNA	
	Tectiviridae	Nonenveloped, isometric	Linear dsDNA	

Table 1: Taxonomy of Bacteriophages

FAMILY	PROPERTIES	SHAPE
Myoviridae	Contractile tail	
Siphoviridae	Noncontractile long tail,	
Podoviridae	Short tail	
Microviridae	ssDNA (C), 27 nm, 12 knoblike capsomers	
Corticoviridae	dsDNA (C), complex capsid, lipids, 63 nm	
Tectiviridae	dsDNA (L), inner lipid vesicle, pseudo-tail, 60 nm	
Leviviridae	ssRNA (L), 23 nm, like poliovirus	
Cystoviridae	dsRNA (L), segmented, lipidic envelope, 70–80 nm	
Inoviridae	ssDNA (C), filaments or rods, 85–1950 x 7 nm	
Plasmaviridae	dsDNA (C), lipidic envelope, no capsid, 80 nm	

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History

In 1896, Ernest Hanbury Hankin reported that something in the waters of the Ganges and Yamuna rivers in India had marked antibacterial action against cholera and could pass through a very fine porcelain filter In 1915, British bacteriologist Frederick Twort, superintendent of the Brown Institution of London, discovered a small agent that infected and killed bacteria. He believed the agent must be one of the following:

1. a stage in the life cycle of the bacteria;
2. an enzyme produced by the bacteria themselves; or
3. a virus that grew on and destroyed the bacteria.

Twort's work was interrupted by the onset of World War I and shortage of funding. Independently, French-Canadian microbiologist Félix d'Hérelle, working at the Pasteur Institute in Paris, announced on 3 September 1917, that he had discovered "an invisible, antagonistic microbe of the dysentery bacillus". For d'Hérelle, there was no question as to the nature of his discovery: "In a flash I had understood: what caused my clear spots was in fact an invisible microbe … a virus parasitic on bacteria." D'Hérelle called the virus a bacteriophage or bacteria-eater (from the Greek *phagein* meaning to eat). He also recorded a dramatic account of a man suffering from dysentery who was restored to good health by the bacteriophages.[8] It was D'Herelle who conducted much research into bacteriophages and introduced the concept of phage therapy.[9]

In 1969, Max Delbrück, Alfred Hershey and Salvador Luria were awarded the Nobel Prize in Physiology and Medicine for their discoveries of the replication of viruses and their genetic structure.]

Structure

Bacteriophages come in different sizes and shapes but most of them have the same basic features: a head or capsid and a tail. A bacteriophage's head structure, regardless of its size or shape, is made up of one or more proteins which protectively coats the nucleic acid. Though there are some phages that don't have a tail, most of them do have one attached to its head structure. It is a hollow tube through which the nucleic acid passes through when the bacteriophage infects a host cell. Some of the more complex phages such as T4 have a tail with a base plate as well as one or more tail fibers that aid the phage in attaching itself to a bacterial cell

How Bacteriophages Work

In order to infect a host cell, the bacteriophage attaches itself to the bacteria's cell wall, specifically on a receptor found on the bacteria's surface. Once it becomes tightly bound to the cell, the bacterial virus injects its genetic material (its nucleic acid) into the host cell. Depending on the type of phage, one of two cycles will occur – the lytic or the lysogenic cycle. During a lytic cycle, the phage will make use of the host cell's chemical energy as well as its biosynthetic machinery in order to produce phage nucleic acids (phage DNA and phage mRNA) and phage proteins. Once the production phase is finished, the phage nucleic acids and structural proteins are then assembled. After a while, certain proteins produced within the cell will cause the cell wall to lyse, allowing the assembled phages within to be released and to infect other bacterial cells.

Viral reproduction can also occur through the lysogenic cycle. The main difference between the two types of cycles is that during lysogeny, the host cell is not destroyed or does not undergo lysis. Once the host cell is infected, the phage DNA integrates or combines with the bacterial chromosome, creating the prophage. When the bacterium reproduces, the prophage is replicated along with the host chromosomes. Thus, the daughter cells also contain the prophage which carries the potential of producing phages. The lysogenic cycle can continue indefinitely (daughter cells with prophage present within continuing to replicate) unless exposed to adverse conditions which can trigger the termination of the lysogenic state and cause the expression of the phage DNA and the start of the lytic cycle. These adverse conditions include exposure to UV or mutagenic chemicals and desiccation.

Applications

Bacteriophages have several applications. In some countries such as Russia and other Eastern European nations, phages are used therapeutically for the treatment of pathogenic bacterial infections that are resistant to antibiotics. Also known as phage therapy, this method involves the use of a phage to destroy the infective bacteria such as E. coli or salmonella. Bacteriophage is also used in identifying pathogenic bacteria (also called

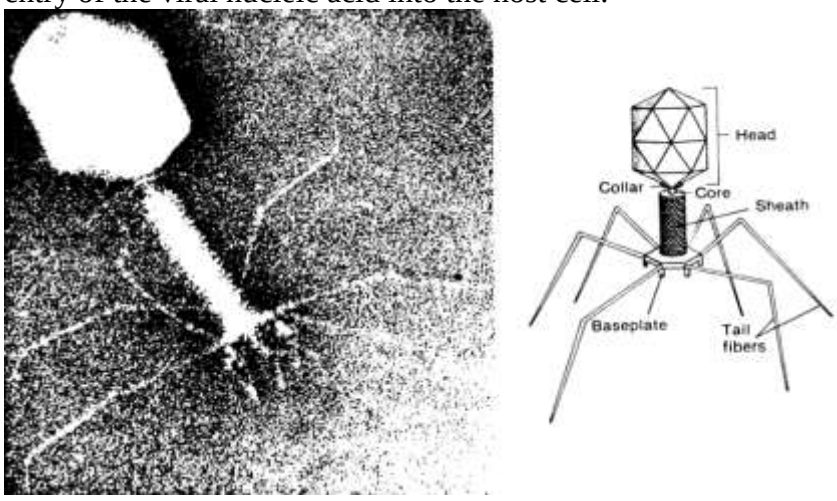
phage typing) in diagnostic laboratories. One other use for bacteriophages is for killing specific bacteria found in food. For example, LISTEX by Micreos is made up of bacteriophages that can kill the L. monocytogenes bacteria in cheese.Viruses that attack bacteria were observed by Twort and d'Herelle in 1915 and 1917. They observed that broth cultures of certain intestinal bacteria could be dissolved by addition of a bacteria-free filtrate obtained from sewage. The lysis of the bacterial cells was said to be brought about by a virus which meant a "filterable poison" ("virus" is Latin for "poison"). Probably every known bacterium is subject to infection by one or more viruses or "bacteriophages" as they are known ("phage" for short, from Gr. "phagein" meaning "to eat" or "to nibble"). Most research has been done on the phages that attack *E. coli*, especially the T-phages and phage lambda.

Like most viruses, bacteriophages typically carry only the genetic information needed for replication of their nucleic acid and synthesis of their protein coats. When phages infect their host cell, the order of business is to replicate their nucleic acid and to produce the protective protein coat. But they cannot do this alone. They require precursors, energy generation and ribosomes supplied by their bacterial host cell.

Bacterial cells can undergo one of two types of infections by viruses termed **lytic infections** and **lysogenic (temperate)** infections. In *E. coli*, lytic infections are caused by a group seven phages known as the T-phages, while lysogenic infections are caused by the phage lambda.

Lytic Infections

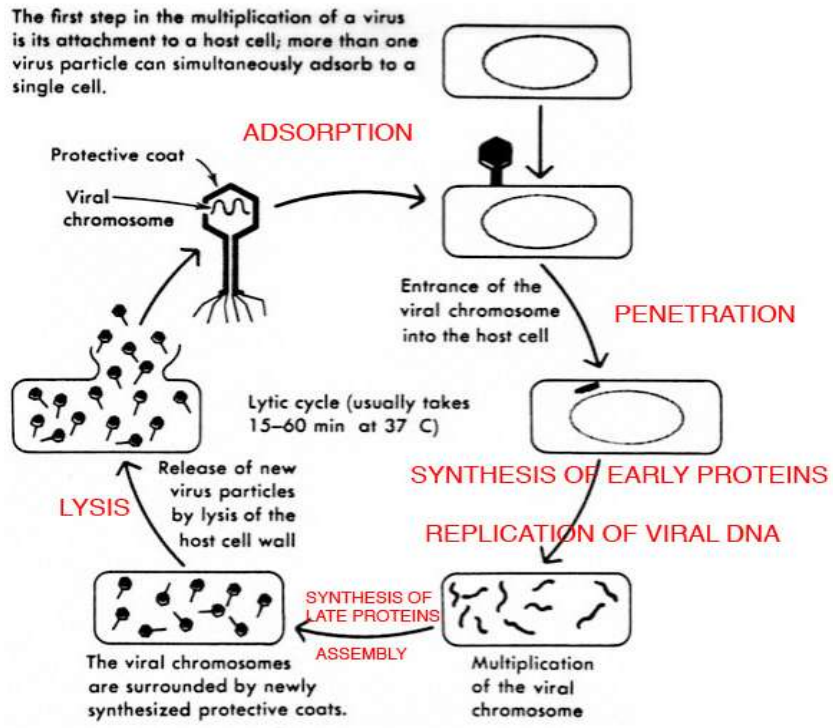
The T-phages, T1 through T7, are referred to as lytic phages because they always bring about the lysis and death of their host cell, the bacterium *E. coli*. T-phages contain double-stranded DNA as their genetic material. In addition to their protein coat or capsid (also referred to as the "head"), T-phages also possess a tail and some related structures. A diagram and electron micrograph of bacteriophage T4 is shown below. The tail includes a core, a tail sheath, base plate, tail pins, and tail fibers, all of which are composed of different proteins. The tail and related structures of bacteriophages are generally involved in attachment of the phage and securing the entry of the viral nucleic acid into the host cell.



Left. Electron Micrograph of bacteriophage T4. Right. Model of phage T4. The phage possesses a genome of linear ds DNA contained within an icosahedral head. The tail consists of a hollow core through which the DNA is injected into the host cell. The tail fibers are involved with recognition of specific viral "receptors" on the bacterial cell surface.

Before viral infection, the cell is involved in replication of its own DNA and transcription and translation of its own genetic information to carry out biosynthesis, growth and cell division. After infection, the viral DNA takes over the machinery of the host cell and uses it to produce the nucleic acids and proteins needed for production of new virus particles. Viral DNA replaces the host cell DNA as a template for both replication (to

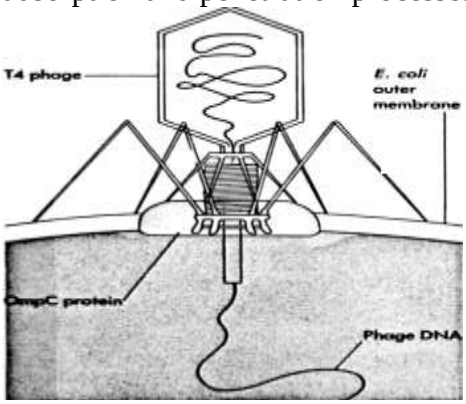
produce more viral DNA) and transcription (to produce viral mRNA). Viral mRNAs are then translated, using host cell ribosomes, tRNAs and amino acids, into viral proteins such as the coat or tail proteins. The process of DNA replication, synthesis of proteins, and viral assembly is a carefully coordinated and timed event. The overall process of lytic infection is diagrammed in the figure below. Discussion of the specific steps follows.



The lytic cycle of a bacterial virus, e.g. bacteriophage T4.

The first step in the replication of the phage in its host cell is called **adsorption**. The phage particle undergoes a chance collision at a chemically complementary site on the bacterial surface, then adheres to that site by means of its tail fibers.

Following adsorption, the phage injects its DNA into the bacterial cell. The tail sheath contracts and the core is driven through the wall to the membrane. This process is called penetration and it may be both mechanical and enzymatic. Phage T4 packages a bit of lysozyme in the base of its tail from a previous infection and then uses the lysozyme to degrade a portion of the bacterial cell wall for insertion of the tail core. The DNA is injected into the periplasm of the bacterium, and generally it is not known how the DNA penetrates the membrane. The adsorption and penetration processes are illustrated below.



Adsorption, penetration and injection of bacteriophage T4 DNA into an*E. coli* cell. T4 attaches to an outer membrane porin protein, ompC.

Immediately after injection of the viral DNA there is a process initiated called **synthesis of early proteins**. This

refers to the transcription and translation of a section of the phage DNA to make a set of proteins that are needed to replicate the phage DNA. Among the early proteins produced are a repair enzyme to repair the hole in the bacterial cell wall, a DNAase enzyme that degrades the host DNA into precursors of phage DNA, and a virus specific DNA polymerase that will copy and replicate phage DNA. During this period the cell's energy-generating and protein-synthesizing abilities are maintained, but they have been subverted by the virus. The result is the **synthesis of several copies of the phage DNA**.

The next step is the synthesis of late proteins. Each of the several replicated copies of the phage DNA can now be used for transcription and translation of a second set of proteins called the **late proteins**. The late proteins are mainly structural proteins that make up the capsomeres and the various components of the tail assembly. Lysozyme is also a late protein that will be packaged in the tail of the phage and be used to escape from the host cell during the last step of the replication process.

Having replicated all of their parts, there follows an **assembly** process. The proteins that make up the capsomeres assemble themselves into the heads and "reel in" a copy of the phage DNA. The tail and accessory structures assemble and incorporate a bit of lysozyme in the tail plate. The viruses arrange their escape from the host cell during the assembly process.

While the viruses are assembling, lysozyme is being produced as a late viral protein. Part of this lysozome is used to escape from the host cell by lysing the cell wall peptiodglycan from the inside. This accomplishes the **lysis of the host cell** and the **release of the mature viruses**, which spread to nearby cells, infect them, and complete the cycle. The life cycle of a T-phage takes about 25-35 minutes to complete. Because the host cells are ultimately killed by lysis, this type of viral infection is referred to as lytic infection.

Lysogenic Infections

Lysogenic or temperate infection rarely results in lysis of the bacterial host cell. Lysogenic viruses, such as lambda which infects *E. coli*, have a different strategy than lytic viruses for their replication. After penetration, the virus DNA integrates into the bacterial chromosome and it becomes replicated every time the cell duplicates its chromosomal DNA during normal cell division. The life cycle of a lysogenic bacteriophage is illustrated below.

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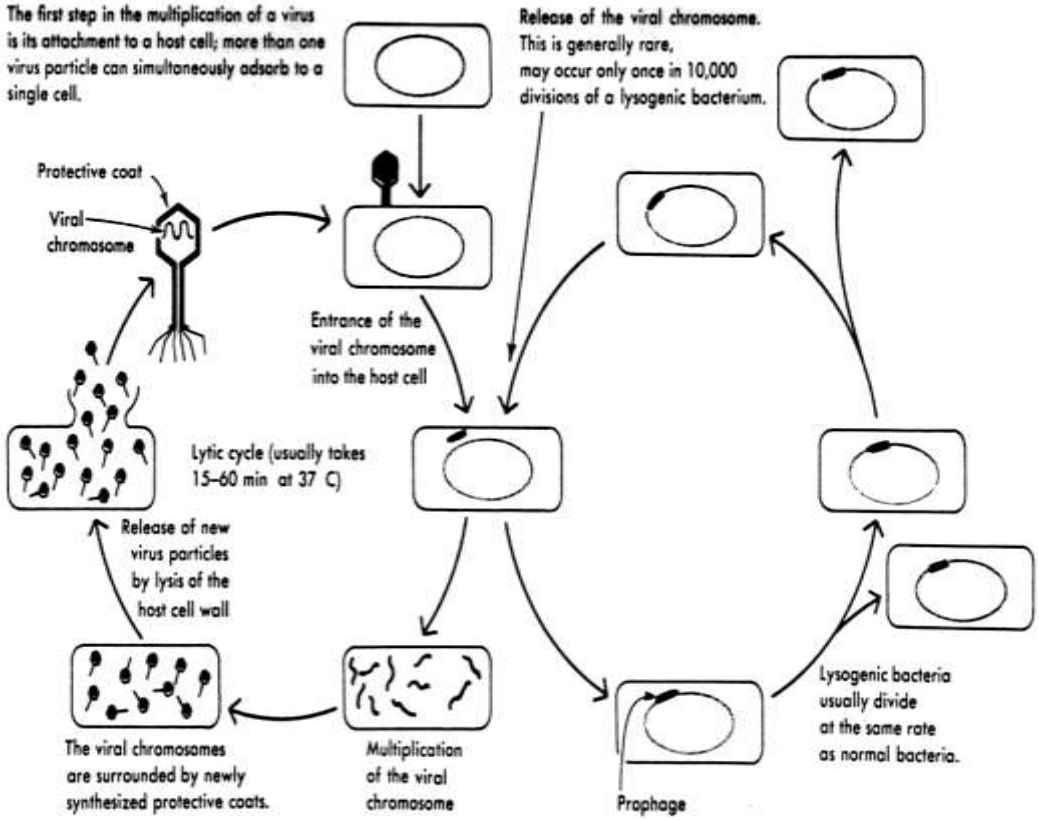

The lysogenic cycle of a temperate bacteriophage such as lambda.

Temperate viruses usually do not kill the host bacterial cells they infect. Their chromosome becomes integrated into a specific section of the host cell chromosome. Such phage DNA is called **prophage** and the host bacteria are said to be **lysogenized**. In the prophage state all the phage genes except one are repressed. None of the usual early proteins or structural proteins are formed.

The phage gene that is expressed is an important one because it codes for the synthesis of a **repressor** molecule that prevents the synthesis of phage enzymes and proteins required for the lytic cycle. If the synthesis of the repressor molecule stops or if the repressor becomes inactivated, an enzyme encoded by the prophage is synthesized which excises the viral DNA from the bacterial chromosome. This excised DNA (the phage genome) can now behave like a lytic virus, that is to produce new viral particles and eventually lyse the host cell (see diagram above). This **spontaneous derepression** is a rare event occurring about one in 10,000 divisions of a lysogenic bacterium., but it assures that new phage are formed which can proceed to infect other cells.

Usually it is difficult to recognize lysogenic bacteria because lysogenic and nonlysogenic cells appear identical. But in a few situations, the prophage supplies genetic information such that the lysogenic bacteria exhibit a new characteristic (new phenotype), not displayed by the nonlysogenic cell, a phenomenon called **lysogenic conversion**. Lysogenic conversion has some interesting manifestations in pathogenic bacteria that only exert certain determinants of virulence when they are in a lysogenized state. Hence, *Corynebacterium diphtheriae* can only produce the toxin responsible for the disease if it carries a temperate virus called phage beta. Only lysogenized streptococci produce the erythrogenic toxin (pyrogenic exotoxin) which causes the skin rash of scarlet fever; and some botulinum toxins are synthesized only by lysogenized strains of *C. botulinum*.

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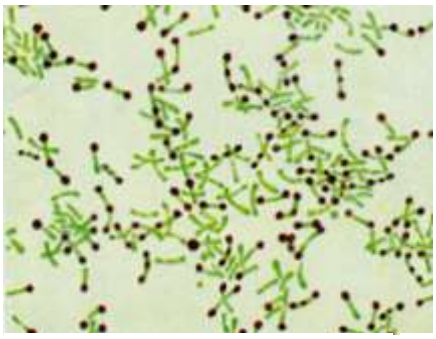

***Corynebacterium diphtheriae* only produces diphtheria toxin when lysogenized by beta phage.*C. diphtheriae* strains that lack the prophage do not produce diphtheria toxin and do not cause the disease diphtheria. Surprisingly, the genetic information for production of the toxin is found to be on the phage chromosome, rather than the bacterial chromosome.**

A similar phenomenon to lysogenic conversion exists in the relationship between an animal tumor virus and its host cell. In both instances, viral DNA is incorporated into the host cell genome, and there is a coincidental change in the phenotype of the cell. Some human cancers may be caused by viruses which establish a state in human cells analogous to lysogeny in bacteria.

Phage therapy

Phages were discovered to be antibacterial agents and were used in the former Soviet Republic of Georgia (pioneered there by Giorgi Eliava with help from the co-discoverer of bacteriophages, Felix d'Herelle) and the United States during the 1920s and 1930s for treating bacterial infections. They had widespread use, including treatment of soldiers in the Red Army. However, they were abandoned for general use in the West for several reasons:

- Medical trials were carried out, but a basic lack of understanding of phages made these invalid.[11]
- Antibiotics were discovered and marketed widely. They were easier to make, store and to prescribe.
- Former Soviet research continued, but publications were mainly in Russian or Georgian languages and were unavailable internationally for many years.
- Clinical trials evaluating the antibacterial efficacy of bacteriophage preparations were conducted without proper controls and were methodologically incomplete preventing the formulation of important conclusions.

Their use has continued since the end of the Cold War in Georgia and elsewhere in Central and Eastern Europe. Globalyz Biotech is an international joint venture that commercializes bacteriophage treatment and its various applications across the globe. The company has successfully used bacteriophages in administering Phage therapy to patients suffering from bacterial infections, including: Staphylococcus (including MRSA), Streptococcus, Pseudomonas, Salmonella, skin and soft tissue, gastrointestinal, respiratory, and orthopedic infections. In 1923, the Eliava Institute was opened in Tbilisi, Georgia, to research this new science and put it into practice.

The first regulated, randomized, double-blind clinical trial was reported in the Journal of Wound Care in June 2009, which evaluated the safety and efficacy of a bacteriophage cocktail to treat infected venous leg ulcers in human patients. The FDA approved the study as a Phase I clinical trial. The study's results demonstrated the safety of therapeutic application of bacteriophages but did not show efficacy. The authors explain that the use of certain chemicals that are part of standard wound care (e.g. lactoferrin or silver) may have interfered with bacteriophage viability.[12] Another controlled clinical trial in Western Europe (treatment of ear infections caused by *Pseudomonas aeruginosa*) was reported shortly after in the journal Clinical Otolaryngology in August 2009.[13] The study concludes that bacteriophage preparations were safe and effective for treatment of chronic ear infections in humans. Additionally, there have been numerous animal and other experimental clinical trials evaluating the efficacy of bacteriophages for various diseases, such as infected burns and wounds, and cystic fibrosis associated lung infections, among others. Meanwhile, bacteriophage researchers are developing engineered viruses to overcome antibiotic resistance, and engineering the phage genes responsible

for coding enzymes which degrade the biofilm matrix, phage structural proteins and also enzymes responsible for lysis of bacterial cell wall.
D'Herelle "quickly learned that bacteriophages are found wherever bacteria thrive: in sewers, in rivers that catch waste runoff from pipes, and in the stools of convalescent patients." This includes rivers traditionally thought to have healing powers, including India's Ganges River

Phage therapy is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections. Phage therapy is an alternative to antibiotics being developed for clinical use by research groups in Eastern Europe and the U.S. After having been extensively used and developed mainly in former Soviet Union countries for about 90 years, phage therapies for a variety of bacterial and poly microbial infections are now becoming available on an experimental basis in other countries, including the U.S. The principles of phage therapy have potential applications not only in human medicine, but also in dentistry, veterinary science, food science and agriculture.

An important benefit of phage therapy is derived from the observation that bacteriophages are much more specific than most antibiotics that are in clinical use. Theoretically, phage therapy is harmless to the eucaryotic host undergoing therapy, and it should not affect the beneficial normal flora of the host. Phage therapy also has few, if any, side effects, as opposed to drugs, and does not stress the liver. Since phages are self-replicating in their target bacterial cell, a single, small dose is theoretically efficacious. On the other hand, this specificity may also be disadvantageous because a specific phage will only kill a bacterium if it is a match to the specific subspecies. Thus, phage mixtures may be applied to improve the chances of success, or clinical samples can be taken and an appropriate phage identified and grown.

Phages are currently being used therapeutically to treat bacterial infections that do not respond to conventional antbiotics, particularly in the country of Georgia. They are reported to be especially successful where bacteria have constructed a biofilm composed of a polysaccharide matrix that antibiotics cannot penetrate.
What comes to mind when you hear the word “bacteria”? Most people, if not all, will answer “disease,” “sickness,” or “bad for the health.” What not all people know is there are actually both good and bad bacteria and some bacterial species are probiotic – bacteria that are helpful to its host. In fact, bacterial infections can be treated with bacteriophages: viruses that have the ability to infect and fight harmful bacteria, culminating in their destruction. Bacteriophage or phage therapy is therefore very useful in various fields like medicine, veterinary science, dentistry, and even agriculture.

History of Phage Therapy

Bacteriophages were discovered by two people: the English bacteriologist Frederick Twort in 1915 and the French-Canadian microbiologist Felix d’Herelle in 1917. Immediately after their discovery, the thought of using phages to fight bacterial infections was already apparent. D’Herelle began testing the therapeutic effects that phages may have on chickens and cows first and the tests were successful. Eventually, human tests were conducted and the development of phage therapy became more extensive especially with the foundation of the Eliavia Institute in 1923; the pharmaceutical company Eli Lilly began the commercialization of phage therapy in the US during the 1940s. During the Second World War, phages were used to treat bacterial diseases among soldiers of the Soviet Union, particularly gangrene and dysentery. The development of antibiotics in the 1950s led to a temporary setback on phage therapy as the use of antibiotics became more favourable. However, there has been a renewed interest in the development and employment of phage therapy in more applications.

Advantages over Antibiotics

Viruses and bacteria evolve over time and can develop a resistance to antibiotics. In theory, this resistance can also apply to phages, but it may be less difficult to overcome compared to antibiotics.
Because phages are target specific, meaning only a one or very few bacterial strains are targeted upon, it is easier to develop new phages than new antibiotics. A time period of only a few days or weeks is needed to acquire new phages for resistant strains of bacteria, whereas it can take years to obtain new antibiotics. When

resisting bacteria evolve, the assigned phages also evolve, so when super bacterium appears, an equivalent super phage fights it as long as the phage is derived from the same environment.

Compared to antibiotics, phages go deeper into the infected area. Antibiotics, on the other hand, have concentration properties that quickly decrease as they go below the surface of the infection. The replication of phages is concentrated on the infected area where they are needed the most, while antibiotics are metabolized and removed from the body. In addition, secondary resistance does not happen among phages, but happens quite often among antibiotics. Secondary resistance is acquired and occurs when there aren't enough blood drug levels.

Certain infections in people and experimentally infected animals have been proven to be more effectively treated with phage therapy than using antibiotics. Since 1966, the average success rate of studies that used phages in various ways (systematically, topically, intravenously, or orally) is from 80 to 95%, with minimal or no allergic and/or gastrointestinal side effects. The infections studied are from *E. coli*, *Acinetobacter*, *Psuedomonas,* and *Staphylococcus aureus*. Multiple side effects like allergies, intestinal disorders, and yeast infections have been observed when using antibiotics.

Applications

Fighting and destroying bacterial infections (both in humans and animals) are the primary applications of phage therapy, but it can also be employed for other uses. It can be the key to fighting the NDM-1, a gene that can be included in the DNA of bacteria, enabling them to resist antibiotics. Waste water from sewage systems are not really considered waste because it is a rich source of phage strains for various kinds of bacteria that lead to the most up-to-date medicines. Skin grafting for extensive wounds, trauma, burns, and skin cancer can also be improved by using phage therapy to lessen the *Psuedomonas aeruginosa* infection. Some experiments for cells in tissue culture have also discovered antitumor agents in phages. Bacteria cause food to spoil faster, and phages have been studied for their potential to increase the freshness of food and decrease the incidents of food spoilage.

Phage therapy has many other potential benefits and giving it ample support can pave the way to a healthier future.

Replication

Bacteriophages may have a lytic cycle or a lysogenic cycle, and a few viruses are capable of carrying out both. With *lytic phages* such as the T4 phage, bacterial cells are broken open (lysed) and destroyed after immediate replication of the virion. As soon as the cell is destroyed, the phage progeny can find new hosts to infect. Lytic phages are more suitable for phage therapy. Some lytic phages undergo a phenomenon known as lysis inhibition, where completed phage progeny will not immediately lyse out of the cell if extracellular phage concentrations are high. This mechanism is not identical to that of temperate phage going dormant and is usually temporary.

In contrast, the *lysogenic cycle* does not result in immediate lysing of the host cell. Those phages able to undergo lysogeny are known as temperate phages. Their viral genome will integrate with host DNA and replicate along with it relatively harmlessly, or may even become established as a plasmid. The virus remains dormant until host conditions deteriorate, perhaps due to depletion of nutrients; then, the endogenous phages (known as prophages) become active. At this point they initiate the reproductive cycle, resulting in lysis of the host cell. As the lysogenic cycle allows the host cell to continue to survive and reproduce, the virus is replicated in all of the cell's offspring. An example of a bacteriophage known to follow the lysogenic cycle and the lytic cycle is the phage lambda of *E. coli*.[16]

Sometimes prophages may provide benefits to the host bacterium while they are dormant by adding new functions to the bacterial genome in a phenomenon called lysogenic conversion. Examples are the conversion of harmless strains of*Corynebacterium diphtheriae* or *Vibrio cholerae* by bacteriophages to highly virulent ones, which cause Diphtheria orcholera, respectively.[17][18] Strategies to combat certain bacterial infections by targeting these toxin-encoding prophages have been proposed.[19]

Attachment and penetration

To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella. This specificity means a bacteriophage

can infect only certain bacteria bearing receptors to which they can bind, which in turn determines the phage's host range. Host growth conditions also influence the ability of the phage to attach and invade them.[20] As phage virions do not move independently, they must rely on random encounters with the right receptors when in solution (blood, lymphatic circulation, irrigation, soil water, etc.).

Myovirus bacteriophages use a hypodermic syringe-like motion to inject their genetic material into the cell. After making contact with the appropriate receptor, the tail fibers flex to bring the base plate closer to the surface of the cell; this is known as reversible binding. Once attached completely, irreversible binding is initiated and the tail contracts, possibly with the help of ATP present in the tail,[3] injecting genetic material through the bacterial membrane. Podoviruses lack an elongated tail sheath similar to that of a myovirus, so they instead use their small, tooth-like tail fibers enzymatically to degrade a portion of the cell membrane before inserting their genetic material.

Synthesis of proteins and nucleic acid

Within minutes, bacterial ribosomes start translating viral mRNA into protein. For RNA-based phages, RNA replicase is synthesized early in the process. Proteins modify the bacterial RNA polymerase so it preferentially transcribes viral mRNA. The host's normal synthesis of proteins and nucleic acids is disrupted, and it is forced to manufacture viral products instead. These products go on to become part of new virions within the cell, helper proteins that help assemble the new virions, or proteins involved in cell lysis. Walter Fiers (University of Ghent, Belgium) was the first to establish the complete nucleotide sequence of a gene (1972) and of the viral genome of bacteriophage MS2 (1976).[21]

Virion assembly

In the case of the T4 phage, the construction of new virus particles involves the assistance of helper proteins. The base plates are assembled first, with the tails being built upon them afterward. The head capsids, constructed separately, will spontaneously assemble with the tails. The DNA is packed efficiently within the heads. The whole process takes about 15 minutes.

Release of virions

Phages may be released via cell lysis, by extrusion, or, in a few cases, by budding. Lysis, by tailed phages, is achieved by an enzyme calledendolysin, which attacks and breaks down the cell wall peptidoglycan. An altogether different phage type, the filamentous phages, make the host cell continually secrete new virus particles. Released virions are described as free, and, unless defective, are capable of infecting a new bacterium. Budding is associated with certain *Mycoplasma* phages. In contrast to virion release, phages displaying a lysogenic cycle do not kill the host but, rather, become long-term residents as prophage.

Genome structure

Given the millions of different phages in the environment, phages genomes come in a variety of forms and sizes. RNA phage such as MS2 have the smallest genomes of only a few kilobases. However, some DNA phages such as T4 may have large genomes with hundreds of genes.

Bacteriophage genomes can be highly mosaic, i.e. the genome of many phage species appear to be composed of numerous individual modules. These modules may be found in other phage species in different arrangements.Mycobacteriophages – bacteriophages with mycobacterial hosts – have provided excellent examples of this mosaicism. In these mycobacteriophages, genetic assortment may be the result of repeated instances of site-specific recombination and illegitimate recombination (the result of phage genome acquisition of bacterial host genetic sequences. It should be noted, however, that evolutionary mechanisms shaping the genomes of bacterial viruses vary between different families and depend on the type of the nucleic acid, characteristics of the virion structure, as well as the mode of the viral life cycle.

Systems biology

Phages often have dramatic effects on their hosts. As a consequence, the transcription pattern of the infected bacterium may change considerably. For instance, infection of *Pseudomonas aeruginosa* by the temperate phage PaP3 changed the expression of 38% (2160/5633) of its host's genes. Many of these effects are probably indirect, hence the challenge becomes to identify the direct interactions among bacteria and phage.[24]

Several attempts have been made to map Protein–protein interactions among phage and their host. For instance, bacteriophage lambda was found to interact with its host E. coli by 31 interactions. However, a large-scale

study revealed 62 interactions, most of which were new. Again, the significance of many of these interactions remains unclear, but these studies suggest that there are most likely several key interactions and many indirect interactions whose role remains uncharacterized.

In the environment

Metagenomics has allowed the in-water detection of bacteriophages that was not possible previously.

Bacteriophages have also been used in hydrological tracing and modelling in river systems, especially where surface water and groundwater interactions occur. The use of phages is preferred to the more conventional dye marker because they are significantly less absorbed when passing through ground waters and they are readily detected at very low concentrations. Non-polluted water may contain ca. 2×10^8 bacteriophages per mL.

Other areas of use

Since 2006, the United States Food and Drug Administration (FDA) and United States Department of Agriculture (USDA) have approved several bacteriophage products. LMP-102 (Intralytix) was approved for treating ready-to-eat (RTE) poultry and meat products. In that same year, the FDA approved LISTEX (developed and produced by Micreos) using bacteriophages on cheese to kill *Listeria monocytogenes* bacteria, giving them generally recognized as safe (GRAS) status.[29] In July 2007, the same bacteriophage were approved for use on all food products.[30] In 2011 USDA confirmed that LISTEX is a clean label processing aid and is included in USDA.[31] Research in the field of food safety is continuing to see if lytic phages are a viable option to control other food-borne pathogens in various food products.

In 2011 the FDA cleared the first bacteriophage-based product for in vitro diagnostic use. The KeyPath MRSA/MSSA Blood Culture Test uses a cocktail of bacteriophage to detect *Staphylococcus aureus* in positive blood cultures and determine methicillin resistance or susceptibility. The test returns results in about 5 hours, compared to 2–3 days for standard microbial identification and susceptibility test methods. It was the first accelerated antibiotic susceptibility test approved by the FDA.

Government agencies in the West have for several years been looking to Georgia and the former Soviet Union for help with exploiting phages for counteracting bioweapons and toxins, such as anthrax and botulism. Developments are continuing among research groups in the US. Other uses include spray application in horticulture for protecting plants and vegetable produce from decay and the spread of bacterial disease. Other applications for bacteriophages are as biocides for environmental surfaces, e.g., in hospitals, and as preventative treatments for catheters and medical devices before use in clinical settings. The technology for phages to be applied to dry surfaces, e.g., uniforms, curtains, or even sutures for surgery now exists. Clinical trials reported in the *Lancet* show success in veterinary treatment of pet dogs with otitis.

Phage display is a different use of phages involving a library of phages with a variable peptide linked to a surface protein. Each phage's genome encodes the variant of the protein displayed on its surface (hence the name), providing a link between the peptide variant and its encoding gene. Variant phages from the library can be selected through their binding affinity to an immobilized molecule (e.g., botulism toxin) to neutralize it. The bound, selected phages can be multiplied by reinfecting a susceptible bacterial strain, thus allowing them to retrieve the peptides encoded in them for further study.

The SEPTIC bacterium sensing and identification method uses the ion emission and its dynamics during phage infection and offers high specificity and speed for detection.

Phage-ligand technology makes use of proteins, which are identified from bacteriophages, characterized and recombinantly expressed for various applications such as binding of bacteria and bacterial components (e.g. endotoxin) and lysis of bacteria

Cultural References

- In 1925 in the Pulitzer Prize-winning novel *Arrowsmith*, Sinclair Lewis fictionalized the discovery and application of bacteriophages as a therapeutic agent.
- The 1999 Greg Bear novel *Darwin's Radio* deals with an epidemic in the form of long-dormant sections of human DNA, introduced in prehistoric times by lysogenic bacteriophages, which begin to express themselves. The sequel, *Darwin's Children*, takes place in the post-epidemic world.

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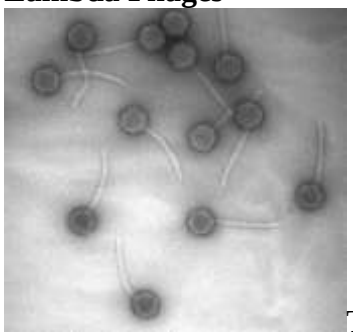
- The *Stargate Atlantis Legacy* novel *The Third Path* deals with a virulent bacteriophage that has turned a bacterial solvent into a deadly plastic-consuming threat with the potential to be fatal to humans. Part of the novel focuses on the characters efforts to stop the bacteriophage, ultimately creating another one to destroy the first.

Lambda Phages



The lambda phage, also called *Enterobacteria phage λ* and colphage λ, is a type of temperate bacteriophage or bacterial virus that infects the *Escherichia coli* (E. coli) species of bacteria. The virus may be housed in the genome of its host via lysogeny.

History of Lambda Phage

In 1950, Esther Lederberg, an American microbiologist, was performing experiments on E. coli mixtures. She happened to observe streaks of mixtures of two types of E. coli strains that seemed to have been nibbled on and had viral plaque. One E. coli strain had been treated with ultraviolet light, so the damage was more apparent. It was later determined that this was caused by bacterial viruses, which replicated and spread resulting in cell destruction. The discovery led to the employment of Lambda phage as a model organism in microbial genetics as well as in molecular genetics.

Structure

A lambda phage has a head measuring around 50-60 nanometers in diameter and a flexible tail that is around 150 nanometers long and may contain tail fibers.

The head consists of various proteins and over a thousand protein molecules including X1, X2, B, B*, E, D, and W. The head functions as a capsid that contains its genome, which contains 48,490 base pairs of double-stranded linear DNA. This number also includes 12-base single-stranded parts at its 5' ends. The single-stranded parts are known as sticky sites and are also called the cos site, which encircles the DNA in the host cytoplasm. Hence, when in circular form, the phage genome is comprised of 48,502 pairs in length. The weight of the genome is estimated to be 32×10^6 Da, which is around half of the weight of the phage.

The tail has a 135 nanometer-tube that is hollow and contains a conical cap which is around 15 nanometers. The tail's inner diameter is around 3 nanometers, while on the outside, it is around 9-18 nanometers depending on the knob-like structures that give the tail a rough appearance.

Life cycle

When E. coli is infected with a lambda phage, two cycles may happen: lytic or lysogenic. The lytic cycle happens when progeny phage particles are produced. The lytic cycle is the more common life cycle that comes after most infections. The first step of this cycle is the attachment of the phage to the host cell, injecting its DNA into the cell. Nucleic acid from the phage is replicated, and the phage's genes are expressed, allowing the production of phage proteins. The phage proteins are assembled into phage particles, which are released when the host cell undergoes lysis (it breaks down). The lysis is mediated by lysis genes *S, R, Rz,* and *Rz1* which, upon expression, yield proteins that work together to break down the host bacterium's cell wall.This mode of lambda replication typically yields many phage particles.

The lysogenic cycle, in contrast, does not produce a huge number of progeny phage or break down the host cell. Instead, the λ DNA recombines with its host's genome to produce a prophage. This typically is the favoured pathway when unfavourable environmental conditions prevent intense replication of the bacterial cells. Like the lytic cycle, the first step of the lysogenic cycle is also the attachment of the phage and the injection of its DNA into the host cell. The phage DNA then integrates with the host chromosome, producing an integrated DNA combination called the prophage DNA. Host cells that carry this DNA are said to be in the lysogenic state. The

prophage DNA is replicated along each time the host bacterial cell replicates itself, producing more cells, each with a copy of the prophage DNA. When these cells are exposed to certain chemicals or to ultraviolet light, phage induction happens; the prophage DNA is then cut out of the host genome and proceeds to the lytic cycle.

Applications

The lambda phage has different applications, most of which are related to DNA cloning. This is because lambda phage can be used as a vector for generating recombinant DNA, which are combined DNA sequences that result from using laboratory techniques like molecular cloning to assemble genetic material from several sources. The site-specific recombinase of lambda phage can be used for shuffling cloned DNAs via the gateway cloning system, a molecular biology technique that ensures the effective transfer of DNA fragments between plasmids.

The lambda phage's ability to mediate genetic recombincation is due to its red operon, which is a functioning unit of genomic DNA that has a cluster of genes controlled by a promoter or a single regulatory signal. This red operon can be expressed to yield the proteins red alpha (or exo), beta, and gamma, which can be used in recombination-mediated genetic engineering, a method commonly employed in bacterial genetics, generation of target vectors, and DNA modification. Undoubtedly, the lambda phage is a powerful genetic tool that can be used in many important studies.

Temperate Phages

A bacteriophage is a kind of virus that can infect and replicate itself inside bacterial cells. The virus has a protein-encapsulated DNA or RNA genome and can have simple or complex anatomies. There are many types of bacteriophages including M13, T phage, lambda phage, MS2, G4, and Phix174.

One of the characteristics of bacteriophages is their temperateness. Temperateness refers to the ability of some bacteriophages, particularly lambda phage, to choose between two cycles: lysogenic or lytic. "Temperance" generally refers to the moderation of actions, and in the case of phages, moderation is seen through the ability to not express anti-bacterial virulence.

Viral reproduction

Viruses cannot multiply through the division of cells because they are acellular (they do not have cells). Instead, they seek a host cell in which they replicate and assemble themselves using the metabolism and machinery of the host cell. Different species of viral populations undergo different viral life cycles, but for temperate phages, as previously mentioned, they must pick between two.

The lytic-lysogeny decision

Decision making isn't just done by people; it is also done by temperate phages as they need to choose between two different life cycles, productive (lytic) or reductive (lysogenic). There is a predominance of lytic among temperate phages, as induction can cause lysogenic to convert to lytic.

However, in most cases, temperate phages reel toward the lysogenic cycle especially when phage absorption in the infected bacteria is apparent. It is inferred that other local bacteria are undergoing the same phage infection, making the bacteria decrease in density. Because of this "crisis," the go-to cycle is lysogenic.

On the other hand, when there is an abundance of uninfected bacteria, undergoing the lytic cycle is preferable because to increase the number of healthy bacteria, phages that have productive infections are needed.

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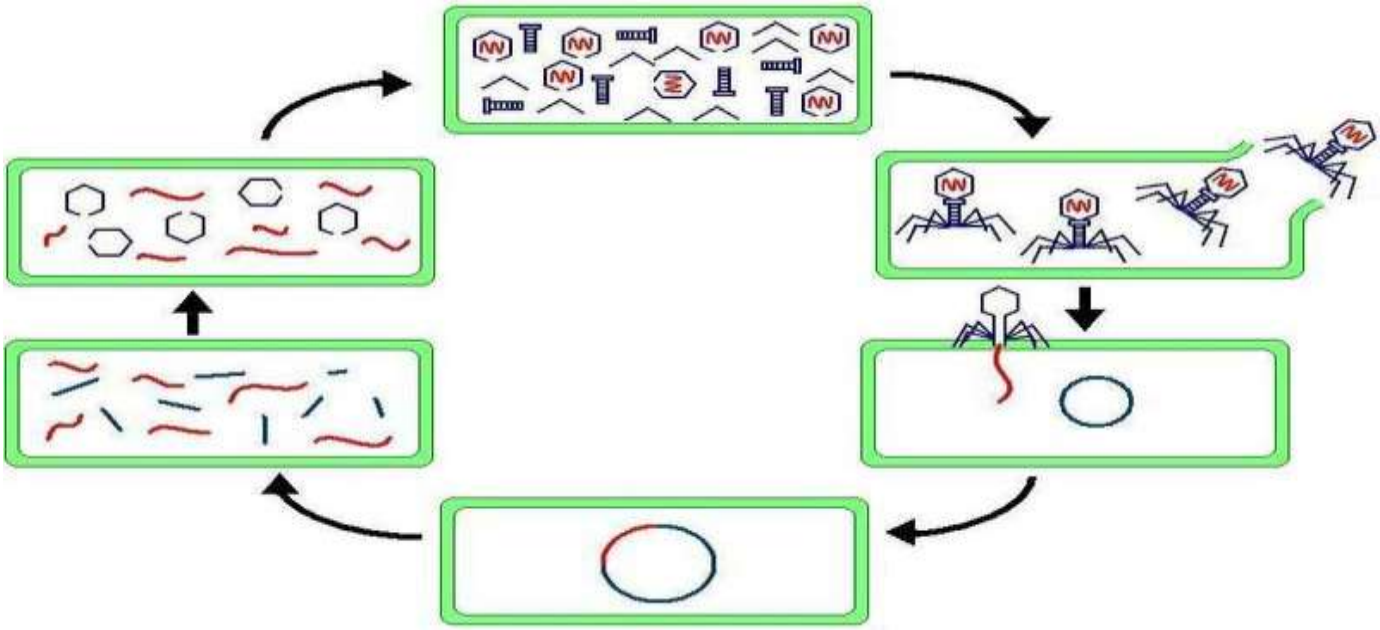

Lysogenic cycle

In the lysogenic cycle, the genomes of temperate phages are not expressed. However, they are integrated into the genome of the bacteria and produce prophages, which are created without disrupting the bacterial cell. Moreover, because of this integration, passive replication of the bacteriophage occurs when daughter bacterial cells are produced. These prophage-containing bacteria cells are called lysogens – phages that can exist as dormant DNA within its host cell. These lysogens have the ability to stay in the lysogenic cycle for a very long time, but through induction, they can be directed to the lytic cycle at any point in time. When induction occurs, prophage DNA is cut off from the bacterial genome and coat proteins are produced via transcription and translation of the prophage DNA for the regulation of lytic growth.

Lytic cycle

The lytic cycle is similar to the lysogenic cycle in that the host DNA machinery is used to replicate the phage, but the phage is considered a separate molecule from the host DNA. When a temperate phage undergoes the lytic cycle, it becomes a virulent phage. The infected cell and its membrane disintegrate as the viral DNA, which is considered a separate molecule from the bacterial cell, replicates separately from the DNA of the host bacteria, eventually overwhelming it.

The lytic cycle is divided into different stages. The first stage is the penetration in which the virus enters the host cell and injects its nucleic acids into it, releasing genetic material (either DNA or RNA) and infecting the cell. Viral components are then produced using the machinery of the host cell, culminating in the biosynthesis of mRNA and protein production. The host cell begins to copy the viral nucleic acids, which combine with viral proteins to produce phage particles within the cell. When there are already too many viral particles within the host, its membrane splits and the released viruses begin infecting other cells.

Applications

Temperate phages have various biological and molecular applications. They can be used to genetically manipulate eukaryotic cells, especially species that have large genomes like plants and mammals. Gene therapy, manipulation of cell lines, and construction of transgenic organisms can also employ phage enzymes. The temperate phage Mu-1 has a DNA-modifying function, which is of great importance especially in virology. Various food and biotechnology products and chemicals also employ the bacterial fermentation of phages.

In most laboratories, temperate phages are considered more of lytic phages because most lytic-lysogenic decisions result in the former. However, whether phages are lytic or lysogenic, it is apparent that even they are capable of making a decision, particularly for replication

Phage Display

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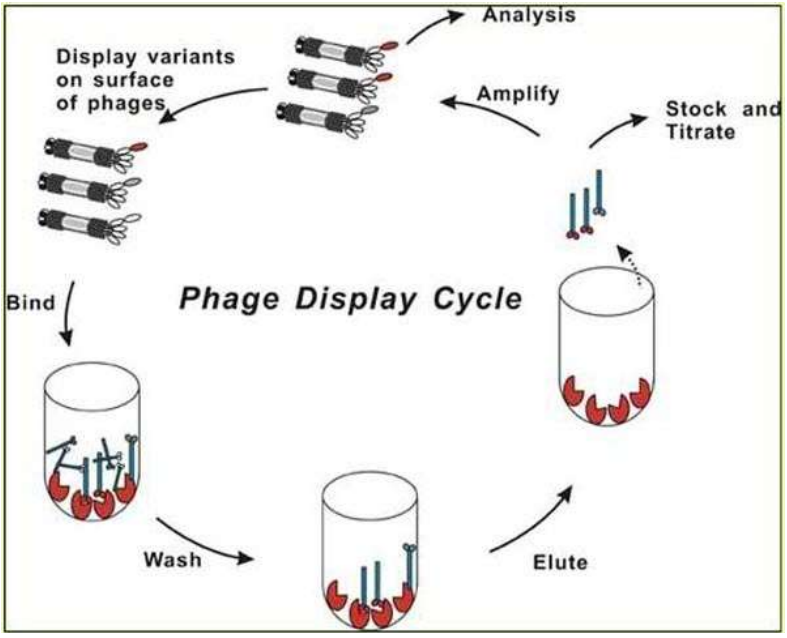

One of the laboratory techniques employed in studying different protein interactions is Phage Display. With this in vitro screening method, protein ligands and macromolecules can be easily identified and interactions between protein and protein, peptide and protein, & DNA and protein can be studied further.

History of Phage Display

The first described instance of Phage Display occurred in 1985, when George P. Smith fused a peptide with a gene III from a filamentous phage. He filed a patent detailing the process of generating phage display libraries in the same year. Eventually, further development of Phage Display technology led by different groups from the MRC Laboratory of Molecular Biology, as well as from The Scripps Research Institute, led to the possibility of displaying proteins for the purpose of therapeutic protein engineering. The technique has been continuously improved to screen and amplify huge collections of proteins showing the connection of phenotype and genotype better.

Structure

A filamentous phage has a diameter of around 6.5 nanometers, with a length that depends on the size of its genome. It comes from a huge family of bacterial viruses that also infect other forms of bacteria. It contains a small genome with an intergenic region containing the necessary sequences for the replication and encapsidation of DNA.

A phage particle consists of five coat proteins. The particle has a hollow tube that houses so many copies of the primary coat protein. There are also binding interactions between the adjacent subunits' hydrophobic midsections. One end of the particle is blunt, and the other is sharp. The blunt end contains plenty of copies of the two tiniest ribosomally translated proteins, while the sharp end contains around only 5 copies of the pIII and pVI genes, which are necessary for the detachment of the phage from the cell membrane.

How it works

Phage Display is a method wherein a library of phage particles that express very diverse peptides is generated. The objective is to choose those that will bind a desired target; the target can be a protein, a peptide, or a piece of DNA.

The most often used vector to build a random peptide display is the filamentous phage M13. In this display, the DNA which encodes the peptide or protein of interest is integrated into the pIII or pVI gene. To make sure that the fragments are completely inserted into the three possible reading frames, multiple cloning sites are sometimes employed, allowing the proper translation of the cDNA in its correct frame. The DNA hybrid and the phage gene are then put inside E. coli bacterial cells. Examples of these bacterial cells include XL1-Blue E. coli, SS320, TG1, and ER2738. The peptide or protein of interest is eventually expressed in either the minor or major coat protein

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If another kind of vector is used, for example, a phagemid vector or simplified display construct vector, a helper phage must infect the E. coli cells; otherwise, the phage particles will not be separated from the E. coli cells. A helper phage activates the packaging of the phage DNA and assembles the mature virions with their corresponding protein fragments, which are included in the outer coating of the minor or major protein coat.

The generated phage library is then screened by addition into a microtiter plate containing immobilised target proteins or DNA sequences. Phages displaying a protein that bind to one target will remain, while the other phages can be discarded through washing. The remaining phage particles can be used to multiply the phage by infecting them into bacteria, thus increasing the diversity of the peptide display library.

Applications

The fast isolation of particular ligands through phage display has a wide variety of applications like epitope mapping, analyzing different protein interactions, vaccine development, drug design, and therapeutic target validation. Phage display is also used to pick inhibitors for the active and allosteric sites of G-protein binding modulatory peptides, enzymes, and receptor antagonists and agonists.

Determining the proper protein partners can be useful to identify the functions of various proteins. For drug discovery and design, Phage Display is employed in protein engineering or in vitro protein evolution. Therapeutic targeting with phage display is also primarily used to diagnose and determine tumour antigens, which is useful for cancer research.

Antibody Phage Display significantly improved the discovery and development of antibody drugs. Phage display for antibody libraries paved the way for rapid vaccine design and therapy. These libraries are used to learn more about the human immune system and to create human antibodies in vitro with the use of diverse synthetic substances.

Phage Display can be used in conjunction with other techniques, and with enough support and studies, more applications for it can be discovered.

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15MBU502
III BSC MICROBIOLOGY
VIROLOGY

Unit III Q	Opt 1	Opt 2	Opt 3	Opt 4	Opt 5	Opt 6	Answer
_____	Host pref	Morpholo	Physical r	Chemical nature of virion const			Chemical nature of v
The ____	fimbriae	flagellae	hemaglutt	neuraminidase			neuraminidase
Negative	use it to t	have to m	use it to n	none of the above			use it to terminate tra
Which of	Attachme	Penetratio	Attachme	Attachment, release, biosynthes			Attachment, penetrat
What can	Patient se	Anti-polio	Polio caps	Colored substrate			Anti-polio antibody
The nucle	envelope	covering	Membron	capsid			envelope
Which typ	alpha	beta	Both (a) a	gamma			Both (a) and (b)
Fluoresce	Subacute	Herpes sir	Rabies	hepatitis			Herpes simplex ence
Viral mat	exposed c	found ma	anchor the	part of the nucleoprotein core o			exposed on the surfa
The micro	viruses	bacteria	fungi	algae			viruses
The two r	fat and pr	nucleic ac	carbohydr	fat and carbohydrate			nucleic acid and prot
The transc	RNA viru	ds DNA v	ss DNA v	NA viruses			ds DNA viruses
What doe	rRNA	tRNA	mRNA	RNA			mRNA
Which of	Type of c	Type of n	Presence	Symmetry			Presence of an envel
The reovi	10 differe	8 differen	5 differen	7 different segments of dsRNA			8 different segments
Which typ	α	β	γ	γ and β			γ and β
_____	Temperat	Adsorbed	RNA pha	DNA phage			Temperate virus
Which of	Toxin pro	Toxin pro	Antigenic	toxic chemical shift			Antigenic variation i
T-even ph	electrosta	hydropho	covalent b	bivalent bonds			electrostatic interact
The filam	lipopolysa	the cell w	the tip of	the cell membrane			the cell wall
A bacteria	Cconcatar	polymeriz	restriction	lysogeny			restriction
Intracellu	prokaryot	chromoso	inclusion	cytocidal bodies			cytocidal bodies
Viral RNA	cytoplasm	nucleus	mitochon	lysozomes			cytoplasmic matrix
Which of	Hepatitis	Herpes sir	Varicella-	Cytomegalovirus			Hepatitis B virus
Viroids ar	single-stra	double-str	single-stra	double-stranded RNA			double-stranded DN
Which of	Prions	Viroids	virions	Virinos			Prions
Transfer c	plasmode	cytodesm	protodesm	cytomorpha			plasmodesmata
Intracytop	Rabies vir	Vaccinia	Fowlpox	HIV			Fowlpox virus
What is th	Synthesis	Productio	Both (a) a	biosynthesis			Synthesis of protein
Which of	Rhinoviru	Coronavir	Measles v	CAMV			Rhinovirus
The predo	phospholi	glycolipid	neutral fa	Lipds			glycolipids
Supercoil	DNA gyra	DNA pyra	RNA gyra	RNA pyrase			DNA pyrase
The tissue	aneuploid	protopath	cytopathic	CFE			cytopathic effect
The struct	hyperplas	anaplasia	metastasis	cytostasis			anaplasia
The penet	vitropexis	viropexis	ectodesma	vivopexis			viropexis
Viropexis	DNA gyra	lysosomal	lysosomal	DNA lipase			lysosomal protease
Hepatitis	Caliciviri	Flaviviric	Hepadnav	Coronaviridae			Flaviviridae
Transmiss	Blood	Sexually	Saliva	neonatal			Sexually
The most	superinfe	infection	coinfectio	re infection			infection with HBV

Which of	Blood	Semen	Saliva	pus			Blood
Vertical t	Hepatitis	Hepatitis	Hepatitis	Hepatitis E Virus			Hepatitis B virus
Which of	HBV	HCV	HDV	All of these			HCV
Most relia	ELISA te	Western b	Polymera	ELISA test for IgG anti-HEV			Western blot assay f
Which of	Rabies	Influenza	Polio	Hepatitis			Rabies
Which of	Astroviru	Caliciviru	Hepatitis	Hepatitis D virus			Astrovirus
Which of	dsDNA	ssRNA	ssDNA	dsRNA			dsDNA
The agent	GBA-A	GBV-B	GBV-C	GBV			GBA-A
Negri boc	Paramyxo	Vaccinia	Fowlpox	Rabies virus			Paramyxoviruses
The unco	hyperplas	anaplasia	metastasis	cytostasis			metastasis
The RNA	fibroblast	myoblasts	iris epithe	protoplasts			fibroblasts
Plant viru	ectodesma	endodesm	cytodesm	protodesmata			cytodesmata
The disea	Vaccinia	Smallpox	Cowpox	Chicken pox			Smallpox
The virus	Poliovirus	Coxsackie	Echo viru	HIV			Coxsackieviruses
Which of	Orthopox	Parapoxv	Molluscip	Mesopox			Orthopoxvirus
Which of	Group A	Group C	Group D	Group E			Group A
Which of	Enterovir	Rhinoviru	Hepatovir	HIV			Enterovirus
Transmiss	direct con	sexual co	Indirect	Mechanical			sexual contact
The symn	complex	icosahedr	helical	Cylinderical			complex
Which of	Hepatitis	Hepatitis	Hepatitis E virus				Hepatitis E virus
What is th	Spherical	Polygona	Bullet-sha	Tubular			Bullet-shaped

virion constituents

anscripts when they copy host cell mRNA
tion, biosynthesis, maturation, release

ephalitis
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tein

ope
s of dsRNA and 10 different segments of ssRNA respectively

n Salmonella anatum
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lA

alone

for IgM anti-HEV

2017

III B. Sc Microbiology – Virology
Unit IV

LECTURE PLAN

LECTURE PLAN - UNIT -1V			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	Animal viruses	T3 194 -262
2	1	Plant viruses	T3 133-143
3	1	Avian viruses	R1 550-566
4	1	Mammalian viruses	W1
5	1	Human Viruses	T2264-275
6	1	oncogenic viruses	T4 550-557
7	1	Viriods and prions	T2 401-410
8	1	Revision of Unit IV	
9	1	Unit IV test	
Textbooks :		T1-medical virology -White & fenner, Academic press publishers T2-Modern virology -dimmock-Black well publishing T3-virology-P.saravanan-Mjp publishers T4-Textbook of Microbiology- Paniker- Orient longman publishers	
Reference books:		R1- Medical Microbiology –Jawertz – McGraw hill Publishers	
Website:		W1- www.virologyweebly.com W2 - www.microbiology.com	
Journals:			

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MORPHOLOGY AND GENERAL PROPERTIES OF VIRUSES

INTRODUCTION

Viruses occupy the twilight zone that separates the 'living' from the 'non-living'. They do not have a cellular organization and contain only one type of nucleic acid, either DNA or RNA but never both. The medical importance of viruses lies in their ability to cause a very large number of human diseases. Viral diseases range from minor ailments like common cold to terrifying diseases like rabies and AIDS.

In this chapter, we shall be discussing the morphology and general properties of viruses.

After reading this lesson you will be able to:

- explain the concept of viruses, in relation to other microorganisms
- describe the morphological features of viruses
- explain the multiplication of viruses (replication)
- describe the methods of cultivation of viruses
- explain the classification and naming (nomenclature) of viruses

Concept of Viruses in relation to other Organisms

Viruses occupy the twilight zone that separates the 'living' from the 'non-living'. They do not have a cellular organization and contain only one type of nucleic acid, either DNA or RNA but never both. Viruses are obligate intracellular parasites. They lack the enzymes necessary for protein and nucleic acid synthesis. They are dependent for replication on the synthetic machinery of host cells. They multiply by a complex process and not by binary fission. They are unaffected by antibacterial antibiotics. Viruses cause a wide range of human diseases. They cause infections like common cold, chicken pox, measles, viral encephalitis, rabies and AIDS.

Properties Bacteria Viruses

Cellular organization Present Absent Growth on inanimate media Yes No Binary fission Yes No DNA and RNA Both are present Either DNA or RNA Ribosomes Present Absent Sensitivity to antibacterial Yes No antibiotics

Morphology of Viruses

Size: The extracellular infectious virus particle is called virion. Viruses are much smaller than bacteria. They are too small to be seen under the light microscope. Some large viruses like the poxviruses can be seen under the light microscope when suitably stained. The viruses range in size from 20 nm to 300 nm. Poxviruses are one of the largest viruses and parvoviruses are one of the smallest viruses. The earliest method of estimating the size of virus particles was by passing them through collodion membrane filters of graded porosity. The average pore diameter of the finest filter that permitted passage of the virion gave an estimate of its size. With the development of the ultracentrifuge, a second method became available. From the rate of sedimentation of the virus in the ultracentrifuge, the particle size could be calculated using Stoke's law. The third and the most direct method of measuring virus size is electron microscopy. By this method, both the shape and size of virions can be studied.

Structure, shape and symmetry: The virion consists essentially of a nucleic acid surrounded by a protein coat, the **capsid**. The capsid with the enclosed nucleic acid is called the **nucleocapsid**. The capsid protects the nucleic acid from harmful agents in the environment. It is composed of a large number of capsomers which form its morphological units. The chemical units of the Capsid are polypeptide molecules which are arranged symmetrically. They form a shell around the nucleic acid. The capsid shows two kinds of symmetry – icosahedral (cubical) and helical. An icosahedron is a polygon with 12 vertices and 20 facets or sides. Each facet is in the shape of an equilateral triangle. Two types of capsomers are present in the icosahedral capsid. They are the pentagonal capsomers at the vertices (pentons) and the hexagonal capsomers making up the facets (hexons). There are always 12 pentons but the number of hexons varies with the virus group. Examples of viruses with icosahedral symmetry of the capsid are Adenovirus and Herpes Simplex Virus. In the

nucleocapsids with helical symmetry, the capsomers and nucleic acid are wound together to form a helical or spiral tube, for example tobacco mosaic virus. All viruses do not show the typical icosahedral or helical symmetry. Some, like the poxviruses, show a complex symmetry. Virions may be enveloped or nonenveloped. The envelope of viruses is derived from the host cell membrane. This occurs when the virus is released from the host cell by budding. Protein subunits may be present as projecting spikes on the surface of the envelope. They are called **peplomers**. The influenza virus carries two kinds of peplomers: haemagglutinin and neuraminidase. Haemagglutinin is a triangular spike and neuraminidase is mushroom-shaped. Envelope is sensitive to the action of lipid solvents. Envelopes confer chemical, antigenic and biological properties on viruses.
The overall shape of the virus particle varies in different groups of viruses. Most animal viruses are roughly spherical. The rabies virus is bullet shaped. Poxviruses are brick-shaped.

Chemical properties: Viruses contain only one type of nucleic acid, either DNA or RNA. Viruses are unique because they carry genetic information on RNA. This property is not seen in any other organism in nature. Viruses also contain protein which makes up the capsid. Enveloped viruses contain lipids derived from the host cell membrane. Most viruses do not have enzymes for the synthesis of viral components or for energy production. Some viruses have enzymes, for example the influenza virus has neuraminidase.

Resistance: Viruses are destroyed by heat except a few. They are stable at low temperatures. For long term storage, they are kept at -70°C. A better method for prolonged storage is lyophilisation or freeze-drying. Viruses are inactivated by sunlight, UV rays and ionising radiation. They are, in general, more resistant than bacteria to chemical disinfectants. Phenolic disinfectants have a weak action on viruses.

Multiplication of Viruses
Multiplication of viruses is called viral **replication**. Viruses contain the genetic information for their replication but they lack the enzymes. They depend on host cell machinery for replication. The viral replication cycle can be divided into six phases – adsorption, penetration, uncoating, biosynthesis, maturation and release.
Adsorption: In this phase, the virus gets attached to the host cell. The host cell should have specific receptors on its surface. These receptors recognize viral surface components. This cell-virus interaction helps the virus to attach to the host cell surface.
Penetration: In this phase, the virus enters into the host cell. Bacteria have rigid cell wall. So, viruses which infect bacteria cannot penetrate into the bacterial cell. Only the nucleic acid of the virus enters the bacterial cell. Animal and human cells do not have cell walls. Therefore, whole virus enters the cell. Virus particle may be engulfed by a process called **viropexis**. In case of enveloped viruses, the viral envelope may fuse with the cell membrane of the host cell. Then the nucleocapsid is released into the cytoplasm.
Uncoating: This is the process in which the outer layers and capsid of the virus are removed. This mostly occurs by the action of lysosomal enzymes of the host cell. This can also occur by a viral uncoating enzyme. Finally, the viral nucleic acid is released into the cell.
Biosynthesis: In this phase, the viral nucleic acid and capsid are synthesised. The enzymes necessary in the various stages of viral synthesis, assembly and release are also synthesised. Certain 'regulator proteins' are synthesised. They shut down the normal metabolism of the host cell. They direct the production of viral components. In general, most DNA viruses synthesise their nucleic acid in the host cell nucleus. Exceptions are the poxviruses. They are DNA viruses, but they synthesise all their components in the host cell cytoplasm. Most RNA
viruses synthesise all their components in the cytoplasm. Orthomyxoviruses and some paramyxoviruses are exceptions. They synthesise some components in the host cell nucleus. Biosynthesis consists essentially of the following steps:
1. Transcription of messenger RNA (mRNA) from the viral nucleic acid
2. Translation of mRNA into "early proteins" or "non-structural proteins".
They are enzymes responsible for the synthesis of viral components.

3. Replication of viral nucleic acid
4. Synthesis of "late proteins" or "structural proteins". They are the components of daughter virion capsids.

Maturation: This is the assembly of daughter virions following the synthesis of viral nucleic acid and proteins. It can take place in the host cell nucleus or cytoplasm. Herpesviruses and adenoviruses are assembled in the nucleus.

Picornaviruses and poxviruses are assembled in the nucleus.

Release: Viruses which infect bacteria (bacteriophages) are released by lysis of the infected bacterium. Animal viruses are usually released without cell lysis. Myxoviruses are released by budding from the cell membrane. The host cell is unaffected. Daughter virions are released into the surrounding medium and may infect other cells. In some viruses (for eg. varicella), transmission occurs directly from cell to cell. In this case, there is very little free virus in the medium. The poliovirus causes cell damage and may be released by cell lysis. From the stage of penetration till the appearance of mature daughter virions, the virus cannot be demonstrated inside the host cell. During this period, the virus seems to disappear. This is called the "eclipse phase". The time taken for a single cycle of replication is about 15-30 minutes for bacteriophages. It is about 15- 30 hours for animal viruses. A single infected cell may release a large number of progeny virions.

Classification and naming of viruses

Till about 1950 little was known of the basic properties of viruses. They were named haphazardly, based on the diseases they caused or on the place of their isolation. They were grouped according to affinity to different systems or organs of the body (tropism). So, human viruses were classified as dermotropic, that is those producing skin lesions (smallpox, chickenpox, measles), neurotropic, that is those affecting the nervous system (poliomyelitis, rabies), pneumotropic, that is those affecting the respiratory tract (influenza, common cold) and viscerotropic, that is those affecting visceral organs (hepatitis). Bawden (1941) made the pioneering suggestion that viral nomenclature and classification should be based on the properties of viruses and not upon host responses. From the early 1950s, viruses began to be classified into groups based on their physiochemical and structural features. Nomenclature and classification are now the official responsibility of the International Committee on Taxonomy o of Viruses (ICTV). Viruses are classified into two main divisions based on the type of nucleic acid they possess: riboviruses contain RNA and deoxyriboviruses contain DNA. Further classification is based on other properties like strandedness of nucleic acid, symmetry of nucleic acid, presence of envelope, size and shape of virion and number of capsomeres.

DNA viruses: A few medically important families of DNA viruses are - Herpesviridae, Adenoviridae, Hepadnaviridae, Parvoviridae and Papillomaviridae. The Herpesviridae family consists of enveloped double-stranded DNA viruses having an icosahedral capsid. Examples of this family are herpes simplex virus and varicella zoster virus. Herpes simplex virus causes skin lesions like herpes labialis. It can also cause viral encephalitis. Parvoviridae consists of nonenveloped single-stranded DNA viruses, for example Parvovirus B19. The Hepadnaviridae family includes Hepatitis B virus which is a partially doublestranded DNA virus. Papillomaviridae family includes human papilloma virus which is responsible for causing skin warts.

RNA viruses: Some medically important families of RNA viruses are – Picornaviridae, Orthomyxoviridae and Paramyxoviridae, Flaviviridae, Rhabdoviridae and Retroviridae. Members of the family Picornaviridae are small (20-30 nm), non-enveloped, icosahedral viruses with single-stranded RNA genome. Examples include poliovirus and coxsackievirus. The viruses included in Orthomyxoviridae are enveloped viruses carrying haemagglutinin and neuraminidase peplomers on the envelope. The genome consists of singlestranded RNA in several (eight) pieces. Thus, they have a segmented genome. An example of this family is influenza virus. Flaviviridae consists of enveloped single-stranded RNA viruses. Examples include yellow fever virus, Japanese encephalitis virus and dengue virus. The members of Retroviridae family are enveloped RNA viruses which have a special enzyme called 'reverse transcriptase'.

This enzyme is an RNA dependent DNA polymerase. It is required in the synthesis of DNA from RNA. An example of the Retroviridae family is Human Immunodeficiency Virus (HIV) which causes AIDS (acquired immunodeficiency syndrome).

Based on the mechanism of replication, Baltimore (1970) categorised viruses into seven categories. This is called the Baltimore classification.

1. The genetic material in viruses is:

A. DNA only B. RNA only

C. Either DNA or RNA D. Both DNA and RNA

2. Protein subunits presenting as projecting spikes on the surface of the envelope are called:

A. Capsomeres B. Capsid

C. Nuceocapsid D. Peplomers

3. Which of the following is the correct sequence of viral replication?

A. Penetration, uncoating, adsorption, biosynthesis, maturation and release

B. Adsorption, penetration, uncoating, biosynthesis, maturation and release

C. Biosynthesis, penetration, uncoating, adsorption, maturation and release

D. Adsorption, biosynthesis, maturation, uncoating, penetration and release

4. Methods used for viral cultivation are:

A. Cell culture B. Animal inoculation

C. Embryonated eggs D. All of the above

5. Baltimore classified viruses on the basis of:

A. Diseases caused by them B. Structure

C. Replication mechanism D. Physiochemical properties

Structure and Classification of Viruses

General Concepts

Structure and Function

Viruses are small obligate intracellular parasites, which by definition contain either a RNA or DNA genome surrounded by a protective, virus-coded protein coat. Viruses may be viewed as mobile genetic elements, most probably of cellular origin and characterized by a long co-evolution of virus and host. For propagation, viruses depend on specialized host cells supplying the complex metabolic and biosynthetic machinery of eukaryotic or prokaryotic cells. A complete virus particle is called a virion. The main function of the virion is to deliver its DNA or RNA genome into the host cell so that the genome can be expressed (transcribed and translated) by the host cell. The viral genome, often with associated basic proteins, is packaged inside a symmetric protein capsid. The nucleic acid-associated protein, called nucleoprotein, together with the genome, forms the nucleocapsid. In enveloped viruses, the nucleocapsid is surrounded by a lipid bilayer derived from the modified host cell membrane and studded with an outer layer of virus envelope glycoproteins.

Classification of Viruses

Morphology: Viruses are grouped on the basis of size and shape, chemical composition and structure of the genome and mode of replication. Helical morphology is seen in nucleocapsids of many filamentous and pleomorphic viruses. Helical nucleocapsids consist of a helical array of capsid proteins (protomers) wrapped around a helical filament of nucleic acid. Icosahedral morphology is characteristic of the nucleocapsids of many "spherical" viruses. The number and arrangement of the capsomeres (morphologic subunits of the icosahedron) are useful in identification and classification. Many viruses also have an outer envelope.

Chemical Composition and Mode of Replication: The genome of a virus may consist of DNA or RNA, which may be single stranded (ss) or double stranded (ds), linear or circular. The entire genome may occupy either one nucleic acid molecule (monopartite genome) or several nucleic acid segments (multipartite genome). The different types of genome necessitate different replication strategies.

Nomenclature

Aside from physical data, genome structure and mode of replication are criteria applied in the classification and nomenclature of viruses, including the chemical composition and configuration of the nucleic acid, whether the genome is monopartite or multipartite. The genomic RNA strand of single-stranded RNA viruses is called sense (positive sense, plus sense) in orientation if it can serve as mRNA, and antisense (negative sense, minus sense) if a complementary strand synthesized by a viral RNA transcriptase serves as mRNA. Also considered in viral classification is the site of capsid assembly and, in enveloped viruses, the site of envelopment.

Structure and Function

Viruses are inert outside the host cell. Small viruses, e.g., polio and tobacco mosaic virus, can even be crystallized. Viruses are unable to generate energy. As obligate intracellular parasites, during replication, they fully depend on the complicated biochemical machinery of eukaryotic or prokaryotic cells. The main purpose of a virus is to deliver its genome into the host cell to allow its expression (transcription and translation) by the host cell.

A fully assembled infectious virus is called a virion. The simplest virions consist of two basic components: nucleic acid (single- or double-stranded RNA or DNA) and a protein coat, the capsid, which functions as a shell to protect the viral genome from nucleases and which during infection attaches the virion to specific receptors exposed on the prospective host cell. Capsid proteins are coded for by the virus genome. Because of its limited size (Table 41-1) the genome codes for only a few structural proteins (besides non-structural regulatory proteins involved in virus replication). Capsids are formed as single or double protein shells and consist of only one or a few structural protein species. Therefore, multiple protein copies must self assemble to form the continuous three-dimensional capsid structure. Self assembly of virus capsids follows two basic patterns: helical symmetry, in which the protein subunits and the nucleic acid are arranged in a helix, and icosahedral symmetry, in which the protein subunits assemble into a symmetric shell that covers the nucleic acid-containing core.

TABLE 41-1 Chemical and Morphologic Properties of Animal Virus Families Relevant to Human Disease

		Virion						
Family	Viral Genome: Type, Configuration[a] and Number of Bases per strand (x 10^3)	Shape[b]	Diameter (nm)	Enveloped[c]	Capsid Symmetry	Number of Capsomeres[d]	Site of Capsid Assembly	Enzymes, e.g. Transcriptase present in Virion
Circoviridae	ssDNA, circular; 0.6-1.2	s	17-22	0	Icosahedral	32?	Nucleus	None
Parvoviridae	ssDNA, linear, sense or antisense; 4-6	s	18-26	0	Icosahedral	32	Nucleus	None
Papovaviridae	dsDNA, circular; 5.1 / 7.9	s	45 / 55	0	Icosahedral	72	Nucleus	None
Adenoviridae	dsDNA, linear; 35-40	s	75-80	0	Icosahedral	252	Nucleus	None
Herpesviridae	dsDNA, linear; 124-235	s	120-200	+	Icosahedral	162	Nucleus	Thymidine kinase
Iridoviridae	dsDNA, linear; 170-200	s	125-300	+	Icosahedral	ca. 1,500	Cytoplasm	DNA-dependent RNA polymerase
Poxviridae	dsDNA, linear, covalently closed; 130-370	x	240x300	+	Complex	–	Cytoplasm	DNA-dependent RNA polymerase Protein kinase
Hepadnaviridae	dsDNA, circular, 1 ss-region; 3.0-3.3/2.0	s	40-48	+	Icosahedral	180	Nucleus	DNA-dependent DNA polymerase

TABLE 41-1 Chemical and Morphologic Properties of Animal Virus Families Relevant to Human Disease (continued)

Family	Viral Genome: Type, Configuration[a] and Number of Bases per strand (X 10^3)	Shape[b]	Diameter (nm)	Enveloped[c]	Capsid Symmetry	Number of Capsomeres[d]	Site of Capsid Assembly	Enzymes, e.g. Transcriptase present in Virion
Picornaviridae	ssRNA, linear; 7-8.5	S	22-30	0	Icosahedral	32	Cytoplasm	None
Astroviridae	ssRNA, linear, sense 6.8-7.9	S	28-30	0	Icosahedral	32?	Cytoplasm	None
Caliciviridae	dsRNA, linear, sense 7.4-7.7	S	35-39	0	Icosahedral	90	Cytoplasm	None
Togaviridae	dsRNA, linear, sense 9.7-11.8	S	70	+	Icosahedral	?	Cytoplasm	None
Flaviviridae	dsRNA, linear, sense 10-12	S	45-50	+	Icosahedral	unknown	Cytoplasm	None
Reoviridae	dsRNA, linear, 10-12 segments; 18-23	S	60-80	0	Icosahedral	32 or 92	Cytoplasm	RNA-dependent RNA polymerase
Orthomyxoviridae	dsRNA, linear, 8 molecules, antisense; 10-13.6	s-pleom[e]	80-120	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Paramyxoviridae	dsRNA, linear, antisense; 15	s-pleom	150-300	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Rhabdoviridae	ssRNA, linear, antisense;11-15	U	60x180	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Bunyaviridae	ssRNA, linear, 3 molecules, antisense; 11-20	s-pleom	90-120	+	Helical	–	Cytoplasm	RNA dependent RNA polymerase
Coronaviridae	ssRNA, linear, sense; 30	s-pleom	120-160	+	Helical	–	Cytoplasm	None
Arenaviridae	ssRNA, linear, 2 species + ribosomal RNA; 3.4	s-pleom	110-130	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Retroviridae	ssRNA, linear, inverted dimer of sense strand; 7-11	s-pleom	90-120	+	Icosahedral (type C)	–	Cytoplasm	RNA-dependent DNA polymerase Protease, Integrase
Filoviridae	ssRNA, linear, antisense; 19.1	Bacilli-form[f]	80x800-2,500	+	Helical	–	Cytoplasm	RNA-transcriptase/polymerase

[a]ss = single stranded; ds = double stranded. [b]S = spherical; X = brickshaped or ovoid; U = elongated with parallel sides and a round end; pleom = pleomorphic. [c]Most enveloped viruses are sensitive to lipid solvents. [d]Applicable to viruses with icosahedral symmetry. [e]Filamentous forms also occur.

Table 41-1 Chemical and Morphologic Properties of Animal Virus Families Relevant to Human Disease.

Some virus families have an additional covering, called the envelope, which is usually derived in part from modified host cell membranes. Viral envelopes consist of a lipid bilayer that closely surrounds a shell of virus-encoded membrane-associated proteins. The exterior of the bilayer is studded with virus-coded, glycosylated (trans-) membrane proteins. Therefore, enveloped viruses often exhibit a fringe of glycoprotein spikes or knobs, also called peplomers. In viruses that acquire their envelope by budding through the plasma or another intracellular cell membrane, the lipid composition of the viral envelope closely reflects that of the particular host membrane. The outer capsid and the envelope proteins of viruses are glycosylated and important in determining the host range and antigenic composition of the virion. In addition to virus-specified envelope proteins, budding viruses carry also certain host cell proteins as integral constituents of the viral envelope. Virus envelopes can be considered an additional protective coat. Larger viruses often have a complex architecture consisting of both helical and isometric symmetries confined to different structural components. Small viruses, e.g., hepatitis B virus or the members of the picornavirus or parvovirus family are orders of magnitude more resistant than are the larger complex viruses, e.g. members of the herpes or retrovirus families.

Classification of Viruses

Viruses are classified on the basis of morphology, chemical composition, and mode of replication. The viruses that infect humans are currently grouped into 21 families, reflecting only a small part of the spectrum of the multitude of different viruses whose host ranges extend from vertebrates to protozoa and from plants and fungi to bacteria.

Morphology

Helical Symmetry

In the replication of viruses with helical symmetry, identical protein subunits (protomers) self-assemble into a helical array surrounding the nucleic acid, which follows a similar spiral path. Such nucleocapsids form rigid, highly elongated rods or flexible filaments; in either case, details of the capsid structure are often discernible by electron microscopy. In addition to classification as flexible or rigid and as naked or enveloped, helical nucleocapsids are characterized by length, width, pitch of the helix, and number of protomers per helical turn. The most extensively studied helical virus is tobacco mosaic virus (Fig. 41-1). Many important structural features of this plant virus have been detected by x-ray diffraction studies. Figure 41-2 shows Sendai virus, an enveloped virus with helical nucleocapsid symmetry, a member of the paramyxovirus family.

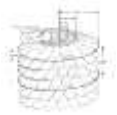

Figure 41-1

The helical structure of the rigid tobacco mosaic virus rod. About 5 percent of the length of the virion is depicted. Individual 17,400-Da protein subunits (protomers) assemble in a helix with an axial repeat of 6.9 nm (49 subunits per three turns). About 5 percent of the length of the virion is depicted. Individual 17,400-Da protein subunits (protomers) assemble in a helix with an axial repeat of 6.9 nm (49 subunits per three turns). Each turn contains a nonintegral number of subunits (16-1/3), producing a pitch of 2.3 nm. The RNA ($2\times1O^6$ Da) is sandwiched internally between adjacent turns of capsid protein, forming a RNA helix of the same pitch, 8 nm in diameter, that extends the length of virus, with three nucleotide bases in contact with each subunit. Some 2,130 protomers per virion cover and protect the RNA. The complete virus is 300 nm long and 18 nm in diameter with a hollow cylindrical core 4 nm in diameter.

Figure 41-2

Fragments of flexible helical nucleocapsids (NC) of Sendai virus, a paramyxovirus, are seen either within the protective envelope (E) or free, after rupture of the envelope. The intact nucleocapsid is about 1,000 nm long and 17 nm in diameter; its pitch

Icosahedral Symmetry

An icosahedron is a polyhedron having 20 equilateral triangular faces and 12 vertices (Fig. 41-3). Lines through opposite vertices define axes of fivefold rotational symmetry: all structural features of the polyhedron repeat five times within each 360° of rotation about any of the fivefold axes. Lines through the centers of opposite triangular faces form axes of threefold rotational symmetry; twofold rotational symmetry axes are formed by lines through midpoints of opposite edges. An icosaheron (polyhedral or spherical) with fivefold, threefold, and twofold axes of rotational symmetry (Fig. 41-3) is defined as having 532 symmetry (read as 5,3,2).

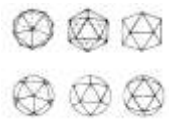

Figure 41-3

Icosahedral models seen, left to right, on fivefold, threefold, and twofold axes of rotational symmetry. These axes are perpendicular to the plane of the page and pass through the centers of each figure. Both polyhedral (upper) and spherical (lower) forms.

Viruses were first found to have 532 symmetry by x-ray diffraction studies and subsequently by electron microscopy with negative-staining techniques. In most icosahedral viruses, the protomers, i.e. the structural polypeptide chains, are arranged in oligomeric clusters called capsomeres, which are readily delineated by negative staining electron microscopy and form the closed capsid shell (Fig. 41-4 a/b). The arrangement of capsomeres into an icosahedral shell (compare Fig. 41-4 with the upper right model in Fig. 41-3) permits the classification of such viruses by capsomere number and pattern. This requires the identification of the nearest pair of vertex capsomeres (called penton: those through which the fivefold symmetry axes pass) and the distribution of capsomeres between them.

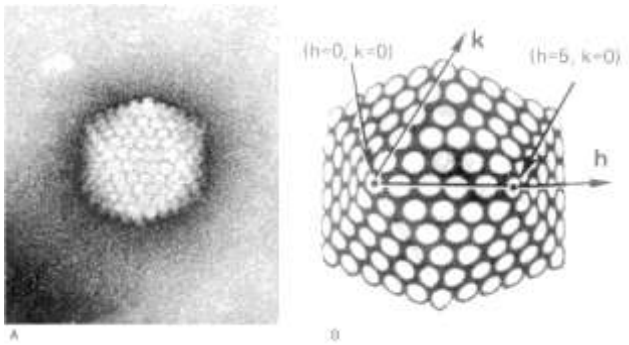

Figure 41-4

Adenovirus after negative stain electron microscopy. (A) The capsid reveals the typical isometric shell made up from 20 equilateral triangular faces. The 252 capsomeres, 12 pentons and the 240 hollow hexon capsomeres are arranged in a T = 25 symmetry

In the adenovirus model in Figure 41-4, one of the penton capsomeres is arbitrarily assigned the indices h = 0, k = 0 (origin), where h and k are the indicated axes of the inclined (60°) net of capsomeres. The net axes are formed by lines of the closest-packed neighboring capsomeres. In adenoviruses, the h and k axes also coincide with the edges of the triangular faces. Any second neighboring vertex capsomere has indices h = 5, k = 0 (or h = 0, k = 5). The capsomere number (C) can be determined to be 252 from the h and k indices and the equation: $C = 10(h^2 + hk + k^2) + 2$. This symmetry and number of capsomeres is typical of all members of the adenovirus family.

Virus Core Structure

Except in helical nucleocapsids, little is known about the packaging or organization of the viral genome within the core. Small virions are simple nucleocapsids containing 1 to 2 protein species. The larger viruses contain in a core the nucleic acid genome complexed with basic protein(s) and protected by a single- or double layered capsid (consisting of more than one species of protein) or by an envelope (Fig. 41-5).

Figure 41-5

Two-dimensional diagram of HIV-1 correlating (immuno-) electron microscopic findings with the recent nomenclature for the structural components in a 2-letter code and with the molecular weights of the virus structural (glyco-) proteins. SU stands for outer surface glycoprotein, TM for transmembrane gp, MA for membrane associated or matrix protein, LI for core-envelope-link, CA for major capsid, NC for nucleocapsid protein, respectively. PR, RT and IN represent the virus-coded enzymes protease, reverse transcriptase and integrase that are functional during the life cycle of a retrovirus

Chemical Composition and Mode of Replication

RNA Virus Genomes

RNA viruses, comprising 70% of all viruses, vary remarkably in genome structure (Fig. 41-6). Because of the error rate of the enzymes involved in RNA replication, these viruses usually show much higher mutation rates than do the DNA viruses. Mutation rates of 10^{-4} lead to the continuous generation of virus variants which show great adaptability to new hosts. The viral RNA may be single-stranded (ss) or double-stranded (ds), and the genome may occupy a single RNA segment or be distributed on two or more separate segments (segmented genomes). In addition, the RNA strand of a single-stranded genome may be either a sense strand (plus strand), which can function as messenger RNA (mRNA), or an antisense strand (minus strand), which is complementary to the sense strand and cannot function as mRNA protein translation. Sense viral RNA alone can replicate if injected into cells, since it can function as mRNA and initiate translation of virus-encoded proteins. Antisense RNA, on the other hand, has no translational function and cannot per se produce viral components.

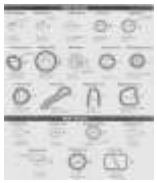

Figure 41-6

Schemes of 21 virus families infecting humans showing a number of distinctive criteria: presence of an envelope or (double-) capsid and internal nucleic acid genome. +, Sense strand; -, antisense strand; ±, dsRNA or DNA; 0, circular DNA; C, number +, Sense strand; -, antisense strand; ±, dsRNA or DNA; 0, circular DNA; C, number of capsomeres or holes, where known; nm, dimensions of capsid, or envelope when present; the hexagon designates the presence of an isometric or icosahedral outline.

DsRNA viruses, e.g., members of the reovirus family, contain 10, 11 or 12 separate genome segments coding for 3 enzymes involved in RNA replication, 3 major capsid proteins and a number of smaller structural proteins. Each segment consists of a complementary sense and antisense strand that is hydrogen bonded into a linear ds molecule. The replication of these viruses is complex; only the sense RNA strands are released from the infecting virion to initiate replication. The retrovirus genome comprises two identical, plus-sense ssRNA molecules, each monomer 7–11 kb in size, that are noncovalently linked over a short terminal region. Retroviruses contain 2 envelope proteins encoded by the env-gene, 4–6 nonglycosylated core proteins and 3 non-structural functional proteins (reverse transcriptase, integrase, protease: RT, IN, PR) specified by the gag-gene (Fig. 41-5). The RT transcribes the viral ssRNA into double-stranded, circular proviral DNA. This DNA, mediated by the viral integrase, becomes covalently bonded into the DNA of the host cell to make possible the subsequent transcription of the sense strands that eventually give rise to retrovirus progeny. After assembly and budding, retroviruses show structural and functional maturation. In immature virions the structural proteins of the core are present as a large precursor protein shell. After proteolytic processing by the viral protease the proteins of the mature virion are rearranged and form the dense isometric or cone-shaped core typical of the mature virion, and the particle becomes infectious.

DNA Virus Genomes

Most DNA viruses (Fig. 41-6) contain a single genome of linear dsDNA. The papovaviruses, comprising the polyoma- and papillomaviruses, however, have circular DNA genomes, about 5.1 and 7.8 kb pairs in size. DsDNA serves as a template both for mRNA and for self-transcription. Three or 2 structural proteins make up the papovavirus capsid: in addition, 5-6 nonstructural proteins are encoded that are functional in virus transcription, DNA replication and cell transformation.

Single-stranded linear DNA, 4–6 kb in size, is found with the members of the Parvovirus family that comprises the parvo-, the erythro- and the dependoviruses. The virion contains 2–4 structural protein species which are differently derived from the same gene product. The adeno-associated virus (AAV, a dependovirus) is incapable of producing progeny virions except in the presence of helper viruses (adenovirus or herpesvirus). It is therefore said to be replication defective. Circular single-stranded DNA of only 1.7 to 2.3 kb is found in members of the Circovirus family which comprise the smallest autonomously propagated viruses. The isometric capsid measures 17 nm and is composed of 2 protein species only.

Virus Classification

On the basis of shared properties viruses are grouped at different hierarchical levels of order, family, subfamily, genus and species. More than 30,000 different virus isolates are known today and grouped in more than 3,600 species, in 164 genera and 71 families. Viral morphology provides the basis for grouping viruses into families. A virus family may consist of members that replicate only in vertebrates, only in invertebrates, only in plants, or only in bacteria. Certain families contain viruses that replicate in more than one of these hosts. This section concerns only the 21 families and genera of medical importance.

Besides physical properties, several factors pertaining to the mode of replication play a role in classification: the configuration of the nucleic acid (ss or ds, linear or circular), whether the genome consists of one molecule of nucleic acid or is segmented, and whether the strand of ss RNA is sense or antisense. Also considered in

classification is the site of viral capsid assembly and, in enveloped viruses, the site of nucleocapsid envelopment. Table 41-1 lists the major chemical and morphologic properties of the families of viruses that cause disease in humans. The use of Latinized names ending in -viridae for virus families and ending in -virus for viral genera has gained wide acceptance. The names of subfamilies end in -virinae. Vernacular names continue to be used to describe the viruses within a genus. In this text, Latinized endings for families and subfamilies usually are not used. Table 41-2 shows the current classification of medically significant viruses.

Current Classification of Major Groups Of viruses of Medical Significance.

In the early days of virology, viruses were named according to common pathogenic properties, e.g. organ tropism and/or modes of transmission, and often also after their discoverers. From the early 1950s until the mid-1960s, when many new viruses were being discovered, it was popular to compose virus names by using sigla (abbreviations derived from a few or initial letters). Thus the name Picornaviridae is derived from pico (small) and RNA; the name Reoviridae is derived from respiratory, enteric, and orphan viruses because the agents were found in both respiratory and enteric specimens and were not related to other classified viruses; Papovaviridae is from papilloma, polyoma, and vacuolating agent (simian virus 40 [SV40]); retrovirus is from reverse transcriptase; Hepadnaviridae is from the replication of the virus in hepatocytes and their DNA genomes, as seen in hepatitis B virus. Hepatitis A virus is classified now in the family Picornaviridae, genus Hepatovirus. Although the current rules for nomenclature do not prohibit the introduction of new sigla, they require that the siglum be meaningful to workers in the field and be recognized by international study groups. The names of the other families that contain viruses pathogenic for humans are derived as follows: Adenoviridae (adeno, "gland"; refers to the adenoid tissue from which the viruses were first isolated); Astroviridae (astron means star); Arenaviridae (arena "sand") describes the sandy appearance of the virion. Bunyaviridae (from Bunyamwera, the place in Africa where the type strain was isolated); Calicivirus (calix, "cup" or "goblet" from the cup-shaped depressions on the viral surfaces); Coronaviridae (corona, "crown") describes the appearance of the peplomers protruding from the viral surface; Filoviridae (from the Latin filum, "thread" or "filament") describes the morphology of these viruses. Herpesviridae (herpes, "creeping") describes the nature of the lesions; Orthomyxoviridae (ortho, "true," plus myxo "mucus," a substance for which the viruses have an affinity; Paramyxoviridae derived from para, "closely resembling" and myxo; Parvoviridae (parvus means, "small"); Poxviridae (pock means, "pustule"); Rhabdoviridae (rhabdo, "rod" describes the shape of the viruses and Togaviridae (toga, "cloak") refers to the tight viral envelope.

Several viruses of medical importance still remain unclassified. Some are difficult or impossible to propagate in standard laboratory host systems and thus cannot be obtained in sufficient quantity to permit more precise characterization. Hepatitis E virus, the Norwalk virus and similar agents that cause nonbacterial gastroenteritis in humans are now assigned to the calicivirus family. The fatal transmissible dementias in humans and other animals (scrapie in sheep and goat; bovine spongiform encephalopathy in cattle, transmissible mink encephalopathy; Kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome in humans are caused by the accumulation of non-soluble amyloid fibrils in the central nervous systems. The agents causing transmissible subacute spongiform encephalopathies have been linked to viroids or virions (i.e. plant pathogens consisting of naked, but very stable circular RNA molecules of about 3-400 bases in size, or infectious genomes enwrapped into a host cell coat) because of their resistance to chemical and

physical agents. According to an alternative theory, the term “prion” has been coined to point to an essential nonviral infectious cause for these fatal encephalopathies—prion standing for self-replicating proteinaceous agent devoid of demonstrable nucleic acid. Some of the transmissible amyloidoses show a familiar pattern and can be explained by defined mutations which render a primary soluble glycoprotein insoluble, which in turn leads to the pathogenomonic accumulation of amyloid fibers and plaques. The pathogenesis of the sporadic amyloidoses, however, is still a matter of highly ambitious research.

15MBU502
III BSC MICROBIOLOGY
VIROLOGY

Unit IVQ	Opt 1	Opt 2	Opt 3	Opt 4	Opt 5	Opt 6	Answer
The natur	heat kille	live attenu	small dos	formalin-inactivated viruses			formalin-inactivated
The agent	fungus	protozoan	prion	virus			prion
Coxsacki	acute hem	muscular	myocardit	gastroenteritis			acute hemorrhagic c
The influ	mutations	it is a poly	the vaccin	none of the above			mutations in the vira
Which of	Poliomye	Yellow fe	Measles	Small pox			Poliomyelitis
What can	Patient se	Anti-polio	Polio caps	Colored substrate			Anti-polio antibody
Influenza	Somatic r	Expressio	Changing	Reassortment of RNA segment			Reassortment of RN
The nucle	envelope	covering	membron	capsid			envelope
Which typ	α	β	Both (a) a	Nothing will be produced			α
Fluoresce	Subacute	Herpes sir	Rabies	AIDS			Subacute sclerosing p
Viral mat	exposed c	found ma	anchor the	part of the nucleoprotein core o			found mainly on nak
Viruses ra	1-100 nm	25-300 nr	10-100 µr	400-1000 nm			25-300 nm
The speci	dog	cat	fowl	cow			dog
Which of	Salivary s	Corneal s	Conjuncti	blood smears			Salivary smears
Hepatocel	Hepatitis	Hepatitis	HSV	HIV			Hepatitis A virus
The defec	hepatitis I	hepatitis C	hepatitis I	hepatitis A			hepatitis D
All of the	Hepatitis	Hepatitis	Hepatitis	Hepatitis D virus			Hepatitis D virus
The diagn	aminotran	detection	both (a) a	detection of IgM anti-HAV by			detection of IgM anti
Which of	It can be g	It is one o	It may cau	nothing change			It can be grown in ce
The virus	Hepatitis	Hepatitis	Hepatitis	Hepatitis E virus			Hepatitis B virus
Which of	HBV	HCV	HDV	HEV			HBV
Which of	HbsAg	HBcAg	HBeAg	HBxAg			HbsAg
The devel	ground sq	hepatitis I	woodchuc	HIV			hepatitis B virus
Which of	Adw	Adr	Ayw	Ayr			Adw
Which of	Rotavirus	Norwalk	Astroviru	Rhinovirus			Rotaviruses
The virus	Coronavi	Astroviru	Norwalk a	Rhinovirus			Coronavirus
Which of	Preparing	Sterilizing	Inoculatin	Incubating			Preparing a culture n
Which of	The posse	A limited	The abilit	The ability of the Rotavirus to l			A limited number of
A structu	The envel	DNA	Capsid	Tail fibers			Capsid
A chemic	Protein	Lipid	DNA	RNA			Protein
A commo	Pentagon	Cube	Icosahedr	Pyramid			Icosahedron
Enterovir	Type of n	Size	Capsid sh	Ability to survive acidic condit			Type of nucleic acid
Viruses th	Togavirus	Herpesvir	Enterovir	Rhinoviruses			Togaviruses
What type	Bacteriop	Animal V	Plant Viru	Fungal Viruses			Bacteriophage
Bacteriop	Immunoa	ELISA	Plaque as	Tissue cell culture			Plaque assays
A type of	Primary c	Continuou	Cell strain	Diploid fibroblast cell			Continuous cell line
Which of	Retroviru	Enterovir	Rhabadov	Adenovirus			Rhabadovirus
What is th	Enterovir	Herpesvir	Arbovirus	Retroviruses			Arboviruses
Protection	Viral end	binding o	Synthesis	Uncoating of nucleic acid			Viral endonuclease a

Which on	Diphtheri	Haemoph	Hepatitis	HIV Vaccine			Diphtheria-pertusis-t
Which on	Blood tra	Fetal cont	Ingestion	Transmission of the virus from			Fetal contact with in
The findi	Bordetella	Respirato	Mycoplas	Epstein-Barr virus			Respiratory syncytial
All of the	Coxsackie	Rhinoviru	Echo viru	Coxsackievirus B			Coxsackievirus A
A divorce	Treatmen	All the pa	The publi	A western blot (immunoblot) te			Treatment with zido
In a chron	Hepatitis	Hepatitis	Hepatitis	Anti-HBsAg			Hepatitis B Surface A
A retrovir	gag	pol	env	onc			gag
When a vi	lysogeny	fermentat	symbiosis	synergism			lysogeny
Usually v	nature of	nucleic ac	capsid sy	diameter of the viroin or nucleo			capsid symmetry
In cancer,	neoplasia	anaplasia	metastasis	oncogenic			neoplasia
Which of	Epstein-B	Human T	Human pa	Human herpes virus 8			Epstein-Barr virus
Virulent a	inhibit ho	inhibit ho	stimulate	degrade host cell DNA			stimulate host cell m
The disea	Vaccinia	Smallpox	Cowpox	Chicken pox			Smallpox
The virus	Poliovirus	Coxsackie	Echo viru	HIV			Coxsackieviruses
Which of	Orthopox	Parapoxv	Molluscip	Mesopox			Orthopoxvirus
Which of	Group A	Group C	Group D	Group E			Group A
Which of	Enterovir	Rhinoviru	Hepatovir	HIV			Enterovirus
Transmiss	direct con	sexual co	Indirect	Mechanical			sexual contact
The symn	complex	icosahedr	helical	Cylinderical			complex
Which of	Hepatitis	Hepatitis	Hepatitis E virus				Hepatitis E virus
What is th	Spherical	Polygona	Bullet-sha	Tubular			Bullet-shaped

viruses

conjunctivitis
l hemagglutinin may allow the virus to evade the immune response elicited by previous vaccines

lA segments from different influenza viruses

panencephalitis
ked viruses

i-HAV by ELISA
ell cultures of primate and human cells

nedium for the growth of microorganisms
capsule types

ıctivity

:etanus (DPT) vaccine
fected blood during childbirth
l virus (RSV)

vudine (azidothymidine, AZT)
Antigen (HbsAg)

acromolecule synthesis

2017

III B. Sc Microbiology –Virology
Unit V

LECTURE PLAN

LECTURE PLAN - UNIT -V			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	Tmv	T1 135-143
2	1	Cmv	T1 144- 149
3	1	Gemini virus	T1 154-160
4	1	HIV	T3 531-561
5	1	Rabies virus	T3 474-482
6	1	Pox virus	T3 346-357
7	1	Herpes virus	T3 317-345
8	1	Influenza virus	T1 277-283
9	1	Hepatitis virus	R1 491-494
10	1	Rhino, adeno and corona virus	T3 391-398
11	1	Revision of Unit V	
12	1	Unit V test	
13	1	Discussion of Old Qps	
Textbooks :		T1-virology-P.saravanan-Mjp publishers T2 -Textbook of Microbiology- Paniker- Orient longman publishers T3 -medical virology -White & fenner, Academic press publishers T4-Modern virology -dimmock-Black well publishing	
Reference books:		R1- Medical Microbiology –Jawertz – McGraw hill Publishers	
Website:		W1 - www.virology.com	
Journals:		J1 - Viruses	

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GENERAL PROPERTIES OF VIRUSES

INTRODUCTION

Viruses occupy the twilight zone that separates the 'living' from the 'non-living'. They do not have a cellular organization and contain only one type of nucleic acid, either DNA or RNA but never both. The medical importance of viruses lies in their ability to cause a very large number of human diseases. Viral diseases range from minor ailments like common cold to terrifying diseases like rabies and AIDS.

In this chapter, we shall be discussing the morphology and general properties of viruses.

After reading this lesson you will be able to:

- explain the concept of viruses, in relation to other microorganisms
- describe the morphological features of viruses
- explain the multiplication of viruses (replication)
- describe the methods of cultivation of viruses
- explain the classification and naming (nomenclature) of viruses

Concept of Viruses in relation to other Organisms

Viruses occupy the twilight zone that separates the 'living' from the 'non-living'. They do not have a cellular organization and contain only one type of nucleic acid, either DNA or RNA but never both. Viruses are obligate intracellular parasites. They lack the enzymes necessary for protein and nucleic acid synthesis. They are dependent for replication on the synthetic machinery of host cells. They multiply by a complex process and not by binary fission. They are unaffected by antibacterial antibiotics. Viruses cause a wide range of human diseases. They cause infections like common cold, chicken pox, measles, viral encephalitis, rabies and AIDS.

Properties Bacteria Viruses

Cellular organization Present Absent Growth on inanimate media Yes No Binary fission Yes No DNA and RNA Both are present Either DNA or RNA Ribosomes Present Absent Sensitivity to antibacterial Yes No antibiotics

Morphology of Viruses

Size: The extracellular infectious virus particle is called virion. Viruses are much smaller than bacteria. They are too small to be seen under the light microscope. Some large viruses like the poxviruses can be seen under the light microscope when suitably stained. The viruses range in size from 20 nm to 300 nm. Poxviruses are one of the largest viruses and parvoviruses are one of the smallest viruses. The earliest method of estimating the size of virus particles was by passing them through collodion membrane filters of graded porosity. The average pore diameter of the finest filter that permitted passage of the virion gave an estimate of its size. With the development of the ultracentrifuge, a second method became available. From the rate of sedimentation of the virus in the ultracentrifuge, the particle size could be calculated using Stoke's law. The third and the most direct method of measuring virus size is electron microscopy. By this method, both the shape and size of virions can be studied.

Structure, shape and symmetry: The virion consists essentially of a nucleic acid surrounded by a protein coat, the **capsid**. The capsid with the enclosed nucleic acid is called the **nucleocapsid**. The capsid protects the nucleic acid from harmful agents in the environment. It is composed of a large number of capsomers which form its morphological units. The chemical units of the Capsid are polypeptide molecules which are arranged symmetrically. They form a shell around the nucleic acid. The capsid shows two kinds of symmetry – icosahedral (cubical) and helical. An icosahedron is a polygon with 12 vertices and 20 facets or sides. Each

facet is in the shape of an equilateral triangle. Two types of capsomers are present in the icosahedral capsid. They are the pentagonal capsomers at the vertices (pentons) and the hexagonal capsomers making up the facets (hexons). There are always 12 pentons but the number of hexons varies with the virus group. Examples of viruses with icosahedral symmetry of the capsid are Adenovirus and Herpes Simplex Virus. In the nucleocapsids with helical symmetry, the capsomers and nucleic acid are wound together to form a helical or spiral tube, for example tobacco mosaic virus. All viruses do not show the typical icosahedral or helical symmetry. Some, like the poxviruses, show a complex symmetry. Virions may be enveloped or nonenveloped. The envelope of viruses is derived from the host cell membrane. This occurs when the virus is released from the host cell by budding. Protein subunits may be present as projecting spikes on the surface of the envelope. They are called **peplomers**. The influenza virus carries two kinds of peplomers: haemagglutinin and neuraminidase. Haemagglutinin is a triangular spike and neuraminidase is mushroom-shaped. Envelope is sensitive to the action of lipid solvents. Envelopes confer chemical, antigenic and biological properties on viruses.
The overall shape of the virus particle varies in different groups of viruses. Most animal viruses are roughly spherical. The rabies virus is bullet shaped. Poxviruses are brick-shaped.

Chemical properties: Viruses contain only one type of nucleic acid, either DNA or RNA. Viruses are unique because they carry genetic information on RNA. This property is not seen in any other organism in nature. Viruses also contain protein which makes up the capsid. Enveloped viruses contain lipids derived from the host cell membrane. Most viruses do not have enzymes for the synthesis of viral components or for energy production. Some viruses have enzymes, for example the influenza virus has neuraminidase.

Resistance: Viruses are destroyed by heat except a few. They are stable at low temperatures. For long term storage, they are kept at -70°C. A better method for prolonged storage is lyophilisation or freeze-drying. Viruses are inactivated by sunlight, UV rays and ionising radiation. They are, in general, more resistant than bacteria to chemical disinfectants. Phenolic disinfectants have a weak action on viruses.

Multiplication of Viruses

Multiplication of viruses is called viral **replication**. Viruses contain the genetic information for their replication but they lack the enzymes. They depend on host cell machinery for replication. The viral replication cycle can be divided into six phases – adsorption, penetration, uncoating, biosynthesis, maturation and release.

Adsorption: In this phase, the virus gets attached to the host cell. The host cell should have specific receptors on its surface. These receptors recognize viral surface components. This cell-virus interaction helps the virus to attach to the host cell surface.

Penetration: In this phase, the virus enters into the host cell. Bacteria have rigid cell wall. So, viruses which infect bacteria cannot penetrate into the bacterial cell. Only the nucleic acid of the virus enters the bacterial cell. Animal and human cells do not have cell walls. Therefore, whole virus enters the cell. Virus particle may be engulfed by a process called **viropexis**. In case of enveloped viruses, the viral envelope may fuse with the cell membrane of the host cell. Then the nucleocapsid is released into the cytoplasm.

Uncoating: This is the process in which the outer layers and capsid of the virus are removed. This mostly occurs by the action of lysosomal enzymes of the host cell. This can also occur by a viral uncoating enzyme. Finally, the viral nucleic acid is released into the cell.

Biosynthesis: In this phase, the viral nucleic acid and capsid are synthesised. The enzymes necessary in the various stages of viral synthesis, assembly and release are also synthesised. Certain 'regulator proteins' are synthesised. They shut down the normal metabolism of the host cell. They direct the production of viral components. In general, most DNA viruses synthesise their nucleic acid in the host cell nucleus. Exceptions are

the poxviruses. They are DNA viruses, but they synthesise all their components in the host cell cytoplasm. Most RNA
viruses synthesise all their components in the cytoplasm. Orthomyxoviruses and some paramyxoviruses are exceptions. They synthesise some components in the host cell nucleus. Biosynthesis consists essentially of the following steps:

1. Transcription of messenger RNA (mRNA) from the viral nucleic acid
2. Translation of mRNA into "early proteins" or "non-structural proteins".
They are enzymes responsible for the synthesis of viral components.
3. Replication of viral nucleic acid
4. Synthesis of "late proteins" or "structural proteins". They are the components
of daughter virion capsids.

Maturation: This is the assembly of daughter virions following the synthesis of viral nucleic acid and proteins. It can take place in the host cell nucleus or cytoplasm. Herpesviruses and adenoviruses are assembled in the nucleus.

Picornaviruses and poxviruses are assembled in the nucleus.

Release: Viruses which infect bacteria (bacteriophages) are released by lysis of the infected bacterium. Animal viruses are usually released without cell lysis. Myxoviruses are released by budding from the cell membrane. The host cell is unaffected. Daughter virions are released into the surrounding medium and may infect other cells. In some viruses (for eg. varicella), transmission occurs directly from cell to cell. In this case, there is very little free virus in the medium. The poliovirus causes cell damage and may be released by cell lysis. From the stage of penetration till the appearance of mature daughter virions, the virus cannot be demonstrated inside the host cell. During this period, the virus seems to disappear. This is called the "eclipse phase". The time taken for a single cycle of replication is about 15-30 minutes for bacteriophages. It is about 15- 30 hours for animal viruses. A single infected cell may release a large number of progeny virions.

PLANT DISEASES CAUSED BY VIRUSES

- Plant viruses consist of a nucleoprotein that multiplies only in the living cells of a host. The presence of viruses in host cells often results in disease.
- 400 or more viruses are known to attack plants (2000 viruses are described for plants, animals, bacteria, etc.). viruses are generally specific, what infects a plant does not cause disease in an animal, and vice versa.
- The first record of a disease that was later found to be caused by a plant virus was on tulips in the 17th century in the Netherlands.
- First experimental demonstration of the infectious nature of viral disease was recorded by Lawrence, who described the transmission of a disease of jasmine by grafting.
- Adolf Mayer (1886) described a disease of tobacco called mosaikkranheit (tobacco mosaic). Disease could be transmitted to healthy plants with sap from diseased plants.
- Dmitrii Iwanowski (1892) demonstrated that the agent in tobacco mosaic was filterable. He demonstrated that the causal agent of tobacco mosaic could pass through a filter that retains bacteria.
- 1898 Martinus Beijerinck - demonstrated that the causal agent was not a microorganism but a *contagium vivum fluidum* (contagious living fluid). He was the first to use the term *virus*, which is the Latin word for poison. He concluded that this was not a toxin, because repeated inoculations of diluted infected sap yielded similar amounts of disease as it was passed from one plant to another. If it had been a toxin, it would eventually be diluted away.
- Loefler and Frosch (1898) described the first filterable infectious agent in animals - the foot-and-mouth disease virus and Walter Reed (1900) - described the first human virus, yellow fever virus.
- In 1929, F. O. Holmes provided a tool by which the virus could be measured by showing that the amount of virus present in a plant sample preparation is proportional to the number of local lesions produced on appropriate host plant leaves rubbed with the contaminated sap.

- 1935 W. M. Stanley isolated and purified some tiny white crystals from leaves of mosaic-infected tobacco plants. He treated healthy plants with TMV, which had been precipitated out of infected tobacco juice with the help of ammonium sulfate and a technique he had developed. The healthy plants contracted tobacco mosaic disease. Due to the high protein content of the purified virus particles, he concluded that the virus was an autocatalytic protein that could multiply within living cells. Although his conclusions were later proved incorrect, Stanley's work merited him receiving the Nobel Prize. He won the Nobel Prize in chemistry in 1946 for this work.
- 1937 - Bawden and Pirie demonstrated that virus consists of protein and nucleic acid (RNA).
- 1939 - Kausche - saw virus particles for the first time with the electron microscope.
- 1955-1960's Much was learned by various workers, regarding the infectivity of viral (TMV) RNA and the structure and arrangement of viral (TMV) coat protein.
- 1971 - T. O. Diener discovered viroids, which only consist of nucleic acids. Smaller than viruses, caused potato spindle tuber disease (250-400 bases long of single-stranded circular molecule of infectious RNA). About a dozen other viroids that cause disease in a variety of plants have been isolated. No viroids have ever been found in animals.
- 1980- Cauliflower mosaic virus, whose genome is a circular double-stranded DNA chromosome, was the first plant virus for which the exact sequence of all its 8,000 base pairs was determined. In 1982, the complete sequence of the bases in the single-stranded tobacco mosaic virus RNA was determined, as were those of smaller viral RNA and of viroids.
- 1986 - Use of transgenic plants to obtain resistance against viruses (TMV).

VIRUS DISEASES OF PLANTS ARE USUALLY DESCRIPTIVE OF THE TYPE OF SYMPTOMS THAT THESE CAUSE IN THE HOST

- For example, the symptoms of specific plant diseases form the basis for the following disease names: tobacco mosaic, turnip crinkle, barley yellow dwarf, ring spot of watermelon, cucumber mosaic, spotted wilt of tomato.
- Some viruses have a broader host range than the name of disease or virus may imply. For example, tobacco mosaic virus (TMV) infects tomato, eggplant, peppers, in addition to tobacco.

PROPERTIES AND MORPHOLOGY OF PLANT VIRUSES

- noncellular, ultramicroscopic particles, that multiply only in living cells. very, very small! (size measured in nanometers).
- most plant viruses consist of protein shells surrounded by a core of positive-stranded nucleic acid (normally ssRNA - nucleotides (guanine, uracil, cytosine, adenine) + 5 carbon sugar called ribose + a phosphate group), but sometimes these viruses contain dsRNA or dsDNA (2 strands of nucleotides with thymine substituted for uracil and deoxyribose instead of ribose).
- 5-40% of virus is nucleic acid 60-95% is protein
- Protein coats or shells can be different shapes, but are normally rod, filamentous, isometric, quasi-isometric/bacilliform or variants of these structures. For example, Tobacco Mosaic and Barley Stripe Mosaic viruses are rods, while broad bean wilt and maize chlorotic dwarf viruses are isometric or more spherical in shape.

VIRUS GENOME

Minimum number of genes in a plant RNA virus could be two: a coat protein and an RNA replicase gene (as is the case with RNA phages). Evidence indicates there are usually 3-5 gene products.

Plant positive-stranded RNA viruses frequently possess divided genomes. In addition, viral genomes are separately encapsulated. Viral genomes consisting of two or three different nucleic acid components, all required for infection are called bipartite, tripartite, or multipartite viruses. More than a single species of genomic RNA.

Multipartite viruses are potentially at an evolutionary disadvantage. Infectivity dilution curve for Alfalfa mosaic virus (requiring B, M, Tb particles for infectivity) is steeper than for tobacco necrosis virus (single particle). Partition of genome could potentially hinder transmission or infection by a virus.

SATELLITE VIRUSES AND RNAs

Kasinis in 1962, described the first satellite viruses. These viruses are serologically unrelated to their helpers and the two genomes exhibit little if any sequence similarity. Satellite viruses are dependent for its replication on the presence of a second, independently replicating virus.
Satellite RNAs have no coat protein of their own and are encapsulated with the help of other viral RNAs.
TRANSMISSION

- Mechanical transmission through sap by plants touching one another, through root grafts, and manhandling.
- Vegetative propagation and grafting.
- Seed, pollen, mites, nematodes, dodder, fungi (carried by zoospores and mycelium) and insects (aphids, leafhoppers, scale insects, thrips, grasshoppers, beetles, whiteflies). For example, cucumber mosaic virus and barley yellow dwarf virus moved by aphids.

DETECTION OF PLANT VIRUSES
Due to the inability to observe plant viruses visually by observing them directly through the light microscope, virologists must resort to the following methods of detecting their presence and in diagnoses.
1. Ability to transmit disease via plant sap by rubbing plant, grafting, dodder or insect transmission.
2. Indexing - indicator plants - sensitive to specific virus and will react a certain way if exposed..
3. Visual inspection with EM.
4. By eliminating possibility that symptoms are not due to other sources (e.g., herbicide, nutritional deficiencies.
5. Serological Tests (ELISA - enzyme-linked immuno sorbent assay).
   Indirect (virus + Ab virus + Enzyme conjugated Ab) and direct (double-antibody sandwich technique) (Ab virus + virus + Enzyme-conjugated Ab).

   1. Virus or Ab virus added to well and these become attached to walls.
   2. Antibody or virus added to well and these attach to their counterpart (i.e., antigen to antibody).
   3. Second antibody with enzyme conjugate attaches to first antibody/virus complex.
   4. Substrate is catalyzed by enzyme and this causes a color change.

ELISA tests are extremely sensitive (small amounts of antisera are needed) results are quantitative, large samples can be run at same time (96 well plates), results can be gathered in a few hours instead of days. ELISAs along with serial dilutions of plant sap and applications of this to the leaves of susceptible hosts (by counting the number of lesions) can be used to quantify the amount of virus present.
MANAGEMENT

- Milk inactivates many viruses - use milk to wash tools/hands. "Milk does a plant body good!" Soap and water work well too!
- Removing diseased plants, killing and removing potential virus vectors (primarily weeds and insects).
- disease-resistant cultivars.
- disease or virus free seed, roots or tubers.
- cross protection (inoculation with a less-virulent strain of a virus protects the plant from a more virulent strain later when exposed to it).
- heat (some viruses are killed at temperatures that will not kill host). For example, dormant propagative organs dipped in hot water (35 C) for few minutes or hours, or by growing plants in greenhouse at 35-40 C for several days, weeks or months may inactivate virus.

TOBACCO MOSAIC

- Caused by Tobacco Mosaic Virus (TMV) worldwide distribution primarily infects tobacco and tomato, but more than 350 species are susceptible.
- tobacco leaves become mottled with light and dark green areas; leave become distorted, puckering or blistering, especially areas of new growth.
- stunting of plant growth. in tomato, mottling of leaves occurs and leaflets become long and pointed.
- TMV is a rod-shaped particle which are 300 nm long by 15-18 nm in diameter. It possesses ssRNA and a protein coat.
- difficult to inactivate, and can survive for 5 years in dead, dried tissues and many months in living plant tissues.

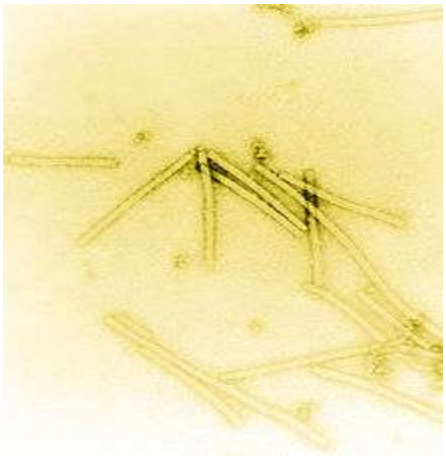
- many strains, that vary in virulence from severe to mild symptoms. virus is spread from plant to plant through injuries caused by crop worker, contaminated equipment and chewing insects.
- virus overwinters in dead plant tissues and debris, on contaminated equipment, in contaminated soil, greenhouse containers, bedding, tools, and in living hosts, including weeds like horsenettle, *Solanum carolinense*, and other crop plants (tomato, pepper, and eggplant).

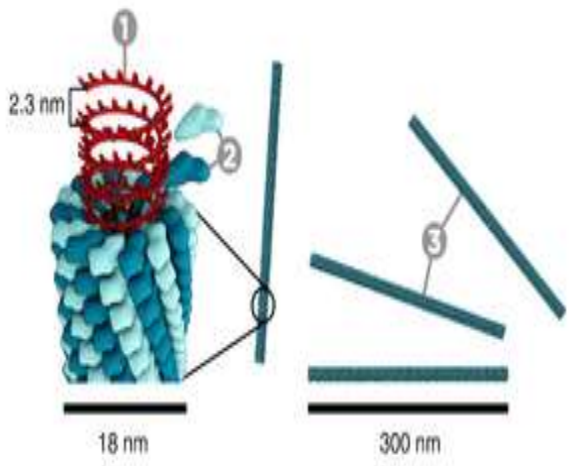
Management of Tobacco Mosaic Disease

- use virus-free seed (tomato seed can by treated with acid or bleach)
- transplant in noninfested soil
- fumigatation with methyl bromide or heated.
- no chewing of tobacco or smoking around seedbeds or in greenhouses.
- to eliminate spreading of virus wash hand with soap and water or milk.
- spraying plants with milk (whole or skim) seems to help reduce
- infections. crop rotation with nonhost crops (corn, rice, other cereal grains).
- resistant cultivars

Tobacco Mosaic Virus: The Prototype Plant Virus

The stability of the TMV virus particle accounts for its having been the first virus to be identified, purified to homogeneity, and then biochemically and biologically characterized.





Small coat protein subunits (capsomeres) aggregate to form a helical protein coat or). The virus particle contains an axial channel that is 4 nm wide and the viral RNA lies within a groove in the surrounding protein helix. The nucleic acid core is not in the axial channel, but passes about halfway between the interior channel and the exterior surface of the rod. The overall particle is rod-shaped, narrow, and rigid. The pitch of the helix is 2.3 nm, and each turn contains 16 1/3 coat protein molecules. A full-length virion contains 130 helical turns. TMV particle is resistant to nucleases and proteolytic enzymes. TMV particles will fall apart in both alkaline and acid solutions. Denaturation is often reversible, as long as temperature and pH are not too extreme. Removal of the denaturant allows the native structure of the viral protein to re-form and near its isoelectric point (pH 4 to 6), the TMV coat protein aggregates to form rod-shaped particles that look exactly like TMV virions. When virus is subjected to neutral pH with either detergents (e.g., SDS) or 6 M urea or by extraction with phenol then RNA can be extracted in an intact form. When isolated TMV RNA are added to native TMV protein, these form stable "reconstituted" virus, which is more stable (stable from pH 3 to 9) then protein alone (unstable below pH of 4 and above pH 6).

Protein and RNA are more infectious than naked RNA alone (nearly 1000 times the amount of naked RNA is required to cause infection).

Proof that the viral RNA was the sole determinant of tobacco mosaic disease was obtained by a mixed reconstitution of RNA from Holmes ribgrass mosaic virus (RMV) with the protein subunits from TMV. Reconstituted virus caused localized lesions on plants instead of a systemic infection and formed new RMV virus (RMV RNA + protein coat containing histidine and methionine - not found in TMV). Refer to Figure 2.13 on page 50 in handout #2

Assembly of Helical Viruses

Aggregates of 33 protein molecules form the double disk. This combines with viral RNA. Attachment of the nucleic acid to the protein aggregate begins at the origin of assembly site (OAS) about 800 nucleotides from the 3' terminus of TMV common strain RNA. Rod growth toward the 5' terminus of the viral RNA is rapid, involving addition of double disks; encapsidation of the 3' terminus proceeds more slowly, through the addition of A protein monomers or small aggregates. Refer to Figure 6.6 in the textbook or to Figure 2.14 on page 50 in handout #2). Cotranslation disassembly - the protein coat is displaced at the 5' end by ribosomes in host cell.

TMV RNA 3' TERMINUS

3' end of TMV RNA ends with the sequence -C-C-C-A and can be charged with an amino acid (histidine). This region is non-coding and be folded into a tRNA-like structure preceded by a series of four pseudoknots. Why? Four possibilities exist.

1. Donating an amino acid during some stage of protein synthesis.
2. Facilitating translation by disrupting base pairing between the 3' and 5' - terminal regions of the viral RNA
3. Acting as a recognition site for the viral replicase to initiate negative-strand synthesis
4. A molecular fossil from the original RNA world where tRNA-like structures tagged RNAs for replication and prevented the uncontrolled loss of nucleotides from third 3' terminus.

Subgenomic mRNAs and translational read-through in TMV replication.

Five open reading frames or ORFs are found in the genome of TMV. Subgenomic mRNAs and translational read-through are two strategies employed by TMV to regulate gene expression.

Plant positive-sense RNA viruses have developed several other mechanisms to facilitate and/or regulate the expression of individual genes. 5 strategies of regulating gene expression.

Environment

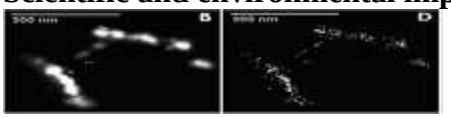
TMV is known as one of the most stable viruses. It has a very wide survival range. As long as the surrounding temperature remains below approximately 40 degrees Celsius, TMV can sustain its stable form. All it needs is a host to infect. If necessary, greenhouses and botanical gardens would provide the most favorable condition for TMV to spread out, due to the high population density of possible hosts and the constant temperature throughout the year.

Treatment and management

One of the common control methods for TMV is sanitation, which includes removing infected plants, and washing hands in between each planting. Crop rotation should also be employed to avoid infected soil/seed beds for at least two years. As for any plant disease, looking for resistant strains against TMV may also be advised. Furthermore, the cross protection method can be administered, where the stronger strain of TMV infection is inhibited by infecting the host plant with mild strain of TMV, similar to the effect of a vaccine.

In the past ten years, the application of genetic engineering on a host plant genome has been developed to allow the host plant to produce the TMV coat protein within their cells. It was hypothesized that the TMV genome will be re-coated rapidly upon entering the host cell, thus it prevents the initiation of TMV replication. Later it was found that the mechanism that protects the host from viral genome insertion is through gene silencing.[20]

Scientific and environmental impact



TMV virus: super resolution light microscopy

The large amount of literature about TMV and its choice for many pioneering investigations in structural biology (including X-ray diffraction), virus assembly and disassembly, and so on, are fundamentally due to the large quantities that can be obtained, plus the fact that it does not infect animals. After growing a few infected tobacco plants in a greenhouse and a few simple laboratory procedures, a scientist can easily produce several grams of the virus. As a result of this, TMV can be treated almost as an organic chemical, rather than an infective agent.

James D. Watson, in his memoir *The Double Helix*, cites his x-ray investigation of TMV's helical structure as an important step in deducing the nature of the DNA molecule.[21]

Investigational uses

Due to its cylindrical shape, high aspect-ratio, self-assembling nature, and ability to incorporate metal coatings (nickel and cobalt) into its shell, TMV is an ideal candidate to be incorporated into battery electrodes. Addition of TMV to a battery electrode increases the reactive surface area by an order of magnitude, resulting in an increase in the battery's capacity by up to six times compared to a planar electrode geometry.[

Cauliflower mosaic virus

Cauliflower mosaic virus (**CaMV**) is a member of the genus *Caulimovirus*, one of the six genera in the *Caulimoviridae* family, which are pararetroviruses that infect plants.[1] Pararetroviruses replicate through reverse transcription just like retroviruses, but the viral particles contain DNA instead of RNA.[2]

Definition

Aphid species *Myzus persicae*

Cauliflower mosaic virus (CaMV) is the type species of the family *Caulimoviridae*. This family is grouped together with *Hepadnaviruses* into the *Pararetrovirus* group due to its mode of replication via reverse transcription of a pre-genomic RNA intermediate.

CaMV infects mostly plants of the *Brassicaceae* family (such as cauliflower and turnip) but some CaMV strains (D4 and W260) are also able to infect *Solanaceae* species of the genera *Datura* and *Nicotiana*. CaMV induces a variety of systemic symptoms such as mosaic, necrotic lesions on leaf surfaces, stunted growth, and deformation of the overall plant structure. The symptoms exhibited vary depending on the viral strain, host ecotype, and environmental conditions.[3]

CaMV is transmitted in a non-circulatory manner by aphid species such as *Myzus persicae*.[4] Once introduced within a plant host cell, virions migrate to the nuclear envelope of the plant cell.

Structure

The CaMV particle is an icosahedron with a diameter of 52 nm built from 420 capsid protein (CP) subunits arranged with a triangulation T = 7, which surrounds a solvent-filled central cavity.[5][6]

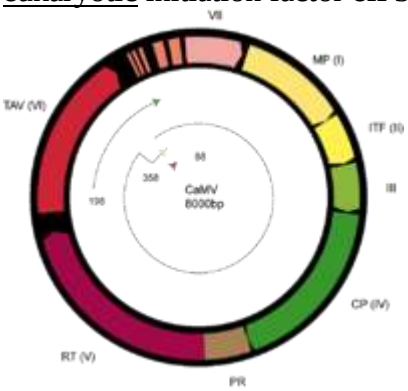
CaMV contains a circular double-stranded DNA molecule of about 8.0 kilobases, interrupted by nicks that result from the actions of RNAse H during reverse transcription. These nicks come from the Met-tRNA, and two RNA primers used in reverse transcription. After entering the host cell, these single stranded "nicks" in the viral DNA are repaired, forming a supercoiled molecule that binds to histones. This DNA is transcribed into a full length, terminally redundant[*clarification needed*], 35S RNA and a subgenomic 19S RNA.

Genome

The promoter of the 35S RNA is a very strong constitutive promoter responsible for the transcription of the whole CaMV genome. It is well known for its use in plant transformation. It causes high levels of gene expression in dicot plants. However, it is less effective in monocots, especially in cereals. The differences in behavior are probably due to differences in quality and/or quantity of regulatory factors. Interestingly, recent study has indicated that the CaMV 35S promoter is also functional in some animal cells, although the promoter elements used are different from those in plants. While this promoter had low activity compared to canonical animal promoters, levels of reporter products were significant. This observation suggests that the 35S promoter may have potential for use in animals.[7]

The promoter was named CaMV 35S promoter ("35S promoter") because the coefficient of sedimentation of the viral transcript, whose expression is naturally driven by this promoter, is 35S. It is one of the most widely used,

general-purpose constitutive promoters. It was discovered at the beginning of the 1980s, by Chua and collaborators at The Rockefeller University.
The 35S RNA is particularly complex, containing a highly structured 600 nucleotide long leader sequence with six to eight short open reading frames (ORFs).[8][9][10]
This leader is followed by seven tightly arranged, longer ORFs that encode all the viral proteins. The mechanism of expression of these proteins is unique, in that the ORF VI protein (encoded by the 19S RNA) controls translation reinitiation of major open reading frames on the polycistronic 35S RNA, a process that normally only happens on bacterial mRNAs. TAV function depends on its association with polysomes and eukaryotic initiation factor eIF3.[11]



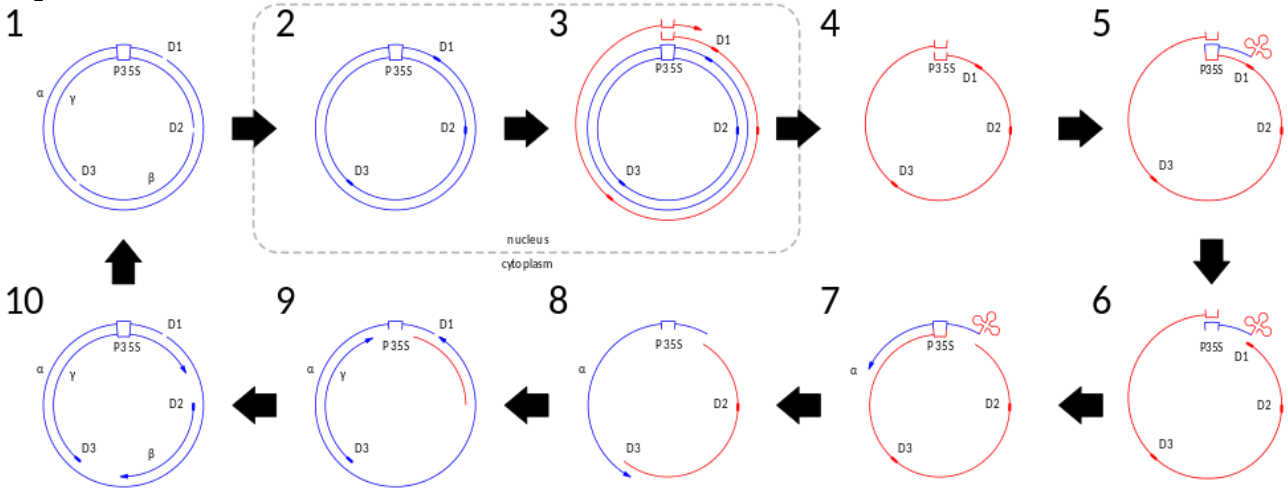
- ORF II - Insect Transmission Factor
- ORF III - Structural Protein, DNA-Binding Capabilities
- ORF IV - Capsid Protein
- ORF V - Protease, Reverse Transcriptase and RNaseH
- ORF VI - Translational Activator, Inclusion Body Formation/Trafficking; Possibly more functions (See Below)
- ORF VII - Unknown (Appears to not be required for infection)

Genomic map of CaMV

- ORF I - Movement Protein

In addition to its functions regarding translational activation and formation of inclusion bodies, P6 has been shown to interact with a number of other CaMV proteins, such as P2 and P3, suggesting that it may also contribute in some degree to viral assembly and aphid-mediated transmission. In addition, P6 has been shown to bind to P7; investigating interactions between the two may help to elucidate the as yet unknown function of P7.[12]
Another interesting function of P6 involves modification of host NONEXPRESSOROFPATHOGENESISRELATED1 (NPR1) during the course of infection. NPR1 is an important regulator of salicylic acid (SA) and jasmonic acid (JA)-dependent signaling, and is most closely associated with crosstalk between the two. Modification of NPR1 serves to inhibit plant cells' defensive responses by preventing SA-dependent signaling; modified NPR1 can properly traffic to the nucleus and bind the PR-1 promoter, but is unable to initiate transcription. Because active NPR1 is required for accumulation of SA, this leads to a further depletion of SA. Whereas regulation of SA-dependent signaling by P6-modified NPR1 is localized to the nucleus, regulation of JA-dependent signaling is cytoplasmic in nature and involves the COI1 pathway. In contrast to that of SA, JA-dependent signaling is increased in the presence of modified NPR1.[13]

Replication



CaMV replicates by reverse transcription:

1. Viral particles enter a plant cell and are unencapsidated. At this stage the viral DNA consists of three fragments, one on the – strand (α) and two on the + strand (β and γ) which are imperfectly assembled into a circular genome with three gaps or discontinuities (D1, D2, and D3).
2. The viral DNA enters the nucleus where the discontinuities are filled in. At this point the viral DNA also associates with host histones, forming a minichromosome (not shown).
3. The host DNA-dependent RNA polymerase transcribes from the 35S promoter all the way around the viral genome, surpassing the 35S promoter. (This creates two copies of the 35S promoter in the resulting RNA.) Transcription also initiates at the 19S promoter (not shown).
4. The viral RNAs pass into the host cytoplasm where they are transcribed.
5. The 3′ end of a $tRNA^{fMet}$ anneals to a site corresponding to discontinuity 1 (D1) near the 5′ end of the 35S RNA.
6. The $tRNA^{fMet}$ primes synthesis, by the viral reverse transcriptase (encoded by ORF V), of a new α strand.
7. RNase H removes the RNA from the DNA–RNA duplex, leaving behind the DNA.
8. This new DNA binds the 35S promoter at the 3′ end of the RNA template and synthesis of the α strand of DNA continues and RNase H continues to degrade RNA complexed to DNA.
9. Synthesis of the α strand completes. RNase H activity exposes purine-rich regions at the position of discontinuity 3 (D3), which primes the synthesis of the γ DNA strand.
10. RNase H activity exposes purine-rich regions at the position of discontinuity 2 (D2), which primes the synthesis of the β DNA strand. When the new γ strand of DNA reaches the 5′ end of the new α strand it switches to the 5′ end of the new α strand, recreating discontinuity 1 (D1). When the new γ strand of DNA reaches the 5′ end of the new β strand, it displaces the primer and some of the newly synthesized β strand, resulting in the recreation of discontinuity 2 (D2). When the new β strand of DNA reaches the 5′ end of the new γ strand, it displaces the primer and some of the newly synthesized γ strand, resulting in the recreation of discontinuity 3 (D3).

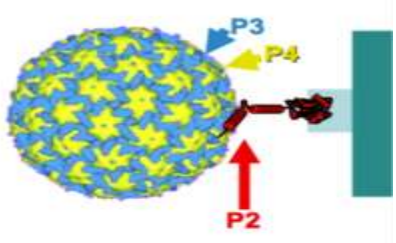
At this point the new viral genome can either be packaged into capsids and released from the cell or they can be transported by movement proteins into an adjacent, uninfected cell.[14]

The Cauliflower mosaic virus promoter (CaMV 35S) is used in most transgenic crops to activate foreign genes which have been artificially inserted into the host plant. It is inserted into transgenic plants in a form which is different from that found when it is present in its natural *Brassica* plant hosts. This enables it to operate in a wide range of host-organism environments which would otherwise not be possible.

CaMV contains about 8 kb double-strand DNA genome and produces spherical particles. CaMV infections are systemic, and even its DNA is infectious when inoculated on abraded plant surfaces. The CaMV genome has 8 tightly packed genes, of which only two small genes, genes II and VII, are nonessential; as a result, only these two genes can be replaced/deleted without a loss of infectivity. In addition, modified CaMV genomes exceeding the natural genome size (8024 bp) by even a few hundred bp are not packaged into virions. These two factors seriously limit the size of DNA insert clonable in CaMV. The bacterial dihydrofolate reductase DHFR gene has been successfully cloned into the CaMV genome, in place of gene II, and has been successfully expressed in plants.

Molecular Mechanisms of Vector-Mediated CaMV Transmission

The virus is acquired from an infected host during feeding by the aphid vector. To occur, a transmissible complex is composed of virions and protein P2 located in the vector's stylets. The P2 N-terminal domain recognizes a protein receptor located at the tip of the stylet and the P2 C-terminal domain binds to the P3-decorated virions.[15]



Transmissible complex of CaMV

The mode of acquisition by the vector is controlled by the tissue and intracellular-specific localization of P2. This protein is only found in epidermis and parenchyma cells. Moreover, in these cells, P2 is localized in single viral electron-lucent inclusion bodies (ELIB).[16] In host cells, viral protein P2 and P3 are first produced in numerous viral factories (electron-dense inclusion bodies), and are later exported and co-localize with microtubules, before concentrating in ELIB. CaMV specifically uses the microtubules to form the transmissible body and thus enable vector transmission.[17] The complete molecular characterization and study of this virus was not carried further.

Evasion of Plant Defenses

Cauliflower mosaic virus possesses a number of mechanisms that allow it to counteract host plant cell defenses. While the pregenomic 35S RNA is responsible for genome replication by reverse transcriptase, it also contains a non-coding 600 base pair leader sequence that serves as an important mRNA for the production of factors involved in viral counter-defense. A number of hosts of CaMV possess small RNA-based viral silencing mechanisms that serve to limit viral infection. The products of the aforementioned 600-bp sequence are viral small RNAs (vsRNA) of 21, 22, and 24 nucleotides in length that serve as decoys, binding and inactivating effectors of host silencing machinery, such as Argonaute 1 (AGO1). As proof-of-principle, experimental overexpression of these vsRNAs allows for increased viral accumulation in infected plants.[18]

Concerns About Use of CaMV 35S Promoter in Transgenic Plants

Recently, some concerns have been raised about using the CaMV 35S promoter for expression in transgenic plants because sequence overlap exists between this promoter and the coding sequences of P6. Fifty four transgenic events certified for release in the USA contain up to 528 bp of ORF VI (encoding C-terminal domains of P6).[19] As P6 is a multifunctional protein whose full range of functions is unknown, there is some concern that expression of one or more of its domains may have unforeseen consequences in the transgenic organisms. Recent studies have attempted to determine what length of CaMV 35S promoter has the least chance of inadvertently producing P6 domains, while still retaining full promoter activity. As one might expect, using shorter promoter lengths decreases the number of P6 domains included and also decreases the likelihood of unwanted effects.[19]

Geminiviridae

Geminiviridae is a family of plant viruses. There are currently 325 species in this family, divided among 7 genera. Diseases associated with this family include: bright yellow mosaic, yellow mosaic, yellow mottle, leaf curling, stunting, streaks, reduced yields.[1][2] They have single-stranded circular DNA genomes encoding genes that diverge in both directions from a virion strand origin of replication (i.e. geminivirus genomes are ambisense). According to the Baltimore classification they are considered class II viruses. It is the largest known family of single stranded DNA viruses.

Mastrevirus transmission is via various leafhopper species (e.g. maize streak virus and other African streak viruses are transmitted by *Cicadulina mbila*), curtoviruses and the only known topocuvirus species, *Tomato pseudo-curly top virus*, are transmitted by treehopper species (e.g.*Tomato pseudo-curly top virus* is transmitted by the treehopper *Micrutalis malleifera*), and begomoviruses are transmitted by the whitefly species, *Bemisia tabaci*.

These viruses are responsible for a significant amount of crop damage worldwide. Epidemics of geminivirus diseases have arisen due to a number of factors, including the recombination of different geminiviruses coinfecting a plant, which enables novel, possibly virulent viruses to be developed. Other contributing factors include the transport of infected plant material to new locations, expansion of agriculture into new growing areas, and the expansion and migration of vectors that can spread the virus from one plant to another.[3]

Virology

The genome can either be a single component between 2500–3100 nucleotides, or, in the case of some begomoviruses, two similar-sized components each between 2600 and 2800 nucleotides. They have elongated, geminate capsids with two incomplete T=1 icosahedra joined at the missing vertex. The capsids range in size from 18–20 nm in diameter with a length of about 30 nm. Begomoviruses with two component (i.e. bipartite) genomes have these components separated into two different particles both of which must usually be transmitted together to initiate a new infection within a suitable host cell.

Genus	Structure	Symmetry	Capsid	Genomic Arrangement	Genomic Segmentation
Eragrovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Curtovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Begomovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Segmented
Becurtovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Topocuvirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Turncurtovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Mastrevirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite

Taxonomy

Group: ssDNA

Order: Unassigned

Several additional genera have been proposed: Baminivirus, Nimivirus and Niminivirus.[4]

Some viruses have yet to be assigned a genus: one of these is the Grapevine Cabernet Franc-associated virus/Grapevine red blotch-associated virus/Grapevine redleaf-associated virus.[5]

Replication

Drawing of geminiviruses

Geminivirus genomes encode only a few proteins; thus, they are dependent on host cell factors for replication: these include factors such as DNA polymerase—and probably repair polymerases—in order to amplify their genomes, as well as transcription factors. Geminiviruses replicate via a rolling circle mechanism like bacteriophages such as M13, and many plasmids. Replication occurs within the nucleus of an infected plant cell. First the single-stranded circular DNA is converted to a double-stranded circular intermediate. This step involves the use of cellular DNA repair enzymes to produce a complementary negative-sense strand, using the viral genomic or plus-sense DNA strand as a template. The next step is the rolling circle phase, where the viral strand is cleaved at a specific site situated within the origin of replication by the viral Rep protein in order to initiate replication.[6] This process in a eukaryotic nucleus can give rise to concatemeric double-stranded forms of replicative intermediate genomes, although double-stranded unit circles can be isolated from infected plants and cells. New single-stranded DNA forms of the virus genome (plus-sense) are probably formed by interaction of the coat protein with replicating DNA intermediates, as genomes lacking a CP gene do not form ssDNA. The ssDNA is packaged into germinate particles in the nucleus. It is not clear if these particles can then leave the nucleus and be transmitted to surrounding cells as virions, or whether ssDNA associated with coat protein and a movement protein is the form of the genome that gets trafficked from cell to cell via the plasmodesmata.[7] These viruses tend to be introduced into and initially infect differentiated plant cells, via the piercing mouthparts of the vector insect: however, these cells generally lack the host enzymes necessary for DNA replication, making it difficult for the virus to replicate. To overcome this block geminiviruses can induce plant cells to reenter the cell cycle from a quiescent state so that viral replication can occur.[8]

Genus	Host Details	Tissue Tropism	Entry Details	Release Details	Replication Site	Assembly Site	Transmission

Genus	Host Details	Tissue Tropism	Entry Details	Release Details	Replication Site	Assembly Site	Transmission
Eragrovirus	Plants	None	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Treehopper; leafhopper
Curtovirus	Dicotyledonous plants	Phloem-limited	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Beet leefhopper
Begomovirus	Dicotyledonous plants	Phloem; sieve; phloem-limited	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Bemisia tabaci whiteflies
Becurtovirus	Spinach	Phloem; sieve; phloem-limited	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Viral movement; contact
Topocuvirus	Dicotyledonous plants	None	Cell receptor endocytosis	Budding	Nucleus	Nucleus	Leafhopper
Turncurtovirus	Turnip	None	Cell receptor endocytosis	Budding	Nucleus	Nucleus	Leafhopper
Mastrevirus	Monocots[9]	None	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Leafhopper

Evolution

These viruses may have evolved from a phytoplasma plasmid.[10] Geminiviruses are capable of horizontal gene transfer of genetic information to the plant host.[11]

HIV

"AIDS virus" redirects here. For the computer virus, see AIDS (computer virus).

The **human immunodeficiency virus** (**HIV**) is a lentivirus (a subgroup of retrovirus) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS).[1][2] AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype.[3] Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

HIV infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells.[4] HIV infection leads to low levels of $CD4^+$ T cells through a number of mechanisms, including pyroptosis of abortively infected T cells,[5] apoptosis of uninfected bystander cells,[6] direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by CD8 cytotoxic lymphocytes that recognize infected cells.[7] When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections.

Virology

Classification

Comparison of HIV species

Species	Virulence	Infectivity	Prevalence	Inferred origin
HIV-1	High	High	Global	Common chimpanzee
HIV-2	Lower	Low	West Africa	Sooty mangabey

HIV is a member of the genus *Lentivirus*,[8] part of the family *Retroviridae*.[9] Lentiviruses have many morphologies and biological properties in common. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period.[10] Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses. Upon entry into the target cell, the viral RNA genome is converted (reverse transcribed) into double-stranded DNA by a virally encoded reverse transcriptase that is transported along with the viral genome in the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors.[11] Once integrated, the virus may become latent, allowing the virus and its host cell to avoid detection by the immune system. Alternatively, the virus may be transcribed, producing new RNA genomes and viral proteins that are packaged and released from the cell as new virus particles that begin the replication cycle anew.

Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed both LAV and HTLV-III. It is more virulent, more infective,[12] and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of those exposed to HIV-2 will be infected per exposure. Because of its relatively poor capacity for transmission, HIV-2 is largely confined to West Africa.[13]

Structure and genome

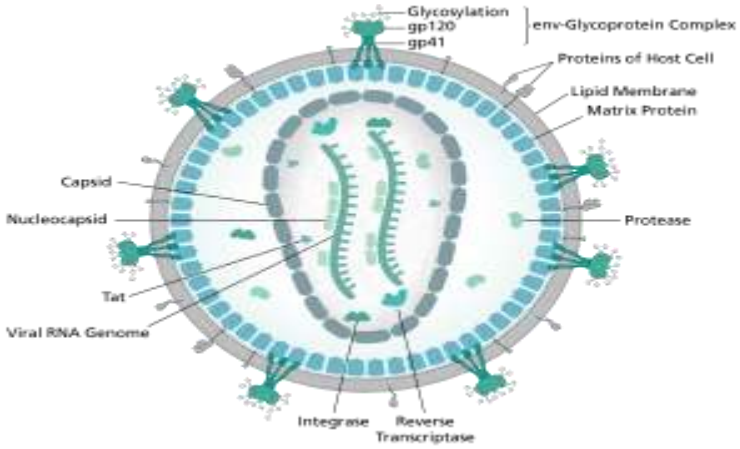
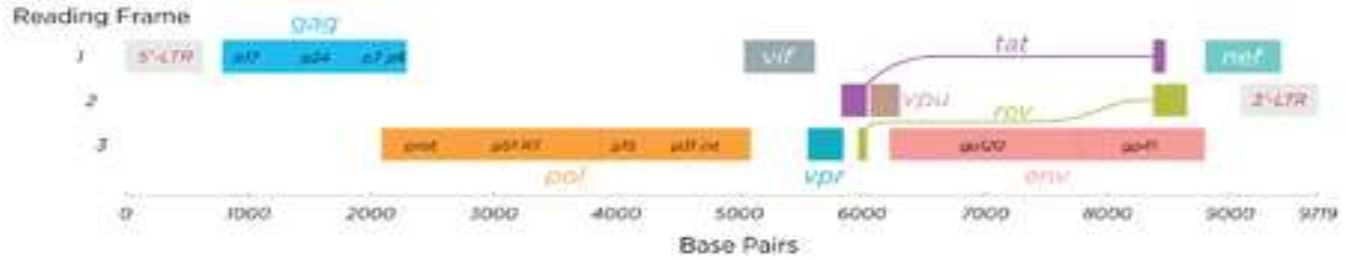


Diagram of HIV virion

HIV is different in structure from other retroviruses. It is roughly spherical[14] with a diameter of about 120 nm, around 60 times smaller than a red blood cell.[15] It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24.[16] The single-stranded RNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle.[16]

This is, in turn, surrounded by the viral envelope, that is composed of the lipid bilayer taken from the membrane of a human cell when the newly formed virus particle buds from the cell. The viral envelope contains proteins from the host cell and relatively few copies of the HIV Envelope protein,[16] which consists of a cap made of three molecules known as glycoprotein (gp) 120, and a stem consisting of three gp41 molecules which anchor the structure into the viral envelope.[17][18] The Envelope protein, encoded by the HIV *env* gene, allows the virus to attach to target cells and fuse the viral envelope with the target cell membrane releasing the viral contents into the cell and initiating the infectious cycle.[17] As the sole viral protein on the surface of the virus, the Envelope protein is a major target for HIV vaccine efforts.[19] Over half of the mass of the trimeric envelope spike is N-linked glycans. The density is high as the glycans shield the underlying viral protein from neutralisation by antibodies. This is one of the most densely glycosylated molecules known and the density is sufficiently high to prevent the normal maturation process of glycans during biogenesis in the endoplasmic and Golgi apparatus.[20][21] The majority of the glycans are therefore stalled as immature 'high-mannose' glycans not

normally present on secreted or cell surface human glycoproteins.[22] The unusual processing and high density means that almost all broadly neutralising antibodies that have so far been identified (from a subset of patients that have been infected for many months to years) bind to or, are adapted to cope with, these envelope glycans.[23]
The molecular structure of the viral spike has now been determined by X-ray crystallography[24] and cryo-electron microscopy.[25] These advances in structural biology were made possible due to the development of stable recombinant forms of the viral spike by the introduction of an intersubunit disulphide bond and an isoleucine to proline mutation in gp41.[26] The so-called SOSIP trimers not only reproduce the antigenic properties of the native viral spike but also display the same degree of immature glycans as presented on the native virus.[27] Recombinant trimeric viral spikes are promising vaccine candidates as they display less non-neutralising epitopes than recombinant monomeric gp120 which act to suppress the immune response to target epitopes.[28]



Structure of the RNA genome of HIV-1
The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and nine genes (*gag*, *pol*, and *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, and sometimes a tenth *tev*, which is a fusion of tat env and rev), encoding 19 proteins. Three of these genes, *gag*, *pol*, and *env*, contain information needed to make the structural proteins for new virus particles.[16] For example, *env* codes for a protein called gp160 that is cut in two by a cellular protease to form gp120 and gp41. The six remaining genes, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (or *vpx* in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease.[16]
The two Tat proteins (p16 and p14) are transcriptional transactivators for the LTR promoter acting by binding the TAR RNA element. The TAR may also be processed into microRNAs that regulate the apoptosis genes ERCC1 and IER3.[29][30] The Rev protein (p19) is involved in shuttling RNAs from the nucleus and the cytoplasm by binding to the RRE RNA element. The Vif protein (p23) prevents the action of APOBEC3G (a cellular protein that deaminates Cytidine to Uridine in the single stranded viral DNA and/or interferes with reverse transcription[31]). The Vpr protein (p14) arrests cell division at G2/M. The Nef protein (p27) down-regulates CD4 (the major viral receptor), as well as the MHC class I and class II molecules.[32][33][34]
Nef also interacts with SH3 domains. The Vpu protein (p16) influences the release of new virus particles from infected cells.[16] The ends of each strand of HIV RNA contain an RNA sequence called the long terminal repeat (LTR). Regions in the LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or the host cell. The Psi element is involved in viral genome packaging and recognized by Gag and Rev proteins. The SLIP element (TTTTTT) is involved in the frameshift in the Gag-Pol reading frame required to make functional Pol.[16]

Tropism

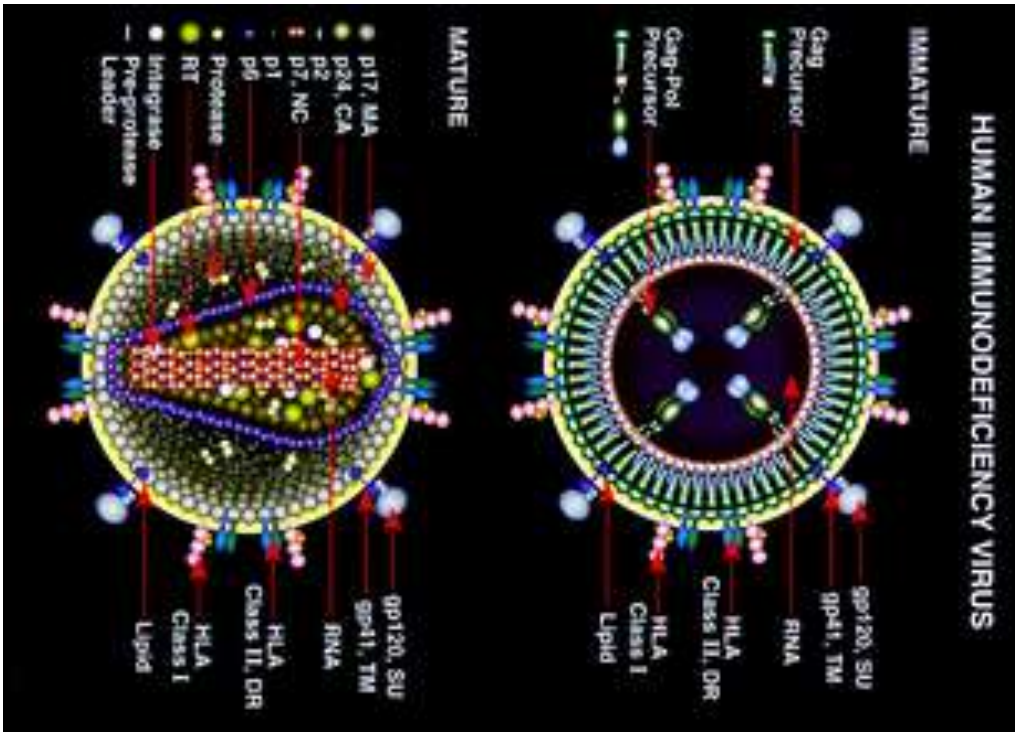


Diagram of the immature and mature forms of HIV

The term viral tropism refers to the cell types a virus infects. HIV can infect a variety of immune cells such as $CD4^+$ T cells, macrophages, and microglial cells. HIV-1 entry to macrophages and $CD4^+$ T cells is mediated through interaction of the virion envelope glycoproteins (gp120) with the CD4 molecule on the target cells and also with chemokine coreceptors.[17][35]

Macrophage (M-tropic) strains of HIV-1, or non-syncytia-inducing strains (NSI; now called R5 viruses[36]) use the β-chemokine receptor CCR5 for entry and are, thus, able to replicate in macrophages and $CD4^+$ T cells.[37] This CCR5 coreceptor is used by almost all primary HIV-1 isolates regardless of viral genetic subtype. Indeed, macrophages play a key role in several critical aspects of HIV infection. They appear to be the first cells infected by HIV and perhaps the source of HIV production when $CD4^+$ cells become depleted in the patient. Macrophages and microglial cells are the cells infected by HIV in the central nervous system. In tonsils and adenoids of HIV-infected patients, macrophages fuse into multinucleated giant cells that produce huge amounts of virus.

T-tropic isolates, or syncytia-inducing (SI; now called X4 viruses[36]) strains replicate in primary $CD4^+$ T cells as well as in macrophages and use the α-chemokine receptor, CXCR4, for entry.[37][38][39] Dual-tropic HIV-1 strains are thought to be transitional strains of HIV-1 and thus are able to use both CCR5 and CXCR4 as co-receptors for viral entry.

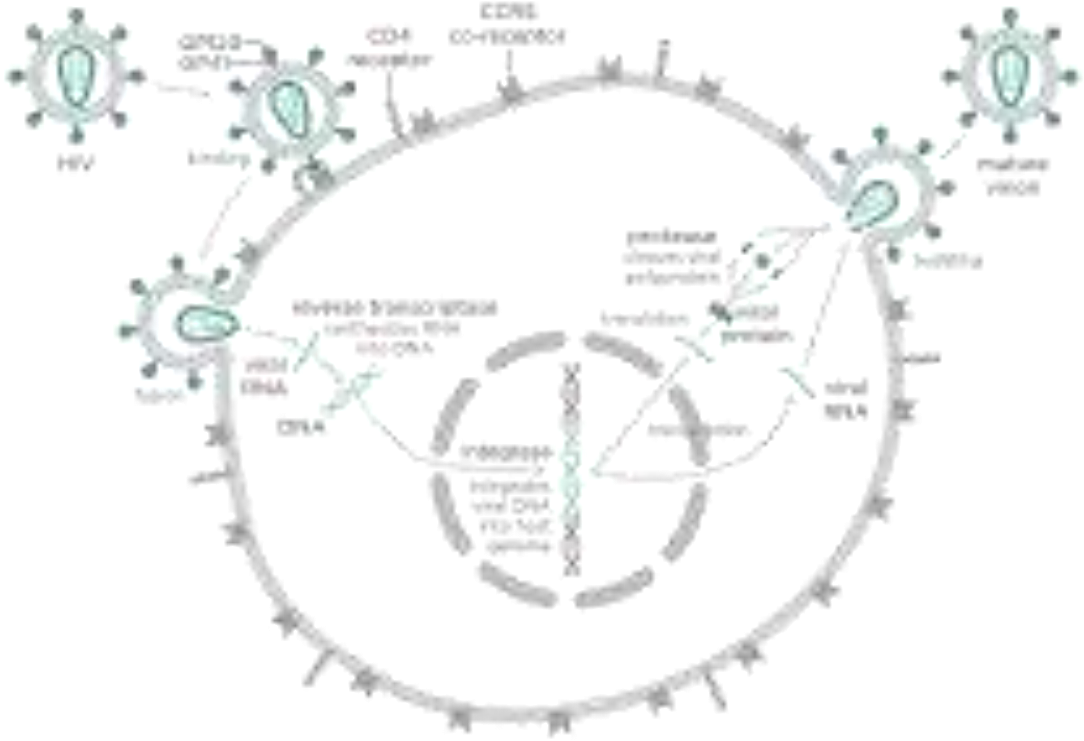
The α-chemokine SDF-1, a ligand for CXCR4, suppresses replication of T-tropic HIV-1 isolates. It does this by down-regulating the expression of CXCR4 on the surface of these cells. HIV that use only the CCR5 receptor are termed R5; those that use only CXCR4 are termed X4, and those that use both, X4R5. However, the use of coreceptor alone does not explain viral tropism, as not all R5 viruses are able to use CCR5 on macrophages for a productive infection[37] and HIV can also infect a subtype of myeloid dendritic cells,[40] which probably constitute a reservoir that maintains infection when $CD4^+$ T cell numbers have declined to extremely low levels. Some people are resistant to certain strains of HIV.[41] For example, people with the CCR5-Δ32 mutation are resistant to infection with R5 virus, as the mutation stops HIV from binding to this coreceptor, reducing its ability to infect target cells.

Sexual intercourse is the major mode of HIV transmission. Both X4 and R5 HIV are present in the seminal fluid, which is passed from a male to his sexual partner. The virions can then infect numerous cellular targets and disseminate into the whole organism. However, a selection process leads to a predominant transmission of the R5 virus through this pathway.[42][43][44] How this selective process works is still under investigation, but one model is that spermatozoa may selectively carry R5 HIV as they possess both CCR3 and CCR5 but not CXCR4 on their surface[45] and that genital epithelial cells preferentially sequester X4 virus.[46] In patients infected with

subtype B HIV-1, there is often a co-receptor switch in late-stage disease and T-tropic variants appear that can infect a variety of T cells through CXCR4.[47] These variants then replicate more aggressively with heightened virulence that causes rapid T cell depletion, immune system collapse, and opportunistic infections that mark the advent of AIDS.[48] Thus, during the course of infection, viral adaptation to the use of CXCR4 instead of CCR5 may be a key step in the progression to AIDS. A number of studies with subtype B-infected individuals have determined that between 40 and 50 percent of AIDS patients can harbour viruses of the SI and, it is presumed, the X4 phenotypes.[49][50]

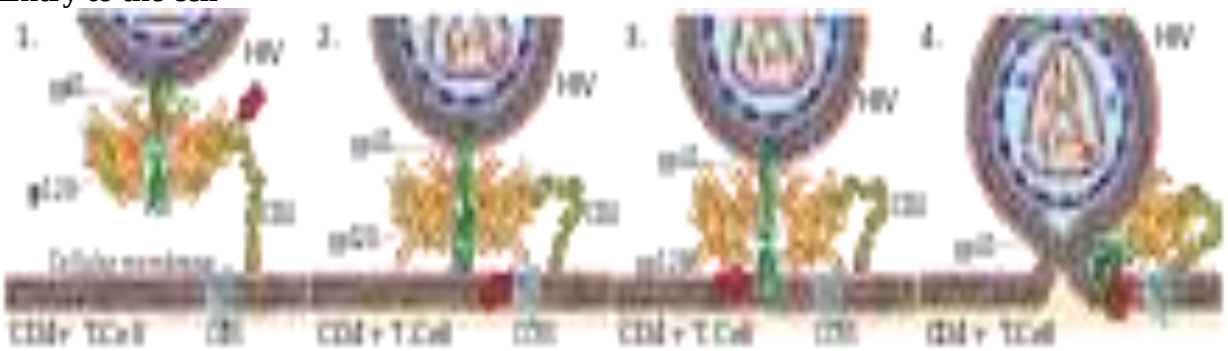
HIV-2 is much less pathogenic than HIV-1 and is restricted in its worldwide distribution. The adoption of "accessory genes" by HIV-2 and its more promiscuous pattern of coreceptor usage (including CD4-independence) may assist the virus in its adaptation to avoid innate restriction factors present in host cells. Adaptation to use normal cellular machinery to enable transmission and productive infection has also aided the establishment of HIV-2 replication in humans. A survival strategy for any infectious agent is not to kill its host but ultimately become a commensal organism. Having achieved a low pathogenicity, over time, variants more successful at transmission will be selected.[51]

Replication cycle



The HIV replication cycle

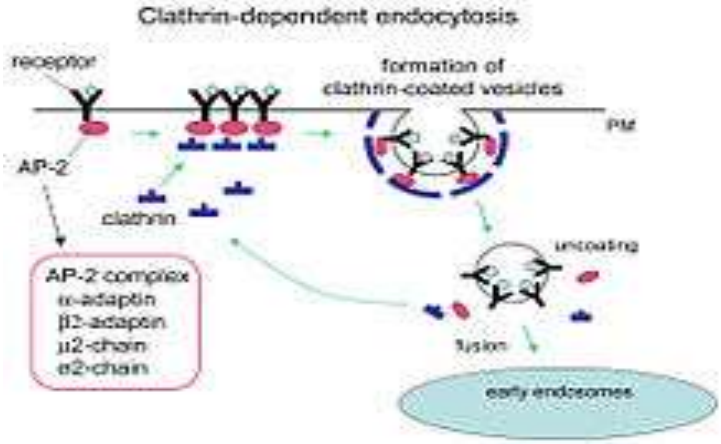
Entry to the cell



Mechanism of viral entry

1. Initial interaction between gp120 and CD4. **2.** Conformational change in gp120 allows for secondary interaction with CCR5. **3.** The distal tips of gp41 are inserted into the cellular membrane. **4.** gp41 undergoes

significant conformational change; folding in half and forming coiled-coils. This process pulls the viral and cellular membranes together, fusing them.
The HIV virion enters macrophages and $CD4^+$ T cells by the adsorption of glycoproteins on its surface to receptors on the target cell followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell.[52][53]
Entry to the cell begins through interaction of the trimeric envelope complex (gp160 spike) and both CD4 and a chemokine receptor (generally either CCR5 or CXCR4, but others are known to interact) on the cell surface.[52][53] gp120 binds to integrin $\alpha_4\beta_7$ activating LFA-1 the central integrin involved in the establishment of virological synapses, which facilitate efficient cell-to-cell spreading of HIV-1.[54] The gp160 spike contains binding domains for both CD4 and chemokine receptors.[52][53]
The first step in fusion involves the high-affinity attachment of the CD4 binding domains of gp120 to CD4. Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, exposing the chemokine binding domains of gp120 and allowing them to interact with the target chemokine receptor.[52][53] This allows for a more stable two-pronged attachment, which allows the N-terminal fusion peptide gp41 to penetrate the cell membrane.[52][53] Repeat sequences in gp41, HR1, and HR2 then interact, causing the collapse of the extracellular portion of gp41 into a hairpin. This loop structure brings the virus and cell membranes close together, allowing fusion of the membranes and subsequent entry of the viral capsid.[52][53]
After HIV has bound to the target cell, the HIV RNA and various enzymes, including reverse transcriptase, integrase, ribonuclease, and protease, are injected into the cell.[52][*not in citation given*] During the microtubule-based transport to the nucleus, the viral single-strand RNA genome is transcribed into double-strand DNA, which is then integrated into a host chromosome.
HIV can infect dendritic cells (DCs) by this CD4-CCR5 route, but another route using mannose-specific C-type lectin receptors such as DC-SIGN can also be used.[55] DCs are one of the first cells encountered by the virus during sexual transmission. They are currently thought to play an important role by transmitting HIV to T-cells when the virus is captured in the mucosa by DCs.[55] The presence of FEZ-1, which occurs naturally in neurons, is believed to prevent the infection of cells by HIV.[56]

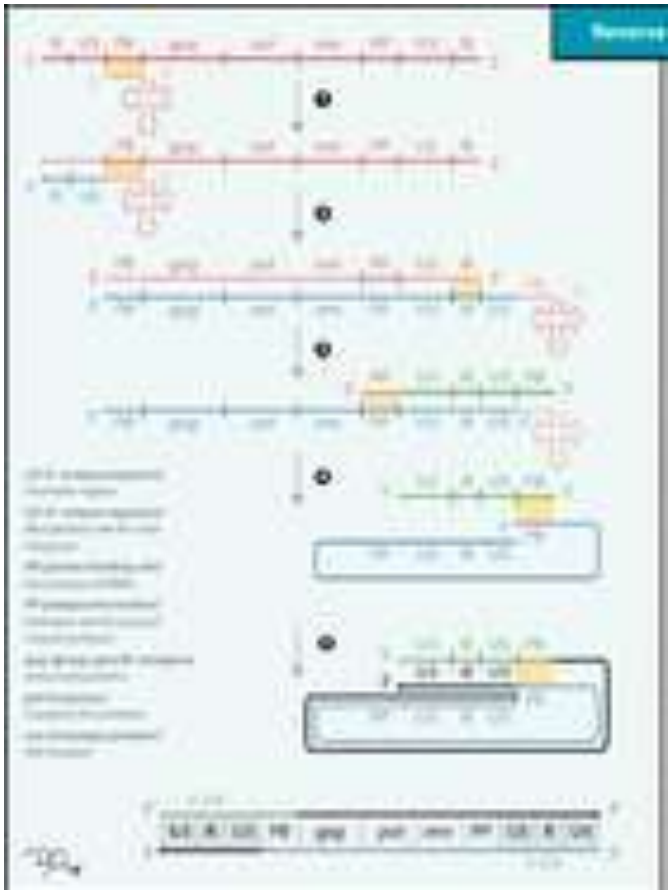


Clathrin-dependent endocytosis

HIV-1 entry, as well as entry of many other retroviruses, has long been believed to occur exclusively at the plasma membrane. More recently, however, productive infection by pH-independent, clathrin-dependent endocytosis of HIV-1 has also been reported and was recently suggested to constitute the only route of productive entry.[57][58][59][60][61]

Replication and transcription

Shortly after the viral capsid enters the cell, an enzyme called *reverse transcriptase* liberates the single-stranded (+)RNA genome from the attached viral proteins and copies it into a complementary DNA (cDNA) molecule.[62] The process of reverse transcription is extremely error-prone, and the resulting mutations may cause drug resistance or allow the virus to evade the body's immune system. The reverse transcriptase also has ribonuclease activity that degrades the viral RNA during the synthesis of cDNA, as well as DNA-dependent DNA polymerase activity that creates a sense DNA from the *antisense* cDNA.[63] Together, the cDNA and its complement form a double-stranded viral DNA that is then transported into the cell nucleus. The integration of the viral DNA into the host cell's genome is carried out by another viral enzyme called *integrase*.[62]



Reverse transcription of the HIV genome into double strand DNA

This integrated viral DNA may then lie dormant, in the latent stage of HIV infection.[62] To actively produce the virus, certain cellular transcription factors need to be present, the most important of which is NF-κB (NF kappa B), which is upregulated when T-cells become activated.[64] This means that those cells most likely to be killed by HIV are those currently fighting infection.

During viral replication, the integrated DNA provirus is transcribed into RNA, some of which then undergo RNA splicing to produce mature mRNAs. These mRNAs are exported from the nucleus into the cytoplasm, where they are translated into the regulatory proteins Tat (which encourages new virus production) and Rev. As the newly produced Rev protein accumulates in the nucleus, it binds to full-length, unspliced copies of virus RNAs and allows them to leave the nucleus.[65] Some of these full-length RNAs function as new copies of the virus genome, while others function as mRNAs that are translated to produce the structural proteins Gag and Env. Gag proteins bind to copies of the virus RNA genome to package them into new virus particles.[66]

HIV-1 and HIV-2 appear to package their RNA differently[*citation needed*]. HIV-1 will bind to any appropriate RNA[*citation needed*]. HIV-2 will preferentially bind to the mRNA that was used to create the Gag protein itself.[67]

Recombination

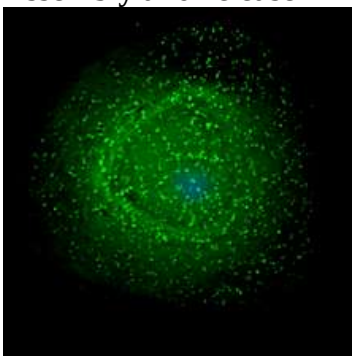
Two RNA genomes are encapsidated in each HIV-1 particle (see Structure and genome of HIV). Upon infection and replication catalyzed by reverse transcriptase, recombination between the two genomes can occur.[68][69] Recombination occurs as the single-strand (+)RNA genomes are reverse transcribed to form DNA. During reverse transcription the nascent DNA can switch multiple times between the two copies of the viral RNA. This form of recombination is known as copy-choice. Recombination events may occur throughout the genome. From 2 to 20 events per genome may occur at each replication cycle, and these events can rapidly shuffle the genetic information that is transmitted from parental to progeny genomes.[69]

Viral recombination produces genetic variation that likely contributes to the evolution of resistance to anti-retroviral therapy.[70] Recombination may also contribute, in principle, to overcoming the immune defenses of the host. Yet, for the adaptive advantages of genetic variation to be realized, the two viral genomes packaged in individual infecting virus particles need to have arisen from separate progenitor parental viruses of differing genetic constitution. It is unknown how often such mixed packaging occurs under natural conditions.[71]

Bonhoeffer et al.[72] suggested that template switching by the reverse transcriptase acts as a repair process to deal with breaks in the ssRNA genome. In addition, Hu and Temin[68] suggested that recombination is an adaptation for repair of damage in the RNA genomes. Strand switching (copy-choice recombination) by reverse transcriptase could generate an undamaged copy of genomic DNA from two damaged ssRNA genome copies. This view of the adaptive benefit of recombination in HIV could explain why each HIV particle contains two complete genomes, rather than one. Furthermore, the view that recombination is a repair process implies that the benefit of repair can occur at each replication cycle, and that this benefit can be realized whether or not the two genomes differ genetically. On the view that that recombination in HIV is a repair process, the generation of recombinational variation would be a consequence, but not the cause of, the evolution of template switching.[72]

HIV-1 infection causes chronic ongoing inflammation and production of reactive oxygen species.[73] Thus, the HIV genome may be vulnerable to oxidative damages, including breaks in the single-stranded RNA. For HIV, as well as for viruses generally, successful infection depends on overcoming host defensive strategies that often include production of genome-damaging reactive oxygen. Thus, Michod et al.[74] suggested that recombination by viruses is an adaptation for repair of genome damages, and that recombinational variation is a byproduct that may provide a separate benefit.

Assembly and release



HIV assembling on the surface of an infected macrophage.

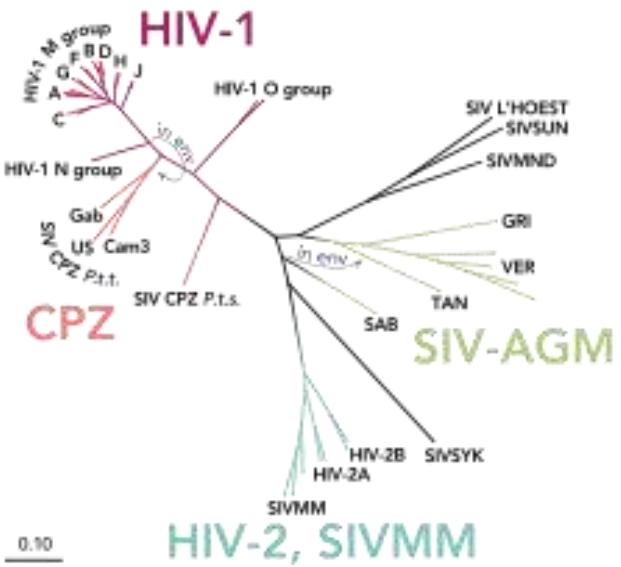
The final step of the viral cycle, assembly of new HIV-1 virions, begins at the plasma membrane of the host cell. The Env polyprotein (gp160) goes through the endoplasmic reticulum and is transported to the Golgi complex where it is cleaved by furin resulting in the two HIV envelope glycoproteins, gp41 and gp120.[75] These are transported to the plasma membrane of the host cell where gp41 anchors gp120 to the membrane of the infected cell. 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. The budded virion is still immature as the gag polyproteins still need to be cleaved into the actual matrix, capsid and nucleocapsid proteins. This cleavage is mediated by the also packaged viral protease and can be inhibited by antiretroviral drugs of the protease inhibitor class. The various structural components then assemble to produce a mature HIV virion.[76] Only mature virions are then able to infect another cell.

Spread within the body

HIV is now known to spread between CD4+ T cells by two parallel routes: cell-free spread and cell-to-cell spread, i.e. it employs hybrid spreading mechanisms.[77] In the cell-free spread, virus particles bud from an infected T cell, enter the blood/extracellular fluid and then infect another T cell following a chance encounter.[77] HIV can also disseminate by direct transmission from one cell to another by a process of cell-to-cell spread. Two pathways of cell-to-cell transmission have been reported. Firstly, an infected T cell can transmit virus directly to a target T cell via a virological synapse.[54][78] Secondly, an antigen presenting cell (APC) can also transmit HIV to T cells by a process that either involves productive infection (in the case of macrophages) or capture and transfer of virions *in trans* (in the case of dendritic cells).[79] Whichever pathway is used, infection by cell-to-cell transfer is reported to be much more efficient than cell-free virus spread.[80] A number of factors contribute to this increased efficiency, including polarised virus budding towards the site of cell-to-cell contact, close apposition of cells which minimizes fluid-phase diffusion of virions, and clustering of HIV entry receptors on the target cell to the contact zone.[78] Cell-to-cell spread is thought to be particularly important in lymphoid tissues where CD4+ T lymphocytes are densely packed and likely to frequently

interact.[77] Intravital imaging studies have supported the concept of the HIV virological synapse *in vivo*.[81] The hybrid spreading mechanisms of HIV contribute to the virus's ongoing replication against antiretroviral therapies.[77][82]

Genetic variability



The phylogenetic tree of the SIV and HIV

HIV differs from many viruses in that it has very high genetic variability. This diversity is a result of its fast replication cycle, with the generation of about 10^{10} virions every day, coupled with a high mutation rate of approximately 3×10^{-5} per nucleotide base per cycle of replication and recombinogenic properties of reverse transcriptase.[83][84][85]

This complex scenario leads to the generation of many variants of HIV in a single infected patient in the course of one day.[83] This variability is compounded when a single cell is simultaneously infected by two or more different strains of HIV. When simultaneous infection occurs, the genome of progeny virions may be composed of RNA strands from two different strains. This hybrid virion then infects a new cell where it undergoes replication. As this happens, the reverse transcriptase, by jumping back and forth between the two different RNA templates, will generate a newly synthesized retroviral DNA sequence that is a recombinant between the two parental genomes.[83] This recombination is most obvious when it occurs between subtypes.[83]

The closely related simian immunodeficiency virus (SIV) has evolved into many strains, classified by the natural host species. SIV strains of the African green monkey (SIVagm) and sooty mangabey (SIVsmm) are thought to have a long evolutionary history with their hosts. These hosts have adapted to the presence of the virus,[86] which is present at high levels in the host's blood but evokes only a mild immune response,[87] does not cause the development of simian AIDS,[88] and does not undergo the extensive mutation and recombination typical of HIV infection in humans.[89]

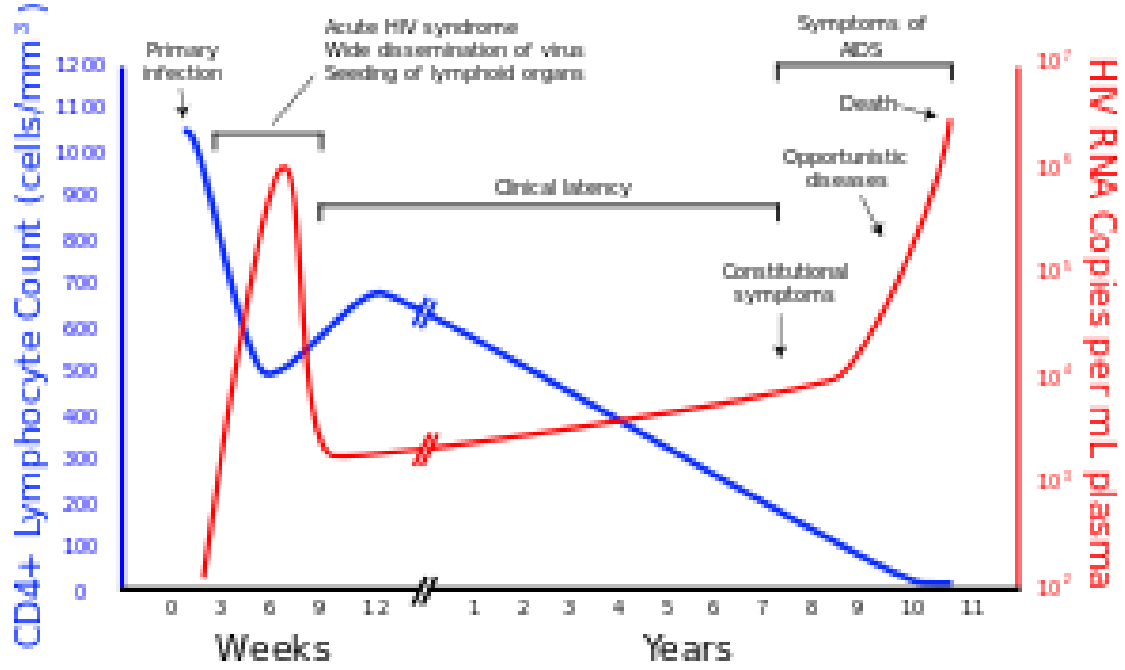
In contrast, when these strains infect species that have not adapted to SIV ("heterologous" hosts such as rhesus or cynomologus macaques), the animals develop AIDS and the virus generates genetic diversity similar to what is seen in human HIV infection.[90] Chimpanzee SIV (SIVcpz), the closest genetic relative of HIV-1, is associated with increased mortality and AIDS-like symptoms in its natural host.[91] SIVcpz appears to have been transmitted relatively recently to chimpanzee and human populations, so their hosts have not yet adapted to the virus.[86] This virus has also lost a function of the Nef gene that is present in most SIVs. For non-pathogenic SIV variants, Nef suppresses T-cell activation through the CD3 marker. Nef's function in non-pathogenic forms of SIV is to downregulate expression of inflammatory cytokines, MHC-1, and signals that affect T cell trafficking. In HIV-1 and SIVcpz, Nef does not inhibit T-cell activation and it has lost this function. Without this function, T cell depletion is more likely, leading to immunodeficiency.[91][92]

Three groups of HIV-1 have been identified on the basis of differences in the envelope (*env*) region: M, N, and O.[93] Group M is the most prevalent and is subdivided into eight subtypes (or clades), based on the whole genome, which are geographically distinct.[94] The most prevalent are subtypes B (found mainly in North America and Europe), A and D (found mainly in Africa), and C (found mainly in Africa and Asia); these

subtypes form branches in the phylogenetic tree representing the lineage of the M group of HIV-1. Coinfection with distinct subtypes gives rise to circulating recombinant forms (CRFs). In 2000, the last year in which an analysis of global subtype prevalence was made, 47.2% of infections worldwide were of subtype C, 26.7% were of subtype A/CRF02_AG, 12.3% were of subtype B, 5.3% were of subtype D, 3.2% were of CRF_AE, and the remaining 5.3% were composed of other subtypes and CRFs.[95] Most HIV-1 research is focused on subtype B; few laboratories focus on the other subtypes.[96] The existence of a fourth group, "P", has been hypothesised based on a virus isolated in 2009.[97] The strain is apparently derived from gorilla SIV (SIVgor), first isolated from western lowland gorillas in 2006.[97]

HIV-2's closest relative is SIVsm, a strain of SIV found in sooty mangabees. Since HIV-1 is derived from SIVcpz, and HIV-2 from SIVsm, the genetic sequence of HIV-2 is only partially homologous to HIV-1 and more closely resembles that of SIVsm.[*citation needed*][98]

Diagnosis



A generalized graph of the relationship between HIV copies (viral load) and CD4 counts over the average course of untreated HIV infection; any particular individual's disease course may vary considerably.

CD4$^+$ T cell count (cells per µL)

HIV RNA copies per mL of plasma

Many HIV-positive people are unaware that they are infected with the virus.[99] For example, in 2001 less than 1% of the sexually active urban population in Africa had been tested, and this proportion is even lower in rural populations.[99] Furthermore, in 2001 only 0.5% of pregnant women attending urban health facilities were counselled, tested or receive their test results.[99] Again, this proportion is even lower in rural health facilities.[99] Since donors may therefore be unaware of their infection, donor blood and blood products used in medicine and medical research are routinely screened for HIV.[100]

HIV-1 testing is initially by an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to HIV-1. Specimens with a nonreactive result from the initial ELISA are considered HIV-negative unless new exposure to an infected partner or partner of unknown HIV status has occurred. Specimens with a reactive ELISA result are retested in duplicate.[101] If the result of either duplicate test is reactive, the specimen is reported as repeatedly reactive and undergoes confirmatory testing with a more specific supplemental test (e.g., western blot or, less commonly, an immunofluorescence assay (IFA)). Only specimens that are repeatedly reactive by ELISA and positive by IFA or reactive by western blot are considered HIV-positive and indicative of HIV infection. Specimens that are repeatedly ELISA-reactive occasionally provide an indeterminate western blot result, which may be either an incomplete antibody response to HIV in an infected person or nonspecific reactions in an uninfected person.[102]

HIV deaths in 2014.[103]

- Nigeria (15.76%)
- South Africa (12.51%)
- India (11.50%)
- Tanzania (4.169%)
- Mozambique (4.061%)
- Zimbabwe (3.49%)
- Cameroon (3.09%)
- Indonesia (3.04%)
- Kenya (2.98%)
- Uganda (2.97%)
- Malawi (2.94%)
- DR Congo (2.17%)
- Ethiopia (2.11%)
- Other (29.21%)

Although IFA can be used to confirm infection in these ambiguous cases, this assay is not widely used. In general, a second specimen should be collected more than a month later and retested for persons with indeterminate western blot results. Although much less commonly available, nucleic acid testing (e.g., viral RNA or proviral DNA amplification method) can also help diagnosis in certain situations.[101] In addition, a few tested specimens might provide inconclusive results because of a low quantity specimen. In these situations, a second specimen is collected and tested for HIV infection.

Modern HIV testing is extremely accurate. A single screening test is correct more than 99% of the time.[104][*needs update*] The chance of a false-positive result in standard two-step testing protocol is estimated to be about 1 in 250,000 in a low risk population.[104] Testing post exposure is recommended initially and at six weeks, three months, and six months.[105]

The latest recommendations of the CDC show that HIV testing must start with an immunoassay combination test for HIV-1 and HIV-2 antibodies and p24 antigen. A negative result rules out HIV exposure, while a positive one must be followed by an HIV-1/2 antibody differentiation immunoassay to detect which is present. This gives rise to four possible scenarios:

- 1. HIV-1 (+) & HIV-2 (-): HIV-1 antibodies detected
- 2. HIV-1 (-) & HIV-2 (+): HIV-2 antibodies detected
- 3. HIV-1 (+) & HIV-2 (+): HIV antibodies detected
- 4. HIV-1 (-) or indeterminate & HIV-2 (-): Nucleic acid test must be carried out to detect the acute infection of HIV-1 or its absence.[106]

An updated algorithm published by the CDC in June 2014 recommends that diagnosis starts with the p24 antigen test. A negative result rules out infection, while a positive one must be followed by an HIV-1/2 antibody differentiation immunoassay. A positive differentiation test confirms diagnosis, while a negative or indeterminate result must be followed by nucleic acid test (NAT). A positive NAT result confirms HIV-1 infection whereas a negative result rules out infection (false positive p24).[107]

Research

HIV/AIDS research includes all medical research that attempts to prevent, treat, or cure HIV/AIDS, as well as fundamental research about the nature of HIV as an infectious agent and AIDS as the disease caused by HIV. Many governments and research institutions participate in HIV/AIDS research. This research includes behavioral health interventions, such as research into sex education, and drug development, such as research into microbicides for sexually transmitted diseases, HIV vaccines, and antiretroviral drugs. Other medical research areas include the topics of pre-exposure prophylaxis, post-exposure prophylaxis, circumcision and HIV, and accelerated aging effects.

History

Discovery

AIDS was first clinically observed in 1981 in the United States.[108] The initial cases were a cluster of injection drug users and gay men with no known cause of impaired immunity who showed symptoms of *Pneumocystis carinii* pneumonia (PCP), a rare opportunistic infection that was known to occur in people with very compromised immune systems.[109] Soon thereafter, additional gay men developed a previously rare skin cancer called Kaposi's sarcoma (KS).[110][111] Many more cases of PCP and KS emerged, alerting U.S. Centers for Disease Control and Prevention (CDC) and a CDC task force was formed to monitor the outbreak.[112] The earliest retrospectively described case of AIDS is believed to have been in Norway beginning in 1966.[113]

In the beginning, the CDC did not have an official name for the disease, often referring to it by way of the diseases that were associated with it, for example, lymphadenopathy, the disease after which the discoverers of HIV originally named the virus.[114][115] They also used *Kaposi's Sarcoma and Opportunistic Infections*, the name by which a task force had been set up in 1981.[116] In the general press, the term *GRID*, which stood for gay-related immune deficiency, had been coined.[117] The CDC, in search of a name, and looking at the infected communities coined "the 4H disease," as it seemed to single out homosexuals, heroin users, hemophiliacs, and Haitians.[118][119] However, after determining that AIDS was not isolated to the gay community,[116] it was realized that the term GRID was misleading and *AIDS* was introduced at a meeting in July 1982.[120] By September 1982 the CDC started using the name AIDS.[121]


Robert Gallo, co-discoverer of HIV

In 1983, two separate research groups led by Robert Gallo and Luc Montagnier independently declared that a novel retrovirus may have been infecting AIDS patients, and published their findings in the same issue of the journal *Science*.[122][123] Gallo claimed that a virus his group had isolated from a person with AIDS was strikingly similar in shape to other human T-lymphotropic viruses (HTLVs) his group had been the first to isolate. Gallo's group called their newly isolated virus HTLV-III. At the same time, Montagnier's group isolated a virus from a patient presenting with swelling of the lymph nodes of the neck and physical weakness, two classic symptoms of AIDS. Contradicting the report from Gallo's group, Montagnier and his colleagues showed that core proteins of this virus were immunologically different from those of HTLV-I. Montagnier's group named their isolated virus lymphadenopathy-associated virus (LAV).[112] As these two viruses turned out to be the same, in 1986, LAV and HTLV-III were renamed HIV.[124]

Origins

Both HIV-1 and HIV-2 are believed to have originated in non-human primates in West-central Africa, and are believed to have transferred to humans (a process known as zoonosis) in the early 20th century.[125][126]

HIV-1 appears to have originated in southern Cameroon through the evolution of SIV(cpz), a simian immunodeficiency virus (SIV) that infects wild chimpanzees (HIV-1 descends from the SIV(cpz) endemic in the chimpanzee subspecies *Pan troglodytes troglodytes*).[127][128] The closest relative of HIV-2 is SIV (smm), a virus of the sooty mangabey (*Cercocebus atys atys*), an Old World monkey living in litoral West Africa (from southern Senegal to western Côte d'Ivoire).[13] New World monkeys such as the owl monkey are resistant to HIV-1 infection, possibly because of a genomic fusion of two viral resistance genes.[129] HIV-1 is thought to have jumped the species barrier on at least three separate occasions, giving rise to the three groups of the virus, M, N, and O.[130]


Left to right: the African green monkey source of SIV, the sooty mangabey source of HIV-2, and the chimpanzee source of HIV-1

There is evidence that humans who participate in bushmeat activities, either as hunters or as bushmeat vendors, commonly acquire SIV.[131] However, SIV is a weak virus, and it is typically suppressed by the human immune system within weeks of infection. It is thought that several transmissions of the virus from individual to individual in quick succession are necessary to allow it enough time to mutate into HIV.[132] Furthermore, due to its relatively low person-to-person transmission rate, it can only spread throughout the population in the presence of one or more of high-risk transmission channels, which are thought to have been absent in Africa prior to the 20th century.

Specific proposed high-risk transmission channels, allowing the virus to adapt to humans and spread throughout the society, depend on the proposed timing of the animal-to-human crossing. Genetic studies of the virus suggest that the most recent common ancestor of the HIV-1 M group dates back to circa 1910.[133] Proponents of this dating link the HIV epidemic with the emergence of colonialism and growth of large colonial African cities, leading to social changes, including a higher degree of sexual promiscuity, the spread of prostitution, and the concomitant high frequency of genital ulcer diseases (such as syphilis) in nascent colonial cities.[134] While transmission rates of HIV during vaginal intercourse are typically low, they are increased many fold if one of the partners suffers from a sexually transmitted infection resulting in genital ulcers. Early 1900s colonial cities were notable due to their high prevalence of prostitution and genital ulcers to the degree that as of 1928 as

many as 45% of female residents of eastern Leopoldville were thought to have been prostitutes and as of 1933 around 15% of all residents of the same city were infected by one of the forms of syphilis.[134]
An alternative view holds that unsafe medical practices in Africa during years following World War II, such as unsterile reuse of single use syringes during mass vaccination, antibiotic, and anti-malaria treatment campaigns, were the initial vector that allowed the virus to adapt to humans and spread.[132][135][136]
The earliest well documented case of HIV in a human dates back to 1959 in the Belgian Congo.[137] The virus may have been present in the United States as early as the mid-to-late 1950s, as a sixteen-year-old male presented with symptoms in 1966 died in 1969.

Rabies virus

Rabies virus is a neurotropic virus that causes rabies in humans and animals. Rabies transmission can occur through the saliva of animals and less commonly through contact with human saliva. Rabies virus, like many rhabdoviruses, has an extremely wide host range. In the wild it has been found infecting many mammalian species, while in the laboratory it has been found that birds can be infected, as well as cell cultures from mammals, birds, reptiles and insects.[1]
The rabies virus has a cylindrical morphology and is the type species of the *Lyssavirus* genus of the *Rhabdoviridae* family. These viruses are enveloped and have a single stranded RNA genome with negative-sense. The genetic information is packaged as a ribonucleoprotein complex in which RNA is tightly bound by the viral nucleoprotein. The RNA genome of the virus encodes five genes whose order is highly conserved. These genes code for nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the viral RNA polymerase (L).[2] The complete genome sequences range from 11,615 to 11,966 nt in length.[3]
All transcription and replication events take place in the cytoplasm inside a specialized "virus factory", the *Negri body* (named after Adelchi Negri[4]). These are 2–10 μm in diameter and are typical for a rabies infection and thus have been used as definite histological proof of such infection.[5]

Structure

Lyssaviruses have helical symmetry, so their infectious particles are approximately cylindrical in shape. They are characterized by an extremely broad host spectrum ranging from plants to insects and mammals; human-infecting viruses more commonly have icosahedral symmetry and take shapes approximating regular polyhedra. The rabies genome encodes five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and polymerase (L). All rhabdoviruses have two major structural components: a helical ribonucleoprotein core (RNP) and a surrounding envelope. In the RNP, genomic RNA is tightly encased by the nucleoprotein. Two other viral proteins, the phosphoprotein and the large protein (L-protein or polymerase) are associated with the RNP. The glycoprotein forms approximately 400 trimeric spikes which are tightly arranged on the surface of the virus. The M protein is associated both with the envelope and the RNP and may be the central protein of rhabdovirus assembly.[6]
The rabies virus has a bullet like shape with a length of about 180 nm and a cross-sectional diameter of about 75 nm. One end is rounded or conical and the other end is planar or concave. The lipoprotein envelope carries knob-like spikes composed of Glycoprotein G. Spikes do not cover the planar end of the virion (virus particle). Beneath the envelope is the membrane or matrix (M) protein layer which may be invaginated at the planar end. The core of the virion consists of helically arranged ribonucleoprotein.

Genome Organization

The rhabdovirus virion is an enveloped, rod- or bullet-shaped structure containing five protein species.The nucleoprotein (N) coats the RNA at the rate of one monomer of protein to nine nucleotides, forming a nucleocapsid with helical symmetry. Associated with the nucleocapsid are copies of P (phosphoprotein) and L (large) protein. The L protein is well named, its gene taking up about half of the genome. Its large size is justified by the fact that it is a multifunctional protein. The M (matrix) protein forms a layer between the nucleocapsid and the envelope, and trimers of G (glycoprotein) form spikes that protrude from the envelope.The genomes of all rhabdoviruses encode these five proteins. Many rhabdoviruses encode one or more proteins in addition to these

15MBU502
III BSC MICROBIOLOGY
VIROLOGY

Unit Vqu	Opt 1	Opt 2	Opt 3	Opt 4	Opt 5	Opt 6	Answer
The hepa	DNA-dep	reverse tr	Rnase H	DNA ligase			DNA-dependent DN
Which of	Hepatitis	Herpes si	Varicella-	Cytomegalovirus			Hepatitis B virus
Viroids a	single-str	double-str	single-str	double-stranded RNA			single-stranded DNA
_____	Fanny He	Rous	Pasteur	Reed			Fanny Hesse
_____	Jaco Henl	Lymc	Khorana	Kreb			Jaco Henk
Complem	Wasserm	Fleming	Ricketts	Bordet			Wasserman
The first s	adsorptio	absorptio	penetratic	replication			adsorption
Which of	Hepatitis	Hepatitis	Varicella-	Herpes simplex virus type 2			Varicella-Zoster vir
The viral	genome a	capsid an	envelope	capsomere and genome			genome and capsid
Which of	Prions	Viroids	virions	Virinos			Prions
_____	Host pref	Morphol	Physical r	Chemical nature of virion cons			Host preference
The _____	fimbriae	flagellae	hemagglu	neuraminidase			neuraminidase
Intracellu	prokaryot	chromoso	inclusion	cytocidal bodies			cytocidal bodies
Viral RN	cytoplasm	nucleus	mitochon	lysozomes			cytoplasmic matrix
Which fa	Reovirida	Baculovir	Iridovirid	Poxviridae			Baculoviridae
Vaccinati	Ricketts	Louis Pas	Bordet	Ehrlich			Louis Pasteur
_____	Fanny He	Rous	Louis Pas	Twort			Louis Pasteur
Anaerobi	Robert K	John Neec	Louis Pas	Ferdinand Cohn			John Needham
The causa	Robert K	Paul Ehrl	Louis Pas	Metchnihoff			Louis Pasteur
Which of	Vaccine f	Vaccinati	Discovery	Isolation of bacteria responsibl			Isolation of bacteria
The theor	Spallanza	Louis Pas	John Tyn	Jenner			Spallanzani
The first	Rabies	TMV	T4	Pox			TMV
Present da	Carolus L	Robert K	Alexande	Pasteur			Carolus Linnaeus
_____	Classifica	Distributi	Corrulatic	Differentiation			Classification
Berkefeld	asbestos	aluminium	sand and c	Copper			asbestos
Asbestos i	magnesiu	mercuric s	manganou	mercapto ethanol			manganous chloride
Phenol wa	Louis Past	John Tyn	Joseph list	Robert koch			Joseph lister
The widel	ethylene	chlorine	formaldeh	carbon-di-oxide			formaldehyde
An examp	gamma ra	UV rays	X- rays	sun rays			UV rays
The viral	lysogeny	spontaneo	lytic phas	induction			lysogeny
Which of	Viruses h	All viruse	All viruse	Viruses probably arose from sn			Viruses have been su
Which of	Hepatitis	Hepatitis	Hepatitis	Hepatitis D virus			Hepatitis E virus
In the sim	hexon	polyhedra	icosahedr	helical			hexon
The size	centimete	micromet	nanomete	millimeters			nanometers
The proph	λ phage e	λ DNA	Phage Mu	Phage Mn			Phage Mu
Enzyme r	Human in	Epstein-B	Influenza	Adenovirus			Influenza virus
Lysozym	immediat	late genes	delayed e	Early genes			late genes
Which of	HeLa	HEp-2	KB	ML2			HeLa
The repre	immunity	immunity	operon re	Operon depresssor			immunity repressor

Which of	Herpes	Influenza	Measles	HSV			Measles
Group E	single stra	double str	single stra	double stranded RNA			double stranded RNA
The micro	Agar	Peplone	Yeast extr	Tryptone			Agar
- - - - - - is	Nutrient A	Tryptone a	Macconke	Mnnitol salt agar			Tryptone agar
Give an ex	Hot air ov	Autoclave	waterbath	Filter			Filter
Ultra viole	300-400nr	200-300nr	100-400nr	50-100nm			100-400nm
Which pro	Ultra soni	Ionization	Pasteuriza	Tyndallization			Ionization radiation
In ultra so	Cavitation	Ionization	Pasteuriza	Tyndallization			Cavitation
The proce	Tyndalliza	Pasteuriza	Disinfecti	Sterilization			Disinfection
Which phe	Ipan	Mum	Dial	Phisohex			Ipan
__ is the ti	Steam	hot air	L-value	D- value			D- value
Name the	Uranium-(	Cobalt-60	Tween-80	Cl			Cobalt-60
The onco	how chen	how virus	how virus	Retransform of tumor cells			how viruses transfor
In cell cul	nuclear py	transform	syncytium	rounding and aggregation of ce			syncytium formation
A change	ultraviole	chemicals	irradiation	alcohol			alcohol
The viral	spontaneo	inductive	resultant i	spontaneous infection			spontaneous inducti
The lysog	immunity	immunity	operon re	Lac operon			immunity repressor
The capso	protomers	caproprot	bprocapsi	depressor			caproprotein
In order t	the capsid	the host c	the genon	the host cell must lack a cell m			the genome must be
Which of	Rubella v	Yellow fe	Hepatitis	Herpes virus			Yellow fever virus
Which of	Hepatitis	T cell lym	Epstein-B	Adeno virus			T cell lymphotronic
Which of	Denaturat	Enzyme t	Pressure	Retro virus			Denaturation
Ultra viole	DNA	ribosomes	cell wall	cytoplasm			DNA
Membrane	cellulose r	poly carbo	cellulose c	cellulose			Cellulose nitrate
Capillary p	Irradation	filtration	evaporatio	respiration			filtration
The phage	the proph	lytic infec	Both (a) a	Lysogenic cycle			the prophage
Which ca	Helical	Icosahedr	Complex	Cylinder			Icosahedral
Contracti	T3	T2	P22	P322			T2
The bacte	A	B	C	D			D
The proce	infection	integratio	repressior	induction			infection
Area of ly	pock	plaque	pox	colony			plaque
The proca	ladder	framing	scaffoldin	form			scaffolding
Which of	Phage stru	Proteins t	Proteins i	lipids			Proteins that help wit

A polymerase

us

responsible for chicken cholera

ccessfully grown in pure cultures in test tubes

A

m normal cells into tumor cells

on

released in the cytoplasm

virus type I

h phage assembly without becoming part of the virion structure

Reg. No. : ----------
[15MBU502]

KARPAGAM ACADEMY OF HIGHER EDUCATION
KARPAGAM UNIVERSITY
(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
B.Sc. DEGREE EXAMINATION, July 2017
DEPARTMENT OF MICROBIOLOGY

I INTERNAL TEST - FIFTH SEMESTER

VIROLOGY

Time: 2 hours **Maximum: 50 marks**
Date / Session : **Class: III BSc MB**

PART-A

Multiple Choice Questions **20 x 1 = 20 marks**

1. What RBCs are used in haemagglutination assay?
   A. Human B. Monkey
   C. Sheep D. Dog
2. Which metal is chelated in BCA
   A. Cs B.Mg
   C. Cu D. Ag
3._____blotting is used for detection of RNA
   A. Western B. Southern
   C. Northern D. Eastern
4. Which enzyme plays a key role in PCR.
   A. Ligase B. Protease
   C. Polymerase D. Helicase
5. Real time PCR analysis id performed with help of process called_____
   A. RIA B. HIA
   C. FRET D. SRID
6. The upcoming powerful technology is _____
   A. Biotechnology B. Molecular technology
   C. Microarray technology D. Immuno technology
7. Plaque formation can be seen from _____
   A. 5 to 10 days B. 3 to 10 days
   C. 3 to 5 days D. 3 to 14 days
8._____ membrane is used in blotting
   A. Cellulose B. Nitrocellulose
   C. Sulfocellulose D.Ferricellulose
9. qPCR means_____
   A.Quantification PCR B. Qualifying PCR
   C. Quantitation PCR D. Quality PCR
10. TCID 50 means_____
   A. Time consumed infective dose B. Tissue culture infectious dose
   C. Tissue culture infective dose D. Time consumed infectious dose
11. For propagation, viruses depend on_____cells
   A. Host B. Other
   C. Own D. Neighbour

12. Monopartite genomes means

A.One nucleic acid B. Two nucleic acid

C. Multiple nucleic acid D. No nucleic acid

13. Proteins associated with nucleic acid is called as

A. Proteins B. Nucleous

C. Nucleoproteins D. Capsid

14. Envelope comes from____

A. Virus B. Host

C. Protein D. Nucleic acid

15. Single type of capsomeres stacked around a central axis form a _____

A.Capsid B. Protein coat

C. Nucleocapsid D. Nucleic acid

16. Virus which requires second virus for its replication is called as_____

A. Defective virus B. Helical structure

C. Complex virus D. Provirus

17. Size of Filo virus is

A.80 & 400 nm B.18 & 40 nm

C.80 & 40 nm D. 18 & 400 nm

18. Virus is classified based on _____

A.DNA B. RNA

C. DNA & RNA D. Host

19. _____ symmetry is seen in animal virus

A.Helical B. Icosahedral

C.Radical D. Spiral

20. Capsomers o the triangular faces are surrounded by six others are called as _____

A. Tetrads B. Trions

C. Hexons D. Pentons

Part B

Answer all the questions **3x10 = 30 marks**

21. A. Explain in short about the viral cultivation methods.

Or

B. Write in detail about the old and modern classification of viruses

22. A. Explain in short about the viral purification and separation process.

Or

B. Write in detail about the assay of viruses based on serology.

23. A. Give a detailed note on viral replication

Or

B. What are the structure of viruses and its symmetry?

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Reg. No. : ----------

[15MBU502]

KARPAGAM ACADEMY OF HIGHER EDUCATION
KARPAGAM UNIVERSITY
(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
B.Sc. DEGREE EXAMINATION, August 2017
DEPARTMENT OF MICROBIOLOGY

II INTERNAL TEST - FIFTH SEMESTER

VIROLOGY

Time: 2 hours **Maximum: 50 marks**
Date / Session : **Class: III BSc MB**

PART-A

Multiple Choice Questions **20 x 1 = 20 marks**

11. The size of viruses is usually measured in
A. Centimetres B. Micrometers
C. Nanometers D. Millimeters

2. The temperate phage that has no site specificity for insertion and may even be able to insert multiple copies of their DNA into a single bacterial chromosome is
A. λ phage enzyme B. λ DNA
C. Phage Mu D. Phage Mn

3. Enzyme neuraminidase is carried by which of the following viruses?
A. HIV B. EBV
C. Influenza virus D. Adenovirus

4. Lysozyme (endolysin) which will lyse the bacterial cell, releasing the mature virions is present in
A. Immediate early phage gene B. Late genes
C. Delayed early genes D. Mutated genes

5. Which of the following is continuous cell line?
A. HeLa B. HEp-2
C. KB D. ML

6. Repressor protein, since the cell is resistant to lysis from externally infecting phage, is also called
A.Immunity repressor B. Immunity operon
C. Operon repressor D. Lac operon

7. Which of the following virus is susceptible to chloroform?
A. Herpes B. Influenza
C. Measles D. Human viruses

8. Group E phages have
A. ssDNA B. ds DNA
C. ssRNA D. ds DNA

9. The temperate phage possesses a gene that codes for a repressor protein which makes the cell resistant to lysis initiated by
A. Prophage B. Lytic infection by other viruses
C. Prophage and Lyticphages D. Virus

10. The bacterial viruses having head made up of large capsomeres, but no tail is morphologically classified as
A. A B. B
C. C D. D

11. The process by which phage reproduction is initiated in lysogenized culture is called
A. Infection B. Integration
C. Repression D. Induction

12. Area of lysis on a bacterial lawn culture produced by a phage is known as
A. Pock B. Plaque
C. Pox D. Colony

13. The procapsid is assembled with the aid of _____ proteins.
    A. Ladder B. Framing
    C. Scaffolding D. Form
14. Which of the following is/are synthesized from late mRNA?
    A. Phage structural proteins
    B. Proteins that help with phage assembly without becoming part of the virion structure
    C. Proteins involved in cell lysis and phage release
    D. Proteins from host
15. Which capsid symmetry is exhibited by most of the phages?
    A. Helical B. Icosahedral
    C. Complex D. Non complex
16. Contractile sheath of the tail is present in which of the following phages?
    A. T3 B. T2
    C. P22 D. T4
17. One of the first enzymes synthesized by many bacteriophage is _____ , an RNA-dependent RNA polymerase.
    A. RNA transcriptase B. RNA polymerase
    C. RNA ligase D. RNA replicase
18. _____ cells are used for traditional viral culture
    A. Human cells B. Kidney cells
    C. Human and kidney cells D. Liver cells
19. Which of the following bacteria can be typed by phage typing method?
    A. *S. aureus* B. *S. typhi*
    C. *V. cholerae* D. *E. coli*
20. _____ protein keeps the prophage dormant and prevents virus reproduction.
    A. Operator B. Promotor
    C. Repressor D. Enhancer

Part B

Answer all the questions **3x10 = 30 marks**

21. A. Give a detailed note on viral genome and viral nomenclature.
    Or
    B. How the viruses are got replicated? Give a note with clear diagram

22. A. What are bacterial viruses? How they infect their host?
    Or
    B. Give a brief note on reproduction of T4 phage.

23. A. What is Bacteriophage? Add a note on its replication.
    Or
    B. Give a brief note on reproduction of Ø X174.

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Reg. No. : ----------

[15MBU502]

KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act 1956)

Eachanari post, Coimbatore – 641 021, Tamil Nadu, India

B.Sc. DEGREE MODEL EXAMINATION, September 2017

DEPARTMENT OF MICROBIOLOGY

FIFTH SEMESTER

VIROLOGY

Time: 3 hours **Maximum Marks : 60 marks**

PART-A

Multiple Choice Questions **20 x 1 = 20 marks**

1. The size of viruses is usually measured in.....
   A. Centimetres B. Micrometers
   C. Nanometers D. Millimeters
2. The temperate phage that has no site specificity for insertion and may even be able to insert multiple copies of their DNA into a single bacterial chromosome is.....
   A. λ phage enzyme B. λ DNA
   C. Phage Mu D. Phage Mn
3. Enzyme neuraminidase is carried by which of the following viruses?
   A. HIV B. EBV
   C. Influenza virus D. Adenovirus
4. Lysozyme (endolysin) which will lyse the bacterial cell, releasing the mature virions is present in.....
   A. Immediate early phage gene B. Late genes
   C. Delayed early genes D. Mutated genes
5. Which of the following is continuous cell line?
   A. HeLa B. HEp-2
   C. KB D. ML
6. Repressor protein, since the cell is resistant to lysis from externally infecting phage, is also called
   A.Immunity repressor B. Immunity operon
   C. Operon repressor D. Lac operon
7. Which of the following virus is susceptible to chloroform?
   A. Herpes B. Influenza
   C. Measles D. Human viruses
8. Group E phages have.....
   A. ssDNA B. ds DNA
   C. ssRNA D. ds DNA
9. The temperate phage possesses a gene that codes for a repressor protein which makes the cell resistant to lysis initiated by.....
   A. Prophage B. Lytic infection by other viruses
   C. Prophage and Lyticphages D. Virus
10. The bacterial viruses having head made up of large capsomeres, but no tail is morphologically classified as.....
   A. A B. B
   C. C D. D
11. The process by which phage reproduction is initiated in lysogenized culture is called.....
   A. Infection B. Integration
   C. Repression D. Induction
12. Area of lysis on a bacterial lawn culture produced by a phage is known as.....
   A. Pock B. Plaque
   C. Pox D. Colony
13. The procapsid is assembled with the aid of _____ proteins.
   A. Ladder B. Framing
   C. Scaffolding D. Form

14. Intracytoplasmic inclusion bodies are found in cells infected with

A. Rabies virus B. Vaccinia virus

C. Fowlpox virus D. Cowpox virus

15.Which of the following virus is transmitted by the respiratory route?

A. Rhinovirus B. Coronavirus

C. Measles virus D. HIV

16. Contractile sheath of the tail is present in which of the following phages?

A. T3 B. T2

C. P22 D. T4

17. One of the first enzymes synthesized by many bacteriophage is _____, an RNA-dependent RNA polymerase.

A. RNA transcriptase B. RNA polymerase

C. RNA ligase D. RNA replicase

18. _____ cells are used for traditional viral culture

A. Human cells B. Kidney cells

C. Human and kidney cells D. Liver cells

19. Which of the following bacteria can be typed by phage typing method?

A. *S. aureus* B. *S. typhi*

C. *V. cholerae* D. *E. coli*

20. _____ protein keeps the prophage dormant and prevents virus reproduction.

A. Operator B. Promotor

C. Repressor D. Enhancer

Part B

Answer all the questions **5x8 = 40 marks**

21. A. Explain in short about the viral cultivation methods.

Or

B. Write in detail about the old and modern classification of viruses

22. A. Give a detailed note on viral genome, viral nomenclature and viral symmetry.

Or

B. What is the structure of viruses and its types?

23. A. What is Bacteriophage? Add a note on its replication.

Or

B. Give a brief note on reproduction of Ø X174.

24. A. Explain in detail about the oncogenic viruses

Or

B. Explain the structural composition, replication and transmission of hepatitis virus

25. A. Give a detailed note on TMV.

Or

B. Explain in detail about the HIV.