Semester – II

18MBU201 **VIROLOGY** **(4H –4C)**

Instruction Hours / week: L: 4 T: 0P: 0 **Marks:** Internal: **40** External:**60 T**otal:**100**

End Semester Exam: 3 Hours

COURSE OBJECTIVES

- To study general aspects of viral morphology and classification, replication, interactions and immunity toviruses
- To discuss the application of various immunological and molecular diagnostictools.

COURSE OUTCOME (CO'S)

1. This paper will have clear understanding the role of various in plant, animal and humandisease
2. Candidate able to understand their various mechanisms to enter and escape fromhost.

Unit I- History of viruses

History of viruses.Structure, Classification, nomenclature of viruses.Isolation, purification and cultivation of viruses.Viral assay.Concept of viroids, virusoids, satellite viruses, Virophage and Prions.

Unit II- Bacteriophages

Diversity, classification, one step multiplication curve, lytic and lysogenic phages (lambda phage) concept of early and late proteins, regulation of transcription in lambda phage

Unit III- Transmission of virus

Modes of viral transmission, Salient features of viral Nucleic acid-unusual bases, overlapping genes, splicing genes, terminal redundancy, cohesive ends, capping and tailing.Viral genome Organisation.

Unit IV- Viral multiplication and replication

Viral multiplication and replication-Interaction, and entry, assembly, maturation and release of virions.Oncogenic viruses and its types, mechanism.Viral replication strategies as per Baltimore classification.Prevention and control of viral diseases.

Unit V- Antiviral compounds

Antiviral compounds and their mode of action. Interferon and their mode of action.General principles of viral vaccination.Immunization schedule. Use of viral vectors in cloning and expression, gene therapy and phage display.

SUGGESTED READINGS

1. Dimmock, N.J., Easton., A.L., Leppard, K.N. (2007). Introduction to Modern Virology. 6thedition, Blackwell PublishingLtd.
2. Carte,r J., and Saunders, V. (2007). Virology: Principles and Applications. John Wiley andSons.
3. Flint, S.J., Enquist, L.W., Krug, R.M., Racaniello, V.R., Skalka, A.M. (2004). Principles of Virology, Molecular biology, Pathogenesis and Control. 2nd edition. ASM press WashingtonDC.
4. Levy, J.A., Conrat, H.F., Owens, R.A. (2000). Virology. 3rd edition. Prentice Hall publication, NewJersey.
5. Wagner, E.K., Hewlett, M.J. (2004). Basic Virology. 2nd edition. BlackwellPublishing.
6. Mathews. (2004). Plant Virology. Hull R. Academic Press, NewYork.
7. Nayud, M.V. (2008). Plant Viruses. Tata McGraw Hill,India.
8. Bos, L. (1999) Plant viruses-A text book of plant virology by. BackhuysPublishers.

UNIT I

Duration	Topic	Reference
01	History of viruses	T1:1-72; T2:115-120
01	Structure, Classification, nomenclature of viruses	T2: 31-47; T3:65-78
01	Isolation, purification and cultivation of viruses	T2: 9-28
01	Isolation, purification and cultivation of viruses, Viral assay	
01	Concept of viroids, virusoids, satellite viruses, Virophage and Prions	T1: 55-59
01	Concept of viroids, virusoids, satellite viruses, Virophage and Prions	
01	Unit revision	
	Total hours: 7 h	

TEXTBOOKS

T1: Dimmock, N.J., Easton., A.L., Leppard, K.N. (2007). Introduction to Modern Virology. 6th edition, Blackwell PublishingLtd.

T2: Carte,r J., and Saunders, V. (2007). Virology: Principles and Applications. John Wiley andSons.

T3: Flint, S.J., Enquist, L.W., Krug, R.M., Racaniello, V.R., Skalka, A.M. (2004). Principles of Virology, Molecular biology, Pathogenesis and Control. 2nd edition. ASM press WashingtonDC.

UNIT II

Duration	Topic	Reference
01	Diversity, classification	T2: 229-255
01	one step multiplication curve	W1
01	lytic and lysogenic phages	W2
01	Isolation, purification and cultivation of viruses, Viral assay	
01	concept of early and late proteins	W3
01	regulation of transcription in lambda phage	W4
01	Unit revision	
01	Unit Test	
	Total hours: 7 h	

TEXTBOOKS

T2: Carte,r J., and Saunders, V. (2007). Virology: Principles and Applications. John Wiley andSons.

Web link

W1:http://biotechkhan.wordpress.com/2014/07/01/one-step-multiplication-curve-for-bacteriophage

W2: https://demonstrations.wolfram.com/LifeCycleOfALyticPhageT4Bacteriophage/

W3: https://msu.edu/course/mmg/569/lifecycles.htm

W4: http://www.people.vcu.edu/~elhaij/bnfo301-12/Units/Intro/phage-notes.pdf

UNIT III

Duration	Topic	Reference
01	Modes of viral transmission	T2: 49-58
01	Salient features of viral Nucleic acid	T2: 69-94
01	unusual bases	
01	overlapping genes, splicing genes	
01	terminal redundancy	
01	cohesive ends	
01	capping and tailing	
01	Viral genome Organisation	
01	Unit revision	
	Total hours: 7 h	

TEXTBOOKS

T2: Carte,r J., and Saunders, V. (2007). Virology: Principles and Applications. John Wiley and Sons.

UNIT IV

Duration	Topic	Reference
02	Viral multiplication and replication Interaction, and entry, assembly maturation and release of virions	T1: 172-189 T2: 59-102
01	Oncogenic viruses and its types	T1: 341-362
01	Oncogenic viruses - mechanism	T1: 341-362
01	Viral replication strategies as per Baltimore classification	T1: 102-109
01	Prevention and control of viral diseases	T1: 364-399
01	Unit revision	
	Total hours: 7 h	

TEXTBOOKS

T1: Dimmock, N.J., Easton., A.L., Leppard, K.N. (2007). Introduction to Modern Virology. 6th edition, Blackwell PublishingLtd.

T2: Carte,r J., and Saunders, V. (2007). Virology: Principles and Applications. John Wiley and Sons.

UNIT V

Duration	Topic	Reference
01	Antiviral compounds and their mode of action	T2: 316-325
01	Interferon and their mode of action	T9: 405-408
01	General principles of viral vaccination	T1: 364-399
01	Immunization schedule	T2: 305-312
01	Use of viral vectors in cloning and expression	T10: 525-528
01	Use of viral vectors in gene therapy and phage display	
01	Unit revision	
01	Previous year question paper discussion	
01	Previous year question paper discussion	
01	Previous year question paper discussion	
	Total hours: 11 h	

TEXTBOOKS

T1: Dimmock, N.J., Easton., A.L., Leppard, K.N. (2007). Introduction to Modern Virology. 6th edition, Blackwell PublishingLtd.

T2: Carte,r J., and Saunders, V. (2007). Virology: Principles and Applications. John Wiley andSons.

T9: Rajan.S. 2009, Medical Microbiology, MJP Publishers.

T10: Dubey RC., Maheswari, 2004, A textbook of Microbiology, 1st Edition, S.Chand and Company, New Delhi

.'Unit –I

Syllabus

History of viruses.Structure, Classification, nomenclature of viruses.Isolation, purification and cultivation of viruses.Viral assay.Concept of viroids, virusoids, satellite viruses, Virophage and Prions.

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 20th 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.

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.

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	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-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.

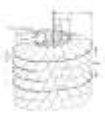


Figure 41-1

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 (2×10^6 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.

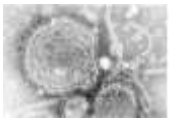


Figure 41-2

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).

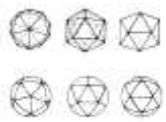


Figure 41-3

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.

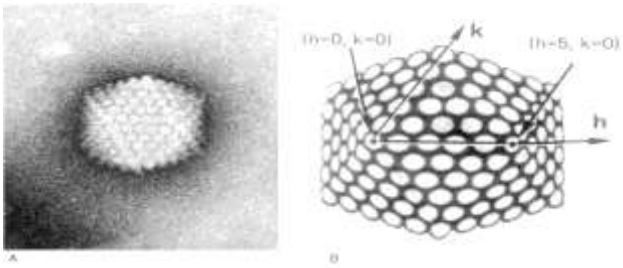


Figure 41-4

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.

Structure

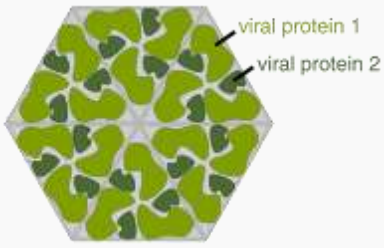
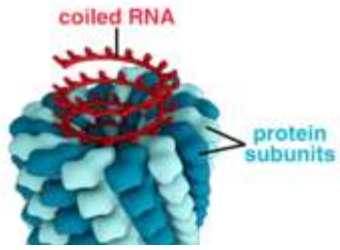
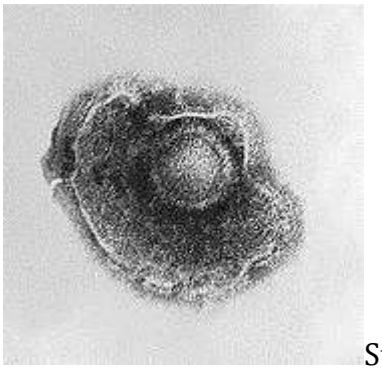


Diagram of how a virus capsid can be constructed using multiple copies of just two protein molecules

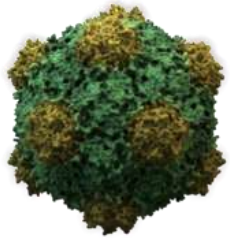


Structure of tobacco mosaic virus: RNA coiled in a helix of repeating protein sub-units

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. 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

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.

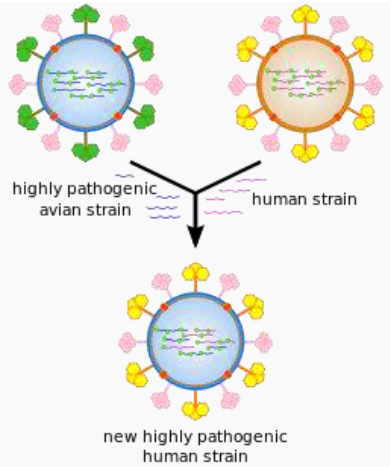
A viral genome, irrespective of nucleic acid type, is almost always either *single-stranded* or *double-stranded*. Single-stranded genomes consist of an unpaired nucleic acid, analogous to one-half of a ladder split down the middle. Double-stranded genomes consist of two complementary paired nucleic 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.

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).

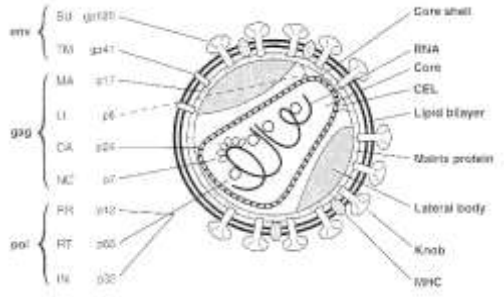


Figure 41-5 -Two-dimensional diagram of HIV-1 correlating (immuno-) electron microscopic findings with the recent nomenclature for the structural components in a 2-letter code and with the molecular weights of the virus structural (glyco-) proteins. SU stands for outer surface glycoprotein, TM for transmembrane 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.

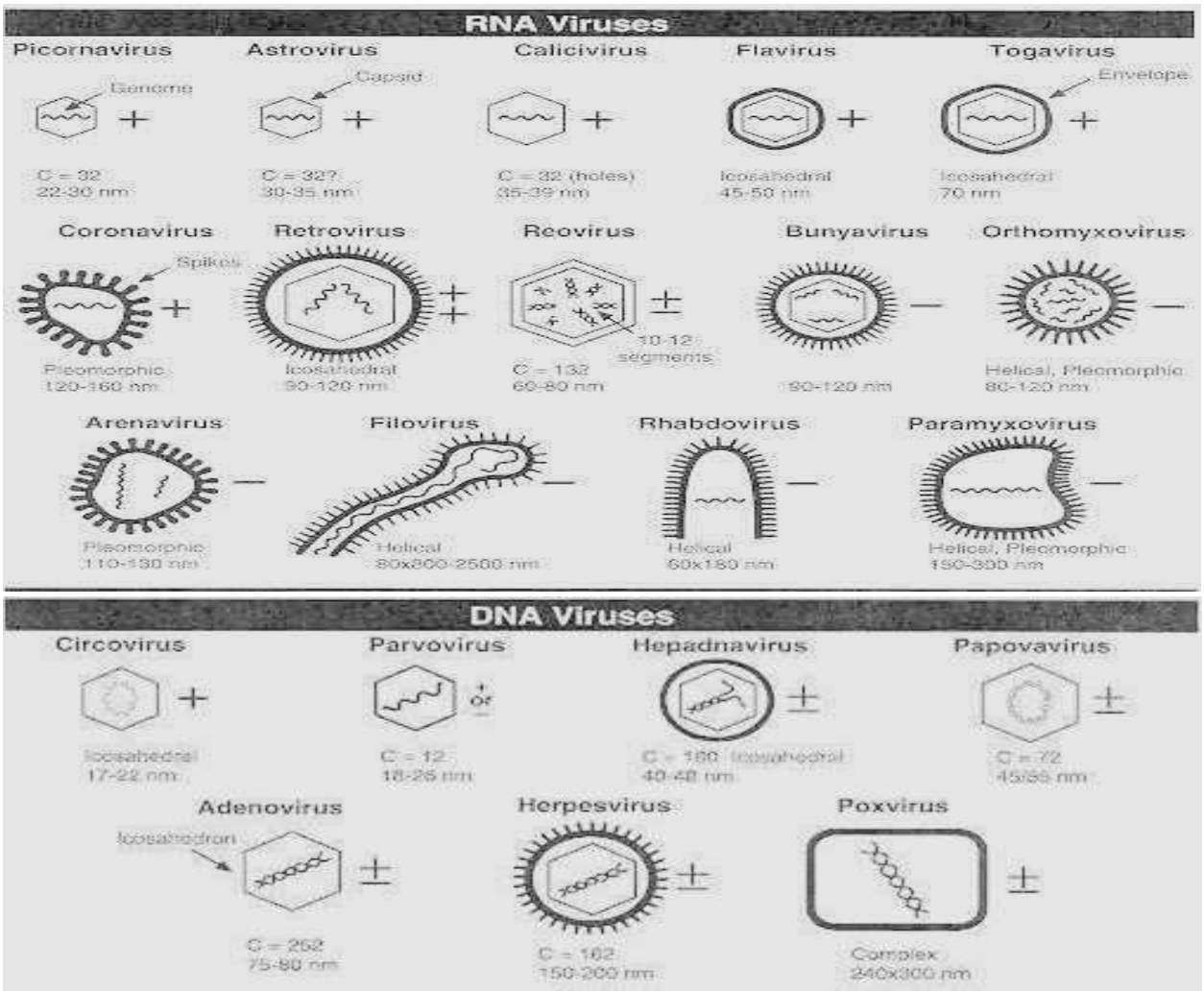


Figure 41-6

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 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.

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

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
	Spomavirinae	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.

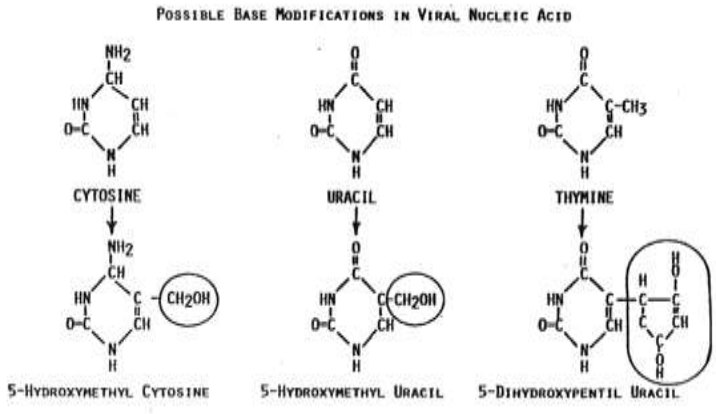
The names of the other families that contain viruses pathogenic for humans are derived as follows: Adenoviridae (adeno, "gland"; refers to the adenoid tissue from which the viruses were first isolated); Astroviridae (astron means star); Arenaviridae (arena "sand") describes the sandy appearance of the 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-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.

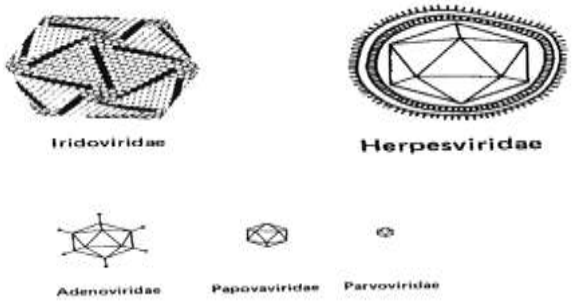
3. Envelope -this is an amorphous structure composed of lipid, protein and carbohydrate which lies to the outside of the capsid.

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.

Morphology (Symmetry)

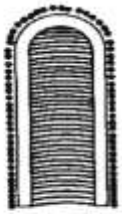
1. Icosahedral -The protomeres aggregate in groups of five or six to form the capsomere. In electron micrographs,
capsomeres are recognized as regularly spaced rings with a central hole. The shape and dimensions of the
icosahedron depends on characteristics of its protomeres. All icosahedral capsids 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.

Poxviridae



Rhabdoviridae

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.

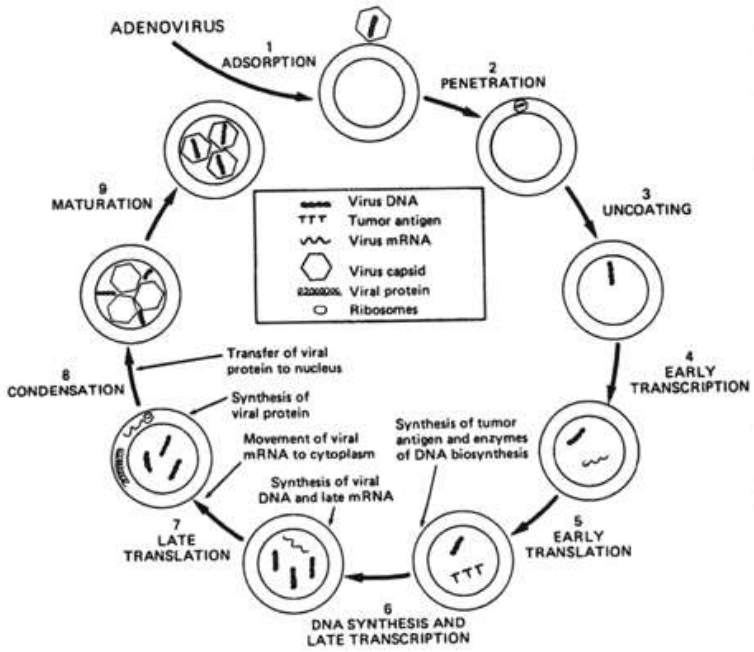
2. **Penetration** -This occurs by one or more processes.

- 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.

Classification of Viruses

Virus classification is the process of naming viruses and placing them into a taxonomic system. Similar to the classification systems used for cellular organisms, virus classification is the subject of ongoing debate and proposals. This is mainly due to the pseudo-living nature of viruses, which is to say they are non-living particles with some chemical characteristics similar to those of life, or non-cellular life. As such, they do not fit neatly into the established biological classification system in place for cellular organisms.

Viruses are mainly classified by phenotypic characteristics, such as morphology, nucleic acid type, mode of replication, host organisms, and the type of disease they cause. The formal taxonomic classification of viruses is the responsibility of the International Committee on Taxonomy of Viruses (ICTV) system, although the Baltimore classification system can be used to place viruses into one of seven groups based on their manner of mRNA synthesis. Specific naming conventions and further classification guidelines are set out by the ICTV.

A catalogue of all the world's known viruses has been proposed; some related preliminary efforts have been accomplished.

Virus species definition

Species form the basis for any biological classification system. The ICTV had adopted the principle that a virus species is a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche. In July 2013, the ICTV definition of species changed to state: "A species is a monophyletic group of viruses whose properties can be distinguished from those of other species by multiple criteria."

ICTV classification

The International Committee on Taxonomy of Viruses began to devise and implement rules for the naming and classification of viruses early in the 1970s, an effort that continues to the present. The ICTV is the only body charged by the International Union of Microbiological Societies with the task of developing, refining, and maintaining a universal virus taxonomy

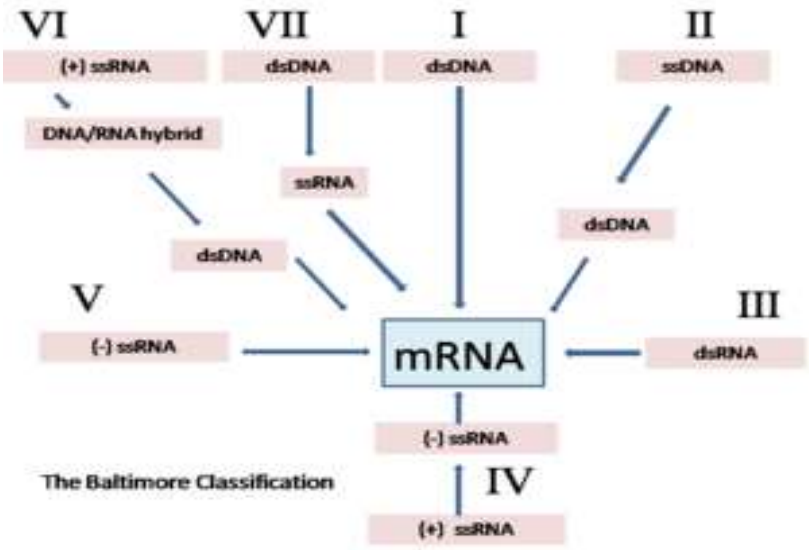
Structure-based virus classification

It has been suggested that similarity in virion assembly and structure observed for certain viral groups infecting hosts from different domains of life (e.g., bacterial tectiviruses and eukaryotic adenoviruses or prokaryotic Caudovirales and eukaryotic herpesviruses) reflects an evolutionary relationship between these viruses. Therefore, structural relationship between viruses has been suggested to be used as a basis for defining higher-level taxa – structure-based viral lineages – that could complement the existing ICTV classification scheme

Baltimore classification

Baltimore classification (first defined in 1971) is a classification system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication. Named after David Baltimore, a Nobel Prize-winning biologist, these groups are designated by Roman numerals. Other classifications are determined by the disease caused by the virus or its morphology, neither of which are satisfactory due to different viruses either causing the same disease or looking very similar. In addition, viral structures are often difficult to determine under the microscope. Classifying viruses according to their genome means that those in a given category will all behave in a similar fashion, offering some indication of how to proceed with further research. Viruses can be placed in one of the seven following groups:[13]

- I: **dsDNA viruses** (e.g. Adenoviruses, Herpesviruses, Poxviruses)
- II: **ssDNA viruses** (+ strand or "sense") DNA (e.g. Parvoviruses)
- III: **dsRNA viruses** (e.g. Reoviruses)
- IV: **(+)ssRNA viruses** (+ strand or sense) RNA (e.g. Picornaviruses, Togaviruses)
- V: **(−)ssRNA viruses** (− strand or antisense) RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
- VI: **ssRNA-RT viruses** (+ strand or sense) RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
- VII: **dsDNA-RT viruses** DNA with RNA intermediate in life-cycle (e.g. Hepadnaviruses)



DNA viruses

Group I: **viruses possess double-stranded DNA. Viruses that cause chickenpox and herpes are found here.**

- **Group II**: viruses possess single-stranded DNA.

Virus family	Examples (common names)	Virion naked/enveloped	Capsid symmetry	Nucleic acid type	Group
1. *Adenoviridae*	Adenovirus, infectious canine hepatitis virus	Naked	Icosahedral	ds	I
2. *Papovaviridae*	Papillomavirus, polyomaviridae, simian vacuolating virus	Naked	Icosahedral	ds circular	I
3. *Parvoviridae*	Parvovirus B19, canine parvovirus	Naked	Icosahedral	ss	II
4. *Herpesviridae*	Herpes simplex virus, varicella-zoster virus, cytomegalovirus, Epstein–Barr virus	Enveloped	Icosahedral	ds	I
5. *Poxviridae*	Smallpox virus, cow pox virus, sheep pox virus, orf virus, monkey pox virus, vaccinia virus	Complex coats	Complex	ds	I
6. Hepadnaviridae	Hepatitis B virus	Enveloped	Icosahedral	circular, partially ds	VII
7. *Anelloviridae*	Torque teno virus	Naked	Icosahedral	ss circular	II

RNA viruses

Group III**: viruses possess double-stranded RNA genomes, e.g. rotavirus.**

- **Group IV**: viruses possess positive-sense single-stranded RNA genomes. Many well known viruses are found in this group, including the picornaviruses (which is a family of viruses that includes well-known viruses like Hepatitis A virus, enteroviruses, rhinoviruses, poliovirus, and foot-and-mouth virus), SARS virus, hepatitis C virus, yellow fever virus, and rubella virus.
- **Group V**: viruses possess negative-sense single-stranded RNA genomes. The deadly Ebola and Marburg viruses are well known members of this group, along with influenza virus, measles, mumps and rabies.

Virus Family	Examples (common names)	Capsid naked/ enveloped	Capsid Symmetry	Nucleic acid type	Group
1. *Reoviridae*	Reovirus, rotavirus	Naked	Icosahedral	ds	III
2. *Picornaviridae*	Enterovirus, rhinovirus, hepatovirus, cardiovirus, aphthovirus, poliovirus, parechovirus, erbovirus, kobuvirus, teschovirus, coxsackie	Naked	Icosahedral	ss	IV
3. *Caliciviridae*	Norwalk virus	Naked	Icosahedral	ss	IV
4. *Togaviridae*	Rubella virus, alphavirus	Enveloped	Icosahedral	ss	IV
5. *Arenaviridae*	Lymphocytic choriomeningitis virus	Enveloped	Complex	ss(-)	V
6. *Flaviviridae*	Dengue virus, hepatitis C virus, yellow	Enveloped	Icosahedral	ss	IV

	fever virus, Zika virus				
7. *Orthomyxoviridae*	Influenzavirus A, influenzavirus B, influenzavirus C, isavirus, thogotovirus	Enveloped	Helical	ss(-)	V
8. *Paramyxoviridae*	Measles virus, mumps virus, respiratory syncytial virus, Rinderpest virus, canine distemper virus	Enveloped	Helical	ss(-)	V
9. *Bunyaviridae*	California encephalitis virus, hantavirus	Enveloped	Helical	ss(-)	V
10. *Rhabdoviridae*	Rabies virus	Enveloped	Helical	ss(-)	V
11. *Filoviridae*	Ebola virus, Marburg virus	Enveloped	Helical	ss(-)	V
12. *Coronaviridae*	Corona virus	Enveloped	Helical	ss	IV
13. *Astroviridae*	Astrovirus	Naked	Icosahedral	ss	IV
14. *Bornaviridae*	Borna disease virus	Enveloped	Helical	ss(-)	V
15. *Arteriviridae*	Arterivirus, equine arteritis virus	Enveloped	Icosahedral	ss	IV

16. *Hepeviridae*	Hepatitis E virus	Naked	Icosahedral	ss	IV
17. *Retroviridae*	HIV	Enveloped			VI

Reverse transcribing viruses[edit]

- **Group VI**: viruses possess single-stranded RNA viruses that replicate through a DNA intermediate. The retroviruses are included in this group, of which HIV is a member.
- **Group VII**: viruses possess double-stranded DNA genomes and replicate using reverse transcriptase. The hepatitis B virus can be found in this group.

Holmes classification

Holmes (1948) used Carl Linnaeus's system of binomial nomenclature to classify viruses into 3 groups under one order, Virales. They are placed as follows:

- **Group I:** *Phaginae* (attacks bacteria)
- **Group II:** *Phytophaginae* (attacks plants)
- **Group III:** *Zoophaginae* (attacks animals)

LHT System of Virus Classification

The LHT System of Virus Classification is based on chemical and physical characters like nucleic acid (DNA or RNA), symmetry (helical or icosahedral or complex), presence of envelope, diameter of capsid, number of capsomers.[14] This classification was approved by the Provisional Committee on Nomenclature of Virus (PNVC) of the International Association of Microbiological Societies (1962).[*citation needed*] It is as follows:

- **Phylum Vira** (divided into 2 subphyla)
    - **Subphylum Deoxyvira** (DNA viruses)
    - **Class Deoxybinala** (dual symmetry)
    - **Order *Urovirales***
    - **Family *Phagoviridae***
        - **Class Deoxyhelica** (helical symmetry)

- **Order *Chitovirales***
- **Family *Poxviridae***
    - **Class Deoxycubica** (cubical symmetry)
- **Order *Peplovirales***
- **Family *Herpesviridae*** (162 capsomeres)
    - **Order *Haplovirales*** (no envelope)
- **Family *Iridoviridae*** (812 capsomeres)
- **Family *Adenoviridae*** (252 capsomeres)
- **Family *Papiloviridae*** (72 capsomeres)
- **Family *Paroviridae*** (32 capsomeres)
- **Family *Microviridae*** (12 capsomeres)
- **Subphylum Ribovira** (RNA viruses)
- **Class Ribocubica**
- **Order *Togovirales***
- **Family *Arboviridae***
    - **Order *Tymovirales***
- **Family *Napoviridae***
- **Family *Reoviridae***
    - **Class Ribohelica**
- **Order *Sagovirales***
- **Family *Stomataviridae***
- **Family *Paramyxoviridae***
- **Family *Myxoviridae***
    - **Order *Rhabdovirales***

- **Suborder *Flexiviridales***

- **Family *Mesoviridae***
- **Family *Peptoviridae***

- **Suborder *Rigidovirales***

- **Family *Pachyviridae***
- **Family *Protoviridae***
- **Family *Polichoviridae***

The Isolation, Cultivation and Identification of Viruses

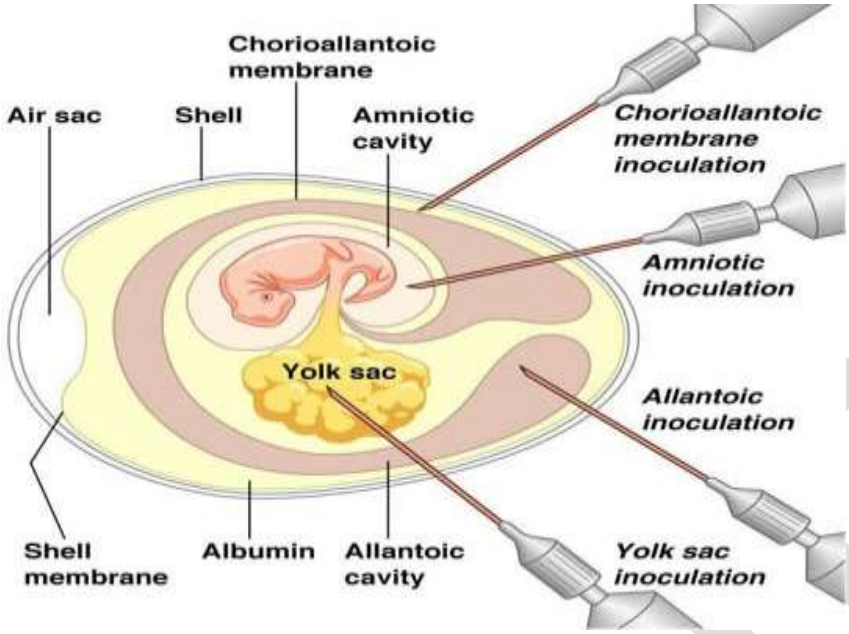
Viruses must be grown in living cells. They can't be grown in culture media or on agar plates alone, they must have living cells to support their replication.
The easiest viruses to grow are bacteriophages (because the easiest cells to grow in the lab are bacteria).

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:
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.

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).



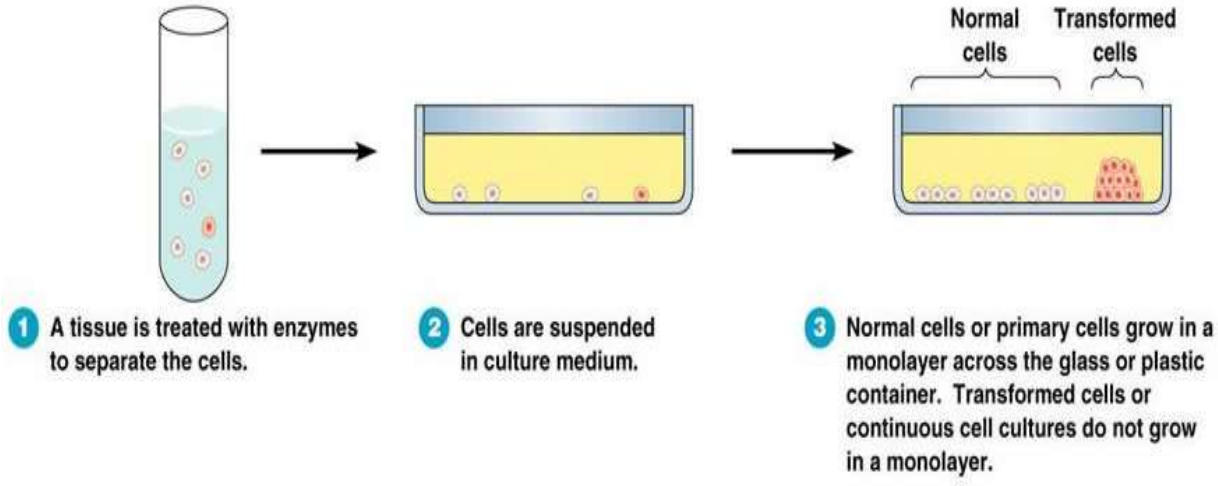
3. Cell culture is a lot cheaper and easier to work with (contamination can be a problem however).

Primary cell lines have a short lifespan in culture – a few generations before reaching senescence.

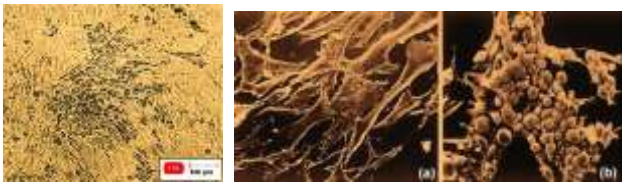
Diploid cell lines are derived from embryos and can grow for up to 100 population doublings before senescence.

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.



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:
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.

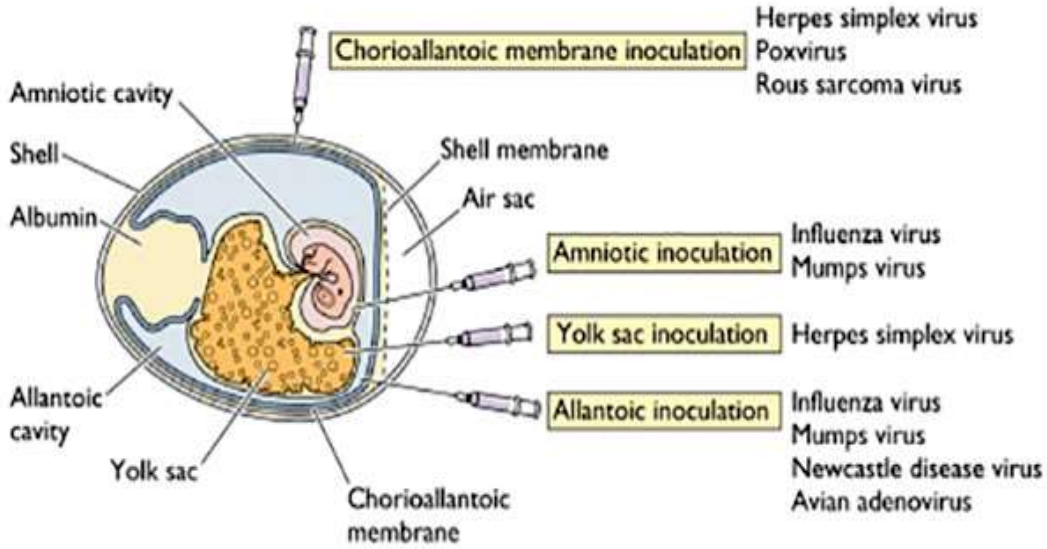
Advantages of Animal Inoculation

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



- 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.
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
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.

Possible questions

Part-B (2 Marks)

1. What are virus and virology?
2. Give a note on early development of virology
3. Explain the classification of viruses?
4. How viruses are differentiated?
5. Give in detail about cultivation of viruses
6. How viruses are purified?
7. Write in detail about the cultivation of animal virus
8. What is DNA virus?
9. What is RNA virus?
10. Give brief account on assay of viruses

Part-C (8 Marks)

1. Write a detailed note on history of Virology.
2. Write a detailed note on Structure of viruses.
3. Write a detailed note on Cultivation of Viruses.
4. Write a detailed note on Classification of Viruses.
5. Write a detailed note Baltimore classification of Viruses.
6. Write a detailed note on Prions, Virusoids.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I B.Sc MB COURSE NAME: VIROLOGY

COURSE CODE: 18MBU201 UNIT: I BATCH-2018-2021

	UNIT - I	Option A	Option B	Option C	Option D	Answers
1	Viruses are_____intracellular parasites.	Obligate	aerobic	anaerobic	Facultative	Obligate
2	Who discovered TMV.	Ivanosky and Beijerinck	Twort and Felix	Edward Jenner	Louis Pasteur	Twort and Felix
3	Which year HIV was discovered	1985	1965	1997	1983	1965
4	Virus means___	pellet	poison	protein	incomplete	poison
5	The complete protein-nucleic acid complex is called as_____	capsid	protein coat	nucleocapsid	nucleic acid	nucleocapsid
6	Virus which requires second virus for its replication is called as_____	defective virus	direct virus	temperate virus	Provirus	defective virus
7	Infectious virus particle	virion	viriod	prion	capsid	virion
8	Virus is classified based on _____-	DNA	RNA	DNA & RNA	Host	DNA & RNA
9	_____ classified virus into seven classes	David Baltimore	Edward jenner	Montangier	Felix	David Baltimore
10	Virus replicates by _____ mechanism	host	own	cell	Direct	host
11	Virus is sensitive to _____	Interleukins	Interferons	antivirals	Antitumours	Interferons
12	_____mice is used for viral inoculation	young	trickling	suckling	Old	suckling
13	_____ is primary detection of virus	CFE	CPE	CDE	CHE	CPE
14	Yolk sac inoculation is used for which virus cultivation.	HIV	Pox	HSV	Influenza	Pox
15	_____ ring culture is done for isolation of Corona virus	lung	liver	trachea	Kidney	trachea
16	At which stage, burst time is calculated	attachment	biosynthesis	uncoating	release	release
17	Advantage of Lytic cycle is/are	replication is fast	replication is slow	host remains live	Generation passes on	replication is fast

18	_____ cells are used for traditional viral culture	human cells	kidney cells	human cells & kidney cells	Plant cells	human cells & kidney cells
19	How viruses are purified	centrifugation	sedimentation	concentration	Dilution	centrifugation
20	Which assay is used for HIV detection	Transformation assay	Endpoint dilution assay	MAGI assay	ELISA	MAGI assay
21	What RBCs are used in haemagglutination assay?	human	monkey	sheep	Dog	sheep
22	Which metal is chelated in BCA	Cs	Mg	Cu	Ag	Cu
23	_____blotting is used for detection of RNA	western	southern	northern	eastern	northern
24	Which enzyme plays a key role in PCR.	ligase	protease	polymerase	helicase	polymerase
25	Real time PCR analysis id performed with help of process called_____	RIA	HIA	FRET	SRID	FRET
26	The upcoming powerful technology is _____	Biotechnology	molecular technology	Microarray technology	immuno technology	Microarray technology
27	Plaque formation can be seen from _____	5 to 10 days	3 to 10 days	3 to 5 days	3 to 14 days	3 to 14 days
28	_____ membrane is used in blotting	cellulose	nitrocellulose	sulfocellulose	ferricellulose	nitrocellulose
29	qPCR means_____	quantification PCR	qualifying PCR	quantitation pCR	quality PCR	quantitation pCR
30	TCID 50 means_____	time consumed infective dose	Tissue culture infectious dose	Tissue culture infective dose	time consumed infectious dose	Tissue culture infectious dose
31	For propagation, viruses depend on_____cells	Host	other	own	neighbour	Host
32	Monopartite genomes means	one nucleic acid	two nucleic acid	multiple nucleic acid	no nucleic acid	one nucleic acid
33	Proteins associated with nucleic acid is called	Proteins	Nucleous	Nucleoproteins	Capsid	Nucleoproteins

	as					
34	Envelope comes from___	virus	host	protein	nucleic acid	host
35	Single type of capsomeres stacked around a central axis form a _____	capsid	protein coat	nucleocapsid	nucleic acid	capsid
36	Virus which requires second virus for its replication is called as_____	defective virus	helical structure	complex virus	Provirus	defective virus
37	Size of Filo virus is	80 & 400 nm	18 & 40 nm	80 & 40 nm	18 & 400 nm	80 & 400 nm
38	Virus is classified based on _____	DNA	RNA	DNA & RNA	Host	DNA & RNA
39	_____ symmetry is seen in animal virus	Helical	Icosahedral	radical	spiral	Icosahedral
40	Capsomers o the triangular faces are surrounded by six others are called as _____	tetrads	trions	hexons	pentons	hexons
41	Minimum number of identical capsomeres required is_____	15	5	12	10	12
42	Which virus is isolated from Chile and Australia	Mega	Mimi	Pandora	Alien	Pandora
43	_____ is size of ss linear DNA	4 - 6 kb	1.7 - 2.3 kb	5.1 - 7.8 kb	6 - 7.8 kb	4 - 6 kb
44	How many non- structural proteins codes for functional in virus transcription	5-8	5-6	5-9	5-7	5-6
45	How many structural proteins will a make up a capsid	3 or 2	3 or 1	3 or 4	3 or 5	3 or 2
46	What is another name of antigenic shift	conjuction	Assortment	recombination	reassortment	reassortment
47	What type of mutation occurs in viral genomes	reverse	frame shift	point	active	point
48	Viruses can enter cells via_____.	penetration	adsoption	Entry	absorption	adsoption
49	During Uncoating stage cellular _____enzymes digest the capsid away from the nucleic acid.	lytic	lipolytic	proteolytic	digestive	proteolytic

50	_____ may be produced during viral replication when the host genome is fragmented	Prions	Virions	Pseudovirions	viriods	Pseudovirions
51	What is the most common cause of aseptic meningitis of viral etiology?	Entero viruses	Herpes viruses	Arbo viruses	Retroviruses	Herpes viruses
52	Protection against influenza A virus in a non immune individual can be achieved through the administration of a drug that interferes with	Viral endonuclease activity	binding of host messenger RNA (mRNA) caps by the viral P1 protein	Synthesis of viral progeny RNA	Uncoating of nucleic acid	Viral endonuclease activity
53	Which one of the following immunizations should be administered immediately after birth?	Diphtheria-pertusis-tetanus (DPT) vaccine	Haemophilus influenzae type b vaccine	Hepatitis B vaccine	HIV Vaccine	Diphtheria-pertusis-tetanus (DPT) vaccine
54	Which one of the following infection routes is most often involved in the neonatal transmission of hepatitis B virus (HBV)?	Blood transfusion	Fetal contact with infected blood during childbirth	Ingestion of the virus via maternal breast milk	Transmission of the virus from hospital personnel during childbirth	Fetal contact with infected blood during childbirth
55	The finding of large, multinucleated, clumps of cells in the bronchial secretions of a 2 year old girl with acute bronchopneumonia suggests that this infection is caused by	Bordetella pertusis	Epstein-Barr virus	Mycoplasma hominis	Respiratory syncytial virus (RSV)	Respiratory syncytial virus (RSV)
56	All of the following picornaviruses are resistant to the acidity of the stomach except:	Coxsackievirus A	Coxsackievirus B	Echo virus	Rhinovirus	Echo virus
57	In a chronic carrier of hepatitis B virus (HBV),	Hepatitis B	Hepatitis B	Hepatitis B e	Anti-HBs Ag	Hepatitis B

	which positive test is most indicative of high infectivity?	Surface Antigen (Hbs Ag)	Core Antigen (Hbc Ag)	Antigen (Hbe Ag)		Surface Antigen (Hbs Ag)

.'Unit –II

Diversity, classification, one step multiplication curve, lytic and lysogenic phages (lambda phage) concept of early and late proteins, regulation of transcription in lambda phage

Bacteriophage

A **bacteriophage** /ˈbækˈtɪər.i.oʊˌfeɪdʒ/ (informally, *phage* /ˈfeɪdʒ/) 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-shaped	Circular 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,	Linear ssRNA	MS2, Qβ

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

Order	Family	Morphology	Nucleic acid	Examples
		isometric		
	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**	

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.

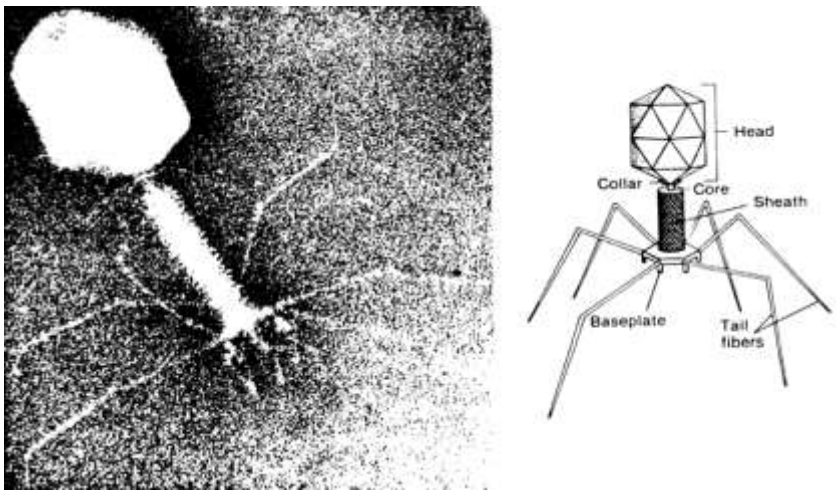
Like most viruses, bacteriophages typically carry only the genetic information needed for replication of their nucleic acid and synthesis of their protein coats. When phages infect their host cell, the order of business is to replicate their nucleic acid and to produce the protective protein coat. But 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.

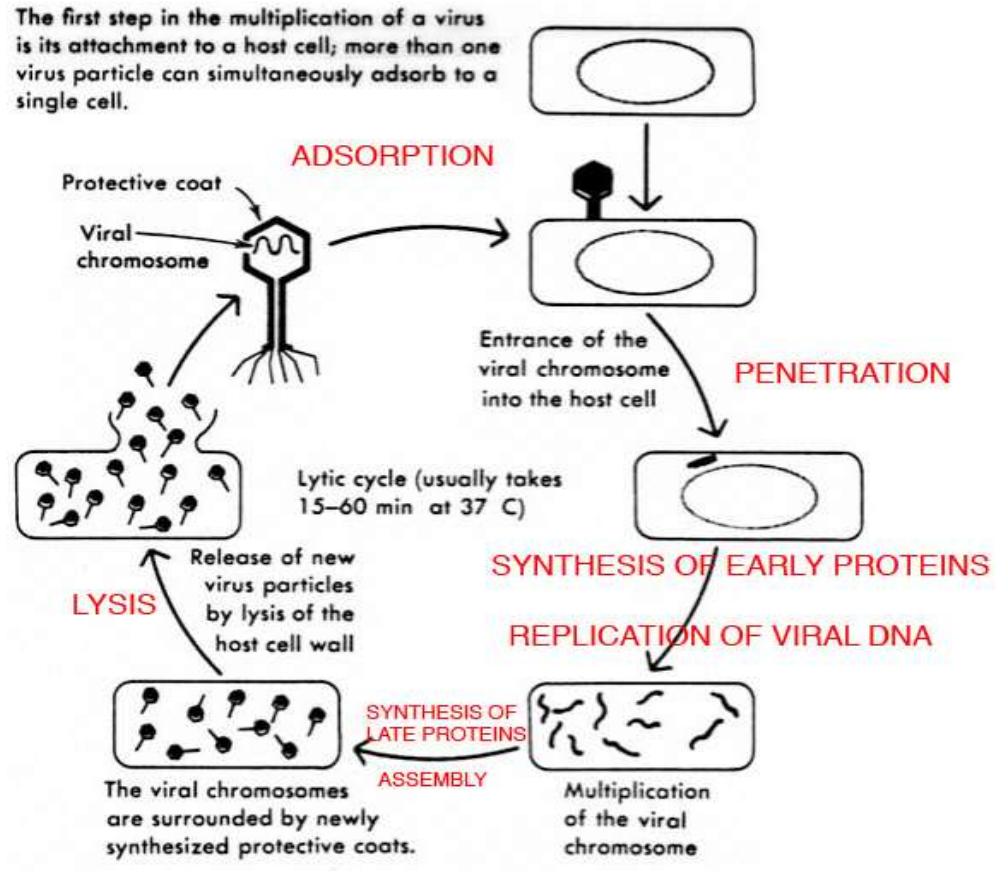
Lytic Infections

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.

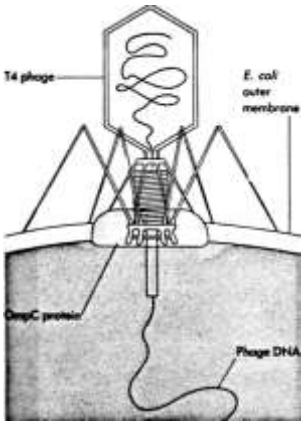
Before viral infection, the cell is involved in replication of its own DNA and transcription and translation of its own genetic information to carry out biosynthesis, growth and cell division. After infection, the viral DNA takes over the machinery of the host cell and uses it to produce the nucleic 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**.

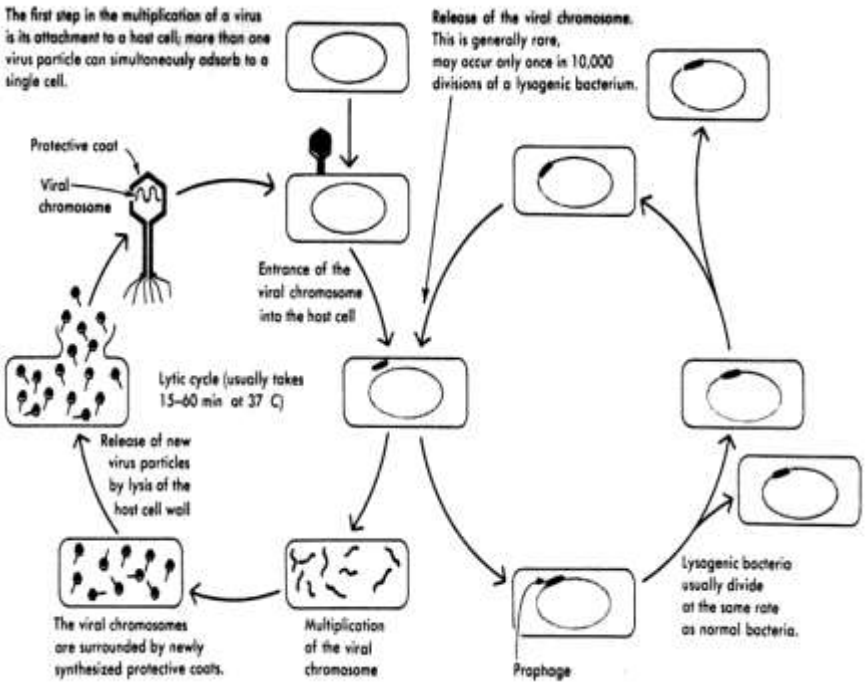
The next step is the synthesis of late proteins. Each of the several replicated copies of the phage DNA can now be used for transcription and translation of a second set of proteins called the **late proteins**. The late proteins are mainly structural proteins that make up the capsomeres and the various 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.



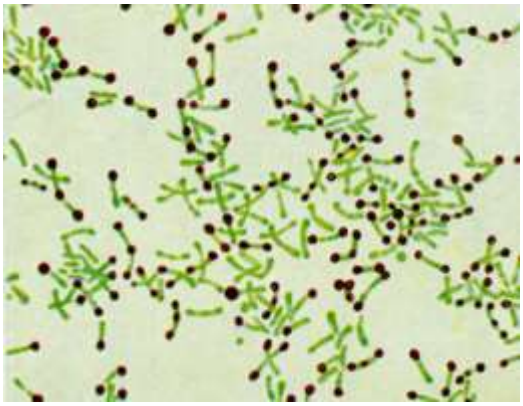
The lysogenic cycle of a temperate bacteriophage such as lambda.

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*.



***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.

The **Double-Stranded DNA (dsDNA) tailed phages**, or Caudovirales, account for 95% of all the phages reported in the scientific literature, and possibly make up the majority of phages on the planet. Nineteen families that infect bacteria and archaeaare currently recognized; of these, 15 have double-stranded DNA genomes.

Under the Baltimore classification scheme, the Caudovirales are group I viruses as they have double-stranded DNA (dsDNA) genomes, which can be anywhere from 18,000 base pairs to 500,000 base pairs in length. The

virus particles have a distinct shape; each virion has an icosohedral head that contains the viral genome, and is attached to a flexible tail by a connector protein. The order encompasses a wide range of viruses, many of which contain genes of similar nucleotide sequence and function. Some tailed bacteriophage genomes can vary quite significantly in nucleotide sequence, however, even among the same genus. Due to their characteristic structure and possession of potentially homologous genes, it is believed these bacteriophages possess a common origin. There are at least 350 recognized species in this order.

Upon encountering a host bacterium, the tail section of the virion binds to receptorson the cell surface and delivers the DNA into the cell by use of an injectisome-like mechanism (an *injectisome* is a nanomachine that evolved for the delivery of proteins by type III secretion). The tail section of the virus punches a hole through the bacterial cell wall and plasma membrane and the genome passes down the tail into the cell. Once inside, the genes are expressed from transcripts made by the host machinery, using host ribosomes. Typically, the genome is replicated by use of concatemers, in which overlapping segments of DNA are made, and then put together to form the whole genome.

Viral capsid proteins come together to form a precursor prohead, into which the genome enters. Once this has occurred, the prohead undergoes maturation by cleavage of capsid subunits to form an icosohedral phage head with 5-fold symmetry. After the head maturation, the tail is joined in one of two ways: either the tail is constructed separately and joined with the connector, or the tail is constructed directly onto the phage head. The tails consist of helix-based proteins with 6-fold symmetry. After maturation of virus particles, the cell is lysed by lysins, holins, or a combination of the two.

Because the lack of homology between the amino acid and DNA sequences of these viruses precludes these from being used as taxonomic markers (as is common for other organisms), the three families here are defined on the basis of morphology. This classification scheme was originated by Bradley in 1969 and has since been extended. All viruses in this order have icosahedral or oblate heads, but differ in the length and contractile abilities of their tails. The Myoviridae have long tails that are contractile, the Podoviridae have short noncontractile tails, and the Siphoviridae have long non-contractile tails. Siphoviridae constitute the majority of the known tailed viruses .

phage T4 is a bacteriophage that infects Escherichia coli bacteria. The T4 phage is a member of the T-even phages, a group including enterobacteriophages T2 and T6. T4 is capable of undergoing only a lytic lifecycle and not the lysogenic lifecycle.

Genome and structure

The T4 phage's double-stranded DNA genome is about 169 kbp long[1] and encodes 289 proteins. The T4 genome is terminally redundant and is first replicated as a unit, then several genomic units are recombined end-to-end to form a concatemer. When packaged, the concatemer is cut at unspecific positions of the same length, leading to several genomes that represent circular permutations of the original.[2] The T4 genome bears eukaryote-like intron sequences.

Translation

The Shine-Dalgarno sequence GAGG dominates in bacteriophage T4 early genes, whereas the sequence GGAG is a target for the T4 endonuclease RegB that initiates the early mRNA degradation.

Virus particle structure

T4 is a relatively large phage, at approximately 90 nm wide and 200 nm long (most phages range from 25 to 200 nm in length). The DNA genome is held in an icosahedral head, also known as a capsid. The T4's tail is hollow so that it can pass its nucleic acid into the cell it is infecting after attachment. The tail attaches to a host

cell with the help of tail fibres. The tail fibres are also important in recognizing host cell surface receptors, so they determine if a bacterium is within the phage's host range.

The structure of the 6 megadalton T4 baseplate that comprises 127 polypeptide chains of 13 different proteins (gene products 5, 5.4, 6, 7, 8, 9, 10, 11, 12, 25, 27, 48 and 53) has recently been described in atomic detail. An atomic model of the proximal region of the tail tube formed by gp54 and the main tube protein gp19 have also been created. The tape measure protein gp29 is present in the baseplate-tail tube complexes, but it could not be modeled.

Infection process

The T4 phage initiates an Escherichia coli infection by binding OmpC porin proteins and Lipopolysaccharide (LPS) on the surface of E. coli cells with its long tail fibers (LTF) A recognition signal is sent through the LTFs to the baseplate. This unravels the short tail fibers (STF) that bind irreversibly to the E. coli cell surface. The baseplate changes conformation and the tail sheath contracts, causing GP5 at the end of the tail tube to puncture the outer membrane of the cell. The lysozyme domain of GP5 is activated and degrades the periplasmic peptidoglycan layer. The remaining part of the membrane is degraded and then DNA from the head of the phage can travel through the tail tube and enter the E. coli cell.

Reproduction

The lytic lifecycle (from entering a bacterium to its destruction) takes approximately 30 minutes (at 37 °C) and consists of:

Adsorption and penetration (starting immediately)
Arrest of host gene expression (starting immediately)
Enzyme synthesis (starting after 5 minutes)
DNA replication (starting after 10 minutes)
Formation of new virus particles (starting after 12 minutes)

After the life cycle is complete, the host cell bursts open and ejects the newly built viruses into the environment, destroying the host cell. T4 has a burst size of approximately 100-150 viral particles per infected host. Complementation, deletion, and recombination tests can be used to map out the rII gene locus by using T4. These bacteriophage infect a host cell with their information and then blow up the host cell, thereby propagating themselves.

Replication and packaging

The rate of DNA replication in a living cell was measured as the rate of phage T4 DNA elongation in phage-infected E. coli.[8] During the period of exponential DNA increase at 37 °C, the rate was 749 nucleotides per second. The mutation rate per base pair per replication during phage T4 DNA synthesis is 1.7 per 10−8,[9] a highly accurate DNA copying mechanism, with only 1 error in 300 copies. The phage also codes for unique DNA repair mechanisms. The T4 DNA packaging motor has been found to load DNA into phage capsids at a rate up to 2000 base pairs per second. The power involved, if scaled up in size, would be equivalent to that of an average automobile engine.

Multiplicity reactivation

Survival curves for phage T4 with DNA damaged by UV (top) or MMC (bottom) after single phage T4 infecting host cells (monocomplexes) or two or more phage T4 simultaneously infecting host cells (multicomplexes).

Multiplicity reactivation (MR) is the process by which two or more virus genomes, each containing inactivating genome damage, can interact within an infected cell to form a viable virus genome. Salvador Luria, while studying UV irradiated phage T4 in 1946, discovered MR and proposed that the observed reactivation of damaged phage occurs by a recombination mechanism. This preceded the confirmation of DNA as the genetic material in 1952 in related phage T2 by the Hershey–Chase experiment.[14]

As remembered by Luria (1984) the discovery of reactivation of irradiated phage (referred to as "multiplicity reactivation") immediately started a flurry of activity in the study of repair of radiation damage within the early phage group . It turned out later that the repair of damaged phage by mutual help that Luria had discovered was only one special case of DNA repair. Cells of all types, not just, bacteria and their viruses, but all organisms studied, including humans, are now known to have complex biochemical processes for repairing DNA damages (see DNA repair). DNA repair processes are also now recognized as playing critical roles in protecting against aging, cancer, and infertility.

MR is usually represented by "survival curves" where survival of plaque forming ability of multiply infected cells (multicomplexes) is plotted against dose of genome damaging agent. For comparison, the survival of phage plaque forming ability of singly infected cells (monocomplexes) is also plotted against dose of genome damaging agent. The top figure shows the survival curves for phage T4 multicomplexes and monocomplexes with increasing dose of UV light. Since survival is plotted on a log scale it is clear that survival of multicomplexes exceeds that of monocomplexes by very large factors (depending on dose). The UV inactivation curve for multicomplexes has an initial shoulder. Other phage T4 DNA damaging agents with shoulders in their multicomplex survival curves are X-rays and ethyl methane sulfonate (EMS). The presence of a shoulder has been interpreted to mean that two recombinational processes are used. The first one repairs DNA with high efficiency (in the "shoulder"), but is saturated in its ability as damage increases; the second pathway functions at all levels of damage. Surviving T4 phage released from multicomplexes show no increase in mutation, indicating that MR of UV irradiated phage is an accurate process.

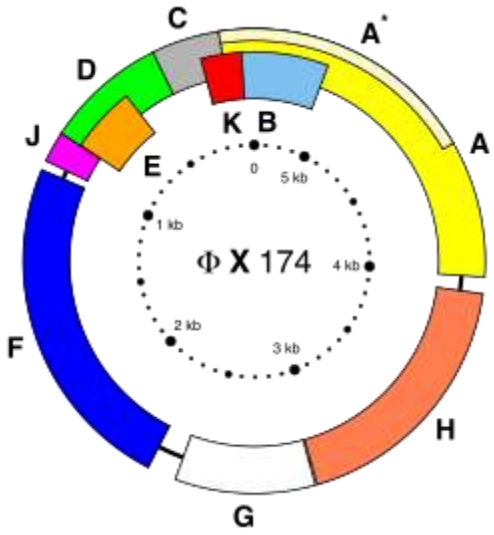
The bottom figure shows the survival curves for inactivation of phage T4 by the DNA damaging agent mitomycin C (MMC). In this case the survival curve for multicomplexes has no initial shoulder, suggesting that only the second recombinational repair process described above is active. The efficiency of repair by this process is indicated by the observation that a dose of MMC that allows survival of only 1 in 1,000 monocomplexes allows survival of about 70% of multicomplexes. Similar multicomplex survival curves (without shoulders) were also obtained for the DNA damaging agents P32 decay, psoralen plus near-UV irradiation (PUVA), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methyl methane sulfonate (MMS) and nitrous acid.

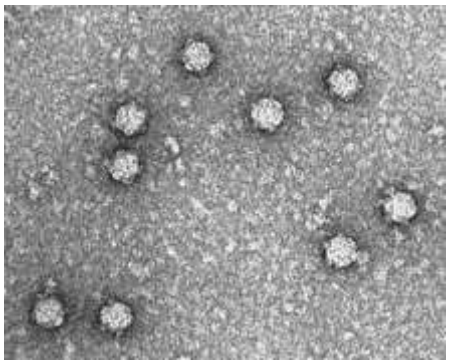
Several of the genes found to be necessary for MR in phage T4 proved to be orthologs for genes essential for recombination in prokaryotes, eukaryotes and archaea. This includes, for instance, T4 gene uvsX[20] which specifies a protein that has three-dimensional structural homology to RecA from Escherichia coli and the homologous protein RAD51 in eukaryotes and RadA in archaea. It has been suggested that the efficient and accurate recombinational repair of DNA damages during MR may be analogous to the recombinational repair process that occurs during meiosis in eukaryotes.

Bacteriophage phiX174

The **phi X 174** (or **ΦX174**) bacteriophage is a virus and was the first DNA-basedgenome to be sequenced. This work was completed by Fred Sanger and his team in 1977. In 1962, Walter Fiers and Robert Sinsheimer had already demonstrated the physical, covalently closed circularity of ΦX174 DNA. Nobel prize winner Arthur Kornberg used ΦX174 as a model to first prove that DNA synthesized in a test tube by purified enzymes could

produce all the features of a natural virus, ushering in the age of synthetic biology. In 2003, it was reported by Craig Venter's group that the genome of ΦX174 was the first to be completely assembled *in vitro* from synthesized oligonucleotides. The ΦX174 virus particle has also been successfully assembled *in vitro*. Recently, it was shown how its highly overlapping genome can be fully decompressed and still remain functional.





This bacteriophage has a [+] circular single-stranded DNA genome of 5386nucleotides encoding 11 proteins. Of these 11 genes, only 8 are essential to viral morphogenesis. The GC-content is 44% and 95% of nucleotides belong to coding genes.

Protein	Copies	Function[1]
A	--	Nicks RF DNA to initiate rolling-circle replication; ligates ends of linear phage DNA to form single-stranded circular DNA
A*	--	Inhibits host cell DNA replication; blocks superinfecting phage; not essential
B	60 in procapsid	Internal scaffolding protein involved in procapsid assembly
C	--	DNA packaging
D	240 in procapsid	External scaffolding protein involved in procapsid assembly
E	--	Host cell lysis
F	60 in virion	Major capsid protein
G	60 in virion	Major spike protein
H	12 in virion	DNA pilot protein (or minor spike protein)
J	60 in virion	Binds to new single-stranded phage DNA; accompanies phage DNA into procapsid
K	--	Optimizes burst size; not essential

Infection begins when G protein binds to lipopolysaccharides on the bacterial host cell surface. H protein (or the DNA Pilot Protein) pilots the viral genome through the bacterial membrane of *E.coli* bacteria (Jazwinski et al. 1975) most likely via a predicted N-terminal transmembrane domain helix (Tusnady and Simon, 2001). However, it has become apparent that H protein is a multifunctional protein (Cherwa, Young and Fane, 2011). This is the only viral capsid protein of ΦX174 to lack a crystal structure for a couple of reasons. It has low aromatic content and high glycine content, making the protein structure very flexible and in addition, individual hydrogen atoms (the R group for glycines) are difficult to detect in protein crystallography. Additionally, H protein induces lysis of the bacterial host at high concentrations as the predicted N-terminal transmembrane helix easily pokes holes through the bacterial wall. By bioinformatics, this protein contains four predicted coiled-coil domains which has a significant homology to known transcription factors. Additionally, it was determined by Ruboyianes et al. (2009) that *de novo* H protein was required for optimal synthesis of other viral proteins. Interestingly, mutations in H protein that prevent viral incorporation, can be overcome when excess amounts of Protein B, the internal scaffolding protein, are supplied.

The DNA is ejected through a hydrophilic channel at the 5-fold vertex (McKenna et al. 1992). It is understood that H protein resides in this area but experimental evidence has not verified its exact location. Once inside the host bacterium, replication of the [+] ssDNA genome proceeds via negative sense DNA intermediate. This is done as the phage genome supercoils and the secondary structure formed by such supercoiling attracts a primosome protein complex. This translocates once around the genome and synthesizes a [-]ssDNA from the positive original genome. [+]ssDNA genomes to package into viruses are created from this by a rolling circle mechanism. This is the mechanism by which the double stranded supercoiled genome is nicked on the negative strand by a virus-encoded A protein, also attracting a bacterial DNA Polymerase to the site of cleavage. DNAP will use the negative strand as a template to make positive sense DNA. As it translocates around the genome it displaces the outer strand of already-synthesised DNA, which is immediately coated by ssBP proteins. The A protein will cleave the complete genome every time it recognises the origin sequence.

As D protein is the most abundant gene transcript, it is the most protein in the viral procapsid. Similarly, gene transcripts for F, J, and G are more abundant than for H as the stoichiometry for these structural proteins is 5:5:5:1.The primosome are protein complexes which attach/bind the enzyme helicase on the template. primosomes gives RNA primers for DNA synthesis to strands.

Phi X is regularly used as a positive control in DNA sequencing due to its relatively small genome size in comparison to other organisms and the extensive work that has been done on it.

A Milestone at the PDB

The 10,000th entry in the Protein Data Bank, the bacteriophage phiX174, is a perfect example of how the science of protein structure has progressed in four decades. In 1960, the world got its first look at the structure of a protein. That first structure was the small protein myoglobin, composed of one protein chain and one heme group--about 1260 atoms in all. By contrast, the 10,000th entry in the PDB contains 420 protein chains and over half a million atoms. Enormous structures like this are not uncommon in the Protein Data Bank. The stakes have risen dramatically since the structure of myoglobin was first revealed.

Animal, Mineral, or Vegetable?

A bacteriophage is a virus that attacks bacteria. The phiX174 bacteriophage attacks the common human bacteria *Escherichia coli*, infecting the cell and forcing it to make new viruses. Do you think that viruses are living organisms? PhiX174 is composed of a single circle of DNA surrounded by a shell of proteins. That's all. It can inject its DNA into a bacterial cell, then force the cell to create many new viruses. These viruses then

burst out of the cell, and go on to hijack more bacteria. By itself, it is like an inert rock. But given the proper bacterial host, it is a powerful reproducing machine. What do you think? Is it alive?

A Molecular Time Bomb

The capsid of phiX174 is designed to find bacterial cells, and then infect them with its DNA. Sixty copies of the capsid protein (colored red here) form a spherical shell around the DNA, and the spike proteins (colored orange here) form 12 pentagonal spikes on the surface. It is thought that the DNA is ejected through the middle of the spikes when the virus infects an *Escherichia coli* cell. The DNA itself encodes 11 genes. In order to fit into this tiny protein shell, however, the DNA is so short that the genes must actually overlap.

Assembling a Virus

As one can imagine, assembling 120 protein chains into a perfectly symmetrical shell is a difficult task. PhiX174 uses special scaffolding proteins to ensure that everything ends up in the right place. The capsid and spike proteins spontaneously form pentagonal units, with five copies of each chain. The scaffolding proteins (shown here in light blue and purple) then arrange these pentagon building-blocks into the whole icosahedron, complete with DNA inside. The scaffolding proteins are small, and bind to the inner and outer surfaces of the pentagons, aligning them one next to the other in the proper orientation.

The DNA Inside

PhiX174 has the distinction of being the first DNA genome sequence that was determined. The virus contains one piece of DNA, 5386 bases long, wrapped into a small circle. In the mature virus, this small circle of DNA is packaged inside the icosahedral protein shell, safe for delivery to an unfortunate bacterial target. The PDB entry 1cd3 includes atomic coordinates for the capsid proteins, but the DNA inside is not included. It does not conform to the beautiful icosahedral symmetry of the capsid, and thus cannot be resolved by x-ray crystallography. We must imagine it packed inside, trapped as the last pentamer closes the capsid.

Exploring the Structure

You can easily look at one of the subunits of this bacteriophage. There are seven separate chains in the PDB file 1cd3 . The spike protein, chain G, is small and compact and the capsid protein, chain F, is large. Both are very stable structures composed of two beta-sheets, forming a structure commonly called a "beta-sandwich." The ribbon diagram shows the chain of the spike protein. Notice how the beta-strands, each depicted with an arrow, arrange side-by-side to form the two sheets. Beta-sandwich structures like this are found in many different viruses.

Four copies of a small scaffolding protein (chains 1, 2, 3 and 4) are arranged in the angle between the capsid and spike proteins, ending up on the outside of the final virus capsid. Another small scaffold protein (chain B) is found on the inside of the capsid, where it assists in the capture of DNA. Compare this detailed atomic view of one subunit of the capsid to the picture of the whole capsid shown above, which contains 60 identical copies of each of these seven proteins.

The pictures were created with RasMol. You can create similar pictures by using one of the viewers on the page for PDB entry 1cd3 .

One step multiplication curve for bacteriophages.

The single-step growth experiment of Ellis and Delbruck demonstrates the cyclic replication of the phage. These authors devised a method to demonstrate only a single step of the many steps of phage replication. Essentially they drastically diluted the mixture after attachment of phage to bacteria, so when the infected cells lysed, no new host cells could be found for a second round of infection. A number of modifications have been

introduced since the original experiment was reported. For instance, instead of diluting the initial bacterium:phage mixture, antibodies specific for the phage attachment apparatus may be added to the mixture to

'neutralize' and thus render all of the unadsorbed phage unable to adsorb to any bacterium.

How do you perform this experiment:

Bacteriophages are infected with a very large number of phage particles: The large number of phage ensures all bacteriem are rapidly infected. The high level of infection is called multiplicity of infection (MOI) and can be achieved with a phage to host ratio of 5 to 10 plaque forming units (PFU) per cell. Adsorption of virions to cells is allowed to proceed for suitable time : To replicate, a virus should induce its host to synthesize components that are necessary for the assembly of new virus particles. The virus accomplishes this process by first attaching to the host (adsorption) and then injecting its nucleic acid into the cell (injection or penetration). The viral DNA can stay free in the cell and be replicated as such, or it can be incorporated into the host chromosome and be replicated simultaneously with it. Viral proteins are next synthesized with the host's machinery under the direction of viral DNA and the new virus particles are assembled mechanically. These particles can find their way out of the cell or lyse the cell and be released into the medium, ready to infect new cells.

The phage/cell mixture is then diluted synchronizing the infection or adding antivirus antiserum. This stop the absorption of virions to cells that are infected and also prevent infection of new cells other than those that has been infected. Antivirus antiserum contains antibodies directed against the virus. It binds and occupy all the attachment sites of the viral particles so that no new bacteria cells can attach a virus. As the infection of bacteriophages is synchronised, the interaction of virus with the cell population can be seen as a single interaction between phage and the cell. In order to visualize the infection over time, samples are removed at specified intervals and plated to quantitate the phage present in the culture.

At the start of the experiment, the plaque count is relatively constant over a time period because each infected bacterium will yield only one plaque. A rise in plaque forming units (pfu) to a plateau level occurs as bacteria are lysed and the newly synthesized phage are released into the medium. These phage particles fail to meet susceptible bacteria (due to the dilution of the adsorption mixture) and thus remain free in the culture fluid. The average number of phage released per bacterium is called the **burst size** and this value may be calculated from the data. The burst size varies in accordance with the specific virus, and may range from 10 to 100 for the DNA transducing phages to approximately 20,000 pfu for the RNA viruses. Plaque assay for bacteriophages are performed by mixing the phage into a layer of bacteria which are spread out as an overlay on the surface of an agar plate. As the plate is incubated, the bacteria grow and they become visible as a turbid layer on the plate. When a phage infects bacteria cells, a zone of Lysis or growth inhibition can occur. This produces clear zone in the bacterial lawn known as **plaque.** Each plaque originates from a single phage particle. If the number of phage particles was monitored during growth, a growth curve could be drawn which would be similar to that of the bacterial growth curve except in the last stage.

The phage growth curve starts with a latent or eclipse period (similar to the bacterial lag phase). During this phase, the infection, adsorption, injection and syntheses of new viral DNA and protein coat occur. The next phase is called the maturation or release stage (similar to the log phase in bacteria) when new phage particles are assembled and released. The cycle can then start over with the infection of new cells. In this manner, the shape of the curve would look step-wise and that is why the process is called "one-step phage growth curve".

Stages of one step multiplication curve:

Eclipse: or initial period can be defined as the time period taken for the appearance of first intracellular phages. No phage particles can be detected during this period as the phages are being uncoated and phage DNA is being injected foe replication.

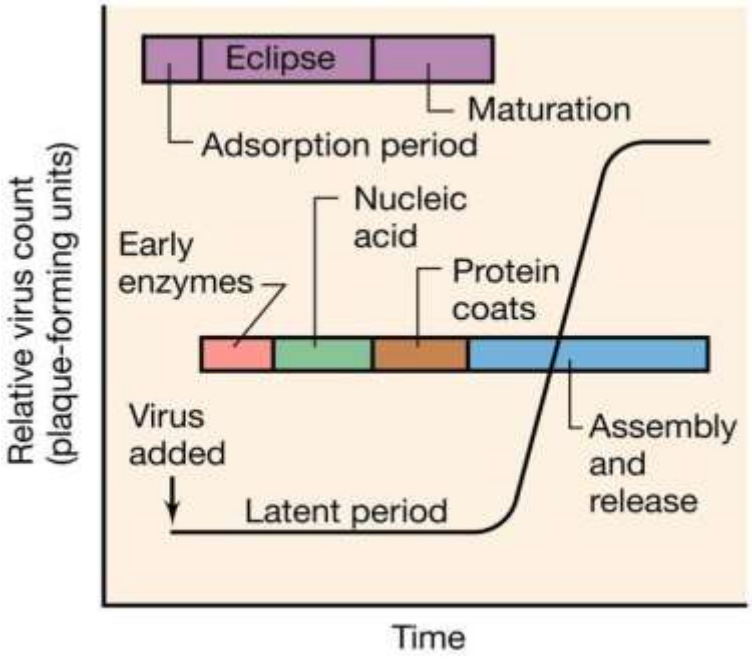
Synthetic period: During synthetic period intracellular particles are being produced. As in the Eclipse period, there are no phage particles released during the synthetic period.

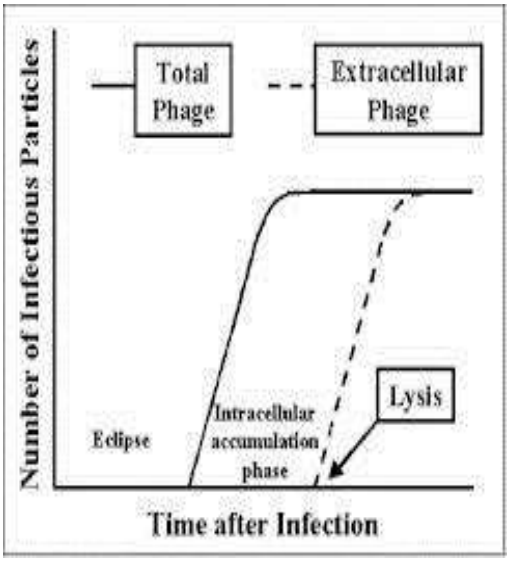
Latent Period: The first two periods are combined in the third period known as latent period. The latent period is described as the time period prior to the release of infection particles or appearance of extra cellular phages.

In the latent period, attachment, entry, replication, transcription, translation and assembly of progeny phages occur.

Rise Period: In this period lysis occurs and extracellular phages appear and they increase in number of concentration of bacteriophages rises.

- **Burst Size:** Average yield of infectious virus per cell is called burst size.
- Burst Size = Final titre of virus / Initial viral titre.
- There is much variation in bursts size between different kind of cells.
- In one study with phage, a burst size of 170 was obtained when growing bacteria cells and value of 20 was obtained with resting bacteria cells. This is because rapid growing cells means, its cell machinery are active and are metabolically active than the resting bacteria cell. So yield of infectious virus increases with growing cells than resting bacteria cell.
- Extra step, **lysis from without (LO):** LO described as an early lysis of bacteria induced by high-multiplicity virion adsorption and that occurs without phage production and leads to killing of bacteria. LO can be induced by adding chloroform, which break open the host cell and the intracellular phages are released. It is an artificial lysis.





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.

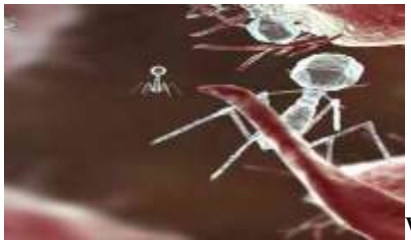
The first regulated, randomized, double-blind clinical trial was reported in the Journal of Wound Care in June 2009, which evaluated the safety and efficacy of a bacteriophage cocktail to treat infected venous leg ulcers in human patients. The FDA approved the study as a Phase I clinical trial. The study's 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 antibiotics, 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

Bacteriophages are also important model organisms for studying principles of evolution and ecology.

Model bacteriophages

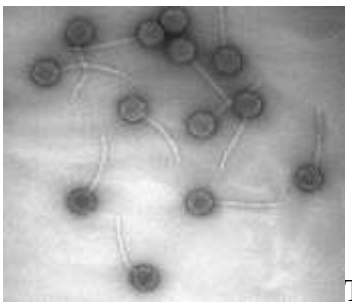
The following bacteriophages are extensively studied:

- λ phage
- T2 phage
- T4 phage (169 kbp genome, 200nm long T7 phage
- T12 phage
- R17 phage
- M13 phage
- MS2 phage (23–25 nm in size
- G4 phage
- P1 phage
- Enterobacteria phage P2
- P4 phage
- Phi X 174 phage
- N4 phage
- Pseudomonas phage Φ6
- Φ29 phage
- 186 phage

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.
- 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 coliphage 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.

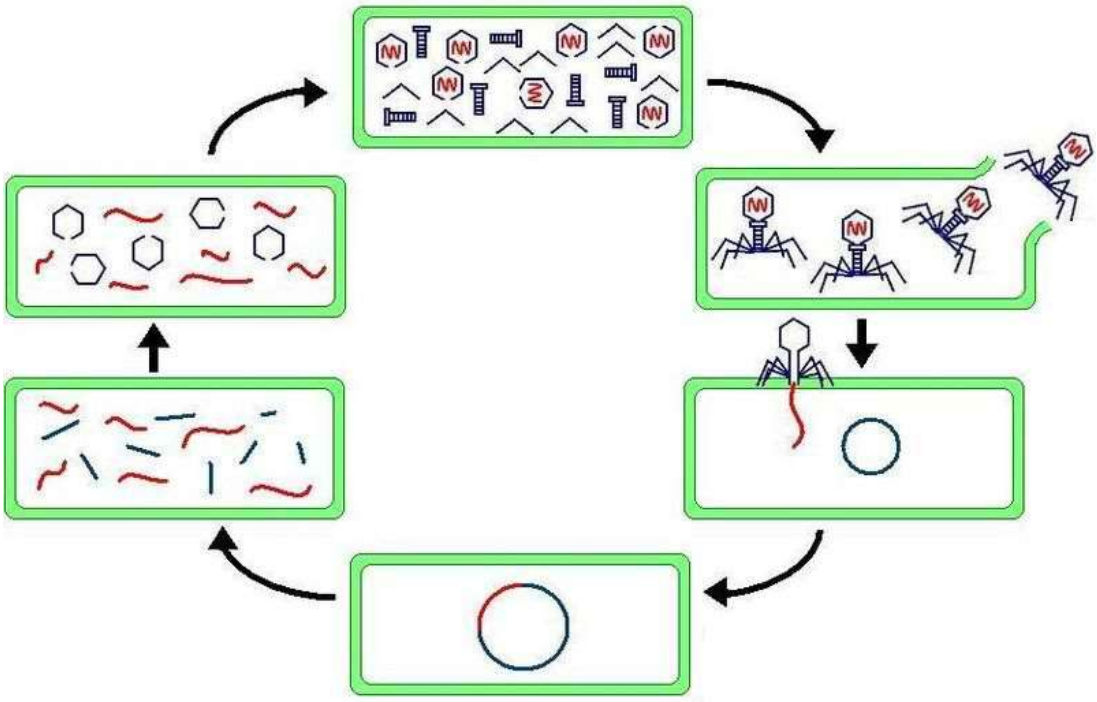
One of the characteristics of bacteriophages is their temperateness. Temperateness refers to the ability of some bacteriophages, particularly lambda phage, to choose between two cycles: lysogenic or lytic. "Temperance" generally refers to the moderation of actions, and in the case of phages, moderation 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.



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

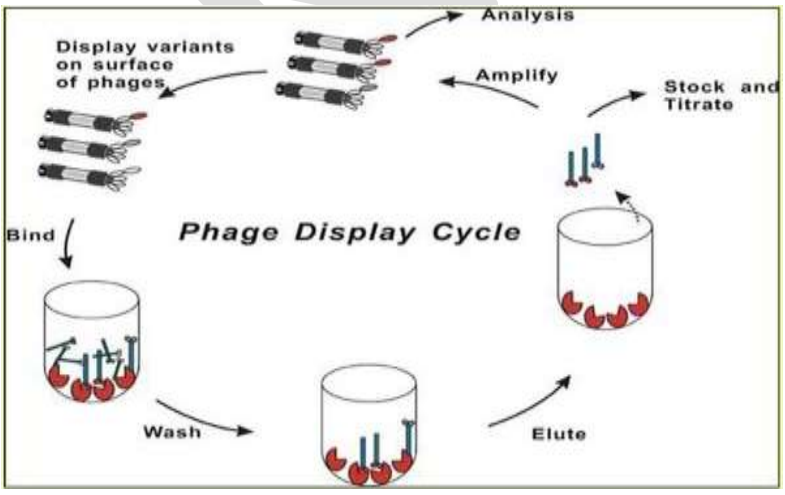
The lytic cycle is similar to the lysogenic cycle in that the host DNA machinery is used to replicate the phage, but the phage is considered a separate molecule from the host DNA. When a temperate phage undergoes the lytic cycle, it becomes a virulent phage. The infected cell and its membrane disintegrate 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



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. 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.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I B.Sc MB COURSE NAME: VIROLOGY

COURSE CODE: 18MBU201 UNIT: II BATCH-2018-2021

	UNIT - II	Option A	Option B	Option C	Option D	Answers
1	Mostly phages are _____ in their shape.	Helical	Icosahedra	tad-pole like	Linear	tad-pole like
2	The head of phage T_4 has a diameter of _____ nm.	65	35	45	55	65
3	The tail has a terminal base plate with totally _____ tail fibres.	3	4	5	6	6
4	The tail of phage T_4 is _____ in length.	10	1000	100	10000	100
5	_____ phage produces lysis of infected cells releasing large number of progeny viruses.	Temperate	Lysogenic	Tryptic	Virulent	Virulent
6	In _____ phage, phage DNA gets integrated into the bacterial chromosome.	Temperate	Lysogenic	Cryptic	Virulent	Temperate
7	The integrated phage is known as _____ .	Coliphage	Prophage	Lytic phage	Prephage	Prophage
8	The bacteria with intetgrated phage is known as _____ .	Lysogen	Colin	Plasmin	Prephage	Lysogen
9	Bacterial lysis occurs without phage multiplication is called _____ .	Lysis	Lysis from without	Lysogenic	Helper phage	Lysis from without
10	_____ phage enzymes weakens the cell wall during replication of phage.	Neuraminidase	Polymerase	Muramidase	cellulose	Muramidase
11	The time interval between the infection of host cell and sudden increase in extracellular virus is called _____.	Eclipse period	Window period	Dormant period	Latent period	Latent period
12	_____ of phage is defined as the highest dilution of phage preparation required to produce confluent lysis.	STD	ISD	RTD	TTD	RTD

13	When phages are applied on a lawn culture of susceptible strain resulting in clear zone, known as _____ after incubation.	Phages	Pock	Plaques	Lysis	Plaques
14	Bacterial cell acquiring new properties after phage infection by the process known as ____ .	Phage Lysis	Phage inversion	Phage immersion	Phage conversion	Phage conversion
15	_____ converts prophage to lytic cycle.	H_2O_2	H_2O	O_2	HO_2	H_2O_2
16	Virus takes _____ time interval from cell adsorption to appearance of newly formed viruses.	20 min	10 min	40 min	60 min	40 min
17	Maintenance of Lysogenic infection is dependent on _____ .	Protein inducer	Protein adsorber	Protein adheser	Protein repressor	Protein repressor
18	The duration of eclipse phase is about _____ in phages.	5-10 min	10-12 min	15-30 min	1-5 min	5-10 min
19	The tail fibre is _____ nm long.	130	120	110	140	130
20	Mostly phages range in size from ---------- ------ nm in length.	20-22	24-200	14-20	15-20	24-200
21	The number of particles released per infected bacteria may be as high as -------- --------	2000	500	1000	100	1000
22	The infectious particle that give rise to a plaque is known as ----------	Plaque forming unit	Pock forming unit	Colony forming unit	Cell forming unit	Plaque forming unit
23	Pfu is ----------	pock forming unit	Plaque forming unit	Plasmid forming unit	Probe forming unit	Plaque forming unit
24	---------- is a phage – coded protein which binds to an operation site on the phage DN	Inducer.	Regulator	Repressor	Operator	Repressor

25	When a lysogenic bacterium is exposed to adverse conditions, the lysogenic state can be ferminated by the process known as ----------	Repression	Regulation	Conversion	Induction	Induction
26	Adverse conditions lead to the production ----------	rec B protein	rec A protein	re C protein	rec D protein	rec A protein
27	Rec A protein is a ---------- enzyme in its activity.	Nucleases	Ligases	Proteases	Kinase	Proteases
28	The lambda phge to enter the lytic (on lysogenic cycle m a cell is determined by the Concentration of the repressor and another phage protein called ---------- in the cell.	Cl	Cro	OR 1	OR 2	Cro
29	A ---------- Protein turns off the synthesis of the repressor and prevents the establishment of lysogeny.	Cl	CRT	Cro	Or 1	Cro
30	In ---------- the United States Food and Drug Administration (FDA) approved using bacteriophages on certain meats to kill the Listeria monocytogenes bacteria, giving GRAS Status	August 2006	September 2006	April 2006	May 2006	August 2006
31	GRAS is ----------	Globally Recorded As Superior	Globally Recorded As safe	Generally recognized as safe	Globally Recognized As safe	Generally recognized as safe
32	Dr. Angela Belcher, founder cambrios Technologies, pioneered the use of ---------- bacteriophage to create nanowires and electrodes.	λ	¢x1 74	Ms 2	M 13	λ

33	The integration of phage λ takes place at a special attachment site in the bacterial genome Called ----------	N	Cro	aHλ	int	aHλ
34	IHF is ----------	Interrupted H factor	Integration host factor	Integrated High factor	Interrupted host factor	Integration host factor
35	Lambda phage was discovered by ---------- in 1951.	Angela Belcher	Fred Sanger	Waher fries	Esther Lederberg	Esther Lederberg
36	---------- is a dimer also known cl protein that regulates the transcription of cl protein and cro protein.	Lambda regulator	Lambda repressor	Lambda inducer	Lambda co inducer	Lambda repressor
37	M 13, a filamentous bacteriophage is ---------- nucleotide long	6207	6307	6407	6507	6407
38	The phage coat is assembled from a 50 amino acid protein called ---------- which is encoded in the phage genome.	pVI	pVIII	p VII	PvI	pVIII
39	The ---------- bacteriophage infects specifically Bacillus Subtitles.	M13	Ms 1	Ms 2	M 12	Ms 2
40	The infection of a bacterium by the naked phage nucleic acid is known as ----------	Transcription	Translation	Transfect ion	Transformative	Transfect ion
41	The presence of high concentrations of upto --------- per ml of phage particles control bacterial population in the particular environment.	10^{-2}	10^{-8}	10^{-3}	10^{-4}	10^{-8}
42	Adsorption is mediated by cofactors such as ----------	Enzyme	Protein	Anions	Cations	Cations
43	Penetration is facilitated by the presence of ---------- on the phage tail that	Ribozyme	Lysozyme	Lipase	Ligase	Lysozyme

	produces a hole in the bacterial wall for the entry of phage DNA					
44	The period in which the number of infectious phages released increases is referred as ---------- Perio	Window	Dormant	Rise	Latent	Rise
45	A lysogenic bacterium is resistant to reinfection by the same (or) related phages is known as	Suppressor immunity	Super infection immunity	Sustainable immunity	Secondary immunity	Super infection immunity
46	The average yield of Progeny phages per infected bacterial cell is known as ----------	Latent period	Small size	Large size	burst size	burst size
47	Assembly of M 13 phage is associated with the membrane of bacteria and requires one Bacterial protein, ----------	redoxin	Bacteriocin	Colicin	thioredoxin	thioredoxin
48	The bacteriophage MS 2 is otherwise known as ----------	Staplylococcus phage M 2	Balillus phage M 2	E. wli phage M 2	Shikgella phage M 2	Balillus phage M 2
49	A ---------- was the first organism to have its DN-based genome to be sequenced in 1977.	M 13 phage	¢ x 714	Ms 2	λ phage	¢ x 714
50	In ---------- Walter fires demonstrated the physical and covalently closed circularity of ¢ x174 DN	1960	1961	1962	1963	1962
51	¢x174 is made of ---------- genes.	11	10	12	13	11
52	---------- is one of the longest DNA-s in phages having icosahedral rea	phage x	phage T4	M 13	M 2	M 2
53	LTF is ----------	Long Tumor formers	Long Terminal fibre	Long Tail fibre	Long Technical	Long Terminal fibre

					former	
54	The lytic life cycle of Bacteriophage T_4 tokes place within ---------- minutes.	10	30	20	2	2
55	A ---------- Bacteriophage has been used as a model in synthetic biology.	T1	T2	T4	T7	T1
56	A ---------- was the first phage typing method developed in 1938.	Vi-phage typing	Bacteriocin typing	HLA typing	MHC typing	Vi-phage typing
57	DVS is ----------	Dissected variable strain	Degenerated variable strain	Degraded Vi-Strains	Desseminated Vi-strains	Degraded Vi-Strains
58	In 1975 ---------- developed a scheme for biotyping to study salmonella typhimunium infections.	Barker et al.	Old et al.	Dudguid et al.	Scarlata et al.	Dudguid et al.
59	There are about ---------- different phage – types of Salmonella typhimurium have been distinguished	252	242	262	232	232

Unit –III

Modes of viral transmission, Salient features of viral Nucleic acid-unusual bases, overlapping genes, splicing genes, terminal redundancy, cohesive ends, capping and tailing.Viral genome Organisation.

Transmission of virus

Outside the host, the infectivity of most viruses is inherently unstable. After being shed from a host animal it is imperative that viruses quickly and efficiently encounter a new host to initiate a fresh infection. The process of transfer between hosts is referred to as transmission and this is an important step in the life cycle of viruses. Knowledge of how viruses are transmitted may enable the cycle to be broken at this stage, preventing further infections.

Since outside the host, the infectivity of most viruses is inherently unstable, this is an important stage in their life cycle. Knowledge of how viruses are transmitted may enable us to break the cycle at this stage and thus prevent further infections. While the routes by which viruses are transmitted between humans are known and can be investigated in the laboratory, the elements that are important in natural transmission are often difficult to investigate and details are poorly understood. All transmission strategies adopted by viruses have in common the ability to circumvent the outer layer of the skin which is impermeable to viruses, and to bring virus into contact with the naked cells. Person-to-person infections are said to take place by *horizontal* transmission, while those from mother to baby are put in a separate category and described as *vertical* transmission. It is no coincidence that most viruses are spread by the respiratory and fecal–oral routes (both examples of horizontal transmission), since, in order to carry out their normal physiological functions, the lungs and small intestine each have a surface of living cells with an area approximately equivalent to that of a tennis-court (400 m^2). It should be remembered that viruses

may be usually transmitted by one particular route, but that other routesmay also be important under certain specific circumstances.

HORIZONTAL TRANSMISSION

Transmission via the respiratory tract

Many virus infections are contracted via the respiratory tract. While many of these cause respiratory infections, other viruses causing generalized infections, such as measles, chicken pox, and smallpox, are contracted by this route (Box 16.1). After virus has multiplied, it either infects additional cells within the same host or escapes from the respiratory tract in liquid droplets that result from our normal activities, such as talking, coughing, and sneezing (and particularly singing). These aerosols are inhaled by others and give rise to a “droplet infection” of a susceptible individual. The size of droplets is important, as those

of very large (>10 μm) diameter rapidly fall to the ground, while the very small droplets (<0.3 μm) dry very quickly, and virus contained within them is inactivated. Thus, the middle-sized range of droplets are those that transmit infection more efficiently. The precise size of these will determine where the droplets are entrapped by the respiratory system of the recipient, since there are baffles lining the nasal cavities that remove the larger airborne particles; the medium sized droplets get as far as the trachea (throat), while the smallest droplets penetrate deep into the lung (Fig. 16.1). However, these particles may be trapped on mucus that is driven upwards by the cilia lining the tubes of the respiratory tract, counter to the incoming air. This is the mucociliary flow. The mucus also serves as a physical barrier that prevents a virus from attaching to its receptors on the surface of cells.

The increase in nasal secretion that accompanies many respiratory infections favors the dispersal of the viruses responsible, and the increase in coughing and sneezing increases the production of infectious aerosols. However, transmission experiments from people infected with a rhinovirus to susceptibles sitting opposite at a table proved singularly unsuccessful. Similar data were obtained with influenza virus in London. Here, chosen families were closely monitored for the transmission of naturally acquired infections. Even in the proximity of a family, an infected spouse (from whom influenza virus could be isolated) frequently did not transmit it to their nonimmune partner. This has led to the suggestions that only particular individuals may shed sufficient virus, produce excess nasal secretion, and/or aerosols containing optimum-sized particles and so act as efficient spreaders of infection, or that, in order to be infected, one has to be in a certain physiological state. Apart from the traditional stories of wet feet predisposing you to catch a cold (not true!), it has been shown that recently bereaved people are particularly susceptible to infectious diseases. Thus, the immune system is influenced by one's state of mind. Neuroimmunology is a developing field of exciting study.

In addition to aerosol transmission via the respiratory tract, it is now believed that some respiratory viruses are spread by contact of droplets with the conjunctiva, the layer of cells covering the eye. Natural drainage to the throat would then carry progeny virus to the respiratory tract. Little information is available, but there is a report of an infection that resulted when a frozen chicken was being dismembered and a particle flew into the cook's eye and resulted in infection. Another variation on standard respiratory transmission is the suspicion that large aerosol droplets that land on solid surfaces can then be transferred from fingers to the conjunctiva or directly into the nose. The importance of airborne aerosols relative to physically borne aerosols in regard to virus transmission remains to be determined.

The dynamics of aerosol infections have been studied in detail by the military, from a biological warfare standpoint, and are known to be highly

complex. There are many germane factors, including the size of the virus-carrying droplets, which determine how long droplets stay airborne and how quickly they dry up and inactivate the infectivity of the virus particles contained therein. The composition of the liquid component of the droplet too affects its size, desiccation, and virus stability. It has been postulated that some infections may not be spread by inhaled droplets, but through large droplets that fall rapidly from the air onto solid surfaces and are then transferred on fingers to the mouth or nose, or to the conjunctiva. There are other possibilities – as some people appear to produce more infected aerosol than others, or more aerosol droplets of a size that is able to spread that virus efficiently, and that this minority of the human population may be responsible for spreading most of the virus. Key points are summarized in Box 16.2.

Environmental factors which result in seasonal variation in the amount of illness or frequency of isolation of a virus are linked with transmission of respiratory infections. Influenza, for example, is a winter disease, occurring around January in the Northern Hemisphere, June in the Southern Hemisphere, but nearly all the year round close to the Equator. Variations both in the environment (e.g. temperature and humidity) and in social behavior (crowding together in winter with poor ventilation) have been identified as factors which could affect virus stability and transmission and hence the seasonal incidence of virus diseases, but a full explanation of these complex phenomena is not yet available. Since viruses can survive for only a limited time outside the cell, it is fair to assume that infected individuals are present continuously somewhere in the population. Interestingly these seasonal patterns for influenza have not been altered by modern mass air travel, which transports thousands of people, many of whom will be incubating the virus, from winter to summer at the other end of the world. However, there is concern that the spread of a new pandemic influenza virus (see Section 17.5) may be enhanced by air travel.

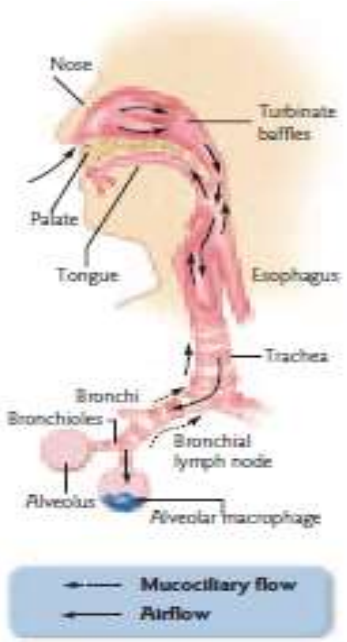


Fig. 16.1 Route taken by aerosol droplets containing virus particles that are breathed into the respiratory tract. Note the defences designed to trap and remove all sorts of small particles: turbinate baffles, the mucociliary flow that runs counter to inspired air, and macrophages. The airways become progressively narrower (here much foreshortened) and end in alveoli, each of which is formed by a single cell. An alveolar macrophage can be seen in one of the alveoli. (Adapted from Mims, C. A., White, D. O. 1984. *Viral Pathogenesis and Immunology.* Blackwell Scientific Publications, Oxford.)

Transmission via the fecal–oral route

Many viruses are ingested with food or water that is contaminated with feces, and infect and multiply in cells of the alimentary tract. This is the fecal–oral route of infection. The surface of the small intestine normally functions to adsorb nutrients and water, and can potentially be infected by viruses. Two well known picornaviruses that invade by this route are poliovirus and hepatitis A virus. Poliovirus infections traditionally result from ingesting sewage-contaminated drinking water, but more recently have come from ingesting the water of swimming pools that were inadequately disinfected. Hepatitis A virus is commonly experienced by travellers as a result of encounters with

drinking sewage-contaminated water, or more subtly when such water has been used to wash fruit or salad vegetables, or used to prepare ice-cubes for drinks. All viruses transmitted via the fecal–oral route are excreted in feces, so their spread is favored by poor sanitation and poor personal hygiene. Many of these viruses grow to very high titers (10^9 infectious units (IU)/ml), but would probably transmit even if there were only 10^6 IU/ml of feces – as just 1 µl of feces contains 10^3 IU. Many of these infections occur in early childhood – not surprising, when it is seen how small children investigate strange objects by putting them in their mouth, and that their personal hygiene is not well developed. Some virus infections (rotavirus) are associated with diarrhea which, though not proven, could be an adaptation to improving virus transmission.

With improvements in sanitation it would be expected that there would be a reduction of disease caused by enteric viruses, and this is the experience. However, there have been some unpleasant surprises. In conditions of poor sanitation, poliovirus infects young children as soon as they can crawl outside the house, but results usually in a subclinical gut infection. With improved sanitation, poliovirus is not contracted until adolescence, and is then associated with an increase in the incidence of paralytic poliomyelitis. This appears to be another example of increased severity of a disease seen when a virus infects an "unnatural" host – in this case the age of the individual is the key difference.

Sexual transmission

Only a few viruses are spread by sexual transmission but, for them, it is a very successful route indeed. The main examples are HIV-1 and hepatitis B virus (HBV), but herpes simplex viruses (HSV) type 2 predominantly but also type 1, and some types of human papilloma virus, are also spread sexually. HIV-1 has only been recognized as the causative agent of AIDS since 1983 and is currently responsible for more than 40 million infections worldwide. AIDS predisposes the infected individuals to both viral and nonviral infections of exaggerated severity, and has a mortality rate approaching 100% (see Chapter 19). HBV is responsible for primary hepatocellular carcinoma in 0.1% of infected individuals, and even with this low incidence rate it the commonest human cancer because the virus is endemic in the very large population of China and the Far East (see Section

14.5). These viruses are transmitted by heterosexual intercourse, both from male to female, and from female to male, and homosexual intercourse. For HIV-1 the risk of infection is marginally less (by two- to threefold) if the female is the carrier. This may be because ejaculate contains not only virus but also around 10^6 lymphocytes, some of which may be infected and producing virus. Virus is also spread by homosexual behavior and the risk for receptive anal intercourse (which is 1/300 to 1/1000) is about the same as that in male–female intercourse when the male is infected. Sexual transmission of infection is affected by sociosexual behavior and spread of these viruses is increased by promiscuity. Rates of HIV-1 infection are stable in some countries, but the worldwide pandemic is moving eastwards and shows no signs of slowing down.

Transmission via urine

Transmission of viruses in urine is rare as urine is usually sterile. However, a few viruses, such as Lassa fever (an African arenavirus) and sin nombre (a North American bunyavirus), are excreted in the urine of their hosts, and are thought to be transmitted in this way. These viruses infect wild mice, but in rural areas at certain times of the year mice enter houses. Infected urine can then contaminate surfaces where food is prepared and virus is ingested. Alternatively, virus-contaminated dust particles may be breathed in. Both viruses cause rare human fatalities. In addition, cytomegalovirus and polyomaviruses are excreted in the urine of small children in large amounts, and may be transmitted by this route.

Transmission by mechanical means

Animal viruses transmitted by mechanical means include those which infect their hosts by various methods that directly puncture the normally impermeable skin layer. This route involves the role of *virus vectors* – animals that transmit the virus to man. Such a relationship is a zoonosis (see Section 16.3). The vectors of virus transmission are usually biting arthropods (mosquitoes which are insects, and ticks which are arachnids) found in tropical parts, which feed on human blood by piercing the skin with their mouth parts. Preparatory to the next meal, the arthropod injects saliva

as an anticoagulant, and in so doing introduces the virus that was picked up from the last blood meal. Viruses spread by arthropods (*arthropod-borne viruses*) are known collectively as *arboviruses* (Box 16.3).

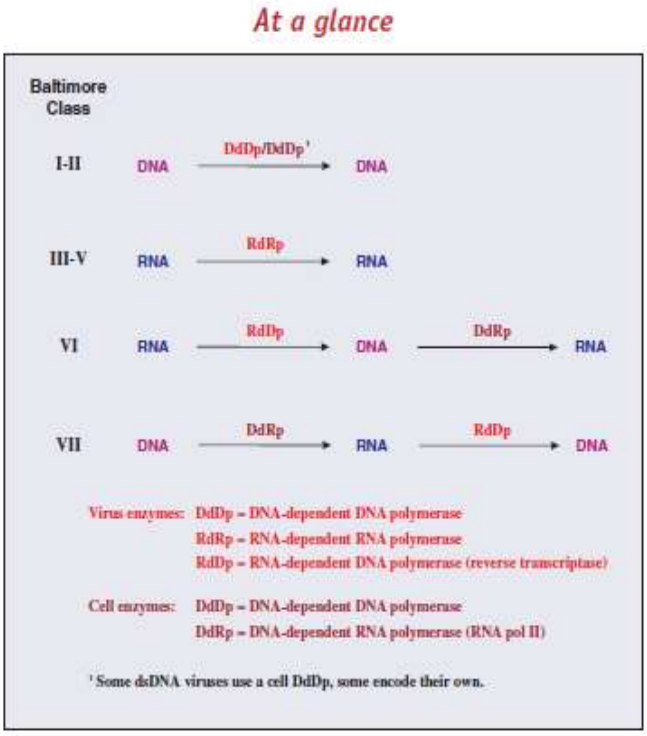
The main groups of animal viruses that are spread by mosquitoes belong to the alpha-, flavi-, reo-, rhabdo-, and bunyavirus groups. Transmission of a virus is specific to a particular mosquito species. Such viruses have a complex life cycle, and are adapted to multiply in the tissues of the invertebrate vector as well as those of the vertebrate host. Although arboviruses are found mostly in the tropics, examples from more temperate regions are the flaviviruses, tick-borne encephalitis virus, that is found in Europe from Austria eastwards, and louping-ill virus of grouse that is spread by *Ixodes* ticks and is found in the northern UK, Ireland, and Norway. Sheep and humans in those regions are occasionally infected with louping-ill virus through bites from infected ticks. Other arthropod-borne animal viruses do not multiply in their vector but are carried passively. For example, myxomavirus (a poxvirus) that causes myxomatosis is spread between rabbit hosts on the contaminated mouth parts of infected mosquitoes in Australia and rabbit fleas in the UK.

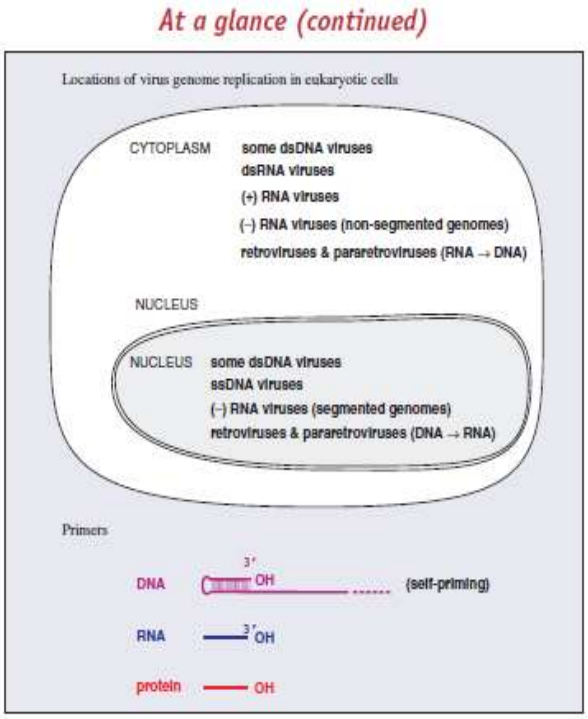
VERTICAL TRANSMISSION

Vertical transmission is the transfer of virus from mother to fetus/baby, in contrast to the horizontal transmission between other individuals. Rubella virus (a togavirus) is the classic example of a vertically transmitted virus, although it is normally spread by the respiratory route. In an adult the infection (German measles) is manifest as a mild skin rash or is subclinical. However, the virus can cross the placenta and multiply in the fetus. As a result the fetus can die or can be born with serious congenital malformations that affect the cardiovascular and central nervous systems, the eyes, and hearing. The risk of malformations arising from rubella virus infection is high in the early stages of pregnancy (up to 80%) and decreases as fetal development proceeds, to become almost no risk by the fifth month of pregnancy or later. Fortunately, there is an excellent live attenuated vaccine (MMR, measles virus, mumps virus and rubella virus combined) that is given to young children and protects them through adulthood. Other viruses which infect the mother and can be passed vertically to her fetus/baby are listed in Table 16.1 together with the outcomes affecting the fetus or newborn infant.

Analysis of the exact route of vertical transmission is not possible as the virus can be transmitted to the zygote from an infected oocyte or sperm, or the zygote can be infected from virus present in cells of the uterus, or via the bloodstream in placental mammals. Any virus that infects the mother while she is producing eggs or offspring can, in theory, be vertically transmitted, while those whose genomes are integrated with that of the reproductive cells of the host cannot help but be transmitted by

Virus genome replication





In this chapter we consider the fifth step of our generalized replication cycle: genome replication. The genome of the infecting virus is replicated so that viral transcription can be amplified and to provide copies of the genome for progeny virions. Generally, DNA viruses copy their genomes directly to DNA and RNA viruses copy their genomes directly to RNA. There are, however, some DNA viruses that replicate their genomes via an RNA intermediate and some RNA viruses that replicate their genomes via a DNA intermediate. The various replication modes of virus genomes are summarized in Figure 7.1. Single-stranded genomes are designated as plus or minus depending on their relationship to the virus mRNA. Plus strand genomes have the same sequence as the mRNA (except that in DNA thymine replaces uracil), while minus-strand genomes have the sequence complementary to the mRNA. Single-stranded DNA is converted to dsDNA prior to copying. There are two classes of viruses with (+) RNA genomes (Figure 7.1). Class IV viruses copy their (+) RNA genomes via a (−) RNA intermediate, while Class VI viruses replicate via a DNA intermediate. The synthesis of DNA from an RNA template (reverse transcription) is

also a characteristic of Class VII viruses. In this chapter we shall look at some general aspects of virus genome replication, and then we shall give individual attention to replication of the genomes of the DNA viruses, the RNA viruses and the reverse transcribing viruses.

Locations of virus genome replication in eukaryotic cells

As we saw in Chapter 5, when viruses infect eukaryotic cells the genomes of some are delivered to the cytoplasm and some are conveyed to the nucleus. The destination of a virus genome, and hence the location in which it is replicated, varies with the type of genome (Table 7.1). The genomes of most DNA viruses are replicated in the nucleus, but those of some dsDNA viruses are replicated in the cytoplasm. The genomes of most RNA viruses are replicated in the cytoplasm, but those of the minus-strand RNA viruses with segmented genomesare replicated in the nucleus. The retroviruses and pararetroviruses are special cases: each replicates RNA to DNA in the cytoplasm and DNA to RNA in the nucleus.

Initiation of genome replication

Each virus genome has a specific sequence where nucleic acid replication is initiated. This sequence is recognized by the proteins that initiate replication. Nucleic acid replication requires priming, which is the first reaction of a nucleotide with an –OH group on a molecule at the initiation site. Replication of the genomes of many RNA viruses (including rotaviruses, and rhabdoviruses) initiates when the first nucleotide of the new strand base pairs with a nucleotide in the viral RNA. The initial nucleotide effectively acts as a primer for RNA replication when its 3_ –OH group becomes linked to the second nucleotide.

Some ssDNA viruses, such as parvoviruses, use self-priming. At the 3_ end of the DNA there are regions with complementary sequences that can base pair (Figure 7.2). The –OH group of the nucleotide at the 3_ end forms a linkage with the first nucleotide, then DNA synthesis proceeds by a rather complex process to ensure that the whole genome is copied. In order to initiate the replication of many DNA genomes, and some RNA genomes, a molecule of RNA or protein is required to act as a primer.

RNA and protein primers

Synthesis of cell DNA commences after a region of the double helix has been unwound by a helicase and

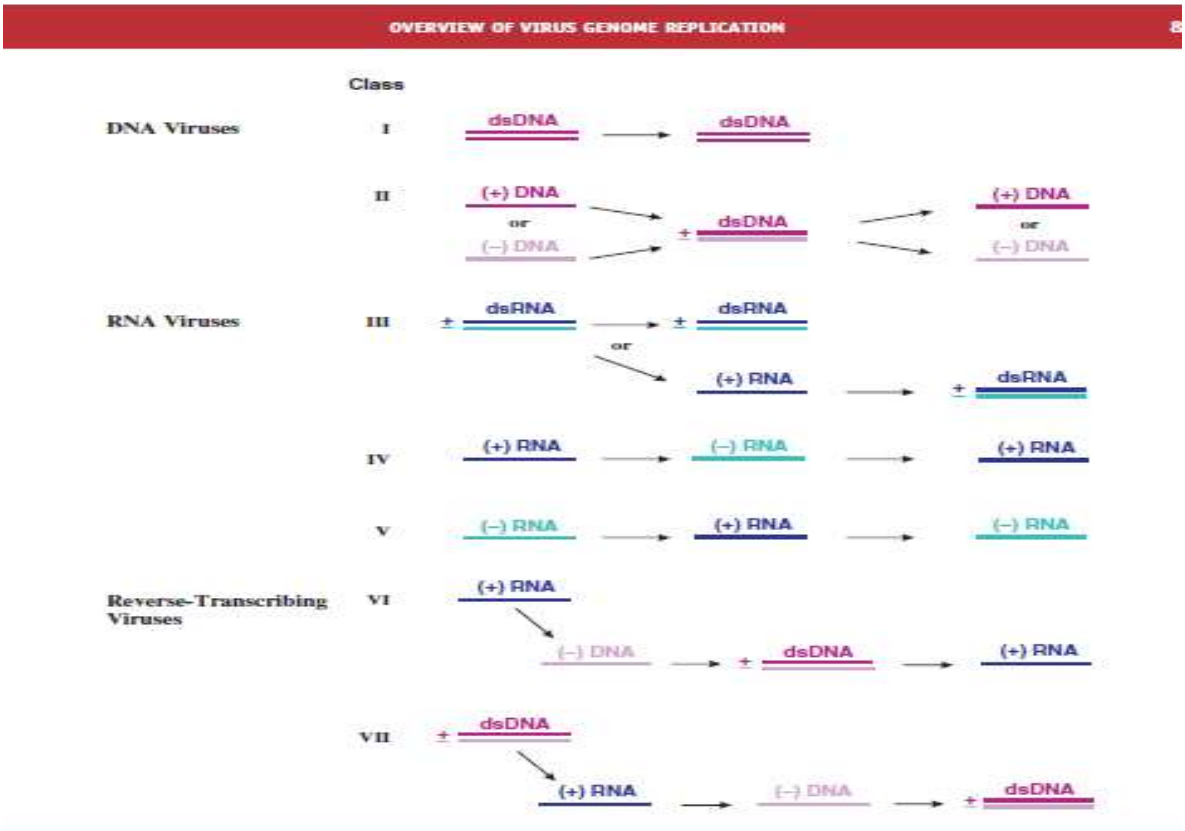
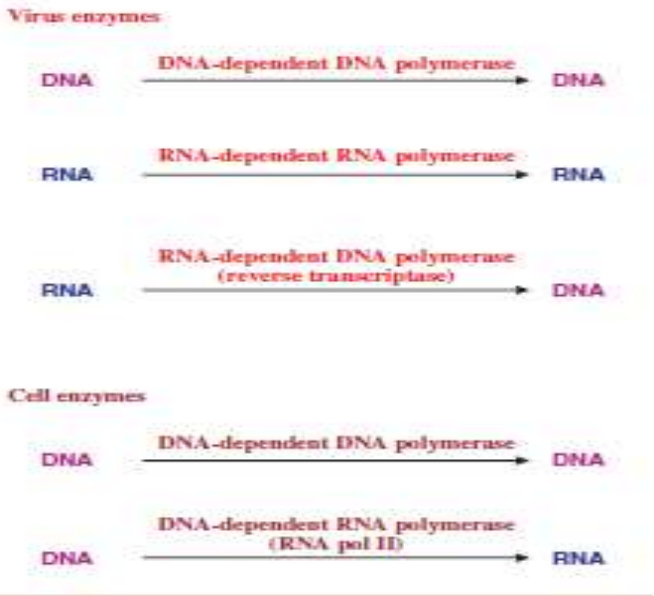


Figure 7.1 *Replication of virus genomes in the seven Baltimore Classes.* (+) RNA and (+) DNA have the same sequence as the mRNA (except that in DNA thymine replaces uracil). (–) RNA and (–) DNA have the sequence complementary to the mRNA (except that in DNA thymine replaces uracil). (+) and (–) strands are not indicated for the dsDNA of the Class I viruses as the genomes of most of these viruses have ORFs in both directions. (+) and (–) strands are indicated for the ssDNA of the Class II viruses. Most of these viruses have either a (+) or a (–) strand genome. Some ssDNA viruses and some ssRNA viruses have ambisense genomes.

after a primase has synthesized short sequences of RNA complementary to regions of the DNA. These RNAs act as primers; one is required for the leading strand, while multiple primers must be synthesized for the Okazaki fragments of the lagging strand. The first nucleotide of a new sequence of DNA is linked to the 3′ –OH group of the primer RNA.

Some DNA viruses also use RNA primers during the replication of their genomes. Some viruses, such as polyomaviruses, use the cell primase to synthesize their RNA primers, while others, such as herpesviruses and phage T7, encode their own primases.

During their replication cycle the retroviruses synthesize DNA from a (+) RNA template (Section 16.3.2). They use a cell transfer RNA to prime (–) DNA synthesis, then they use the 3′ –OH group in a polypurine tract of the partly degraded (+) RNA template to prime (+) DNA synthesis. The retrovirus DNA becomes integrated into a cell chromosome. If the infection is latent and the cell subsequently divides (Section 9.3.1), then the virus DNA is copied along with the cell DNA, using RNA primers synthesized by the cell primase.



Polymerases

The key enzymes involved in virus genome replication are DNA polymerases and RNA polymerases. Many viruses encode their own polymerase, but some use a host cell enzyme (Figure 7.3). A DNA virus requires a DNA-dependent DNA polymerase. Amongst the DNA viruses that replicate in the nuclei of eukaryotic cells, viruses with small genomes (e.g. papillomaviruses) use the cell enzyme, while viruses with large genomes (e.g. herpesviruses) encode their own enzyme. Those DNA viruses that replicate in the cytoplasm must encode their own enzyme. The enzyme that replicates the genome of an RNA virus is often referred to as a replicase; for many RNA viruses this is the same enzyme as that used for transcription (Section 6.3.3). The retroviruses and the pararetroviruses encode reverse transcriptases to transcribe from RNA to DNA, and use the host cell RNA polymerase II to transcribe from DNA to RNA. Many viral polymerases form complexes with other viral and/or cell proteins to produce the active enzyme. Some of these additional proteins are processivity factors, for example an *Escherichia coli* thioredoxin molecule functions as a processivity factor for the DNA polymerase of phage T7.

7.5 DNA replication

The viruses of Class I (dsDNA) and Class II (ssDNA) replicate their genomes via dsDNA. The ssDNA viruses first synthesize a complementary strand to convert the genome into dsDNA.

Each viral DNA has at least one specific sequence (*ori*; replication origin) where replication is initiated. The proteins that initiate DNA replication bind to this site, and amongst these proteins are

- a helicase (unwinds the double helix at that site);
- a ssDNA binding protein (keeps the two strands apart);
- a DNA polymerase.

Viral dsDNA is generally replicated by a process similar to that used by cells to copy their genomes. The basic process and the enzymes involved are outlined in Figure 7.4. Fewer proteins are involved in bacterial systems than in eukaryotic systems; for example, the helicase–primase of phage T7 is a single protein molecule, while that of herpes simplex virus is a complex of three protein species.

DNA synthesis takes place near a replication fork. One of the daughter strands is the leading strand and the other is the lagging strand, synthesized as Okazaki fragments, which become joined by a DNA ligase. After a dsDNA molecule has been copied each of the daughter molecules contains a strand of the original molecule. This mode of replication is known as semi-conservative, in contrast to the conservative replication of some dsRNA viruses (Section 7.6).

Some DNA genomes are linear molecules, while some are covalently closed circles (Section 3.2). Some of the linear molecules are circularized prior to DNA replication, hence many DNA genomes are replicated as circular molecules, for which there are two modes of replication, known as theta and sigma (Figure 7.5). These terms refer to the shapes depicted in diagrams

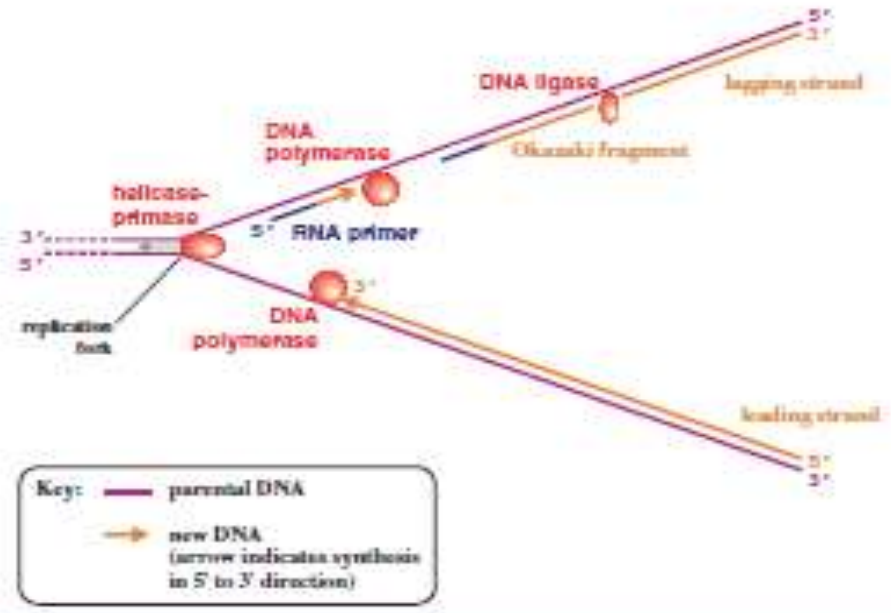


Figure 7.4 *DNA replication.* A helicase–primase unwinds the dsDNA and synthesizes RNA primers that are used by the DNA polymerase to initiate DNA synthesis. The leading strand is synthesized continuously, while the lagging strand is synthesized as Okazaki fragments that are joined together by a DNA ligase.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I B.Sc MB COURSE NAME: VIROLOGY

COURSE CODE: 18MBU201 UNIT: III BATCH-2018-2021

	UNIT - III	Option A	Option B	Option C	Option D	Answers
1	The family Herpesviridae is divided into _____ subfamilites.	3	2	1	4	3
2	The size of Herpes viruses are _____ nm in diameter.	160	140	130	150	150
3	The space between the envelope and the capsid is known as _____ .	Legument	Tegument	Regument	Aegument	Tegument
4	Herpes viruses were described as _____ .	Hot sores	Hot spots	Cold sores	Cold spots	Cold sores
5	The disease caused by HSV – I is _____ .	Neonatal herpes	Cervical carcinoma	Vulvular carcinoma	Eczema herpeticum	Eczema herpeticum
6	Herpetic whitlow is an infection of _____ .	Neck	Leg	Hair	Finger	Finger
7	Tzank cells are otherwise known as _____ .	Multinuclear giant cells	Kupffer cells	Latent cells	Vesicular endothelial cells	Multinuclear giant cells
8	Famciclovir is a drug administered _____ to HSV infections.	Intraneously	Subcutaneously	Orally	Cutaneously	Cutaneously
9	Acyclovir should be given within _____ to all HSV infections.	24 hrs	12 hrs	48 hrs	72 hrs	72 hrs
10	Shingles are caused by _____ virus.	Pox	Varicella	Herpes-zoster	Influenza	Herpes-zoster
11	VZIG is _____ .	Viral zidovidine Immuno globulin	Varicella – zoster Immuno globulin	Viral zoster Immuno globulin	Varicella zidovidine Immunoglobulin	Varicella – zoster Immuno globulin
12	_____ drug is given to CMV infections.	Ganciclovir	Vamciflovir	Acyclovir	Valacyclovir	Ganciclovir
13	'OWL's eye' type large intranuclear inclusion bodies is found in _____ virus.	TMV	EBV	CaMV	CMV	CMV
14	TORCH is panel of test including _____ .	Toxoplasmic cauliflower	Toxoplasmosis Other	Toxoplasmosis Oriented	Toxoplasmosis Other cystic HSV	Toxoplasmosis Other

		mosaic HSV	cytomegalovirus HSV	cytomegalo HSV		cytomegalovirus HSV
15	Tumour of jaw is known as _____ .	Kaposi's Sarcoma	Firboma	Burkittt's Lymphoma	Lymphosarcoma	Burkittt's Lymphoma
16	Paul – Bunnel test is a _____ metho	Direct Examination	Indirect examination Molecular Serological	Molecular	Serological	Indirect examination Molecular Serological
17	VCA is _____ .	Vero Cell Antigen	Viral Capsid Antigen	Viral core A	Viral Capsid A	Vero Cell Antigen
18	EA is _____ .	Early Antigen	E-type Antibody	Type E Antigen Epstein Antibody		Type E Antigen Epstein Antibody
19	EBNA is _____ .	Enveloped B type nucleic acid	Enveloped Bunyavirus Nuclear – Ag	Epstein – Barr Nuclear Antigen	Epstein type B Nucleic acid	Epstein type B Nucleic acid
20	_____ a benign exanthematous disease of childhoo	Burkitt's Lymphoma	Kaposi's Sarcoma	Leukoplakia	Roseola Infantum	Kaposi's Sarcoma
21	In KSHV, 'KS' Stands for _____ Herpes Virus.	Koplik's spot	Kaposi's Sarcoma	Klebsiella sensitive	Kenyen S-type	Kenyen S-type
22	In _____ Rowe and his associates first detected adenoviruses in adenoid tissue.	1923	1933	1943	1953	1943
23	Adenovirus contain icosohedral capsid with _____ capsomers.	212	222	252	242	212
24	Total Number of human adenovirus serotypes is _____ .	47	48	46	49	46
25	Incubation period ranges between _____ days in adenovirus infection.	1 - 2	2 - 3	5 - 7	8 – 9	2 - 3

26	Adenovirus associated virus is known as _____ .	Picornaviruses	Dependoviruses	Reoviruses	Retroviruses	Picornaviruses
27	Burkitt's Lymphoma is caused by _____ family.	Herpesviridae	Papoviridae	Flaviviridae	Reoviridae	Papoviridae
28	Apoptosis is _____ .	Generation Time	Programmed cell death	Programmed cell proliferation	MIC	Programmed cell death
29	_____ is the only human oncogenic retrovirus that encodes a 'tax' protein.	Hepatitis B Virus	HTLV – I	HTLV – II	HCV	HTLV – II
30	'V-one' gene commonly referred as _____ .	Cellular genes	Regulatory genes	Cancer genes	Structural genes	Cancer genes
31	Loss of 'Rb' gene leads to development of _____ .	Myelomas	Sarcomas	Carcinomas	Retinoblastomas	Retinoblastomas
32	In _____, Peyton Rous first described Retroviruses.	1911	1901	1910	1912	1911
33	_____ genes codes for envelope glycoprotein.	V-onc	O onc	Pol	EnV	EnV
34	_____ gene codes for nucleocapsid shell.	V – onc	Gag	Pol	EnV	Gag
35	_____ gene codes for reverse transcriptase.	Pol	Gag	EnV	O onc	Pol
36	_____ gene encodes the products responsible for cell transformation.	V-onc	O onc	C – Src	V – Src	O onc
37	In _____ , Robert Gallo isolated a retrovirus, HTLV – III from AIDS patient.	1982	1983	1984	1985	1984
38	The size of HIV ranges between _____ nm diameter.	60 - 80	50 - 70	190 - 220	90 – 120	90 – 120
39	_____ is cleared into two envelope components gp120 and gp41.	gp121	gp140	gp160	gp141	gp160
40	The tat gene codes for a protein _____ .	p19	p16	p18	p20	p16

41	_____ protein promotes the expression of HIV – I structural proteins.	p16	p18	p19	p20	p19
42	_____ gene codes a protein that downregulate the transcription of HIV genome.	tat	rev	vif	nef	nef
43	Vif is _____ gene.	Virus flexible	virion infectivity factor	virus infection	Virion including factor	virion infectivity factor
44	_____ gene represents the promoting region of the virus.	vpr	vif	vpu	vpx	vpr
45	PGL is also known as _____ .	Burkitt's syndrome	Lymphadenopathy syndrome	Nasopharyngeal syndrome	Polyma like syndrome	Lymphadenopathy syndrome
46	The interval between HIV infection and appearance of antibodies in serum is known as _____ .	Eclipse period	early Eclipse period	Window Period	Late Eclipse period	Window Period
47	Zydovudine is also known as _____ .	Dideoxyinosine	Azidideoxycytidine	Azidodideoxy thymidine	Dideoxyadenine	Azidodideoxy thymidine
48	Which of the following is not the protease inhibitor?	Squinavir	Ritonavir	Indianavir	Ganciclovir	Ganciclovir
49	The Cowpea Mosaic Virus is a Plant mosaic virus belongs to _____ group.	Enterovirus	Rhinovirus	Comovirus	Hepadnavirus	Comovirus
50	CPMV can be readily isolated from_____	Animals	Insects	Human	Plants	Plants
51	_____Particls are used to amplify signals in microarray based sensors	HSV	EBV	HBV	CPMV	CPMV
52	When a Satellite Subviral Agent encodes the coat protein in which it is encapsulated,it is known as ---------- virus.	Polyoma	Satellite	Adeno	Herpes	Satellite

53	MSV is _____	Maize Streak Virus	Master Standard Virus	Mixed Standard Virus	Mixed Steak Virus	Maize Streak Virus
54	MSV is transmitted by a variety of _____ species.	Stemhopper	Leafhopper	Roothopper	Flowerhopper	Leafhopper
55	TYDV is _____	Tri Yield Dwaff Virus	Tobacco Yellow Dwarf Virus	Tri Yellow Dwarf Virus	Tobacco Yield Dwarf Virus	Tobacco Yellow Dwarf Virus
56	The Mastrevirus genome contains _____intergenic regions located at opposite sides of the viral genome	3	4	2	5	2
57	For smooth entry into the plant cells ,Tmv produces protein called_____	P30	P20	P40	P50	P30
58	In TMV ,there are_____RNA nucleotides per protein monomer.	2	3	4	5	3
59	In _____ d' Herelle suggested the name Bacteriophage to bacteria eaters.	1911	1917	1918	1916	1917

Unit IV- Viral multiplication and replication

Viral multiplication and replication-Interaction, and entry, assembly, maturation and release of virions.Oncogenic viruses and its types, mechanism.Viral replication strategies as per Baltimore classification.Prevention and control of viral diseases.

Viral Structure and Replication

Viruses are noncellular genetic elements that use a living cell for their replication and have an extracellular state. Viruses are ultramicroscopic particles containing nucleic acid surrounded by protein, and in some cases, other macromolecular components such as a membranelike envelope.

Outside the host cell, the virus particle is also known as a **virion.** The virion is metabolically inert and does not grow or carry on respiratory or biosynthetic functions.

At present, there are no technical names for viruses. International committees have recommended genus and family names for certain viruses, but the process is still in a developmental stage.

Viruses vary considerably in size and shape. The smallest viruses are about 0.02 μm (20 nanometers), while the large viruses measure about 0.3 μm (300 nanometers). Smallpox viruses are among the largest viruses; polio viruses are among the smallest.

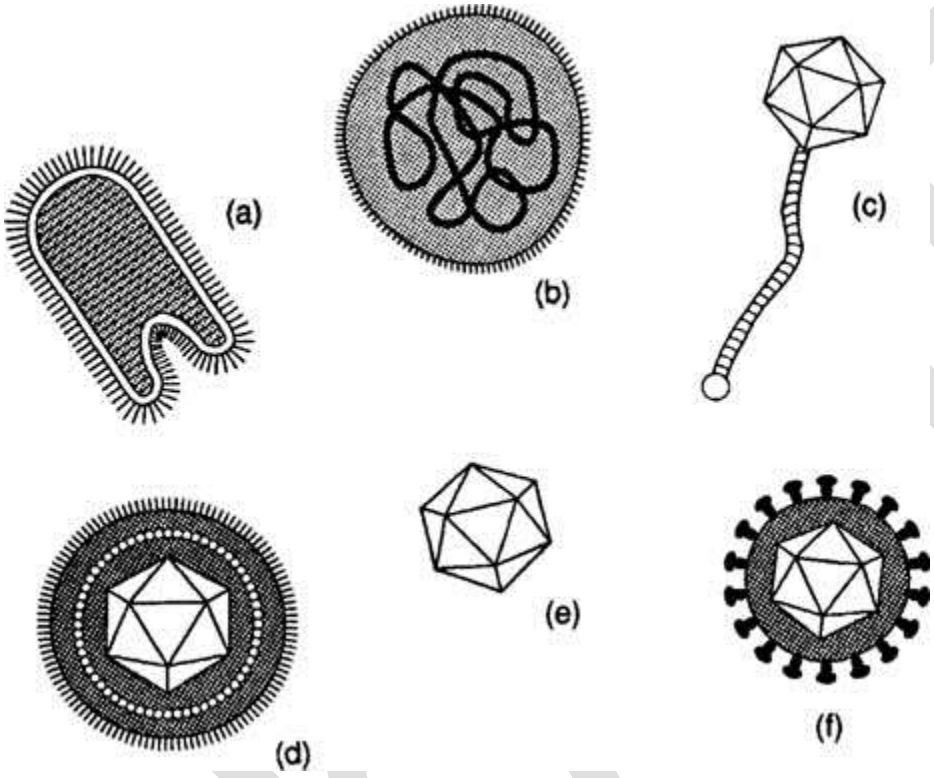
Viral structure. Certain viruses contain ribonucleic acid (RNA), while other viruses have deoxyribonucleic acid (DNA). The nucleic acid portion of the viruses is known as the**genome.** The nucleic acid may be single-stranded or double-stranded; it may be linear or a closed loop; it may be continuous or occur in segments.

The genome of the virus is surrounded by a protein coat known as a **capsid**, which is formed from a number of individual protein molecules called **capsomeres.** Capsomeres are arranged in a precise and highly repetitive pattern around the nucleic acid. A single type of capsomere or several chemically distinct types may make up the capsid. The combination of genome and capsid is called the viral **nucleocapsid.**

A number of kinds of viruses contain **envelopes.** An envelope is a membranelike structure that encloses the nucleocapsid and is obtained from a host cell during the replication process. The envelope contains viral-specified proteins that make it unique. Among the envelope viruses are those of herpes simplex, chickenpox, and infectious mononucleosis.

The nucleocapsids of viruses are constructed according to certain symmetrical patterns. The virus that causes tobacco mosaic disease, for example, has **helical symmetry.** In this case, the nucleocapsid is wound like a tightly coiled spiral. The rabies virus also has helical symmetry. Other viruses take the shape of an

icosahedron, and they are said to have **icosahedral symmetry.** In an icosahedron, the capsid is composed of 20 faces, each shaped as an equilateral triangle (Figure 1). Among the icosahedral viruses are those that cause yellow fever, polio, and head colds.



The envelope of certain viruses is a lipid bilayer containing glycoproteins embedded in the lipid. The envelope gives a somewhat circular appearance to the virus and does not contribute to the symmetry of the nucleocapsid. Projections from the envelope are known as **spikes.** The spikes sometimes contain essential elements for attachment of the virus to the host cell. The virus of AIDS, the human immunodeficiency virus, uses its spikes for this purpose.

Bacteriophages are viruses that multiply within bacteria. These viruses are among the more complex viruses. They often have icosahedral heads and helical tails. The virus that attacks and replicates in *Escherichia coli* has 20 different proteins in its helical tail and a set of numerous fibers and “pins.” Bacteriophages contain DNA and are important tools for viral research.

Viral replication. During the process of **viral replication**, a virus induces a living host cell to synthesize the essential components for the synthesis of new viral particles. The particles are then assembled into the correct structure, and the newly formed virions escape from the cell to infect other cells.

The first step in the replication process is **attachment.** In this step, the virus adsorbs to a susceptible host cell. High specificity exists between virus and cell, and the envelope spikes may unite with cell surface receptors. Receptors may exist on bacterial pili or flagella or on the host cell membrane.

The next step is **penetration** of the virus or the viral genome into the cell. This step may occur by phagocytosis; or the envelope of the virus may blend with the cell membrane; or the virus may "inject" its genome into the host cell. The latter situation occurs with the bacteriophage when the tail of the phage unites with the bacterial cell wall and enzymes open a hole in the wall. The DNA of the phage penetrates through this hole.

The **replication** steps of the process occur next. The protein capsid is stripped away from the genome, and the genome is freed in the cell cytoplasm. If the genome consists of RNA, the genome acts as a messenger RNA molecule and provides the genetic codes for the synthesis of enzymes. The enzymes are used for the synthesis of viral genomes and capsomeres and the assembly of these components into new viruses. If the viral genome consists of DNA, it provides the genetic code for the synthesis of messenger RNA molecules, and the process proceeds.

In some cases, such as in HIV infection (as discussed below), the RNA of the virus serves as a template for the synthesis of a DNA molecule. The enzyme reverse transcriptase catalyzes the DNA's production. The DNA molecule then remains as part of the host cell's chromosome for an unspecified period. From this location, it encodes messenger RNA molecules for the synthesis of enzymes and viral components.

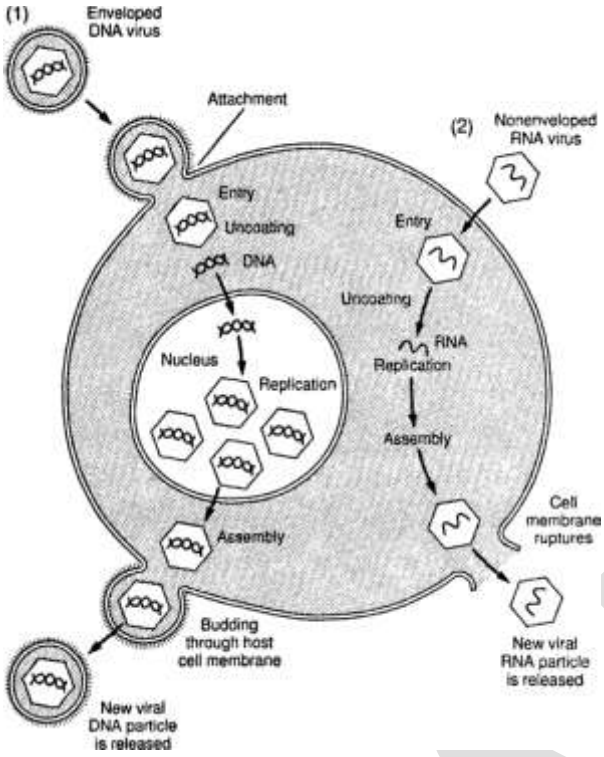


Figure 2

A generalized representation of the replication of two viruses. Replication of a DNA virus is shown in (1); replication of an RNA virus is displayed in (2).

For the **release** of new viral particles, any of a number of processes may occur. For example, the host cell may be "biochemically exhausted," and it may disintegrate, thereby releasing the virions. For enveloped viruses, the nucleocapsids move toward the membrane of the host cell, where they force themselves through that membrane in a process called **budding.** During budding, a portion of cell membrane pinches off and surrounds the nucleocapsid as an envelope. The replication process in which the host cell experiences death is called the **lytic cycle** of reproduction. The viruses so produced are free to infect and replicate in other host cells in the area.

Lysogeny. Not all viruses multiply by the lytic cycle of reproduction. Certain viruses remain active within their host cells for a long period without replicating. This cycle is called the **lysogenic cycle.** The viruses are called **temperate viruses**, or **proviruses**, because they do not bring death to the host cell immediately.

In lysogeny, the temperate virus exists in a latent form within the host cell and is usually integrated into the chromosome. Bacteriophages that remain latent within their bacterial host cell are called **prophages.** This process is a key element in the recombination process known as **transduction.**

Multiplication Within the Host Cell

Viral replication is the term used indicate the formation of biological viruses during the infection process in the target host cells. Viruses must first penetrate and enter the cell before viral replication can occur. From the perspective of the virus, the purpose of viral replication is to allow reproduction and survival of its kind. By generating abundant copies of its genome and packaging these copies into viruses, the virus is able to continue infecting new hosts.

Replication between viruses is varied and depends on the type of genes involved. Most DNA viruses assemble in the nucleus; most RNA viruses develop solely in cytoplasm. Viral populations do not grow through cell division, because they are acellular. Instead, they hijack the machinery and metabolism of a host cell to produce multiple copies of themselves, and they assemble inside the cell.

The life cycle of viruses differs greatly between species but there are six common basic stages:

Attachment is a specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus. For example, HIV can infect only a limited range of human leukocytes. Its surface protein, gp120, specifically interacts only with the CD4 molecule – a chemokine receptor – which is most commonly found on the surface of CD4+ T-

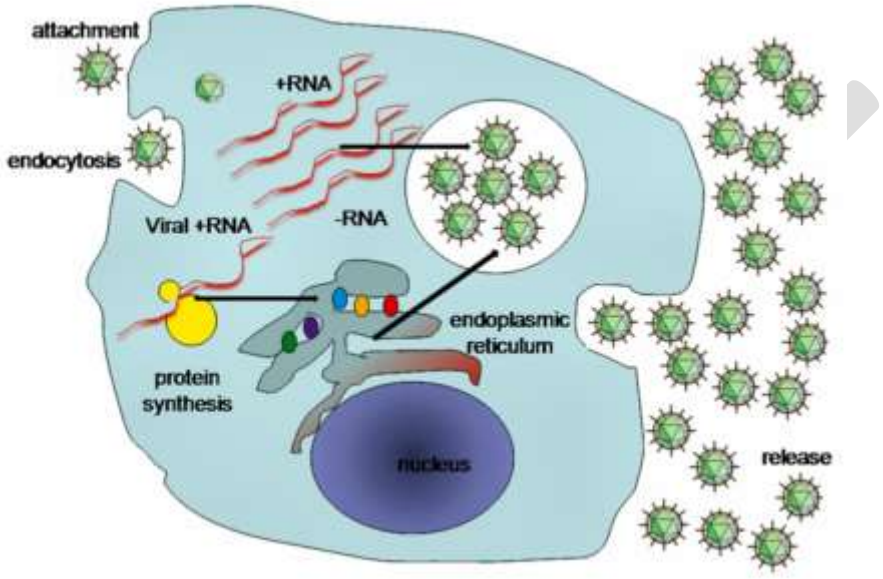
Cells. This mechanism has evolved to favor those viruses that infect only cells within which they are capable of replication. Attachment to the receptor can fore the viral envelope protein to undergo either changes that result in the fusion of viral and cellular membranes, or changes of non-enveloped virus surface proteins that allow the virus to enter.

Penetration follows attachment. Virions enter the host cell through receptor-mediated endocytosis or membrane fusion. This is often called *viral entry*. The infection of plant and fungal cells is different from that of animal cells. Plants have a rigid cell wall made of cellulose, and fungi one of chitin, so most viruses can get inside these cells only after trauma to the cell wall. However, nearly all plant viruses (such as tobacco mosaic virus) can also move directly from cell to cell, in the form of single-stranded nucleoprotein complexes, through pores called plasmodesmata. Bacteria, like plants, have strong cell walls that a virus must breach to infect the cell. However, since bacterial cell walls are much less thick than plant cell walls due to their much smaller size, some viruses have evolved mechanisms that inject their genome into the bacterial cell across the cell wall, while the viral capsid remains outside.

Uncoating is a process in which the viral capsid is removed: This may be by degradation by viral or host enzymes or by simple dissociation. In either case the end-result is the release of the viral genomic nucleic acid.

Replication of viruses depends on the multiplication of the genome. This is accomplished through synthesis of viral messenger RNA

(mRNA) from “early” genes (with exceptions for positive sense RNA viruses), viral protein synthesis, possible assembly of viral proteins, then viral genome replication mediated by early or regulatory protein expression. This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis: “late” gene expression is, in general, of structural or virion proteins.



Hepatitis C virus: A simplified diagram of the Hepatitis C virus replication cycle.

Following the structure-mediated self-assembly of the virus particles, some modification of the proteins often occurs. In viruses such as HIV, this modification (sometimes called maturation) occurs after the virus has been released from the host cell.

Viruses can be released from the host cell by lysis, a process that kills the cell by bursting its membrane and cell wall if present. This is a

feature of many bacterial and some animal viruses. Some viruses undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome. The viral genome is then known as a *provirus* or, in the case of bacteriophages a *prophage*. Whenever the host divides, the viral genome is also replicated. The viral genome is mostly silent within the host; however, at some point the provirus or prophage may give rise to active virus, which may lyse the host cells. Enveloped viruses (e.g., HIV) typically are released from the host cell by budding. During this process the virus acquires its envelope, which is a modified piece of the host's plasma or other internal membrane. The genetic material within virus particles, and the method by which the material is replicated, varies considerably between different types of viruses.

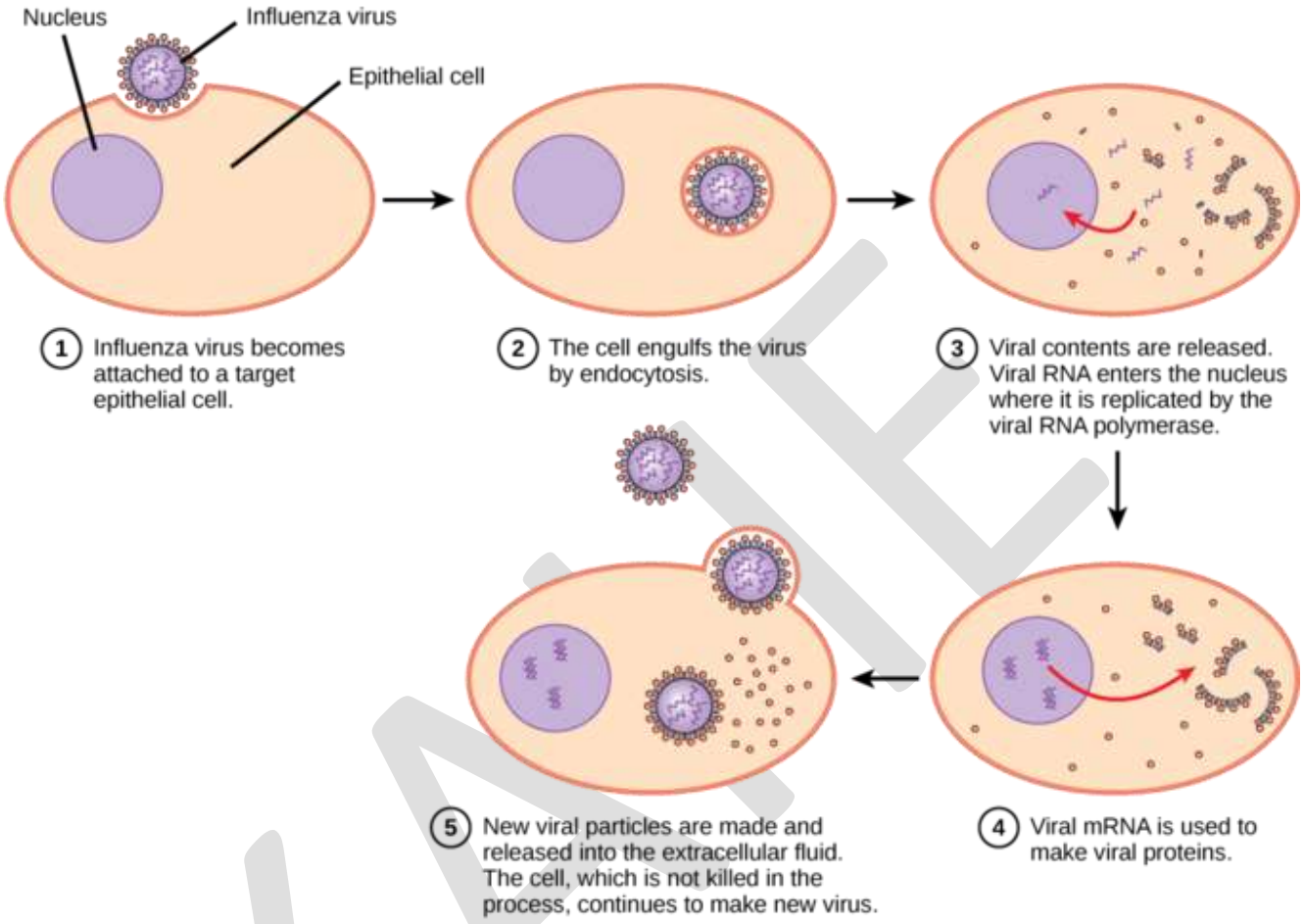
Steps of Virus Infections

Viral infection involves the incorporation of viral DNA into a host cell, replication of that material, and the release of the new viruses.

Steps of Virus Infections

A virus must use cell processes to replicate. The viral replication cycle can produce dramatic biochemical and structural changes in the host cell, which may cause cell damage. These changes, called cytopathic (causing cell damage) effects, can change cell functions or even destroy the cell. Some infected cells, such as those infected by the common cold virus known as rhinovirus, die through lysis (bursting) or apoptosis (programmed cell death or "cell suicide"), releasing all progeny virions at once. The symptoms of viral diseases result from the

immune response to the virus, which attempts to control and eliminate the virus from the body and from cell damage caused by the virus. Many animal viruses, such as HIV (Human Immunodeficiency Virus), leave the infected cells of the immune system by a process known as budding, where virions leave the cell individually. During the budding process, the cell does not undergo lysis and is not immediately killed. However, the damage to the cells that the virus infects may make it impossible for the cells to function normally, even though the cells remain alive for a period of time. Most productive viral infections follow similar steps in the virus replication cycle: attachment, penetration, uncoating, replication, assembly, and release.



Pathway to viral infection: In influenza virus infection, glycoproteins attach to a host epithelial cell. As a result, the virus is engulfed. RNA and proteins are made and assembled into new virions.

Attachment

A virus attaches to a specific receptor site on the host cell membrane through attachment proteins in the capsid or via glycoproteins embedded in the viral envelope. The specificity of this interaction determines the host (and the cells within the host) that can be infected

by a particular virus. This can be illustrated by thinking of several keys and several locks where each key will fit only one specific lock.

Entry

The nucleic acid of bacteriophages enters the host cell naked, leaving the capsid outside the cell. Plant and animal viruses can enter through endocytosis, in which the cell membrane surrounds and engulfs the entire virus. Some enveloped viruses enter the cell when the viral envelope fuses directly with the cell membrane. Once inside the cell, the viral capsid is degraded and the viral nucleic acid is released, which then becomes available for replication and transcription.

Replication and Assembly

The replication mechanism depends on the viral genome. DNA viruses usually use host cell proteins and enzymes to make additional DNA that is transcribed to messenger RNA (mRNA), which is then used to direct protein synthesis. RNA viruses usually use the RNA core as a template for synthesis of viral genomic RNA and mRNA. The viral mRNA directs the host cell to synthesize viral enzymes and capsid proteins, and to assemble new virions. Of course, there are exceptions to this pattern. If a host cell does not provide the enzymes necessary for viral replication, viral genes supply the information to direct synthesis of the missing proteins. Retroviruses, such as HIV, have an RNA genome that must be reverse transcribed into DNA, which then is incorporated into the host cell genome.

To convert RNA into DNA, retroviruses must contain genes that encode the virus-specific enzyme reverse transcriptase, which transcribes an RNA template to DNA. Reverse transcription never occurs in uninfected host cells; the needed enzyme, reverse transcriptase, is only derived from the expression of viral genes within the infected host cells. The fact that HIV produces some of its own enzymes not found in the host has allowed researchers to develop drugs that inhibit these enzymes. These drugs, including the reverse transcriptase inhibitor AZT, inhibit HIV replication by reducing the activity of the enzyme without affecting the host's metabolism. This approach has led to the development of a variety of drugs used to treat HIV and has been effective at reducing the number of infectious virions (copies of viral RNA) in the blood to non-detectable levels in many HIV-infected individuals.

Egress

The last stage of viral replication is the release of the new virions produced in the host organism. They are then able to infect adjacent cells and repeat the replication cycle. As you have learned, some viruses are released when the host cell dies, while other viruses can leave infected cells by budding through the membrane without directly killing the cell.

Tissue Tropism in Animal Viruses

Host tropism refers to the way in which viruses/pathogens determine which cells become infected by a given pathogen.

	UNIT - IV	Option A	Option B	Option C	Option D	Answers
1	A structural component that is found in all viruses is:	The envelope	DNA	Capsid	Tail fibers	Capsid
2	A chemical component that is found in all viruses is:	Protein	Lipid	DNA	RNA	Protein
3	A common polyhedral capsid shape of viruses is a :	Pentagon	Cube	Icosahedron	Pyramid	Icosahedron
4	Enteroviruses differ from rhinoviruses mainly in their:	Type of nucleic acid	Size	Capsid shape	Ability to survive acidic conditions	Capsid shape
5	Viruses that can remain latent (usually in neurons) for many years are most likely:	. Toga viruses	Herpes viruses	Entero viruses	Rhinoviruses	Herpes viruses
6	What types of viruses contain the enzyme lysozyme to aid in their infection?	Bacteriophage	Animal Viruses	Plant Viruses	Fungal Viruses	Animal Viruses
7	Bacteriophage is readily counted by the process of:	Immunoassays	ELISA	Plaque assays	Tissue cell culture	Plaque assays
8	A type of cell culture that can reproduce for an extended number of generations and is used to support viral replication is a :	Primary cell culture	Continuous cell line	Cell strain	Diploid fibroblast	Continuous cell line
9	Which of the following is not an RNA virus?	Retrovirus	Entero virus	Rhabado virus	Adenovirus	Entero virus
10	Which of the following disinfectant is effective against viruses?	Hydrogen peroxide	Hypochlorite	Formaldehyde	chlorine	Formaldehyde
11	Viruses largely lack metabolic machinery of their own to generate	protein	carbohydrate	alcohol	lipids	protein

	energy or to synthesize					
12	Viruses require _____ for growth.	bacteria	plants	animals	living cells	living cells
13	Reverse transcriptase is a useful enzyme to have when	an RNA virus converts its RNA to DNA	there are no host cells present	nutrients are scarce	spikes are forming in the new virus	an RNA virus converts its RNA to DNA
14	The sequence of nucleic acid in a variety of viruses and viral host, will find more similarities	among different viruses than between viruses and their hosts	among different viral hosts than among different viruses	among different viral hosts than between viruses and their hosts	between viruses and their hosts than among different viruses	among different viruses than between viruses and their hosts
15	When a virus enters a cell but does not replicate immediately, the situation is called	lysogeny	fermentation	symbiosis	synergism	lysogeny
16	Viruses are separated into several large groups based primarily on	nature of the host	nucleic acid characteristics	capsid symmetry	diameter of the viroin or nucleocapsid	nature of the host
17	The first step in infection of a host bacterial cells by a phage is	adsorption	absorption	penetration	replication	adsorption
18	Which of the following viruses has not been associated with human cancer?	Hepatitis C virus	Hepatitis B virus	Varicella-Zoster virus	Herpes simplex virus type 2	Varicella-Zoster virus
19	The viral nucleocapsid is the combination of	genome and Capsid	capsid and spikes	envelope and Capsid	capsomere and genome	genome and Capsid
20	Edward Jenner began inoculating humans with material from _____ lesions.	Smallpox	Avianpox	Cowpox	Chickenpox	Cowpox

21	The viruses in an attenuated vaccine	have no genome	continue to replicate	are usually larger than bacteria	is altered with chemicals	have no genome
22	Enveloped viruses have a _____ shape.	icosahedral	helical	roughly spherical	complex	complex
23	The envelope of which of the following viruses is derived from the host cell nucleus?	Paramyxo viruses	Retro viruses	Orthomyxo viruses	Herpes viruses	Retro viruses
24	Which of the following is semi-continuous (diploid) cell line?	HeLa	HEp-2	WI-38	KB	HeLa
25	Plant viruses may be cultivated in	tissue culture	cultures of separated cells	whole plants	human cells	tissue culture
26	The oncogene theory refers to	how chemicals inactivate viruses when applied	how viruses replicate in host cells	how viruses transform normal cells into tumor cells	no change	how viruses transform normal cells into tumor cells
27	In cell culture, measles virus may lead to	nuclear pyknosis	transformation of cells	syncytium formation	rounding and aggregation of cells	syncytium formation
28	A change from lysogeny to lysis is generally not induced by	ultraviolet light	chemicals	irradiation	alcohol	alcohol
29	The viral DNA is removed from the host's chromosomes and the lytic cycle occurs. The process is called	spontaneous induction	inductive infection	resultant induction	spontaneous infection	spontaneous infection
30	The lysogenic state is governed by the activity of the regulatory region of the lambda phage genomes; this region is	immunity repressor	immunity operon	operon repressor	Lac operon	immunity repressor

	termed as					
31	The capsomeres consist of a number of proteins subunits or molecules called	protomers	caproprotein	bprocapsid	capsomers	caproprotein
32	In order for a virus to replicate	the capsid must enter the host cell cytoplasm	the host cell must be undergoing mitosis	the genome must be released in the cytoplasm	the host cell must lack a cell membrane	the genome must be released in the cytoplasm
33	Which of the following viruses belong to family Flaviviridae?	Rubella virus	Yellow fever virus	Hepatitis C virus	Dengue	Dengue
34	Which of the following viruses show/s transformation of infected cells?	Hepatitis B virus	T cell lymphotronic virus type I	Epstein-Barr virus	CAMV	T cell lymphotronic virus type I
35	Which of the following may affect proteins and nucleic acids, but not viruses?	Denaturation	Enzyme treatment	Pressure	Sedimentation	Denaturation
36	The viral DNA of the temperate phage, instead of taking over the functions of the cell's genes, is incorporated into the host DNA and becomes a prophage in the bacterial chromosome, acting as a gene. This happens in	lysogeny	spontaneous induction	lytic phase	Induced induction	lysogeny
37	Which of the following statements is not true of viruses?	Viruses have been successfully grown in pure cultures in test tubes	All viruses are obligatory intracellular parasites	All viruses have either DNA or RNA as their genetic material	Viruses probably arose from small fragments of cellular chromosomes	Viruses have been successfully grown in pure cultures in test tubes

38	Which of the following viruses belong/s to family caliciviridae?	Hepatitis B virus	Hepatitis D virus	Hepatitis E virus	Hepatitis A virus	Hepatitis E virus
39	In the simplest capsid, there is a capsomere at each of the 12 vertices; this capsomere, which is surrounded by five other capsomeres, is termed a	penton	polyhedra	icosahedral	helical	penton
40	The size of viruses is usually measured in	centimeters	micrometers	nanometers	millimeters	nanometers
41	The temperate phage that have no site specificity for insertion and may even be able to insert multiple copies of their DNA into a single bacterial chromosome is	λ phage enzyme	λ DNA	Phage Mu	Phage Mn	Phage Mn
42	Enzyme neuraminidase is carried by which of the following viruses?	Human immunodeficiency virus	Epstein-Barr virus	Influenza virus	Adenovirus	Influenza virus
43	Lysozyme (an endolysin) which will lyse the bacterial cell, releasing the mature virions is present in	immediate early phage gene	late genes	delayed early genes	Early genes	late genes
44	Which of the following is continuous cell line?	HeLa	HEp-2	KB	All of these	HeLa
45	The repressor protein, since the cell is resistant to lysis from externally infecting phage, is also called	immunity repressor	immunity operon	operon repressor	Operon deppressor	immunity repressor
46	Which of the following virus is susceptible to chloroform?	Herpes	Influenza	Measles	HIV	Measles
47	Group E phages have	single stranded DNA	double stranded DNA	single stranded RNA	double stranded RNA	double stranded DNA

48	The temperate phage possesses a gene that codes for a repressor protein which makes the cell resistant to lysis initiated by	phage destruction	lytic infection by other viruses	Both (a) and (b)	lysogenic	lytic infection by other viruses
49	The process by which phage reproduction is initiated in lysogenized culture is called	infection	integration	repression	induction	infection
50	Area of lysis on a bacterial lawn culture produced by a phage is known as	pock	plaque	pox	Colony	plaque
51	The procapsid is assembled with the aid of _____ proteins.	ladder	framing	scaffolding	form	ladder
52	Which of the following is/are synthesized from late mRNA?	Phage structural proteins	Proteins that help with phage assembly without becoming part of the virion structure	Proteins involved in cell lysis and phage release	Complex protein	Proteins involved in cell lysis and phage release
53	Which capsid symmetry is exhibited by most of the phages?	Helical	Icosahedral	Complex	Cylinder	Icosahedral
54	Contractile sheath of the tail is present in which of the following phages?	T3	T2	P22	P322	T2
55	One of the first enzymes synthesized by many bacteriophage is _____ , an RNA-dependent RNA polymerase.	RNA transcriptase	RNA polymerase	RNA ligase	RNA replicase	RNA polymerase
56	In viruses with envelopes	the envelope and the embedded	the envelope is derived from	the envelope is coded by the	the envelope and its	the envelope is coded by the viral

		proteins are encoded by the viral nucleic acid	the host but it contains embedded proteins coded by the viral nucleic acid	viral nucleic acids, but the proteins come from the host's membrane proteins	imbedded proteins are derived from the host's membranes	nucleic acids, but the proteins come from the host's membrane proteins
57	Which of the following bacteria can be typed by phage typing method?	Staphylococcus aureus	Salmonella typhi	Vibrio cholerae	E coli	Salmonella typhi
58	_____ protein keeps the prophage dormant and prevents virus reproduction.	Operator	Promotor	Repressor	Enhancer	Promotor

Unit V- Antiviral compounds

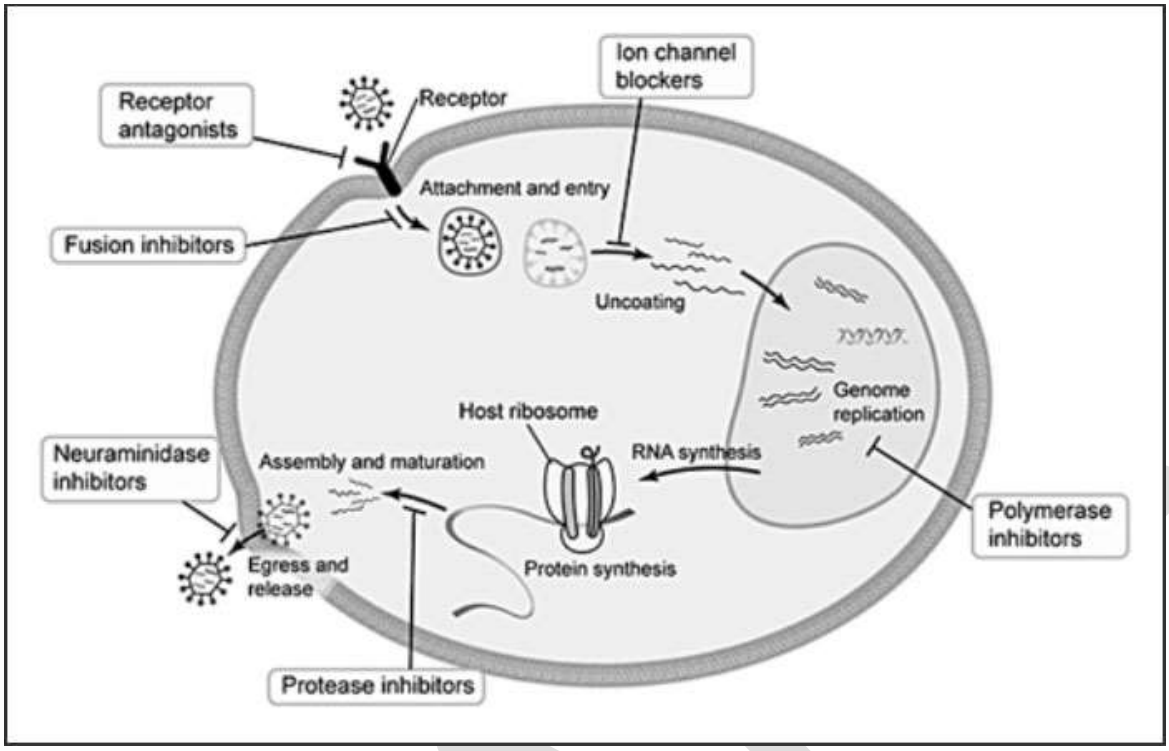
Antiviral compounds and their mode of action. Interferon and their mode of action.General principles of viral vaccination.Immunization schedule. Use of viral vectors in cloning and expression, gene therapy and phage display.

Antiviral compounds

Viruses are obligate intracellular parasites called **virions** and consist of RNA or DNA genomes (Table 4). The viral genome is surrounded by a virus-encoded protein shell **capsid**. In some cases, the capsid is surrounded by an **envelope**, a lipid bilayer membrane that contains additional virus-encoded proteins. With some variations, all virions have the same general **viral life cycle** and each stage is a potential target for pharmacological intervention

Mechanisms of Action of Antiviral Agents

Viral infections are initiated when virions attach to host cells. **Attachment** is mediated by capsid- or envelope-related viral proteins that bind specific receptors on host cell membranes. For example, the HIV envelope contains glycoproteins that mediate binding of the virus to CD4+ T lymphocytes that express CCR5 and/or CXCR4 receptors. A currently available **inhibitor of viral attachment** blocks HIV-specific CCR5 receptors on CD4+ T cell membranes.



Viral entry across host cell membranes into the cytoplasm is mediated by other viral proteins. For example, the HIV envelope contains a protein (gp41) which promotes **fusion** of the viral envelope with target host cell (CD4+ T lymphocyte) membranes. A currently available **inhibitor of viral entry** blocks gp41-mediated fusion of the HIV envelope with the plasma membrane of host CD4+ T lymphocytes.

Viral entry is followed by **uncoating**. This step refers to the removal/degradation or structural modification of the nucleocapsid, which results in the release of the viral genome into host cell cytoplasm and, in the case of DNA genomes, transport into host cell nuclei. Currently available **inhibitors of viral uncoating** block M2 proton channel in influenza A viruses and prevent pH-dependent disassociation of viral matrix proteins from the viral RNA.[10]

Following uncoating, the viral nucleic acid becomes available for **gene expression**, i.e., the **transcription** of viral RNA or DNA genome into mRNA, **translation** of mRNA into viral proteins, and **proteolytic cleavage** of viral polyproteins into their individual protein units. Currently

available **inhibitors of viral gene expression** block HCV-related NS3/4A protease, essential for the expression of functional HCV proteins.[10]

Genome replication requires the generation of ribo- or deoxyribonucleoside triphosphates. Most RNA viruses replicate their genomes in host cell cytoplasm and most DNA viruses replicate their genomes in the nucleus of host cells. **Nucleoside analogues**, which when phosphorylated by viral or cellular kinases are incorporated into the growing viral genome and inhibit polymerase activity.[10] **Non-nucleoside polymerase inhibitors** directly inhibit RNA or DNA polymerases.[10]

The first step in HIV genome replication is reverse transcription, i.e., the viral RNA is first copied into DNA, which is then transcribed into mRNA. **Reverse transcriptase inhibitors** block the transcription of the HIV RNA genome into DNA. The life cycle of the HIV also includes the additional step of integration, a process that binds HIV DNA to host cell DNA. **HIV integrase inhibitors** block the integration of viral genome into host cell genome.

The next step in the viral life cycle is **assembly**, the process in which the immature virions are formed. Assembly is followed by **maturation**. This is the stage in the viral life cycle in which the new virions become infectious. The process involves proteolytic cleavage of one or more capsid- or envelop-related proteins by viral or host cell proteases. Currently available **inhibitors of viral maturation are inhibitors of HIV proteases**.

Most viruses **egress** from infected host cells by cell lysis or by budding through the cell membrane. However, some virions require the additional step of **release**. For example, influenza A and B viruses require viral neuraminidase to effect their release from the extracellular surface of host cell membranes. Currently available **inhibitors of viral release or neuraminidase inhibitors** prevent the detachment the new influenza A and B virions from host cells.[10]

Treatment of Viral Infections

The treatment of most viral infections is primarily the responsibility of physicians (Table 5).[10-13] However, two of the known *Herpesviridae*, HSV-1 and HSV-2 are responsible for **primary** and **recurrent mucocutaneous herpetic infections** and HSV-1 is predominately associated with orolabial infections.[14] Consequently, the diagnosis and management of orolabial herpetic infections fall, to a great extent, within the purview of oral healthcare providers.

Indications for Antiviral Chemotherapy.

Mechanism of action	Drugs*	Indications
Inhibitors of viral attachment	Maravoric	CCR5-tropic HIV-1 infections
Inhibitors of viral entry	Enfuvirtide	HIV infections
	Docosanol	HSV infections
Inhibitors of viral uncoating	Amantadine Rimantadine	Influenza A infections
Inhibitors of viral gene expression	Boceprevir Ledipasvir Ombitasvir Paritaprevir Simeprevir Telaprevir	Chronic HCV (genotype 1) infections
Anti-herpesvirus nucleoside analogs	Acyclovir Valacyclovir	HSV and VZV infections
	Famciclovir Penciclovir	HSV infections
	Idoxuridine Trifulodine Vidarabine	HSV keratitis
	Ganciclovir	HCMV infections

	Valganciclovir	
	Cidofovir	HCMV retinitis
Anti-HBV nucleoside analogs	Adefovir Emtricitabine Entecavir Lamivudine Telbivudine	HBV infections
Anti-HCV nucleoside analogs	Sofosbuvir	Chronic HCV infections
Anti-HIV nucleoside analogues (reverse transcriptase inhibitors)	Abacavir Didanosine Emtricitabine Lamivudine Stavudine Tenofovir Zidovudine	HIV infections
Anti-herpesvirus non-nucleoside DNA polymerase inhibitors	Foscarnet	HSV and HCMV infections
Anti-HIV non-nucleoside reverse transcriptase inhibitors	Delaviridine Efavirenz Etravirine Nevirapine Rilpivirine	HIV infections
Anti-HCV non-nucleoside RNA polymerase inhibitors	Dasabuvir	HCV infections

Inhibitors of viral integration	Dolutegravir Raltegravir Elvitegravir	HIV infections
Inhibitors of viral maturation (protease inhibitors)	Atazanavir Darunavir Fosamprenavir Indinavir Lopinavir Nelfinavir Ritonavir Saquinavir Tipranavir	HIV infections
Inhibitors of viral release	Oseltamivir Zanamivir	Influenza A and B infections

*FDA-approved information on specific antiviral agents is available at *DailyMed* - the website is a user-friendly, look-up-and-download resource that provides comprehensive, up-to-date information on individual drugs.[3]

The most common initial presentation of orolabial HSV-1 infection is **primary herpetic gingivostomatitis** (Figures 9a and 9b). Following primary infection, the virions enter sensory nerve endings and are transported via retrograde axonal transport to regional sensory ganglia, i.e., the trigeminal ganglia. In the trigeminal ganglia the virus establishes latency in neuronal cell bodies and persists in an immunologically shielded state until reactivated.

Herpetic whitlow may occur with either HSV-1 or HSV-2 inoculation into a finger. **Herpetic keratoconjunctivitis** in most adult is likely the result of autoinoculation. Less frequently, primary and recurrent HSV infection of the skin may present as **herpes gladiatorum** or **eczema herpeticum**. A substantial number of erythema multiforme cases are believed to be related to the herpes simplex virus (**herpes-associated erythema multiforme**).

Orolabial herpetic infections in immunocompetent patients are usually self-limiting. Treatment is palliative and supportive directed at controlling fever, dehydration, and pain; and monitoring for

evidence of systemic viremia. Antiviral agents may be prescribed to patients with moderate-to-severe primary herpetic gingivostomatitis; to patients with recurrent orolabial herpetic infections; and to immunocompromised patient, who are inherently at risk of complications (Table 6).[11,12,15-25]

Antiviral Agents for the Treatment and Prevention of Orolabial HSV Infections.[11,12,15-25]

Infection	Drug	Adult dosage
Moderate-to-severe primary herpetic gingivostomatitis • Immunocompetent patients	acyclovir	400 mg, PO, tid for 7-10 days OR 15 mg/kg (oral suspension), PO, 5x/day for 7 days
	famciclovir	500 mg, PO, bid/tid for 7-10 days
	valacyclovir	1 g, PO, bid for 7-10 days
Recurrent orolabial infections • Immunocompetent patient	docosanol*	10% cream, 5x/day until healed
	penciclovir*	1% cream, q2h while awake for 4 days
	acyclovir*	5% cream, 5x/day x 4 days
• Immunocompetent patients • Immunocompromised patient	famciclovir	1500 mg, PO, single dose
	valacyclovir	2 g, PO, 2x/day x 1 day
	acyclovir	400 m, PO, 5x/day for 5 days

Suppression of recurrences • Immunocompromised patients	acyclovir	400 mg,, PO, bid
	valacyclovir	500 mg, PO, once/day
	famciclovir	500 mg, PO, bid
Suppression of dental procedure-related recurrences • Immunocompetent patients	valacyclovir	2 g, PO, 2x/day, the day of procedure then 1 g, PO, 2x/day, the day after procedure
Mucocutaneous herpetic infections • Immunocompromised patients	acyclovir	400 mg, PO, 5x/day for 7/10days
	famciclovir	500 mg, PO, bid for 7-10 days
	valacyclovir	500 – 1000 mg, PO, bid for 7-10 days
Acyclovir-resistant mucocutaneous infections • Immunocompetent patients with moderate-to-severe primary herpetic gingivostomatitis • Immunocompromised patients	foscarnet	40 mg/kg, IV, q8h x 12-21 days

*Immunocompromised patients should not be treated with topical antiviral agents.

The recommendation of **oral acyclovir** for the treatment of moderate-to-severe primary herpetic gingivostomatitis in immunocompetent patients is based on limited-quality evidence.[12,15] In a placebo controlled study, children on acyclovir experienced reduction in the duration of lesions, fever, difficulty eating and drinking, and viral shedding. **Oral valacyclovir** or **famciclovir** are recommended as acceptable alternatives to oral acyclovir.[11]

The recommendation of **oral acyclovir**, **valacyclovir**, or **famciclovir** for the treatment of recurrent herpes labialis in immunocompetent and immunocompromised patients is based on good-quality evidence.[11,12,16-18] The drugs have been found to be effective in reducing viral shedding, pain, and healing time. Based on good-quality evidence, **oral acyclovir**, **valacyclovir**, or **famciclovir** are also recommended for the suppression of frequent recurrent infections.[11,22]

Oral acyclovir, valacyclovir, and famciclovir are well tolerated and associated adverse reactions are similar.[3] Common ADRs include nausea, vomiting, diarrhea, malaise, and headaches. Rare ADRs include myalgia, rash, Stevens-Johnson syndrome, tremors, lethargy, confusion, hallucinations, seizures, and coma. Resistance to acyclovir is uncommon, but HSV strains resistant to acyclovir are also resistant to valacyclovir and famciclovir.[11]

Topical docosanol, 10% cream, a 22-carbon saturated alcohol, is approved by the FDA as an over-the-counter agent for the treatment of recurrent herpes labialis in immunocompetent patients. Based on good-quality evidence the drug is somewhat effective. Treatment within 12 hours of prodromal signs and symptoms decreased pain (2.2 vs. 2.7 days) and healing time (4.1 vs. 4.8 days).[12,22] Common ADRs at the site of application include rash and pruritis.[3]

Topical penciclovir, 1% cream, based on good-quality evidence is helpful in the treatment of herpes labialis in immunocompetent adults.[12,23] The application of penciclovir versus placebo beginning within one hour of the first signs and symptoms of recurrence reduced median duration of pain (4.1 to 3.5 days) and healing time (5.5 to 4.8 days).[24] The drug did not affect the medial time for viral shedding (3 vs. 3 days). Systemic absorption of penciclovir is negligible.

Treatment with **topical acyclovir**, 5% cream, beginning within one hour of the onset of signs and symptoms of recurrence, based on good-quality evidence, has been shown in two parallel and independent trials to reduce the duration of recurrent herpes labialis in immunocompetent patients by about half a day. In study 1, the mean duration of lesions was reduced from 4.8 to 4.3 days. In study 2, the mean duration of lesions was reduced from 5.2 to 4.6 days.

Patients with moderate-to-severe primary herpetic gingivostomatitis and immunocompromised patients with acyclovir-resistant mucocutaneous herpetic infections may respond to **intravenous foscarnet**.[11] Potential ADRs include electrolyte imbalances; seizures; anemia and neutropenia; fever; nausea, vomiting, and diarrhea; headache; and reversible renal dysfunction, especially in patients with inadequate hydration, is not uncommon.

Interferon

Interferons (**IFN**s) are a group of signaling proteins[1] made and released by host cells in response to the presence of several viruses. In a typical scenario, a virus-infected cell will release interferons causing nearby cells to heighten their anti-viral defenses.

IFNs belong to the large class of proteins known as cytokines, molecules used for communication between cells to trigger the protective defenses of the immune system that help eradicate pathogens.[2] Interferons are named for their ability to "interfere" with viral replication[2] by protecting cells from virus infections. IFNs also have various other functions: they activate immune cells, such as natural killer cells and macrophages; they increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens. Certain symptoms of infections, such as fever, muscle pain and "flu-like symptoms", are also caused by the production of IFNs and other cytokines.

More than twenty distinct IFN genes and proteins have been identified in animals, including humans. They are typically divided among three classes: Type I IFN, Type II IFN, and Type III IFN. IFNs belonging to all three classes are important for fighting viral infections and for the regulation of the immune system.

Types of interferon

Based on the type of receptor through which they signal, human interferons have been classified into three major types.

- Interferon type I: All type I IFNs bind to a specific cell surface receptor complex known as the IFN-α/β receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains.[3] The type I interferons present in humans are IFN-α, IFN-β, IFN-ε, IFN-κ and IFN-ω.[4] In general, type I interferons are produced when the body recognizes a virus has invaded it. They are produced by fibroblasts and monocytes. However, the production of type I IFN-α is prohibited by another cytokine known as Interleukin-10. Once released, type I interferons bind to specific receptors on target cells, which leads to expression of proteins that will prevent the virus from producing and replicating its RNA and DNA.[5] Overall, IFN-α can be used to treat hepatitis B and C infections, while IFN-β can be used to treat multiple sclerosis.[2]
- Interferon type II (IFN-γ in humans): This is also known as immune interferon and is activated by Interleukin-12.[2] Furthermore, type II interferons are released by Cytotoxic T cells and T helper cells, type 1 specifically. However, they block the proliferation of T helper cells type two. The previous results in an inhibition of T_h2 immune response and a further induction of T_h1 immune response, which leads to the development of debilitating diseases such as multiple sclerosis.[6] IFN type II binds to IFNGR, which consists of IFNGR1 and IFNGR2 chains and has a different receptor than type I IFN.[2]

- Interferon type III: Signal through a receptor complex consisting of IL10R2 (also called CRF2-4) and IFNLR1 (also called CRF2-12). Although discovered more recently than type I and type II IFNs,[7] recent information demonstrates the importance of Type III IFNs in some types of virus or fungal infections.[8][9][10]

In general, type I and II interferons are responsible for regulating and activating the immune response.[2] Expression of type I and III IFNs can be induced in virtually all cell types upon recognition of viral components, especially nucleic acids, by cytoplasmic and endosomal receptors, whereas type II interferon is induced by cytokines such as IL-12, and its expression is restricted to immune cells such as T cells and NK cells.

Function

All interferons share several common effects: they are antiviral agents and they modulate functions of the immune system. Administration of Type I IFN has been shown experimentally to inhibit tumor growth in animals, but the beneficial action in human tumors has not been widely documented. A virus-infected cell releases viral particles that can infect nearby cells. However, the infected cell can protect neighboring cells against a potential infection of the virus by releasing interferons. In response to interferon, cells produce large amounts of an enzyme known as protein kinase R (PKR). This enzyme phosphorylates a protein known as eIF-2 in response to new viral infections; the phosphorylated eIF-2 forms an inactive complex with another protein, called eIF2B, to reduce protein synthesis within the cell. Another cellular enzyme, RNAse L—also induced by interferon action—destroys RNA within the cells to further reduce protein synthesis of both viral and host genes. Inhibited protein synthesis impairs both virus replication and infected host cells. In addition, interferons induce production of hundreds of other proteins—known collectively as interferon-stimulated genes (ISGs)—that have roles in combating viruses and other actions produced by interferon.[11][12] They also limit viral spread by increasing p53 activity, which kills virus-infected cells by promoting apoptosis.[13][14] The effect of IFN on p53 is also linked to its protective role against certain cancers.[13]

Another function of interferons is to upregulate major histocompatibility complex molecules, MHC I and MHC II, and increase immunoproteasome activity. Higher MHC I expression increases presentation of viral peptides to cytotoxic T cells, while the immunoproteasome processes viral peptides for loading onto the MHC I molecule, thereby increasing the recognition and killing of infected cells. Higher MHC II expression increases presentation of viral peptides to helper T cells; these cells release cytokines (such as more interferons and interleukins, among others) that signal to and co-ordinate the activity of other immune cells.

Interferons, such as interferon gamma, directly activate other immune cells, such as macrophages and natural killer cells.

Virus resistance to interferons

Many viruses have evolved mechanisms to resist interferon activity.[21] They circumvent the IFN response by blocking downstream signaling events that occur after the cytokine binds to its receptor, by preventing further IFN production, and by inhibiting the functions of proteins that are induced by IFN.[22] Viruses that inhibit IFN signaling include Japanese Encephalitis Virus (JEV), dengue type 2 virus (DEN-2) and viruses of the herpesvirus family, such as human cytomegalovirus (HCMV) and Kaposi's sarcoma-associated herpesvirus(KSHV or HHV8).[22][23] Viral proteins proven to affect IFN signaling include EBV nuclear antigen 1 (EBNA1) and EBV nuclear antigen 2 (EBNA-2) from Epstein-Barr virus, the large T antigen of Polyomavirus, the E7 protein of Human papillomavirus (HPV), and the B18R protein of vaccinia virus.[23][24] Reducing IFN-α activity may prevent signaling via STAT1, STAT2, or IRF9 (as with JEV infection) or through the JAK-STAT pathway (as with DEN-2 infection).[22] Several poxviruses encode soluble IFN receptor homologs—like the B18R protein of the vaccinia virus—that bind to and prevent IFN interacting with its cellular receptor, impeding communication between this cytokine and its target cells.[24] Some viruses can encode proteins that bind to double-stranded RNA (dsRNA) to prevent the activity of RNA-dependent protein kinases; this is the mechanism reovirus adopts using its sigma 3 (σ3) protein, and vaccinia virus employs using the gene product of its E3L gene, p25.[25][26][27] The ability of interferon to induce protein production from interferon stimulated genes (ISGs) can also be affected. Production of protein kinase R, for example, can be disrupted in cells infected with JEV.[22] Some viruses escape the anti-viral activities of interferons by gene (and thus protein) mutation. The H5N1 influenza virus, also known as bird flu, has resistance to interferon and other anti-viral cytokines that is attributed to a single amino acidchange in its Non-Structural Protein 1 (NS1), although the precise mechanism of how this confers immunity is unclear.

Interferon therapy

Diseases

Interferon beta-1a and interferon beta-1b are used to treat and control multiple sclerosis, an autoimmune disorder. This treatment is effective for reducing attacks in relapsing-remitting multiple sclerosis and slowing disease progression and activity in secondary progressive multiple sclerosis.[29]

Interferon therapy is used (in combination with chemotherapy and radiation) as a treatment for some cancers.[30] This treatment can be used in hematological malignancy; leukemia and lymphomas including hairy cell leukemia, chronic myeloid leukemia, nodular lymphoma, and cutaneous T-cell lymphoma.[30] Patients with recurrent melanomas receive recombinant IFN-α2b.[31] Both hepatitis B and hepatitis C are treated with IFN-α, often in combination with other antiviral drugs.[32][33] Some of those treated with interferon have a sustained virological response and can eliminate hepatitis virus. The

most harmful strain—hepatitis C genotype I virus—can be treated with a 60-80% success rate with the current standard-of-care treatment of interferon-α, ribavirin and recently approved protease inhibitors such as Telaprevir (Incivek) May 2011, Boceprevir(Victrelis) May 2011 or the nucleotide analog polymerase inhibitor Sofosbuvir (Sovaldi) December 2013.[34] Biopsies of patients given the treatment show reductions in liver damage and cirrhosis. Some evidence shows giving interferon immediately following infection can prevent chronic hepatitis C, although diagnosis early in infection is difficult since physical symptoms are sparse in early hepatitis C infection. Control of chronic hepatitis C by IFN is associated with reduced hepatocellular carcinoma.[35]

Unconfirmed results suggested that interferon eye drops may be an effective treatment for people who have herpes simplex virus epithelial keratitis, a type of eye infection.[36] There is no clear evidence to suggest that removing the infected tissue (debridement) followed by interferon drops is an effective treatment approach for these types of eye infections.[36]Unconfirmed results suggested that the combination of interferon and an antiviral agent may speed the healing process compared to antiviral therapy alone.[36]

When used in systemic therapy, IFNs are mostly administered by an intramuscular injection. The injection of IFNs in the muscle or under the skin is generally well tolerated. The most frequent adverse effects are flu-like symptoms: increased body temperature, feeling ill, fatigue, headache, muscle pain, convulsion, dizziness, hair thinning, and depression. Erythema, pain, and hardness at the site of injection are also frequently observed. IFN therapy causes immunosuppression, in particular through neutropenia and can result in some infections manifesting in unusual ways.[37]

Drug formulations

Several different types of interferons are approved for use in humans. One was first approved for medical use in 1986.[38] For example, in January 2001, the Food and Drug Administration (FDA) approved the use of PEGylated interferon-alpha in the USA; in this formulation, PEGylated interferon-alpha-2b (*Pegintron*), polyethylene glycol is linked to the interferon molecule to make the interferon last longer in the body. Approval for PEGylated interferon-alpha-2a (*Pegasys*) followed in October 2002. These PEGylated drugs are injected once weekly, rather than administering two or three times per week, as is necessary for conventional interferon-alpha. When used with the antiviral drug ribavirin, PEGylated interferon is effective in treatment of hepatitis C; at least 75% of people with hepatitis C genotypes 2 or 3 benefit from interferon treatment, although this is effective in less than 50% of people infected with genotype 1 (the more common form of hepatitis C virus in both the U.S. and Western Europe).[39][40][41] Interferon-containing regimens may also include protease inhibitors such as boceprevir and telaprevir.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I B.Sc MB COURSE NAME: VIROLOGY

COURSE CODE: 18MBU201 UNIT: V BATCH-2018-2021

	UNIT - V	Option A	Option B	Option C	Option D	Answers
1	An example for natural antiviral compounds is _____ .	Zydovudine	Interferon	Acyclovir	Protein kinase	Interferon
2	An example for synthetic antiviral agents is _____ .	Interferon	Interleukin	Acyclovir	Polymerase	Acyclovir
3	In _____, Isaacs and Lindenmann discovered interferon.	1957	1967	1947	1937	1957
4	_____ Interferon is produced by monocytes and B lymphocytes.	β	γ	δ	α	α
5	_____ interferon is produced by fibroblasts and epithelial cells.	γ	β	δ	α	β
6	_____ interferon is produced by activated T-cells.	α	δ	γ	ζ	γ
7	The IFN - β lies on chromosome _____ .	2	3	9	6	9
8	The IFN – γ lies on chromosome _____ .	10	12	11	13	12
9	Interferons are glycoprotein with molecular weight of _____ .	10,000 - 15,000	20,000 - 40,000	50,000 - 60,000	5,000 - 15,000	20,000 - 40,000
10	Interferons is inactivated by _____ enzymes.	polymerase	Ligase	Restriction	Proteolytic	Proteolytic
11	_____ interferon is a lymphokine.	β	α	δ	γ	γ
12	There are totally _____ gene codes for IFN – α	24	23	1	2	23
13	There are totally _____ gene codes for IFN – β	23	2	1	25	2
14	There are totally _____ gene codes for IFN – γ	1	2	3	4	1
15	The molecular weight of IFN – α is _____.	11,000	22,000	14,000	17,000	17,000

16	The molecular weight of IFN – β is _____.	11,000	17,000	16,000	15,000	17,000
17	The molecular weight of IFN – γ is _____.	17,000	11,000	15,000	16,000	17,000
18	The best inducers of α and β interferons production is _____ .	ss DNA	ds DNA	ss RNA	ds RNA	ds RNA
19	Example for mitogens is _____ .	Exotoxins	Endotoxins	Mutant	Aflatoxins	Endotoxins
20	Immune stimulators is otherwise referred as _____ .	Polymerase	Sterile Soil	Sterile water	Mitogens	Mitogens
21	Synthesis of IFN – α is and INF – β takes _____ hours after infection and attaining maximum level of synthesis.	2-5	6-12	15-25	16-24	6-12
22	Type I interferon inhibit _____ .	Cellular protein synthesis	viral protein synthesis	DNA synthesis	RNA synthesis	viral protein synthesis
23	Type II interferon possess _____ activity.	antibacterial	antifungal	antiviral	antihaemolytic	antiviral
24	_____ protein kinase inactivates elongation factor – 2 in protein synthesis.	72 kd	62 kd	52 kd	68 kd	68 kd
25	Induction of _____ inhibit viral replication.	Sodium oxide synthetase	Hydrogen peroxide synthetase	Potassium oxide synthetase	Nitric oxide synthetase	Nitric oxide synthetase
26	_____ bind to gp120 in HIV.	CD4	CD8	Recombinant CD4	Rec CD8	Recombinant CD4
27	ICAM is _____, blocks infectivity of common cold viruses.	Induced choroallantric membrane	Intracellular cell adhesion molecule	Indian council of Atomic membership	Indian council of Academic Microbiology	Intracellular cell adhesion molecule
28	_____ is used as an Immunostimulant.	IFN - β	IFN – γ	IFN – δ	IFN - α	IFN – γ

29	_____ inhibit the fusion of viral envelope with endosome membrance preventing the release of nucleocapsid into the cytoplasm.	Amanladine	Zydovudine	Ganciclovir	Rifampicin	Amanladine
30	_____ agent has a territory structure similar to deoxyadenosine.	Idoxuridine	Amantadine	Xydovudine	Vidarabine	Vidarabine
31	IDU is _____ .	Induced Dermi Uridine	Idoxuridine	Indoluridine	idiotopic uridine	Idoxuridine
32	_____ is a derivative of acyclovir.	Ganciclovir	Valacyclovir	famciclovir	Vidarabine	Ganciclovir
33	_____ is an antiviral drug that cause termination of DNA chain.	Valacyclovir	Amantadine	ICAM	type I IFN	Valacyclovir
34	The enzyme _____ is expressed in HSV infected cells soon after its infection.	Viral polymerase	Viral Ligase	Viral thymidine kinase	Viral kinase	Viral thymidine kinase
35	Zydovudine is otherwise known as _____ .	Acyclovir	Zakitabine	Ribavirin	Azido thymidine	Azido thymidine
36	_____ was the first drug used for the treatment of HIV infection.	Zalcitabine	Zidovudine	Stavudine	Nevirapine	Zidovudine
37	_____ is a nucleoside analogue of thymidine that has greater affinity for reverse transcriptase.	Retrovir	Zalcitabine	Idoxuridine	ribavirin	Zalcitabine
38	AZT, thymidine analogue lacks _____ essential for the formation of 5’ – 3’ diester linkages between nucleic acid molecules.	5’ P	3’ P	5’ OH	3’ OH	3’ OH
39	Dideoxycytidine is otherwise known as _____ .	Retrovir	Zalcitabine	Idoxuridine	Ribavirin	Zalcitabine

40	Dideoxyinosine is otherwise known as _____ .	Idoxuridine	Azidothymidine	Didanosine	Foscarnet	Didanosine
41	_____ is an inhibitor of HIV protease.	Retrovir	Acyclovir	Ritonavir	Famciclovir	Ritonavir
42	_____ is also known as phasphonoformic aci	Foscarnet	Ribavirin	Retrovir	Ritonavir	Foscarnet
43	_____ is an “Broad Spectrum” nucleoside analogue that acts on cellular enzymes important to viral replication.	Zalcitabine	Ribavirin	Acyclovir	Didanosine	Ribavirin
44	Enzyme producing GTP is the initial compound necessary for capping of _____ synthesis.	r RNA	m RNA	s RNA	t RNA	m RNA
45	_____ drug used in the early stages of Hantavirus infection.	Zidovudine	Zalcitabine	Ribavirin	Didanosine	Ribavirin
46	A ---------- deactivates virus particles outside the body.	Fungicide	Bacteriocide	Viricide	Gellicide	Viricide
47	The first experimental antiviral were developed in ---------- to be dealt with Herpesviruses.	1920s	1960s	1950s	1970s	1950s
48	Vaccines attack viruses when they are in ---------- Stage.	Minute particle	Small unit	incomplete particle	complete particle	complete particle
49	Entry blocking drug is ---------- is used to combat influento.	pleconaril	streptomycin	ketaconazole	Amantadine	Amantadine
50	The entry blocker against rninoviruses is ----------.	Streptomycin	Amantadine	Pleconaril	Glucanazole	Pleconaril
51	A ---------- is the first successful antiviral drug effective against herpes virus infection	Acyclovir	amantadine	Lamivudine	pleconaril.	Acyclovir
52	The first antiviral drug to be approved	Amantadine	Gluconazole	Zidovudine	Pleconaril	Zidovudine

	for treating HIV is ----------.					
53	A ---------- is a component of reverse transcriptase that splits the synthesized DNA from the original viral RN	polymerase	Ligase	DNase H	RNase H	RNase H
54	The enzyme ---------- Splices the synthesized DNA into the host cell genome.	integrase	Ligase	polymerase	protease	integrase
55	---------- is used to treat opportunistic eye infections in AIDS patient caused by Cytomegalovirus.	Streptomycin	fomivirsen	Lamivudine	pleconaril	fomivirsen
56	Relenta is the other name for ---------- drug.	Acyclovir	Ribavirin	Zanamivir	Oseltamivir	Zanamivir
57	Tamiflu is otherwise known as ----------	Acyclovir	Oseltamivir	Zanamivir	Ribavirin	Oseltamivir
58	MMR vaccine is ecommended by CDC for Children at the age group between_____	0-1	0 to 9	0 - 6	11 to 12	0 - 6
59	BCG is given for infants at the time of_____	Disease	Birth	Emergency	Maturity	Birth
60	MCV is _____	Measles Vaccine	Mumps Cornea Virus	Measles Cornea Virus	Meningococcal Vaccine	Meningococcal Vaccine

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

[18MBU201]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

DEPARTMET OF MICROBIOLOGY

FIRST INTERNAL EXAMINATION, DECEMBER – 2018

SECOND SEMESTER

VIROLOGY

Time: 2 hours **Maximum: 50 marks**

Date: / /2018 **Class: I B.Sc., MB**

PART-A – (20 x 1 = 20 marks)

(Answer all the questions)

1. Viruses are_____intracellular parasites.
   a. Obligate b. aerobic
   c. anaerobic d. Facultative.
2. Who discovered TMV?
   a. Ivanosky and Beijerinck b. Twort and Felix
   c. Edward Jenner d. Louis Pasteur
3. The complete protein-nucleic acid complex is called as_____.
   a. capsid b. protein coat
   c. nucleocapsid d. nucleic acid
4. Infectious virus particle is known as_____.
   a. virion b. viriod
   c. prion d. capsid
5. Virus replicates by _____ mechanism.
   a. host b. own
   c. cell d. Direct
6. Virus is classified based on _____.
   a. DNA b. RNA
   c. DNA & RNA d. Host
7. Proteins associated with nucleic acid is called as _____.
   a. Proteins b. Nucleous
   c. Nucleoproteins d. Host
8. Yolk sac inoculation is used for _____ virus cultivation.
   a. HIV b. Pox
   c. HSV d. Influenza
9. _____ classified virus into seven classes.
   a. David Baltimore b. Edward jenner
   c. Montangier d. Christian Gram
10. Viruses can enter cells via_____.
    a. penetration b. adsoption
    c. Entry d. absorption
11. Viruses range in size from:
    a. 1-100 nm b. 25-300 nm
    c. 10-100 µm d. 400-1000 nm
12. A chemical component that is found in all viruses is
    a. Protein b. Lipid
    c. DNA d. RNA
13. A structural component that is found in all viruses is_____.
    a. The envelope b. DNA
    c. Capsid d. Tail fibers
14. The viral nucleocapsid is the combination of _____.
    a. genome and Capsid b. capsid and spikes
    c. envelope and Capsid d. capsomere and genome
15. The nucleocapsid is covered by an outer membrane like structure called___.
    a. envelope b. covering
    c. Membronocapsid d. capsid
16. Viroids are composed of_____.
    a. single-stranded DNA b. double-stranded DNA
    c. single-stranded RNA d. double -stranded RNA
17. Which of the following is the agent associated with the development of neurodegenerative disease in livestock and humans?
    a. Prions b. Viroids
    c. virions d. Virinos
18. Viral RNA is replicated in the host cell_____.
    a. cytoplasmic matrix b. nucleus
    c. mitochondria d. lysozomes
19. Virus means____.
    a. pellet b. poison
    c. protein d. incomplete
20. _____mice is used for viral inoculation.
    a. young b. trickling
    c. suckling d. Old

PART – B (3x2 = 6 marks)

(Answer all the questions)

21. Define virology.
22. What is capsid?
23. Define virions.

PART – C (3x8 = 24marks)

Answer all the questions either a or b. (All question carry equal marks)

24. a) Write short notes on the classification of Viruses.

    (or)

    b) Write in detail about the history of viruses.
25. a) Explain in short about the viral cultivation methods .

    (or)

    b) Explain structure of viruses and its symmetry.
26. a) Discuss in detail about Baltimore classification.

    (or)

    b) Give a detailed account on bacteriophage.