# UNIT1:Microbes andFungi

## Viruses

Viruses are infectious agents with both living and nonliving characteristics. They can infect animals,plants,andevenothermicroorganisms.Virusesthatinfectonlybacteriaare called**bacteriophages***(def)*and those that infect onlyfungi aretermed **mycophages***(def)*. There are even some viruses called **virophages** *(def)* that infect other viruses.

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 PasteurandEdward Jennerdeveloped the firstvaccinesto 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 Ivanovskyused one of these filters to show that sap from a diseasedtobacco plantremained infectious to healthy tobacco plants despite having been filtered.MartinusBeijerinckcalled the filtered, infectious substance a "virus" and this discoveryis considered to be the beginning of virology. The subsequent discovery and partial characterization ofbacteriophages byFelix d'Herellefurther catalyzed the field, and by the early 20th century many viruses were discovered.

**LivingCharacteristicsof Viruses**

a. They**reproduce**at afantasticrate, but onlyin livinghost cells.
b. Theycan**mutate.**

**NonlivingCharacteristicsof Viruses**

a. Theyare**acellular**,that is,theycontainno cytoplasmor cellular organelles.
b. They**carry out no metabolism on their own and must replicate using the host cell's metabolic machinery**. In other words, viruses don't grow and divide. Instead, new viral components are synthesized and assembled within the infected host cell.
c. The**vastmajorityofvirusespossess eitherDNAorRNAbut not both**.

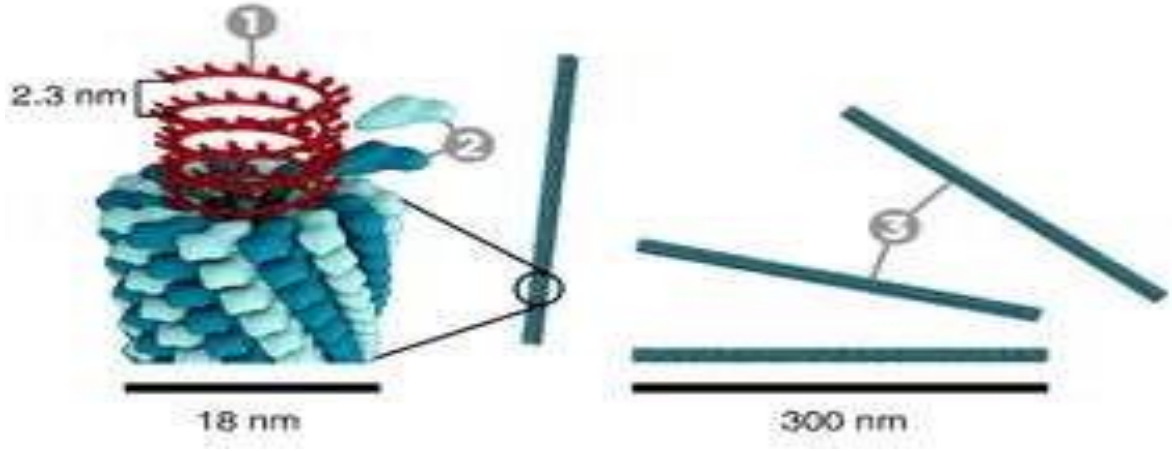
**Recently, viruses have been declared as living entities** based on the large number of protein folds encoded by viral genomes that are shared with the genomes of cells. This indicates that viruses likely arose from multiple ancient cells.

**CriteriaUsedtoDefineaVirus**

a. The vast majorityof viruses contain onlyone type of nucleic acid: DNA or RNA, but not both.

b. They are totally dependent on a host cell for replication. (They are strict intracellular parasites.)
c. Viral components must assemble into complete viruses (virions) to go from one host cell to another.

**Tobacco mosaic virus:**(TMV) is apositive-sensesingle strandedRNA virusthat infects a wide rangeofplants,especiallytobacco andothermembersofthefamilySolanaceae.Theinfectioncauses characteristic patterns, such as "mosaic"-likemottlingand discoloration on theleaves(hence the name). TMV was the first virusever to be discovered. Although it was known from the late 19th century that an infectious diseasewas damaging tobacco crops, it was not until 1930 that the infectious agent was determined to be a virus.




Tobacco mosaic virus has a rod-like appearance. Its capsidis made from 2130moleculesof coat proteinandonemoleculeofgenomicsinglestrandRNA,6400baseslong. Thecoatproteinself-assembles into the rod-like helical structure (16.3 proteins per helix turn) around the RNA which formsahairpinloopstructure(see theelectronmicrographabove).Theproteinmonomerconsists of 158amino acidswhich are assembled into four main alpha-helices, which are joined by a prominent loop proximal to the axis of the virion. Virions are ~300 nm in length and ~18nm in diameter.[12]Negativelystainedelectronmicrophotographsshowadistinctinnerchannelof ~4nm. The RNA is located at a radius of ~6 nm and is protected from the action of cellular enzymes by the coat protein. There are three RNA nucleotides per protein monomer. X-rayfiberdiffractionstructure of the intact virus was studied based on an electron densitymap at 3.6 Å resolution.

## Disease cycle

TMV does not have a distinct overwinteringstructure. Rather, it will over-winter in infected tobaccostalksand leaves inthesoil,onthesurfaceofcontaminatedseed(TMVcanevensurvive in contaminated tobacco products for many years). With the direct contact with host plants through its vectors (normally insects such as aphids and leafhoppers), TMV will go through the infection process and then the replication process.

## Infection

Afteritsmultiplication,itenterstheneighboringcellsthrough plasmodesmata.Foritssmooth entry, TMV produces a 30 kDamovement proteincalled P30 which enlarge the plasmodesmata. TMV most likelymoves from cell-to-cell as a complex of the RNA, P30, and replicase proteins. Itcanalsospreadthrough phloemforlongerdistancemovementwithintheplant.Moreover, TMVcanbetransmittedfromoneplanttoanotherby directcontact.AlthoughTMVdoesnot have defined transmission vectors, the virus can be easily transmitted from the infected hosts to the healthy plants, by human handling.

## Replication

Following entry into its host via mechanical inoculation, TMV uncoats itself to release its viral [+] RNA strand. As uncoating occurs, the MetHel:Pol gene is translated to make the capping enzyme MetHel and the RNA Polymerase. Then the viral genome will further replicate to produce multiple mRNAs via a [-]RNA intermediate primed by the $tRNA_{HIS}$at the [+]RNA 3' end. The resulting mRNAs encode several proteins, including the coat proteinand anRNA-dependent RNA polymerase(RdRp), as well as the movement protein. Thus TMV can replicate its own genome. After the coat protein and RNA genome of TMV have been synthesized, they spontaneously assemble into complete TMV virions in a highly organized process. Theprotomers come together to form disks or 'lockwashers' composed of two layers of protomers arranged in a helix. The helical capsid grows by the addition of protomers to the end of the rod. As the rod lengthens, the RNA passes through a channel in its center and forms a loop at the growing end. In this way the RNA can easily fit as a spiral into the interior of the helical capsid.

## Host andSymptoms

TobaccomosaicvirussymptomsontobaccoTobaccomosaicvirussymptomson orchid

Like other plant pathogenic viruses, TMV has a very wide host range and has different effects depending on the host being infected. The tobacco mosaic virus has been known to cause a production loss for flue cured tobacco of up to two percent in North Carolina·It is known toinfectmembersofnineplantfamilies,andatleast125individualspecies,includingtobacco,tomato,pepper(all members of the useful Solanaceae), cucumbers, and a number of ornamental flowers.There are many different strains. The first symptom of this virus disease is a light green coloration between the veins of young leaves. This is followed quickly by the development of a "mosaic" or mottled pattern of light and dark green areas in the leaves. Rugosity may also be seen where the infected plant leaves display small localized random wrinkles. These symptoms develop quickly and are more pronounced on younger leaves. Its infection does not result in plant death, but if infection occurs early in the season, plants are stunted. Lower leaves are subjected to "mosaic burn" especially during periods of hot and dry weather. In these cases, large dead areas develop in the leaves. This constitutes one of the most destructive phases of tobacco mosaic virus infection. Infected leaves may be crinkled, puckered, or elongated. However, if TMV infects crops like grape and apple, it is almost symptomless.

## Treatmentand management

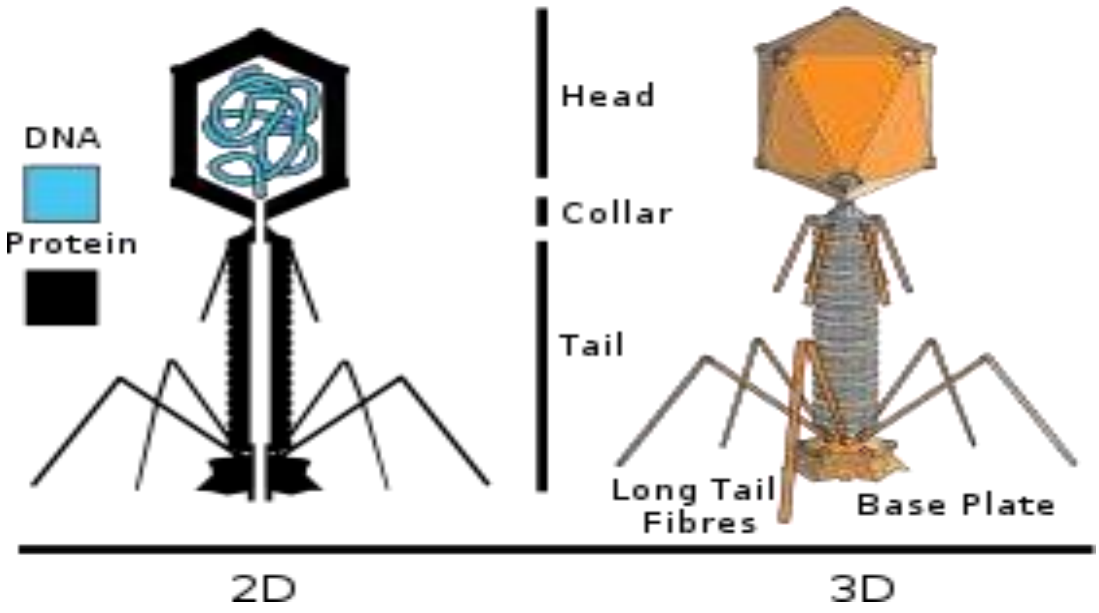
One of the common control methods for TMV is sanitation, which includes removing infected plants, and washing hands in between each planting. Crop rotation should also be employed to avoid infectedsoil/seed beds for at least two years. Asforanyplant disease, looking forresistant strains against TMV may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

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

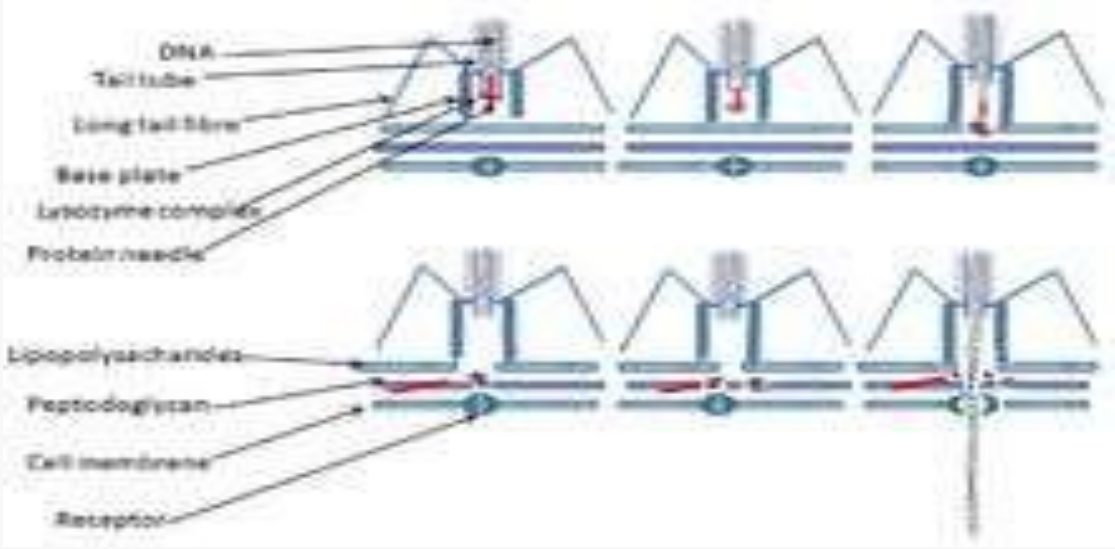
**Bacteriophage**

A**bacteriophage**is avirusthat infects and replicates within abacterium. The term is derived from"bacteria"andthe Greek:φαγεῖν(*phagein*),"todevour".Bacteriophagesarecomposed ofproteinsthatencapsulateaDNAorRNAgenome, and may have relatively simple or elaborate structures. Their genomes may encodeas few as fourgenes, 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 bybacterialhosts, such as soil or the intestines of animals. One of the densest natural sources for phages and other viruses is seawater, where up to $9\times10^8$virionsper 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.Theyareseen as apossibletherapyagainst multi-drug-resistantstrains ofmany bacteria (seephage therapy).Nevertheless, phages of Inoviridaehave been shown to complicate biofilms involved in pneumonia and cystic fibrosis, shelter the bacteria from drugs meant to eradicate disease and promote persistent infection.




**Replication**




**Diagramof theDNAinjection process**

Bacteriophages mayhave alytic cycleor alysogenic cycle, and a few viruses are capable of carrying out both. With *lytic phages*such as theT4 phage, bacterial cells are broken open (lysed) and destroyed after immediate replication of the virion. As soon as the cell is destroyed, thephageprogenycanfindnewhoststoinfect. Lyticphagesaremoresuitablefor phagetherapy. Some lytic phages undergo a phenomenon known as lysis inhibition, where completed phage progenywillnotimmediatelylyseoutofthecellifextracellularphageconcentrationsarehigh.

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 astemperate phages. Their viral genome will integrate with host DNA and replicate along with it relatively harmlessly, or may even become established as aplasmid. The virus remains dormant until host conditions deteriorate, perhapsdue to depletion of nutrients; then, endogenousphages (known asprophages) become active. At this point they initiate the reproductive cycle, resulting in lysis of the host cell. As the lysogenic cycleallowsthehost cell to continueto surviveand reproduce, thevirus is replicatedin all ofthe cell's offspring. An example of a bacteriophage known to follow the lysogenic cycle and thelytic cycle is the phage lambda of *E. coli.*

Sometimes prophages may provide benefits to the host bacterium while they are dormant by adding new functions to the bacterial genomein a phenomenon called lysogenic conversion. Examples are the conversion of harmless strains of *Corynebacteriumdiphtheriae*or*Vibriocholerae*by bacteriophages to highly virulent ones, which cause Diphtheriaorcholera, respectivelyStrategies to combat certain bacterial infections by targeting these toxin-encoding prophages have been proposed.

**Attachmentandpenetration**

**Inthiselectronmicrographof bacteriophagesattachedto abacterialcell,the virusesare the size and shape of coliphage T1.**

To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, includinglipopolysaccharides,teichoicacids,proteins, or evenflagella. This specificity means a bacteriophage can infect onlycertain 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.As phage virions do not move independently, theymust rely on random encounters with the right receptors when in solution (blood, lymphatic circulation, irrigation, soil water, etc.).

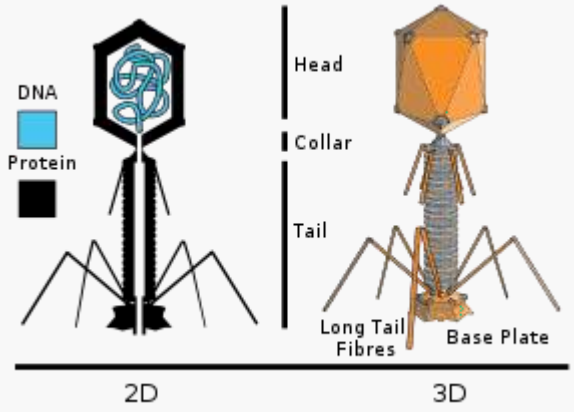
Myovirus bacteriophages use ahypodermic syringe-like motion to inject their genetic material into the cell. After making contact with the appropriate receptor, the tail fibers flex to bring the base plate closer to the surface of the cell; this is known as reversible binding. Once attached completely, irreversible binding is initiated and the tail contracts, possiblywith the help ofATPpresentinthetail, injectinggeneticmaterialthroughthebacterialmembrane. 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.

**Synthesisofproteinsandnucleicacid**

Within minutes, bacterial ribosomesstart translating viral mRNA into protein. For RNA-based phages,RNA replicaseis synthesized early in the process. Proteins modify the bacterial RNApolymeraseso 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)

**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.




**Diagramof a typicaltailedbacteriophagestructure**

**Releaseof virions**

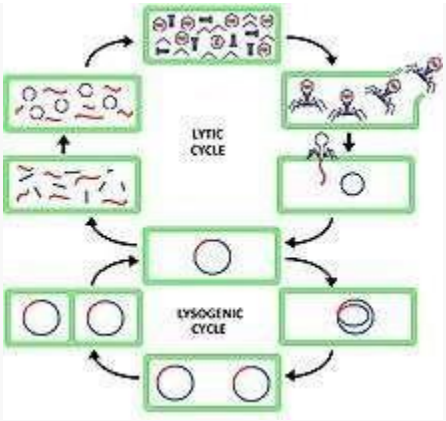
Phages may be released via cell lysis, by extrusion, or, in a few cases, by budding. Lysis, by tailedphages,isachievedbyanenzymecalled endolysin,whichattacksandbreaksdownthecell wallpeptidoglycan. An altogether different phage type, the filamentous phages, make the host cell continually secrete new virus particles. Released virions are described as free, and, unless defective,arecapableofinfectinganewbacterium.Buddingisassociatedwith certain*Mycoplasma*phages. In contrast to virion release, phages displaying a lysogeniccycle do not kill the host but, rather, become long-term residents asprophage.

**Genomestructure**

Given the millions of different phages in the environment, phages genomes come in a variety of forms and sizes. RNA phage such as MS2have 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 highlymosaic, 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 withmycobacterialhosts – have provided excellent examples of this mosaicism. In these mycobacteriophages, genetic assortment may be the result of repeated instances of site-specificrecombinationand illegitimate recombination (the result of phage genome acquisition ofbacterial 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.

Lysogeniccycle




**Lysogeniccycle,comparedtolyticcycle**

**Lysogeny**,orthe**lysogeniccycle**,isoneoftwocyclesofviralreproduction(thelyticcycleisthe other). Lysogeny is characterized by integration of the bacteriophagenucleic acid into the host bacterium's genome or formations of a circular repliconin the bacterium's cytoplasm. In this condition the bacterium continues to live and reproduce normally. The genetic material of the bacteriophage, called aprophage, can be transmitted to daughter cells at each subsequent cell division,andalaterevent(suchasUVradiationorthepresenceofcertainchemicals)canrelease it, causing proliferation of new phages via the lytic cycleLysogenic cycles can also occur ineukaryotes, although the method of DNA incorporation is not fully understood.

The distinction between lysogenic and lytic cycles is that the spread of the viral DNA occurs through the usual prokaryotic reproduction, while the lytic phage is spread through the production of thousands of individual phages capable of surviving and infecting other cells. The keydifferencebetweenthelyticcycleandthelysogeniccycleisthatthelysogeniccycledoesnot lysethehostcell.Phages thatreplicateonlyviathelyticcycleareknownasvirulentphageswhile phages that replicate using both lytic and lysogenic cycles are known astemperate phages.

In the lysogenic cycle, the phage DNA first integrates into the bacterial chromosome to produce the prophage. When the bacterium reproduces, the prophage is also copied and is present in each of the daughter cells. The daughter cells can continue to replicate with the prophage present or the prophage can exit the bacterial chromosome to initiate the lytic cycle.

The**lyticcycle**is one of thetwo cycles of viralreproduction, theotherbeingthelysogeniccycle. The lytic cycle results in the destruction of the infected celland its membrane. A key difference between the lytic and lysogenic phage cycles is that in the lytic phage, the viral DNA exists as a separate molecule within the bacterial cell, and replicates separately from the host bacterialDNA. The location of viral DNA in the lysogenic phage cycle is within the host DNA, therefore in both cases the virus/phage replicates using the host DNA machinery, but in the lytic phage cycle, the phage is a free floating separate molecule to the host DNA.

**Description**

Viruses that onlyuse lytic cycle are called virulentviruses (in contrast totemperateviruses). The lytic cycle is a six-stage cycle. In the first stage, called "penetration," the virus injects its own nucleic acid into a host cell. In some viruses this genetic material is circular and mimics a bacterialplasmid. The virus hijacks the cell's replication and translation mechanisms, using them to make more viruses. Once enough virionshave accumulated, specialized viral proteins are allowedtodissolvethebacterial cellwall.Thecellburstsduetohighinternal osmoticpressure (water pressure) that can no longer be constrained by the cell wall. This releasesprogeny virions into the surrounding environment, where they can go on to infect other cells.

**Penetrating**

Toinfectacell,avirusmust firstenterthecellthroughthe plasmamembraneand(ifpresent)the cell wall. Viruses do so by either attaching to a receptor on the cell's surface or by simple mechanical force.The binding is due to electrostatic interactions and is influenced by pH and presence of ions such as $Mg^{2+}$ and $Ca^{2+}$. The virus then releases its genetic material (eithersingle- or double-strandedRNAorDNA) into the cell. In doing this, the cell becomes infected and can also be targeted by the immune system.

**Biosynthesis**

The virus' nucleic acid uses the host cell’s metabolic machinery to make large amounts of viral components.InthecaseofDNAviruses,theDNA transcribesitselfintomessengerRNA(mRNA) molecules that are then used to direct the cell's ribosomes. One of the first polypeptides to be translated destroys the host's DNA. In retroviruses (which inject an RNA strand), a unique enzyme calledreverse transcriptasetranscribes the viral RNA into DNA, which is then transcribed again into RNA. Once the viral DNA has taken control it induces the host cell'smachinerytosynthesizeviralDNA,proteinandstartsmultiplying.About25minutesafter

initialinfection,approximately200newbacteriophagesareformedandthebacterialcellbursts i.e. it has undergone lysis. Newlyformed phages arereleased to infect other bacteria and another lytic cycle begins. The phage which causes lysis of the host is called a lytic or virulent phageThe biosynthesis is (e.g.T4) regulated in three phases of mRNA production followed by a phase of protein production.

**Earlyphase**

Enzymes modify the host's DNA replication byRNA polymerase. Amongst other modifications, virus T4 changes thesigma factor of the host by producing ananti-sigmafactorso that the host promotorsare not recognized any more but now recognize T4 middle proteins. For protein synthesisShine-Dalgarnosubsequence GAGG dominates an early genes translation.

**Middle phase**

Virusnucleicacid (DNAor RNAdependingon virustype).

**Late phase**

Structuralproteins includingthose fortheheadand the tail.

**Maturationandlysis**

After many copies of viral components are made, they are assembled into complete viruses. The phage then directs production oflysin, an enzyme that breaks down the bacterial cell wall, which allows extracellular fluid to enter the cell. The cell eventually becomes filled with viruses (typically 100-200) and liquid, and bursts, or lyses; thus giving the lytic cycle its name. The new viruses are then free to infect other cells.

***Lyticcyclewithoutlysis***

Some viruses escape the host cell without bursting the cell membrane, but rather bud/extrude off from it by taking a portion of the membrane with them. Because it otherwise is characteristic of the lytic cycle in other steps, it still belongs to this category, although it is sometimes named the Productive Cycle. HIV,influenzaand other viruses that infect eukaryotic organisms generallyuse this method. These group includes all viruses that have a lipid membrane.