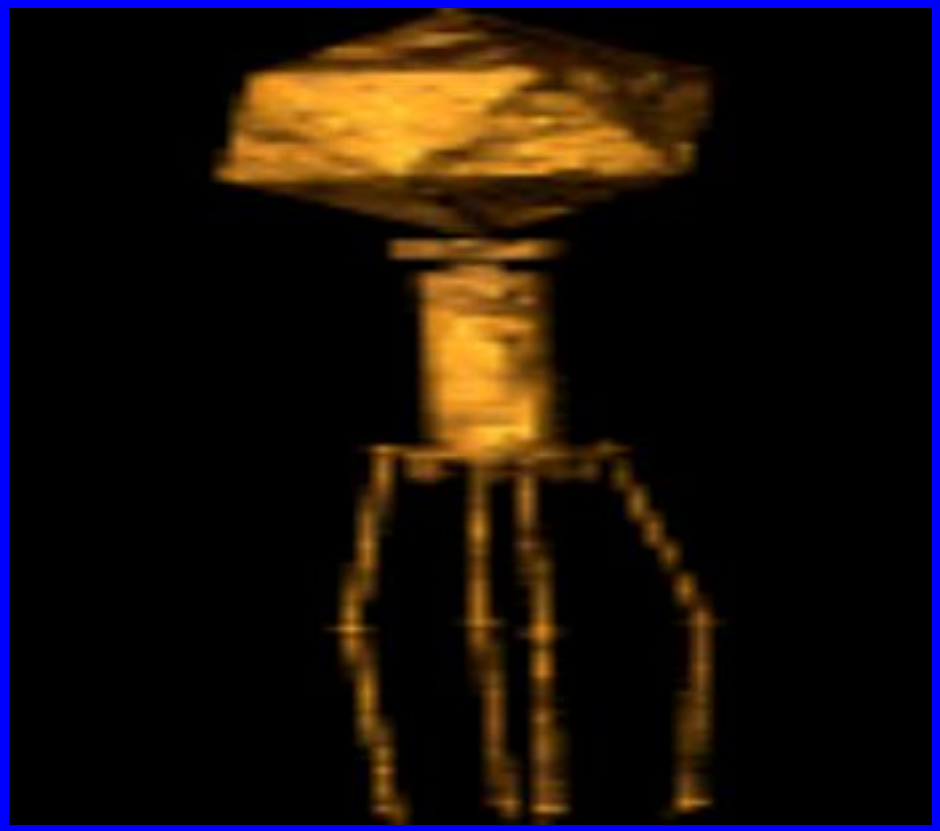
Chapter 5 *Viruses*



Chapter outline

5.1 General Properties of Viruses
5.2 General Features of Virus Reproduction
5.3 Overview of Bacterial Viruses
5.4 Temperate Bacteriophages: Lysogeny and Lambda
5.5 Overview of Animal Viruses
5.6 Pox Viruses
5.7 Adcnoviruses
5.8 Retroviruses
5.9 Viroids and Prions

Concepts

- Viruses are simple, acellular entities consisting of one or more molecules of either DNA or RNA enclosed in a coat of protein. They are reproduced only within living cells and are obligately intracellular parasites
- The nucleic acid strands can be linear, closed cycle, or able to assume either shape.
- Viruses are classified on the bases of their nucleic acid's characteristics, capsid symmetry, the presence or absence of an envelop, their host and other properties.

5.1 General Properties of Viruses

Viruses differ from living cells in at least three ways:

(1) Their simple, acellular organization ,

(2) The absence of both DNA and RNA in the same virion,

(3) Their inability to reproduce independently of cells and carry out cell division as prokaryotes and eukaryotes do.

Viruses can exist in two phases

Extracellular and intracellular

Virion, the extracellular phase, posses few if any enzymes and can not reproduce independently of living cells. In the intracellular phase, viruses exist primarily as replicating nucleic acids that induce host metabolism to synthesize virion components; eventually complete virus particles or virions are released.

Hosts

The particular host range of a virus is determined by the virus's requirements for its specific attachment to the host cell and the availability within the potential host of cellular factors required for viral multiplication.

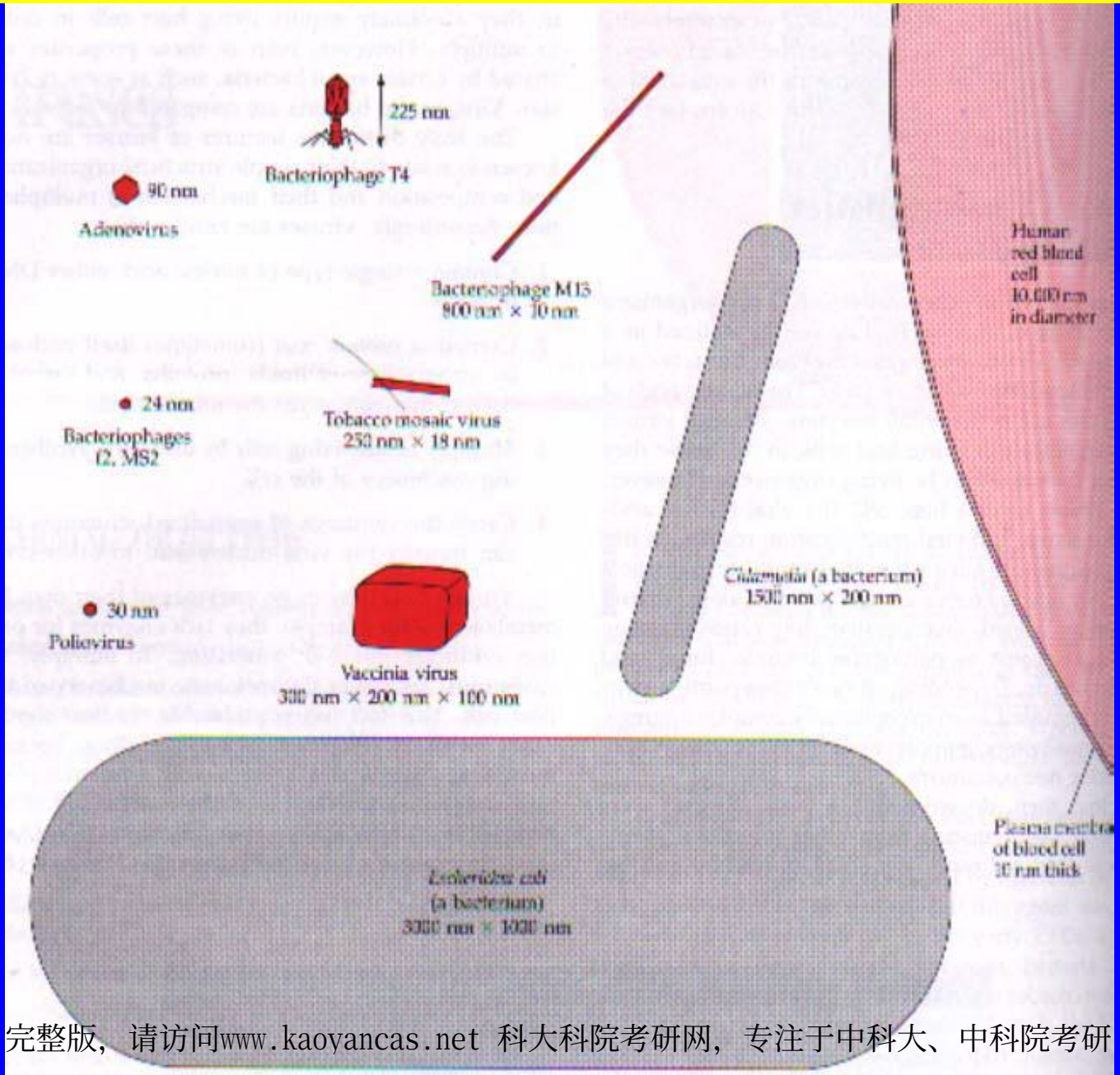
Three main classes - animal viruses, bacterial viruses (bacteriophages), and plant viruses.

Size

Viruses vary considerably in size. Although most are quite a bit smaller than bacteria, some of the larger viruses (such as the smallpox virus) are about the same size as some very small bacteria (such as the mycoplasmas, rickettsias, and chlamydias).

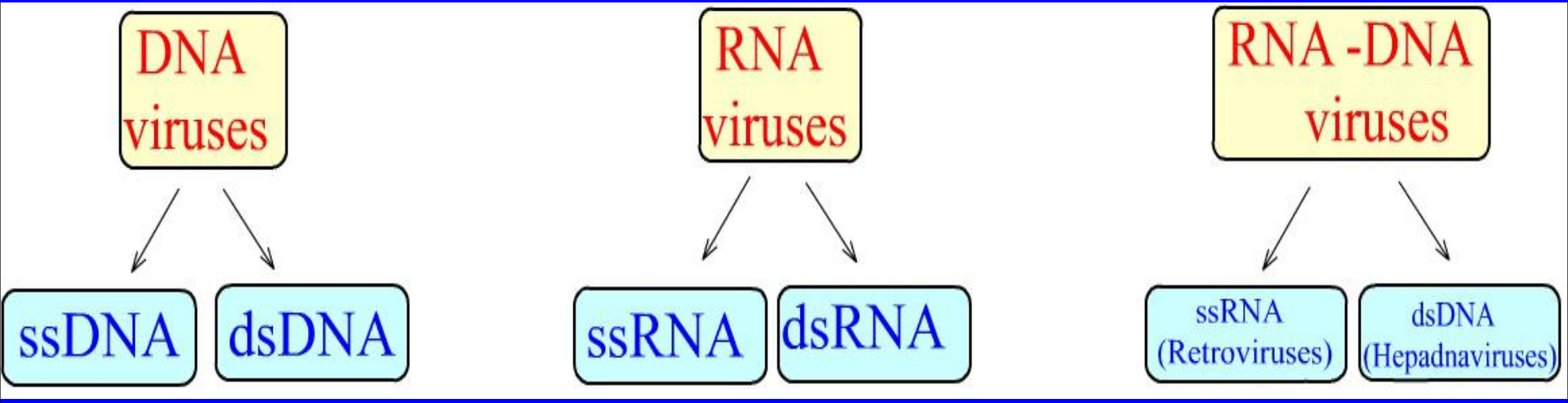
Viruses range from 20 to 300 nm in diameter

The comparative sizes of several viruses and bacteria:

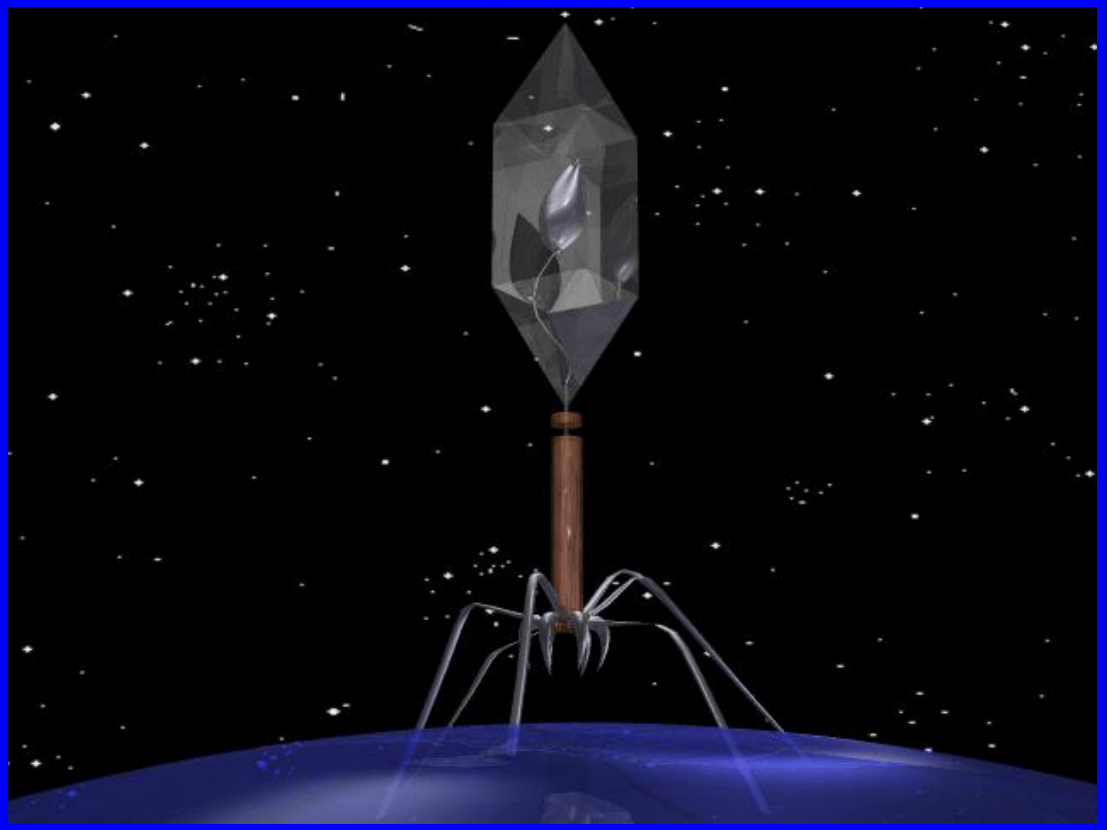


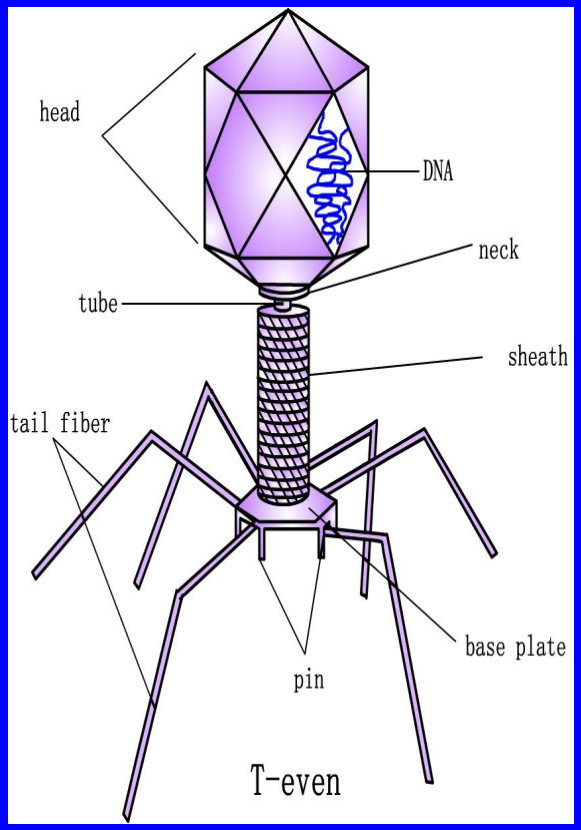
Virus particles (virions) vary widely in size and shape. Viruses are smaller than cells, ranging in size from 0.02 to 0.3 um. Smallpox virus, one of the largest viruses, is about 200 nm in diameter; poliovirus, one of the smallest, is only 28 nm in diameter.

Genome in virion



The genomes of viruses can be composed of either DNA or RNA, and some use both as their genomic material at different stages in their life cycle. However , only one type of nucleic acid is found in the virion of any particular type of virus.

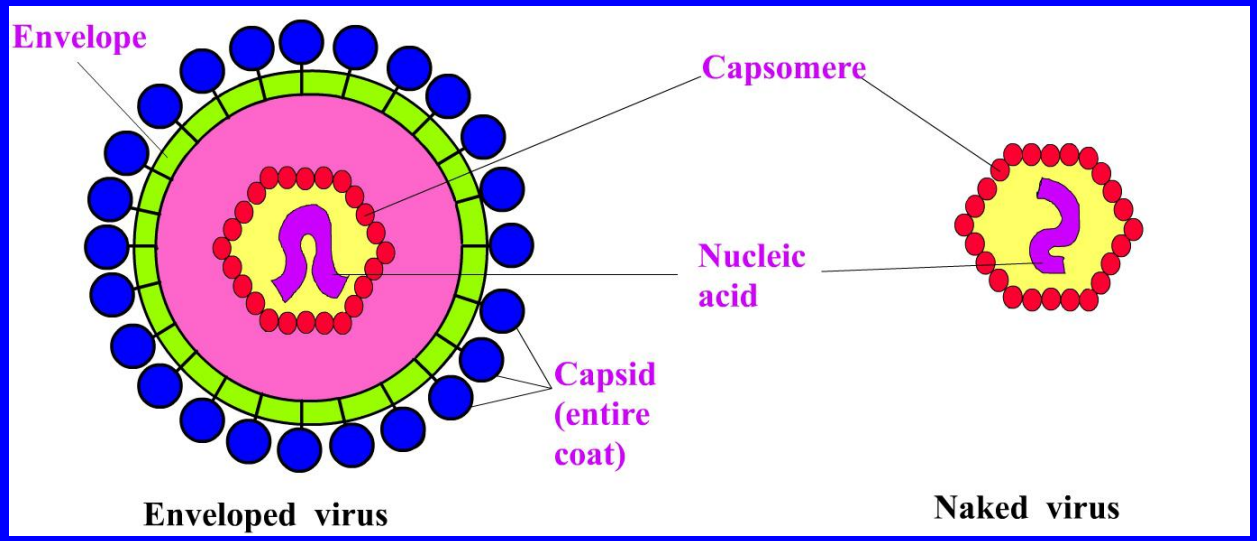




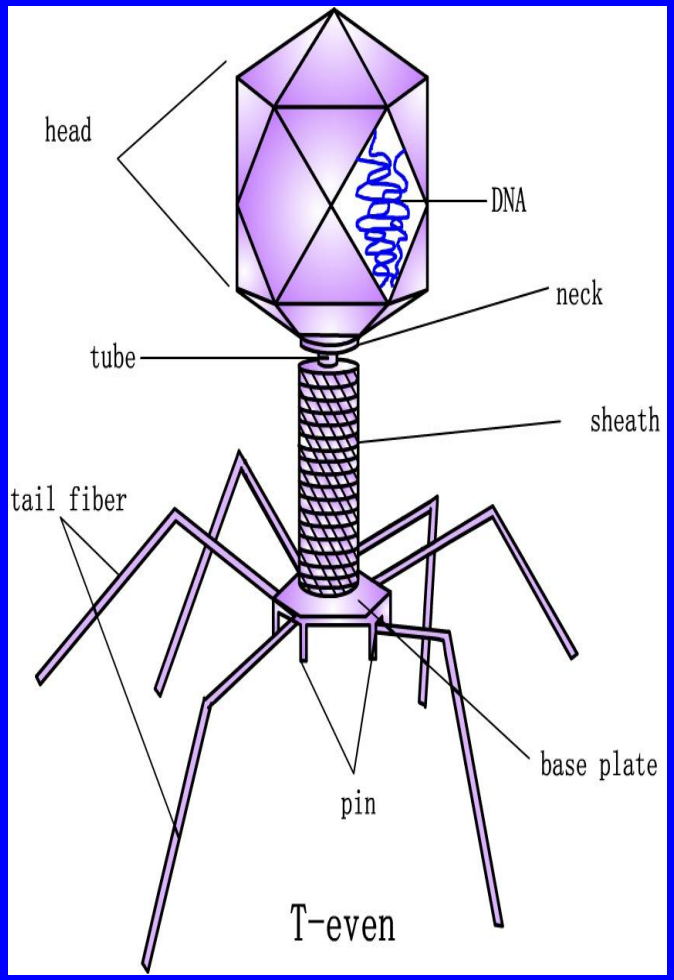
A virus can have either DNA or RNA but never both !!

Structure of viruses

- Most viruses are too small to be seen under light microscope.
- All viruses consists of an RNA or DNA core genome surrounded by a protein coat capsid.
- The combined viral genome and capsid is called the nucleocapsid.



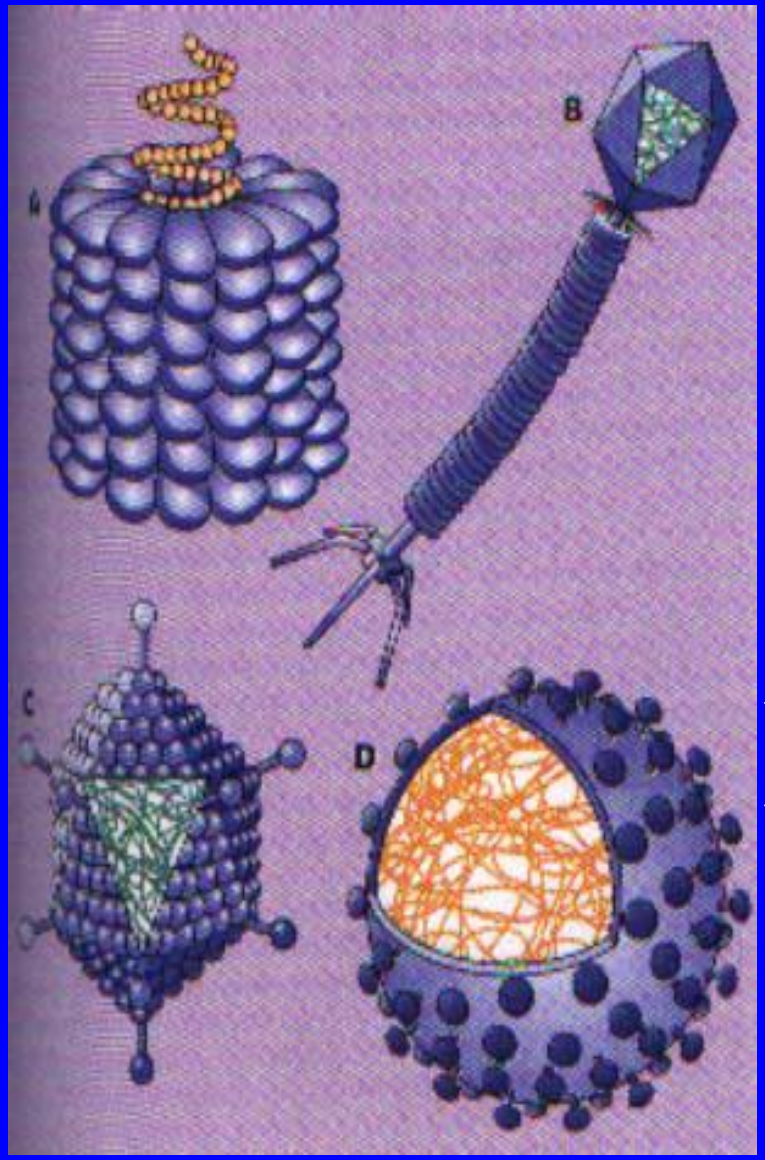
Complex viruses



Some viruses have complicated structures and are called complex viruses. Examples of complex viruses are poxviruses, which do not contain clearly identifiable capsids but have several coats around the nucleic acid. Certain bacteriophages have capsids to which additional structures are attached.

General morphology

Viruses may be classified into several morphological types on the basis of their capsid architecture as revealed by electron microscopy and a technique called x-ray crystallography.



A. Some viruses, such as tobacco mosaic virus, have a helical symmetry with the capsid surrounding an RNA genome.

B. Many viruses that infect bacteria, such as the T-even bacteriophage, have a complex capsid with DNA contained within the head structure.

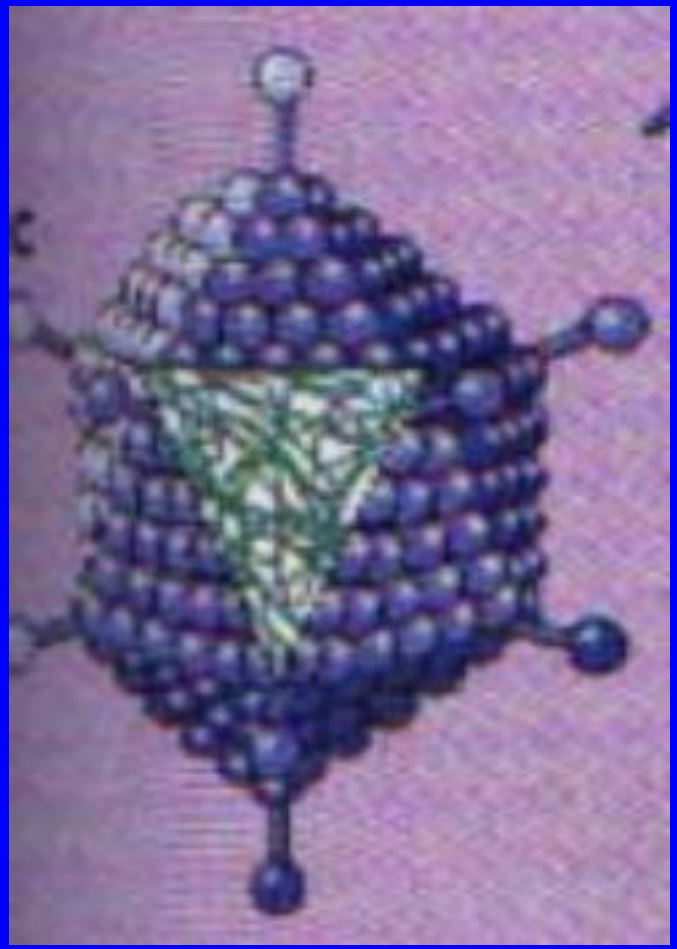
C. Some animal viruses, such as adenovirus, have isometric symmetry and a DNA genome.

D. Others, such as coronavirus, have complex capsids and an envelope with protruding proteins surrounding an RNA genome.

Helical viruses

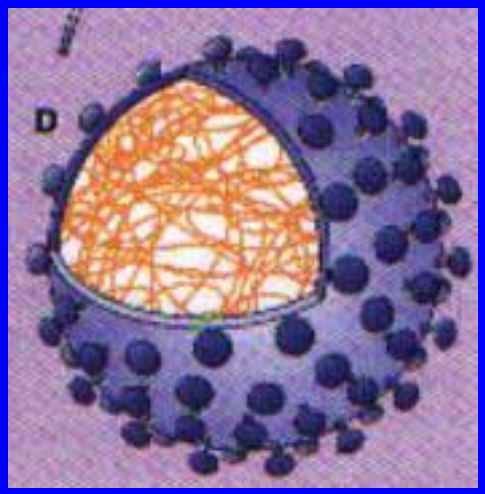
Helical viruses resemble long rods that may be rigid or flexible. Surrounding the nucleic acid, their capsid is a hollow cylinder with a helical structure. An example of a helical virus that is a rigid rod is the tobacco mosaic virus.

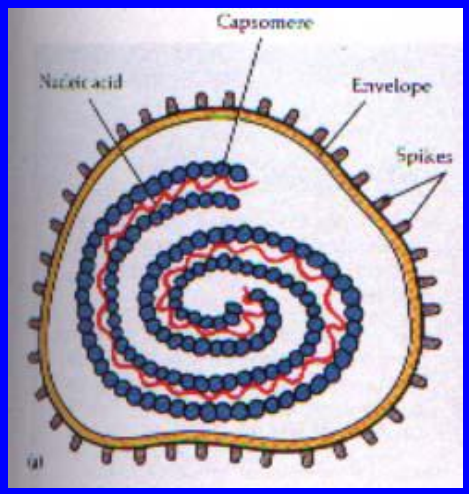
Polyhedral viruses



The capsid of most polyhedral viruses is in the shape of a regular polyhedron with 20 triangular faces and 12 corners. The capsomeres of each face form an equilateral triangle. An example of a polyhedral virus is the adenovirus. Another is the poliovirus.

Enveloped viruses

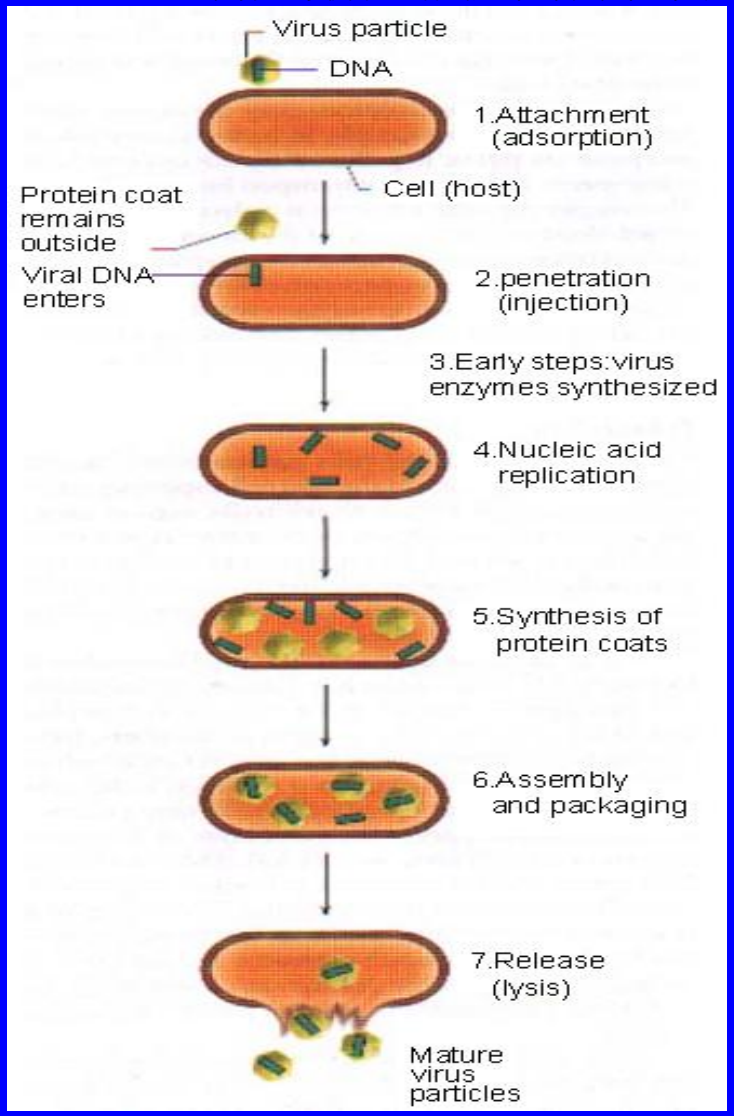




The capsid of viruses is covered by an envelope. Enveloped viruses are roughly spherical but variable in shape. When helical or polyhedral viruses are enclosed by envelopes, they are called enveloped helical and enveloped polyhedral viruses.

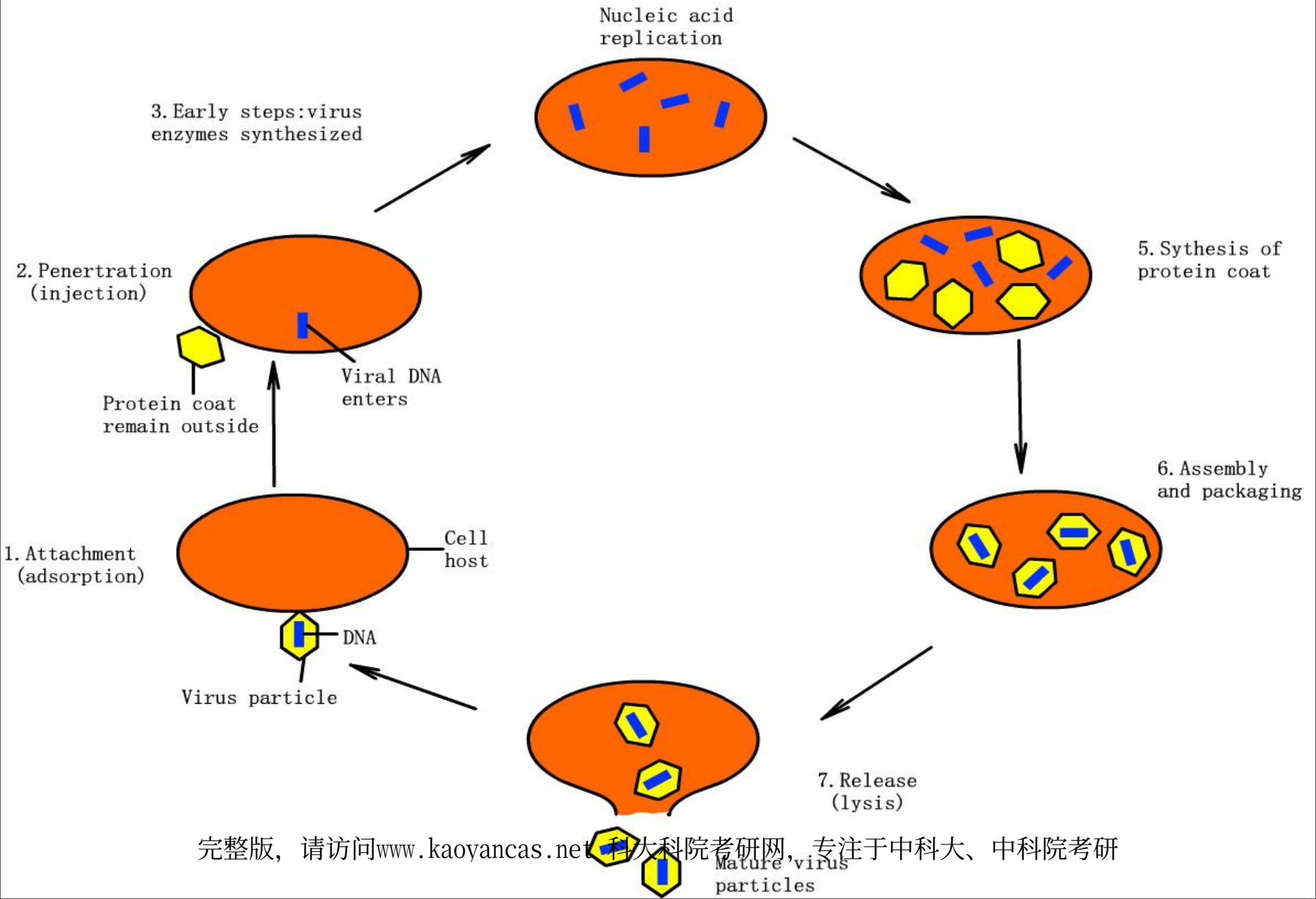
5.2 General Features of Virus Reproduction

The virus must induce a living host cell to synthesize all the components needed to make virus particles. These components must then be assembled into the proper structure, and the new virions must escape from the cell and infect other cells. The phases of this replication process in a bacteriophage can be categorized in seven steps.



1. **Attachment**
2. **Penetration**
3. **Early steps in replication**
4. **Replication**
5. **Synthesisof proteins**
6. **Assembly**
7. **Release**

The Replication Cycle of a Bacterial Virus



Multiplication of bacteriophages

1. Attachment (adsorption) of the virion to a susceptible host cell.

2. Penetration (injection) of the virion or its nucleic acid into the cell.

3. Early steps in replication during which the host cell biosynthetic machinery is altered as a prelude to virus nucleic acid synthesis. Virus-specific enzymes are typically made.

4. Replication of the virus nucleic acid.

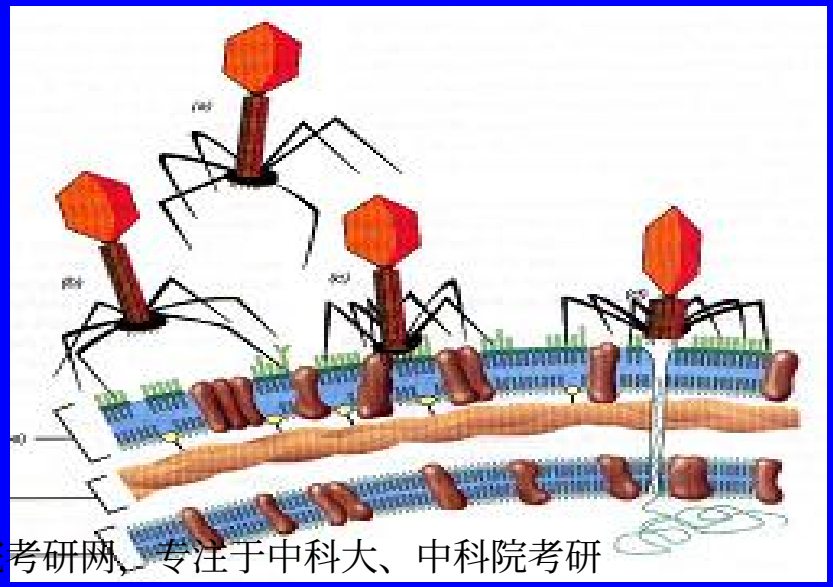
5. Synthesis of proteins used as structural subunits of the virus coat.

6. Assembly of structural subunits (and membrane components in enveloped viruses) and packaging of nucleic acid into new virus particles.

7. Release of mature virions from the cell.

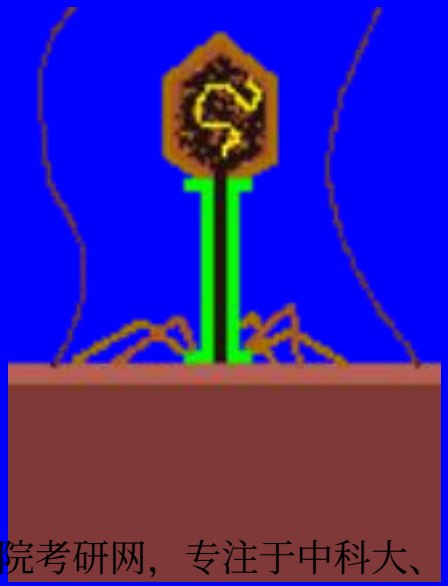
Attachment of phage to host cell :

After a chance collision between phage particles and bacteria, an attachment site on the virus attaches to a complementary receptor site on the bacterial cell.



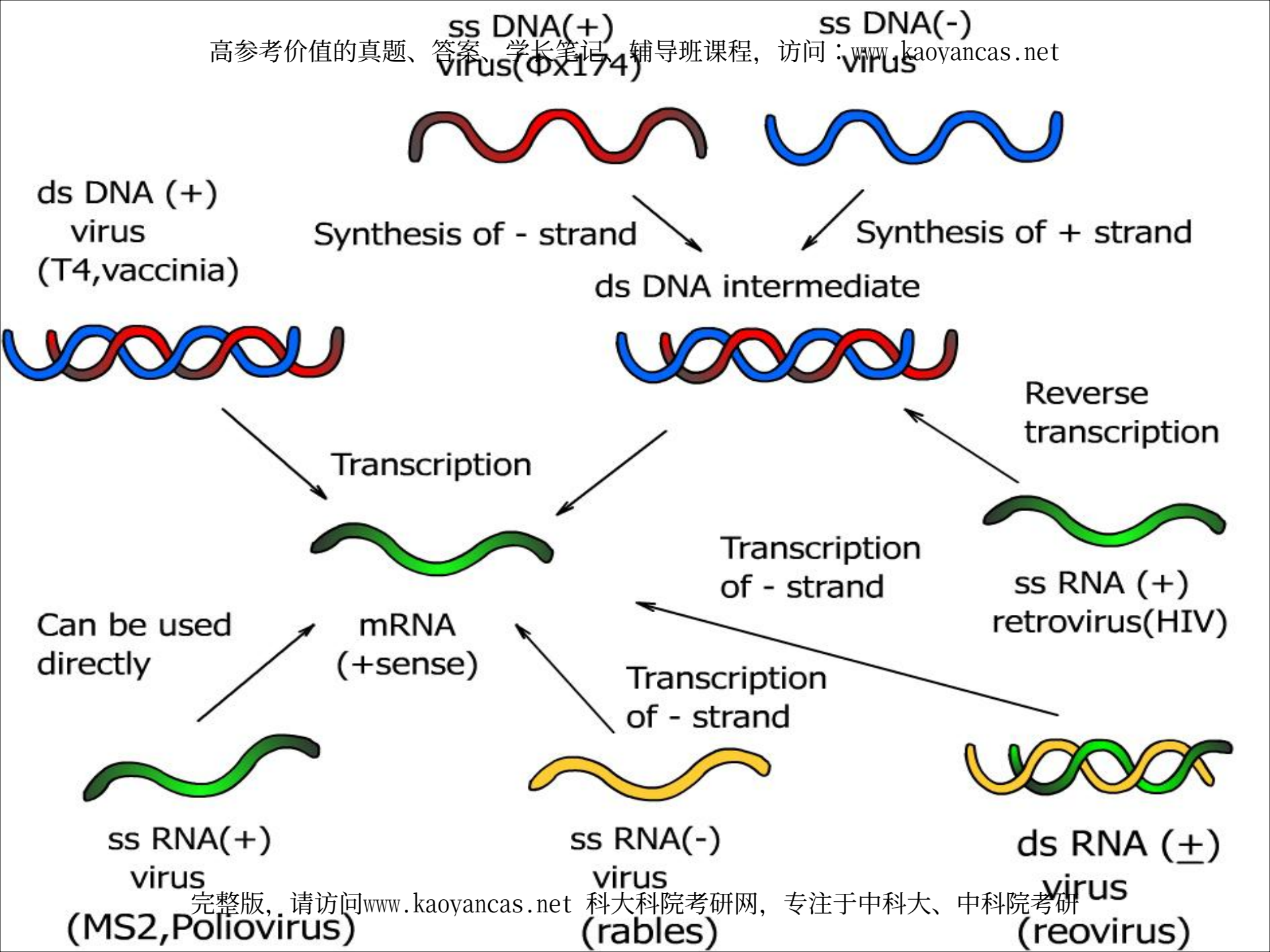
Penetration:

During the process of penetration, the bacteriophage's tail releases an enzyme, p*hage lysozyme,* which breaks down a portion of the bacterial cell wall. then the bacteriophage injects its DNA (nucleic acid) into the bacterium.



Biosynthesis of viral components:

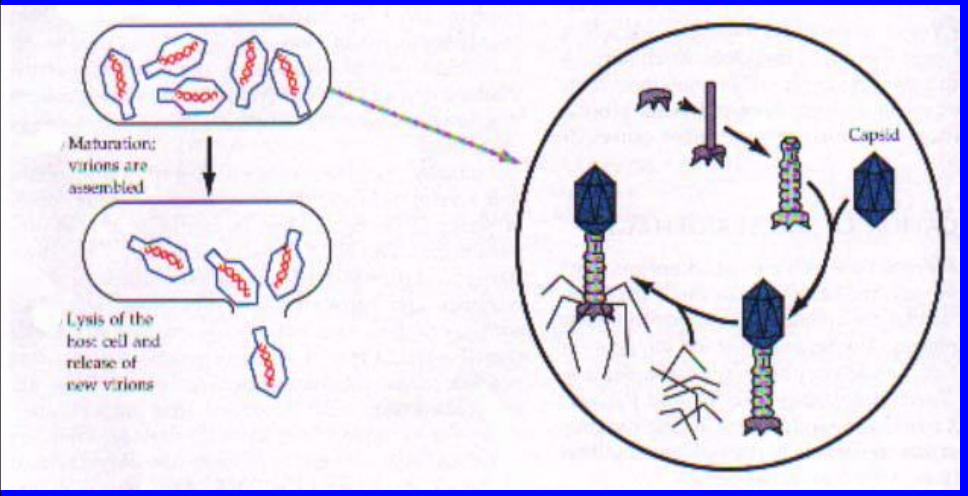
Any RNA transcribed in the cell is mRNA transcribed from phage DNA for biosynthesis of phage enzymes and capsid protein. The host cell's ribosomes, enzymes, and amino acids are used for translation.

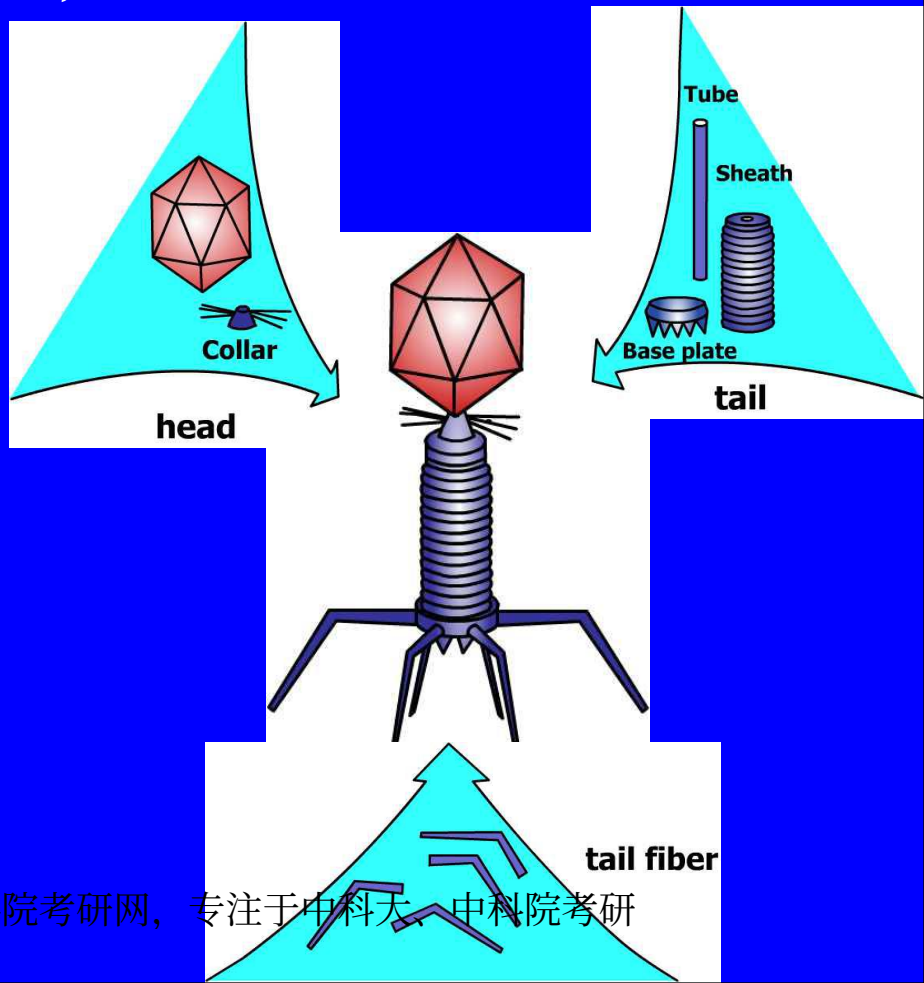


Formation of mRNA after infection of cells by viruses of different types. The chemical sense of the mRNA is considered as plus(+). The sense of the various virus nucleic acids are indicated as+if the same as mRNA, as–if opposite, or as+–if double–stranded. Examples are indicated next to the virus nucleic acid.

Maturation and release:

The phage heads and tails are separately assembled from protein subunits, the head is packaged with phage DNA, and the tail is attached.



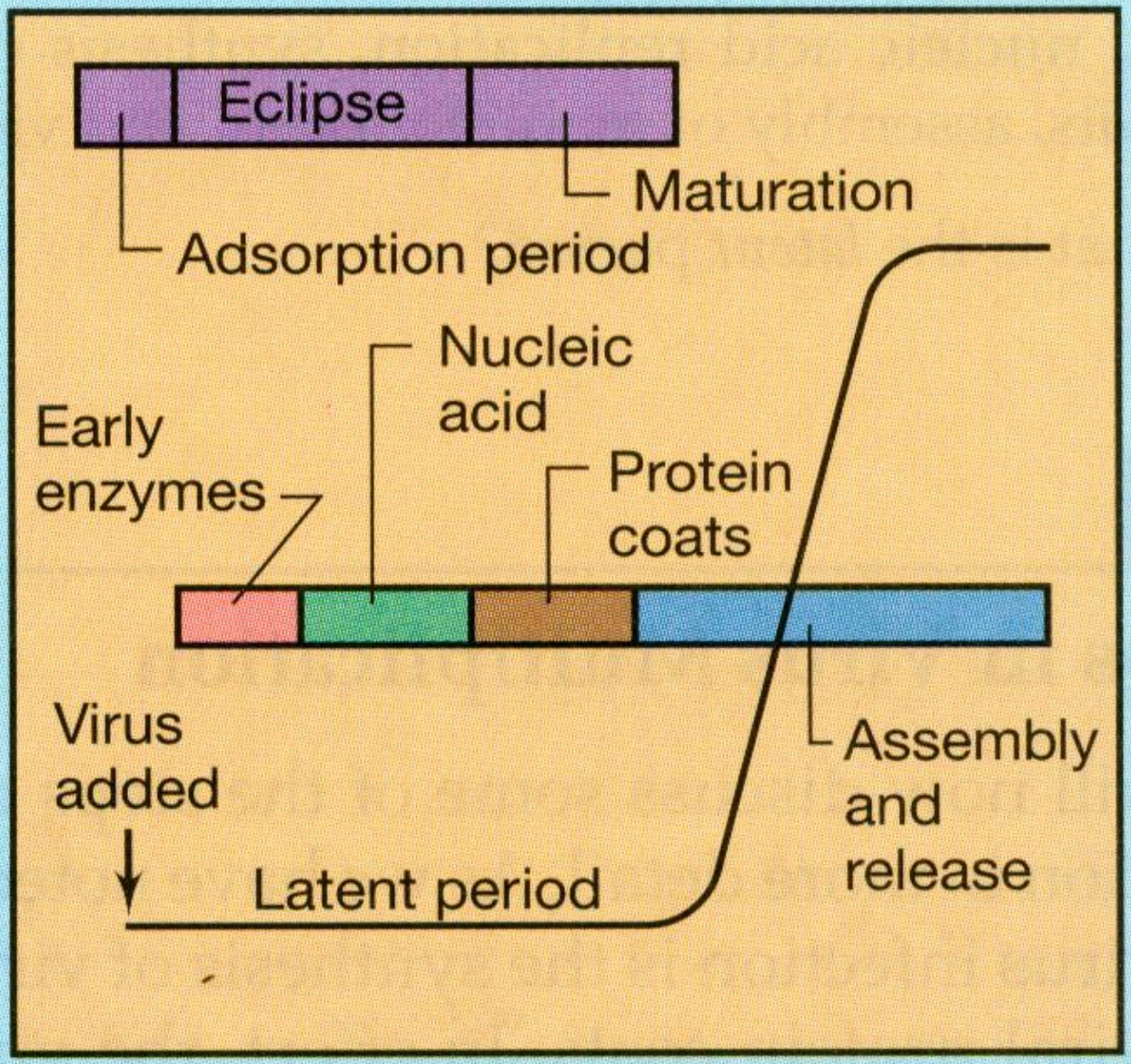


Release:

Lysozyme, whose code is provided by a phage gene, is synthesized within the cell. This enzyme causes a breakdown of the bacterial cell wall, and the newly produced bactedophages are released from the host cell.

The number of newly synthesized phage particles released from a single cell usually ranges from about 50 to 200.

One-step growth curve of virus replication



Following adsorption, the infectivity of the virus particles disappears, a phenomenon called eclipse. This is due to the uncoating of the virus particles.

During the latent period, replication of viral nucleic acid and protein occurs. The maturation period follows, when virus nucleic acid and protein are assembled into mature virus particles. At this time, if the cells are broken up, active virus can be detected.

Finally, release occurs, either with or without cell lysis.

The liming of the one-step growth cycle varies with the virus and host. With many bacterial viruses, the whole cycle may be complete in 20-60 min, whereas with animal viruses 8-40 hr is usually required for a complete cycle.

Eclipse period

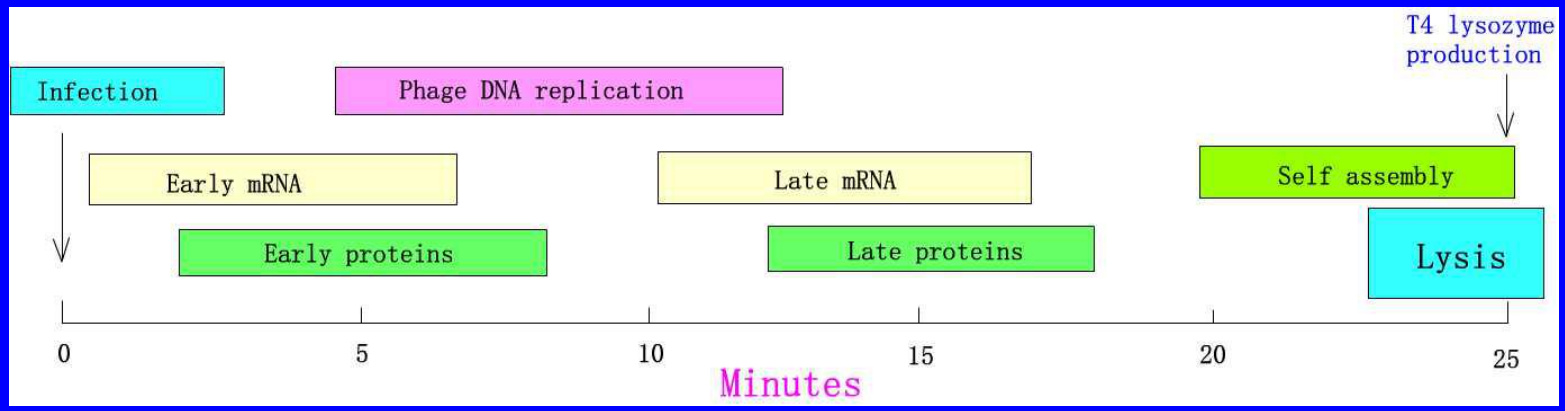
• There are genetic controls that regulate when different regions of phage DNA are transcribed into mRNA during the multiplication cycle.

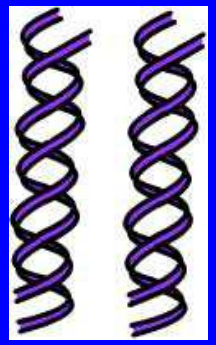
• There are early messages that are translated into early phage proteins, the enzymes used in the synthesis of phage DNA.

- There are late messages that are translated into late phage proteins for the synthesis of capsid proteins.

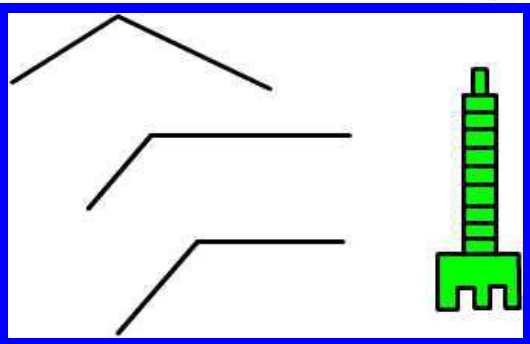
- This control mechanism is mediated by RNA polymerase.

Time course of events in phage T_4 infection

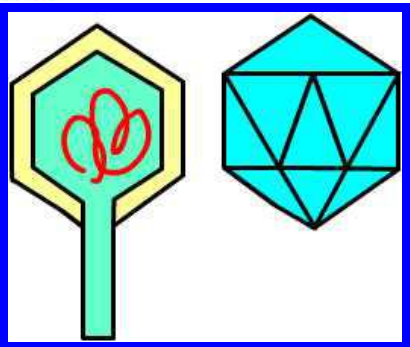




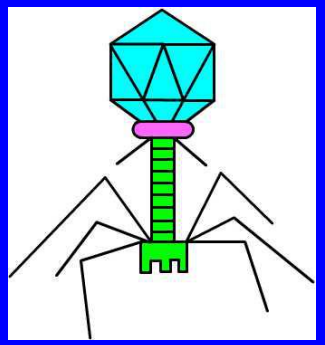
Phage DNA



Tail, collar, base plate, and tail fiber proteins



Phage head proteins



Mature phage particle

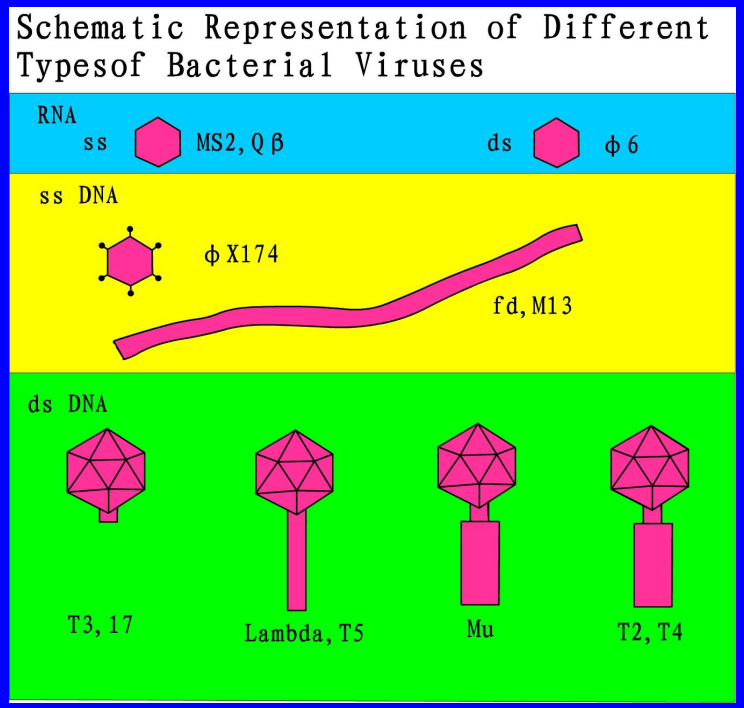
Following injection of DNA, early and middle mRNA is produced that codes for nucleases, DNA polymerase, new phage-specific sigma factors, and various other proteins involved in DNA replication. Late mRNA codes for structural proteins of the phage virion and for T4 lysozyme, needed to lyse the cell and release new phage particles.

5.3 Overview of Bacterial Viruses

Most of the bacterial viruses that have born studied in detail infect bacteria of the enteric group, such as *Escherichia coli* and *Salmonella typhimurium*.

However, viruses are known that infect a variety of prokaryotes, both bacteria and archaea. A few bacterial viruses have lipid envelopes but most do not. However, many bacterial viruses are structurally complex, with head and complex tail structures.

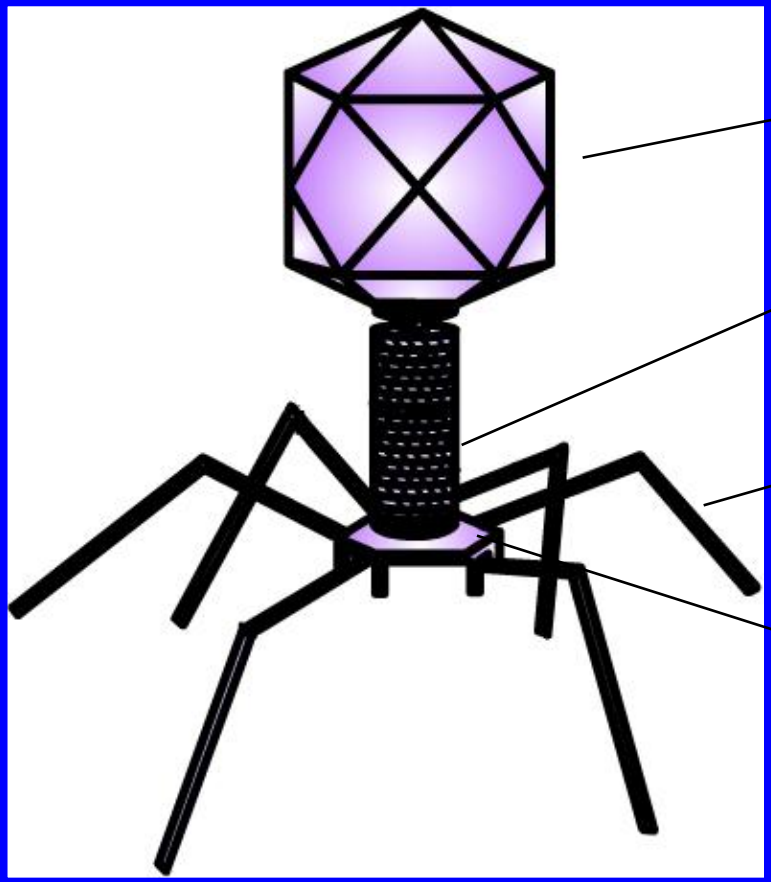
Schematic representations of the main types of bacterial viruses



The structures of M13, Φχ174, MS2, T4, lambda, T7 and Mu. sizes are to approximate scale.

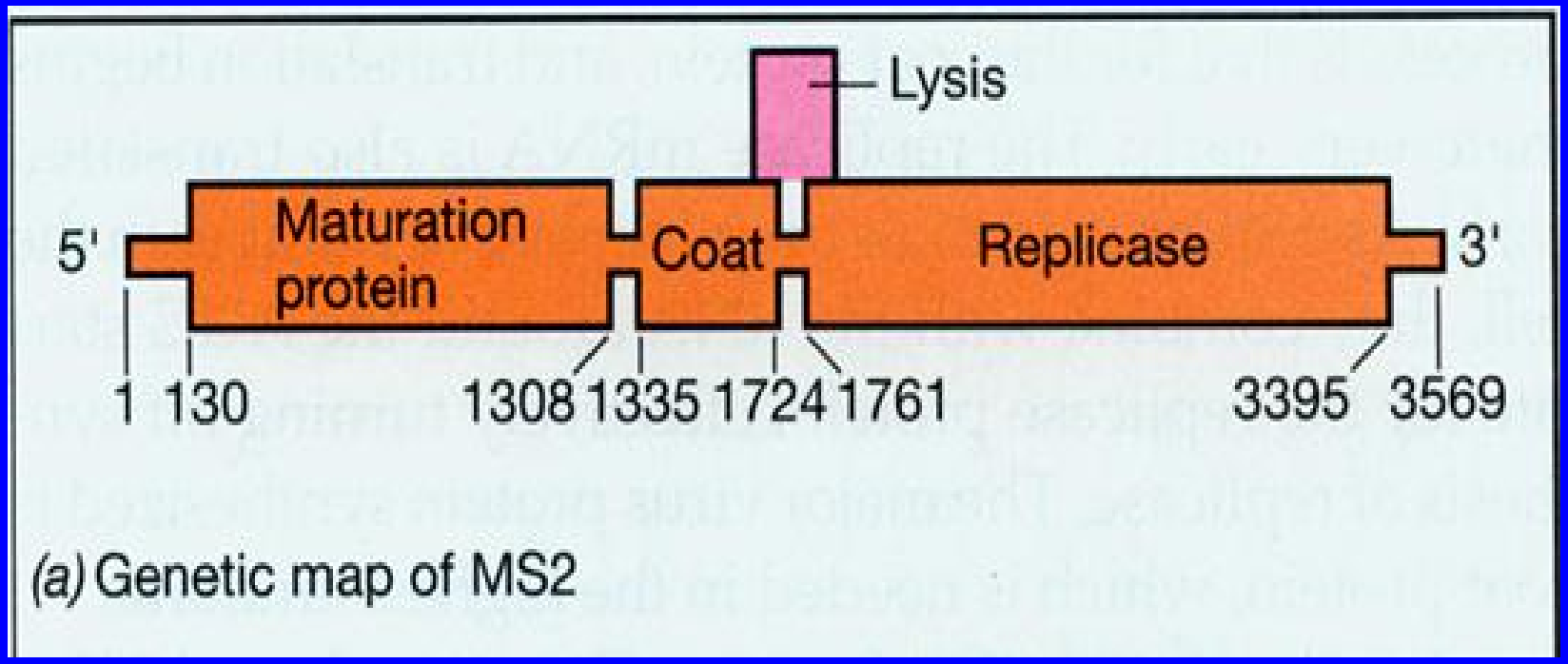
Many viruses are structurally complex, with head and complex tail structures.

There is also a great diversity in the manner in which virus multiplication occurs.

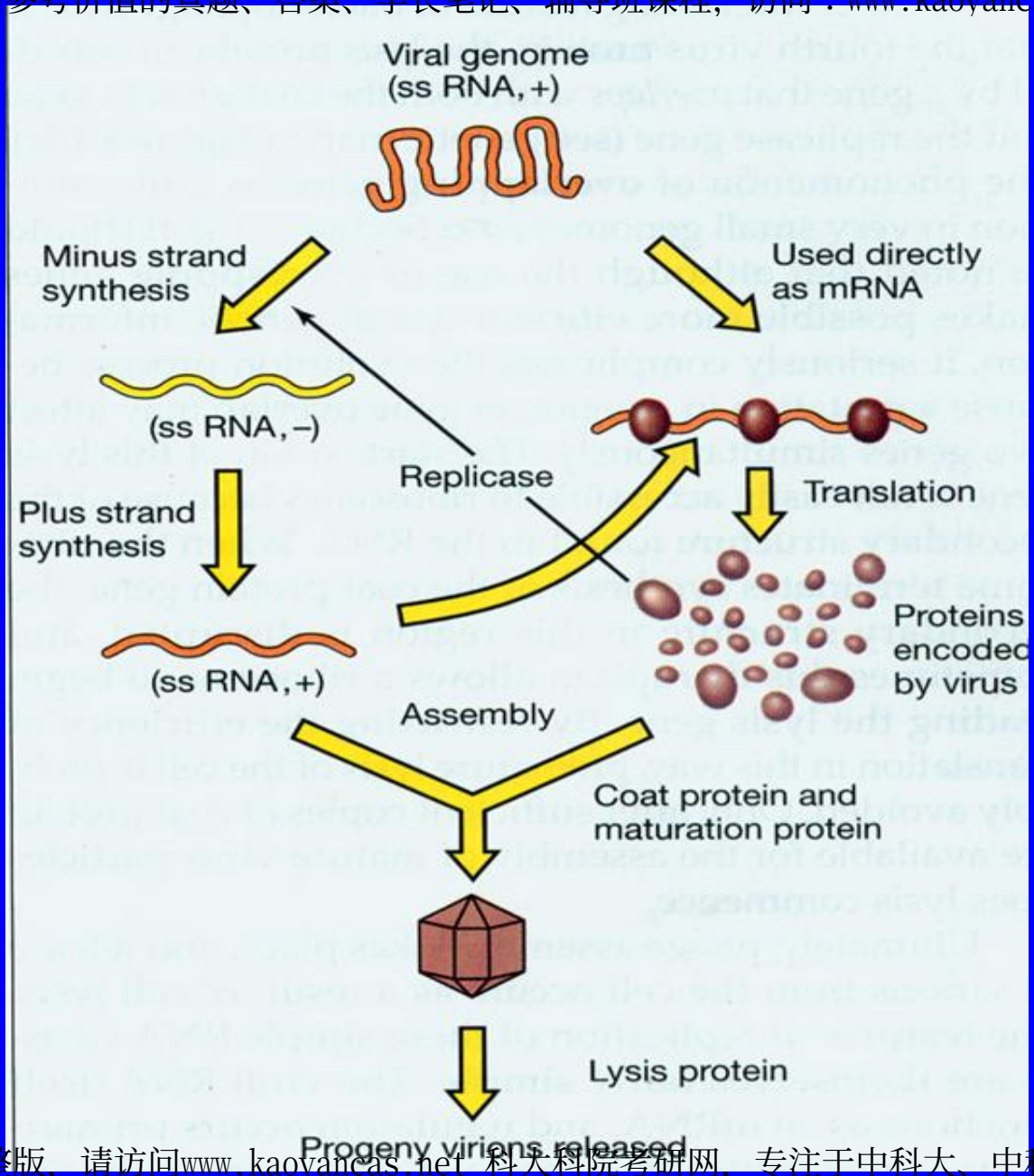


Note the complex structure. The tail components are involved in attachment of virion to the host and infection of the nucleic acid the head is about 85 nm in diameter

Genetic map of MS2



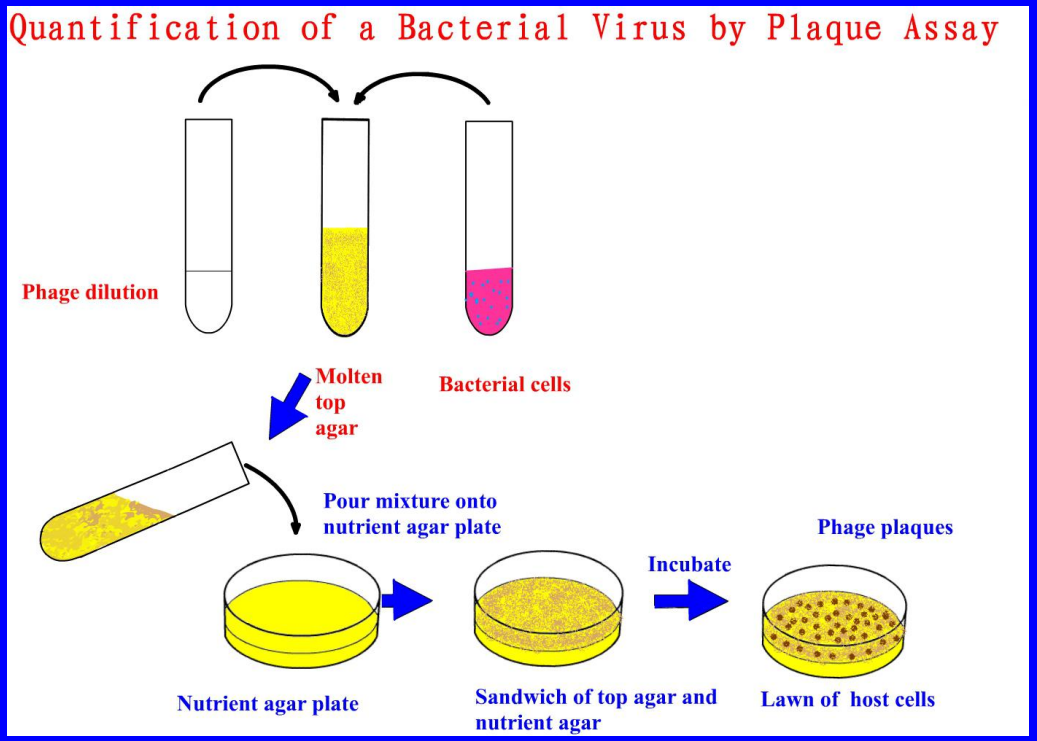
The bacterial RNA viruses are all quite small, about 26 nm in size, and they are all icosahedral, with 180 copies of coat protein per virus particle. The complete nucleotide sequences of several RNA phage genomes are known. The genome of the RNA phage MS2, which infects Escherichia coli, is 3569 nucleotides long. The RNA strand in the virion acts directly as mRNA on entry into the cell.



Flow of events during viral multiplication

The small genome encodes only four proteins. These are the **maturation protein**, **coat protein**, **lysis protein**, and a subunit of **RNA replicase**, the enzyme that brings about replication of the viral RNA. The RNA replicase is a composite protein, composed of the virus-encoded polypeptide and host polypeptides. The virus appears to employ host proteins that have distinct functions and use them to make viral replicase.

Quantification of bacterial virus by plaque assay

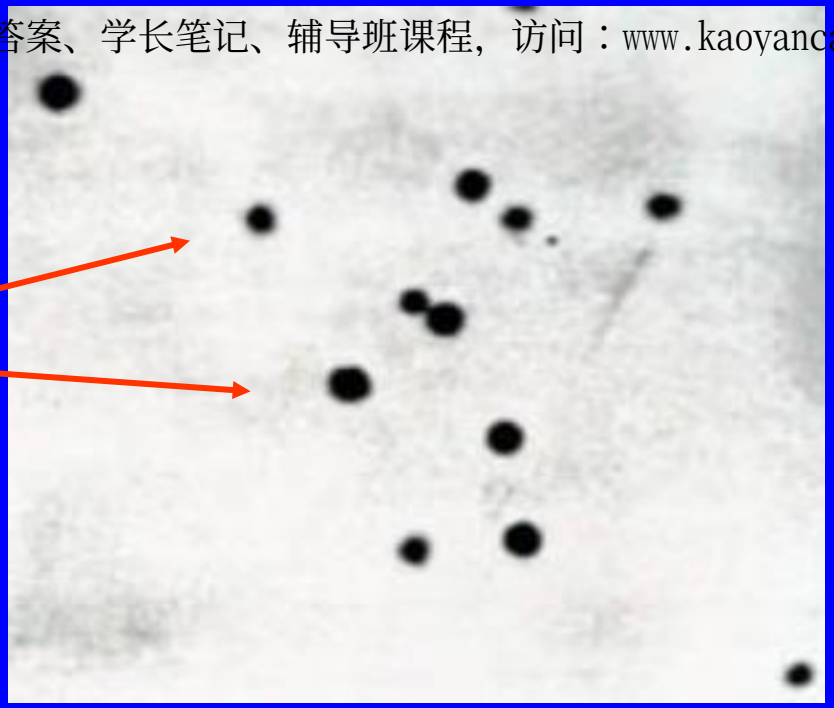


2. The mixture poured on the surface of a nutrient agar plate

3. The host bacteria begin to grow, and after overnight incubation form a lawn of confluent growth.

1. A dilution of a suspension containing the virus material is mixed in a small amount of melted agar with the sensitive host bacteria.

Phage plaques



Photograph of a plate showing plaques formed by bacteriophage on a lawn of sensitive bacteria. The plaques shown are about 1-2 mm in diameter.

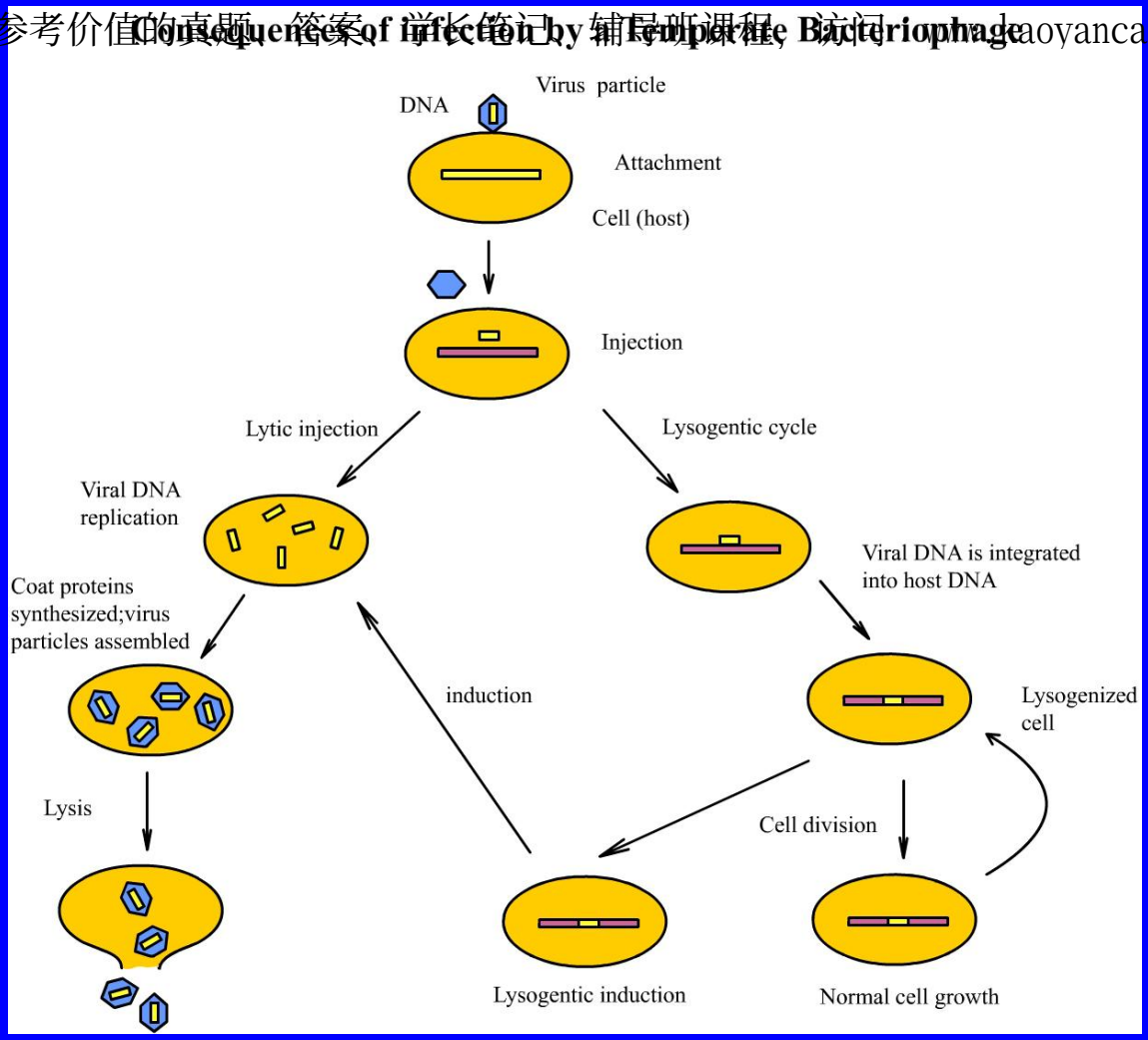
The size of the plaque formed depends on the virus, the host, and conditions of culture.

5.4 Temperate Bacteriophages: Lysogeny and Lambda

Some phages can incorporate their DNA into the host cell's DNA, The phage remains latent and does not cause lysis of the host cell. Such a state is called lysogeny.

Such phages are called lysogenic phages or temperate phages. The participating bacterial host cells are known as lysogenic cells.

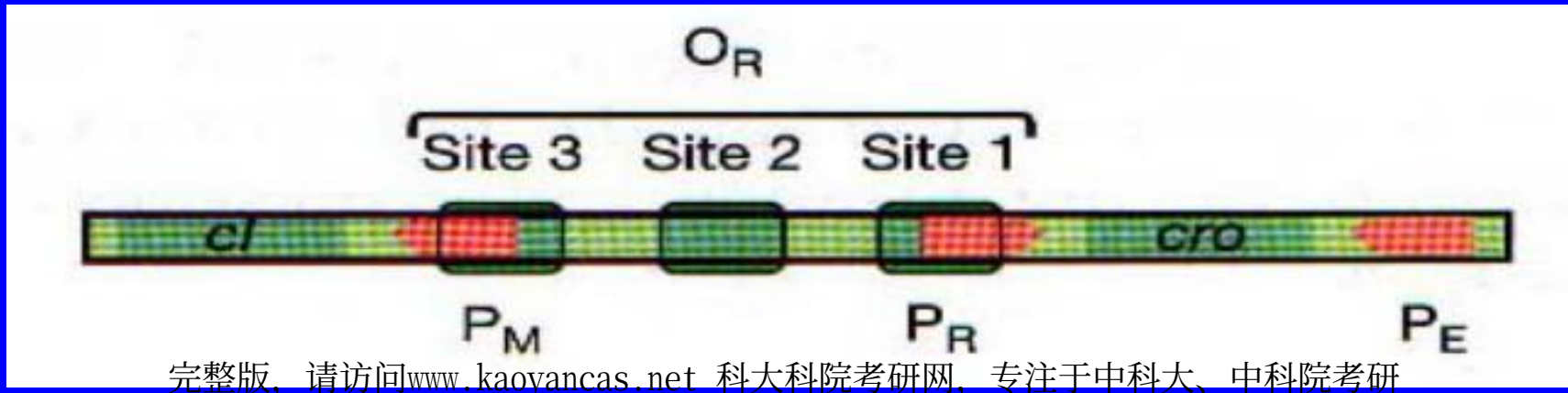
Under certain conditions these bacteria, called lysogens, can spontaneously produce virions of the temperate virus.

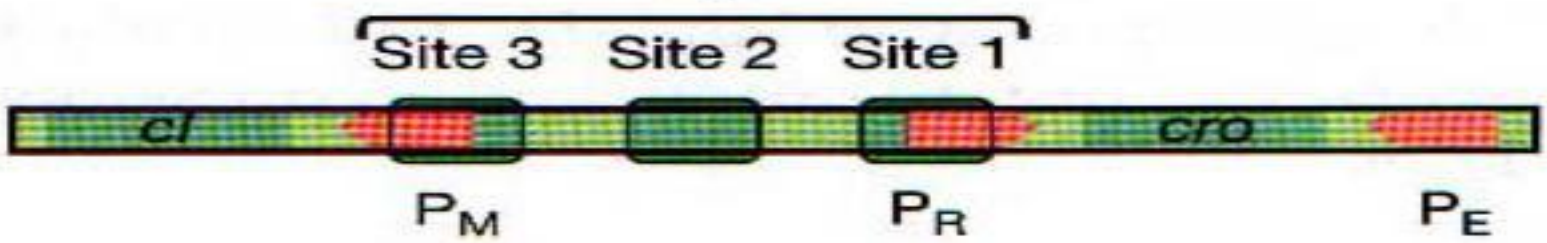


The alternatives on infection are integration of the virus DNA into the host DNA (lysogenization) or replication and release of mature virus (lysis). The lysogenic cell can also be induced to produce mature virus and lyse.

Think and answer following two questions !!

1. What are the two pathways available to temperate virus?
2. Describe how a single protein like the lambda represor can act both as an activator and a represor.





Both regulatory proteins Cro and the lambda represser bind to operator right (OR)on the lambda genome. The Cro protein binds to the three sites in the order site 3, site 2, site 1. The lambda represser binds to these sites in the opposite order. The promoter PR is transcribed on phage entry into the cell. Rightward transcription from this promoter is necessary to produce Cro protein and downstream genes. Leftward transcription from either of the promoters PE or PM is necessary to synthesize the lambda repressor. Both these promoters require activation in order to function.

Lysis or Lysogenization?

Lambda and other temperate viruses have a *genetic switch* that controls whether the lytic pathway or the lysogenic pathway is followed. So far the steps we have outlined for lambda are those for the lytic pathway. We now consider how the genetic switch can be thrown to lead to lysogeny.

Lytic Growth of Lambda After Induction

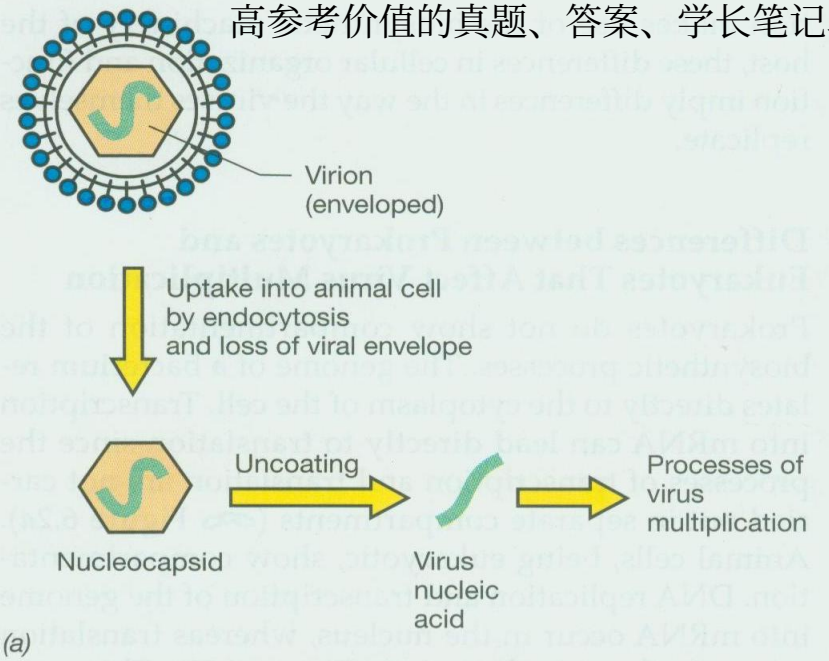
Agents that induce lambda lysogens to produce phage are agents that damage DNA. These include ultraviolet irradiation, X-rays, and DNA-damaging chemicals such as the nitrogen mustards. These agents interfere with the function of the lambda repressor.

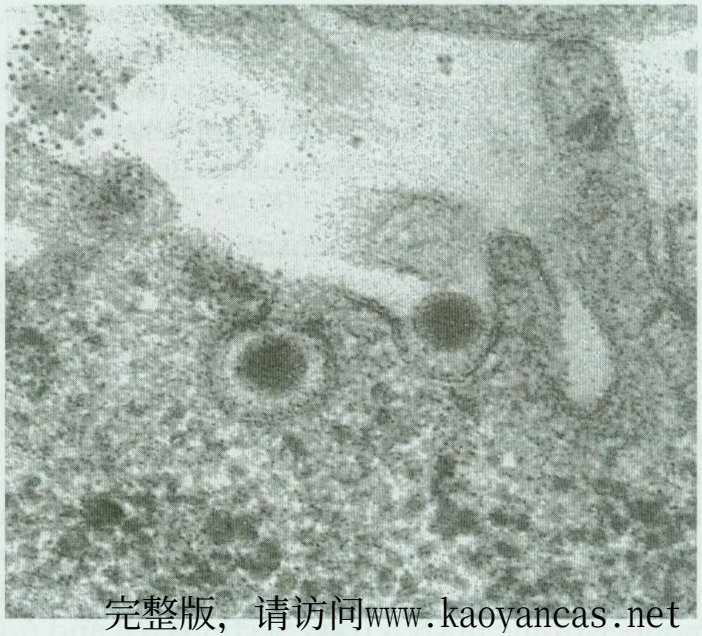
With the lambda represser destroyed, the inhibition of expression of lambda lytic genes is abolished.

5.5 Overview of Animal Viruses

One important group of animal viruses, those called the retroviruses, have both an RNA and a DNA phase of replication.

Retroviruses are especially interesting not only because of their unusual mode of replication but also because they cause such important diseases as certain cancers and acquired immunodeficiency syndrome (AIDS).





Uptake of an enveloped virion by an animal cell.

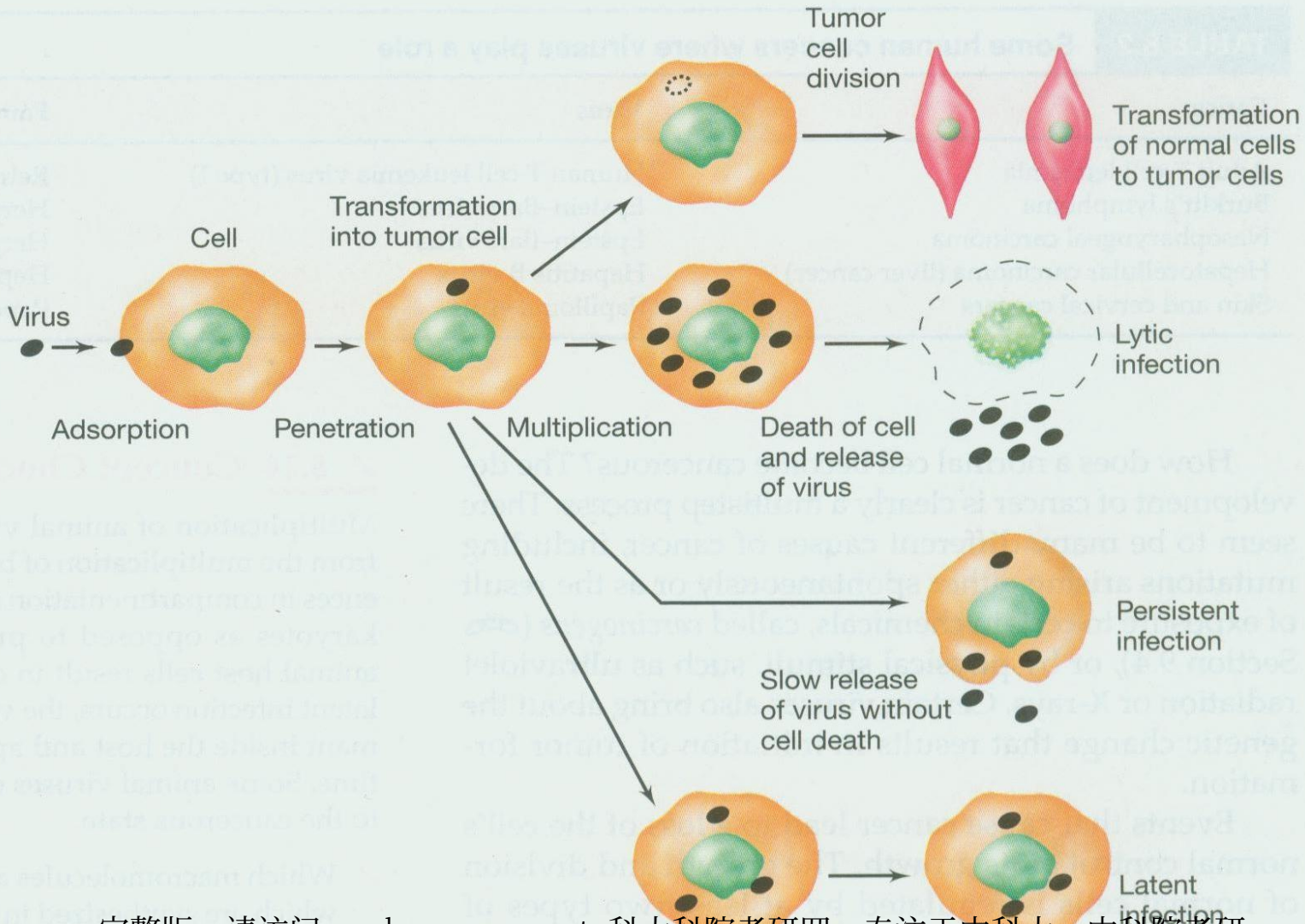
(a)The process by which the viral nucleocapsid is separated from its envelope.

(b)Electron micrograph of adenovirus virions entering a cell. Each particle is about 70 nm in diameter.

Viruses can have varied effects on cells. Lytic infection results in the destruction of the host cell. However, there are several other possible effects .

In the case of enveloped viruses, release of virions, which occurs by a kind of budding process and the host cell may not be lysed. The cell may remain alive and continue to produce virus over a long period of time. Such infections are referred to as persistent infections

Possible effects that animal viruses may have on cells they effect



Viruses and cancer

A number of animal viruses participate in the events that change a cell from a normal one to a cancer or tumor cell . Cancer is a cellular phenomenon of uncontrolled growth. Most cells in a mature animal, although alive, do not divide extensively.

Because cancerous cells in the animal body have fewer growth requirements, they grow profusely, leading to the formation of large masses of cells, called tumors.

How does a normal cell become cancerous?

The development of cancer is clearly a multistep process. There seem to be many different causes of cancer, including mutations arising either spontaneously or as the result of exposure to certain chemicals, called carcinogens, or by physical stimuli, such as ultraviolet radiation or X-rays. Certain viruses also bring about the genetic change that results in initiation of tumor formation.

Some human cancers where viruses play a role

Cancer	Virus	Family	Genome in virion
Adult T-cell leukemia	Human T-cell leukemia virus(type Ⅰ)	Retrovirus	RNA
Burkitt's lymphoma	Epstein-Barr virus	Herpes	DNA
Nasopharyngeal carcinoma	Epstein-Barr virus	Herpes	DNA
Hepatocellular carcinoma(liver cancer)	Hepatitis B virus	Hepadna	DNA
Skin and cervical cancers	Papilloma virus	Papova	DNA

5.6 Pox Viruses

The most complex and largest animal viruses known and have some characteristics that approach those of primitive cells.

The pox viruses are not able to metabolize and thus depend on the host for the complete machinery of protein synthesis.

These viruses are also unique in that they are DNA viruses that replicate in the cytoplasm.

General Properties of Pox Viruses

Smallpox was the first virus to be studied in any detail and was the first virus for which a vaccine was developed .

The pox viruses are very large, so large that they can actually be seen under the light microscope.

Vaccinia virions are taken up into cells via a phagocytic process from which the cores are liberated into the cytoplasm.

5.7 Adenoviruses

The genomes of the adenoviruses consist of linear double-stranded DNA of about 36 kilobase pairs.

Attached in covalent linkage to the 5'-terminus of the DNA is a protein component essential for infectivity of the DNA.

The DNA has inverted terminal repeats of 100-1800 base pairs (this varies with the virus strain).

The DNA of the adenoviruses is six to seven times the size of the DNA of the papovavirus SV40.

Replication of the viral DNA occurs in the nucleus.

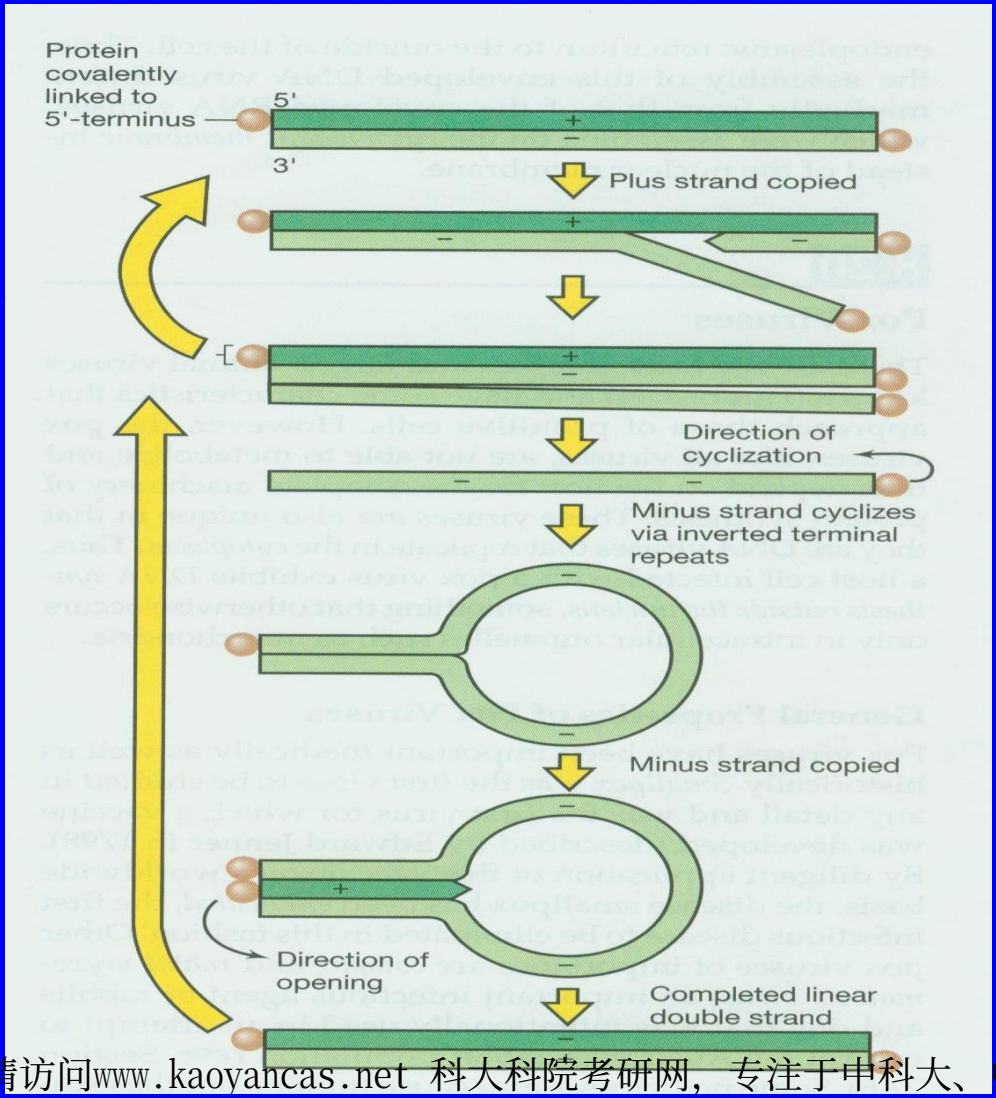
After the virus particle has been transported to the nucleus, the core is released and converted to a viral DNA-histone complex.

Early transcription is carried out by an RNA polymerase of the host, and a number of primary transcripts are made.

The transcripts are spliced, capped, and polyadenylated, giving sever al different mRNAs.

Replication of adenovirus DNA

(See text for details)



5.8 Retroviruses

The retroviruses are RNA viruses, but they replicate by means of a DNA intermediate using the enzyme reverse transcriptase.

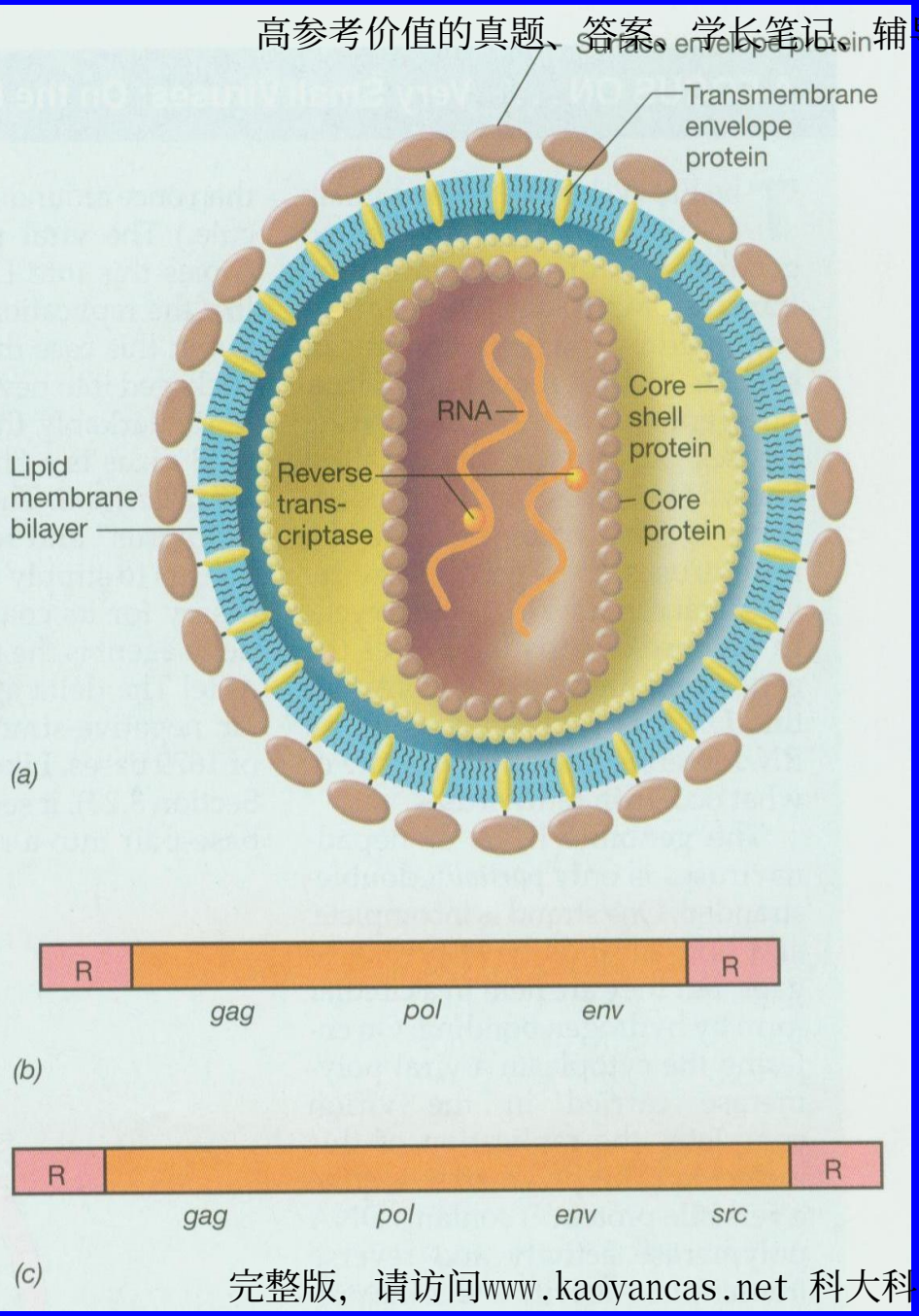
First, they were the first viruses shown to cause cancer and have been studied most extensively for their carcinogenic characteristics.

Second, one retrovirus, the one causing acquired immunodeficiency syndrome (AIDS)

1. They were the first viruses shown to cause cancer and have been studied most extensively for their carcinogenic characteristics.

2. One retrovirus, the one causing acquired immunodeficiency syndrome (AIDS) .

3. The enzyme reverse transcriptase has become a major tool in genetic engineering.



Retrovirus structure and function:

(a)Structure of a retrovirus.
(b) Genetic map of a typical retrovirus genome.
(c)Genetic map of Rous sarcoma virus, a retrovirus that causes malignant tumors in birds. Each end of the genomic RNA contains direct repeats(R), and this RNA also has a 5'-cap and a 3'-poly-A tail. See text for more details.

5.9 Viroids and Prions

Viroids are small, circular, single-stranded RNA molecules that are the smallest known pathogens.

The extracellular form of the viroid is naked RNA-there is no capsid of any kind. Even more interestingly, the RNA molecule contains no protein-encoding genes, and therefore the viroid is totally dependent on host function for its replication.

Prions represent the other extreme from viroids. They have a distinct extracellular form, but the extracellular form seems to be entirely protein. It apparently does not contain any nucleic acid, or if it does, the molecule is not long enough to encode the single kind of protein of which the prion is composed.

The prion protein particle is infectious, and various prions are known to cause a variety of diseases in animals.

Plant Viruses

Tobacco mosaic virus (TMV) as an example

(1) Penetration by the virus of a susceptible plant cell-generally through abrasions or insect bites,

(2) Tincoating of the viral nucleic acid within the plant cell,

(3) Assumption by the viral genome of control of the synthetic activities of the host cell,

(4) Expression of the viral genome so that viral nucleic acid and capsid components are synthesized,

(5) Assembly of the viral particles within the host cell, and,

(6) Release of the complete viral particles from the host plant cell.

Some important characteristics for viral classification

1. Nature of the host-animal, plant, bacterial, insect, fungal

2. Nucleic acid characteristics-DNA or RNA, single or double stranded, molecular weight

3. Capsid symmetry-icosahedral, helical

4. Presence of an envelope and ether sensitivity

5. Diameter of the virion or nucleocapsid

6. Number of capsomers in icosahedral viruses

7. Immunologic properties

8. Intracellular location of viral replication

Recently, the International Committee for Taxonomy of Viruses has developed a uniform classification system and divided viruses into 50 families. The committee places greatest weight on three properties:

(1) nucleic acid type

(2) nucleic acid strandedness

(3) presence or absence of an envelope

Think and try to awnser following questions:

1. Write a paragraph describing the events that occur on an agar plate containing a bacterial lawn when a single bacteriophage particle causes the formation of a bacteriophage plaque.

2. One can divide the replication process of a virus into seven steps. What events are happening in each of these steps?

3. Describe how a restriction endonuclease might play a role in resistance to bacteriophage infection. Why could a restriction endonuclease play such a role whereas a generalized DNase could not?

4. Typically, transfer RNA is used in translation. How-ever, it also plays a role in the replication of retroviralnucleic acid. Explain this role.

5. What is unique about reovirus genomes, and what special problems does this introduce for nucleic acid replication?