

# UNIT-III

## Virus & Fungi



**CENTURION**  
**UNIVERSITY**  
*Shaping Lives...  
Empowering Communities!*

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# Unit III

- Study of morphology, classification, reproduction/replication and cultivation of fungi and virus.
- Classification and mode of action of disinfectants.
- Factors influencing disinfection, antiseptics and their evaluation.
- Evaluation of bacteriostatic and bacteriocidal.
- Sterility testing of products (solids, liquids, ophthalmic and other sterile products) according to IP,BP and USP.

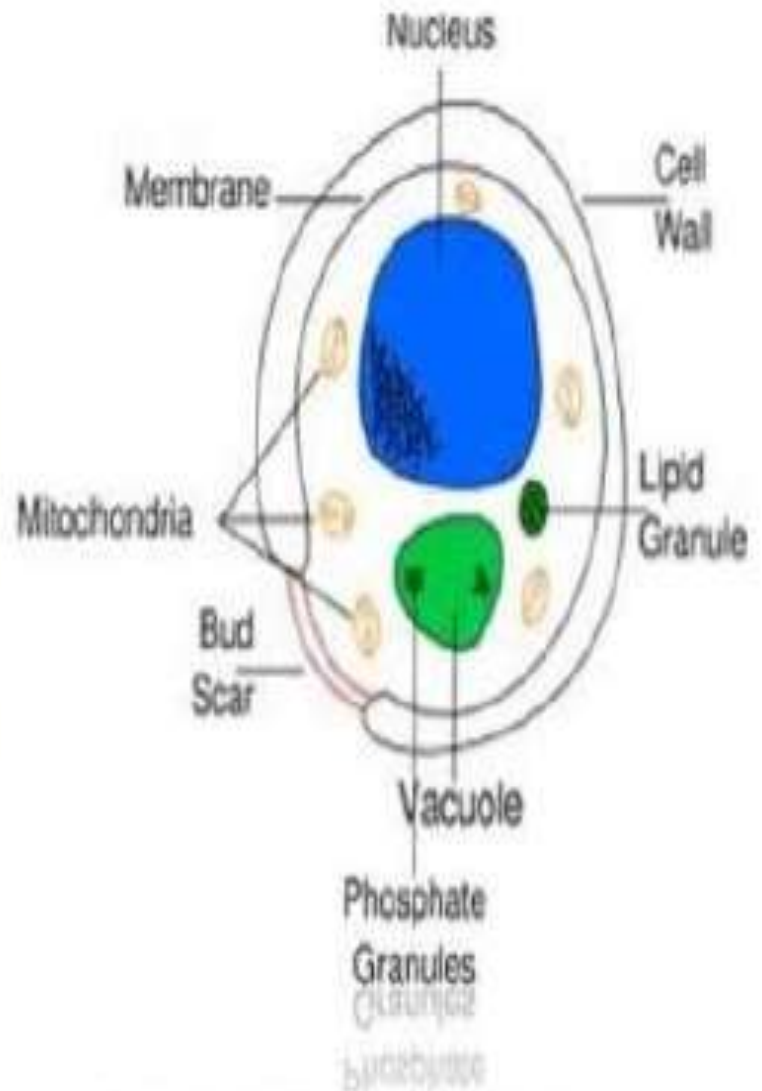
# Introduction to Fungi

- Fungi are heterotrophic organisms which means they require organic compound for nutrition or growth.
- Fungi are **spore-bearing eukaryotes**.
- Fungi may be unicellular or multicellular.
- Fungi includes **moulds and yeast**.
- **Molds– filamentous, multicellular.**
- **Yeast– unicellular.**
- Study of fungi is generally known as **mycology**.

### MUSHROOM



Fungus Diagram: Mushroom



Fungus Diagram: Yeast cell

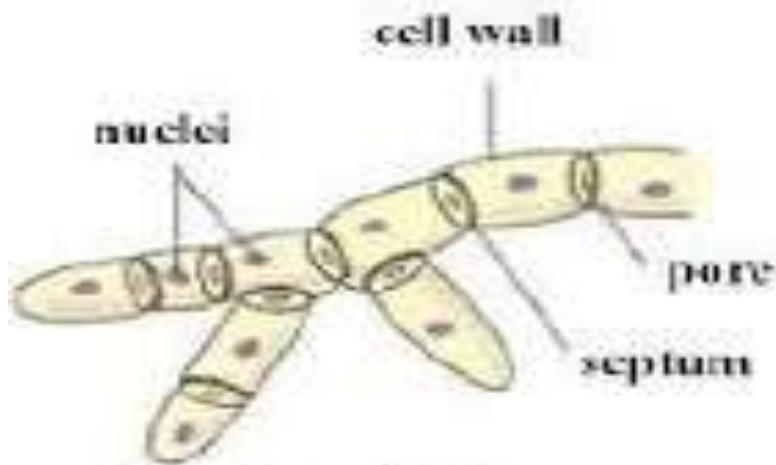
## Distribution

- The fungus occurs in all possible habitats i.e. **aquatic, terrestrial** (which grow in **soil, on dead and decaying material**).
- Some grow on **plants and animals**.
- Fungi also present in the air.
- In fungus **chlorophyll are absent**, so they **depend on other for food**. That is why fungi may be **saprophytes, parasite or symbionts**.

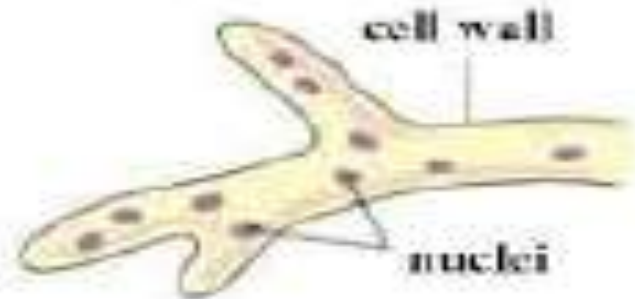
# Morphology

- **Yeasts cells** are generally **larger** than most of the **bacteria**.
- Size of yeast ranging from **1 to 5 micrometers in width** and from **5 to 30 micrometers in length**.
- **Flagella** or **other organelles of locomotion** are **absent** in yeast.
- **Cell wall** constituents of fungi are mainly **chitin and glucans**.
- **Multicellular fungi** are composed of networks of **long filamentous branched** structure called **hyphae**.
- The **hyphae** often **aggregate in a thread like dense network** known as **mycelium**.
- The hyphae may be: **Without crosswalls** as in the case of **lower fungi** or, Divided into **compartment** by formation of **septa** in the **higher fungi**.

- **Hyphae occurs in three forms: Coenocytic or nonseptate, such hyphae have no septa.**
- Septate with uninucleate cells.
- Septate with multinucleate cells.

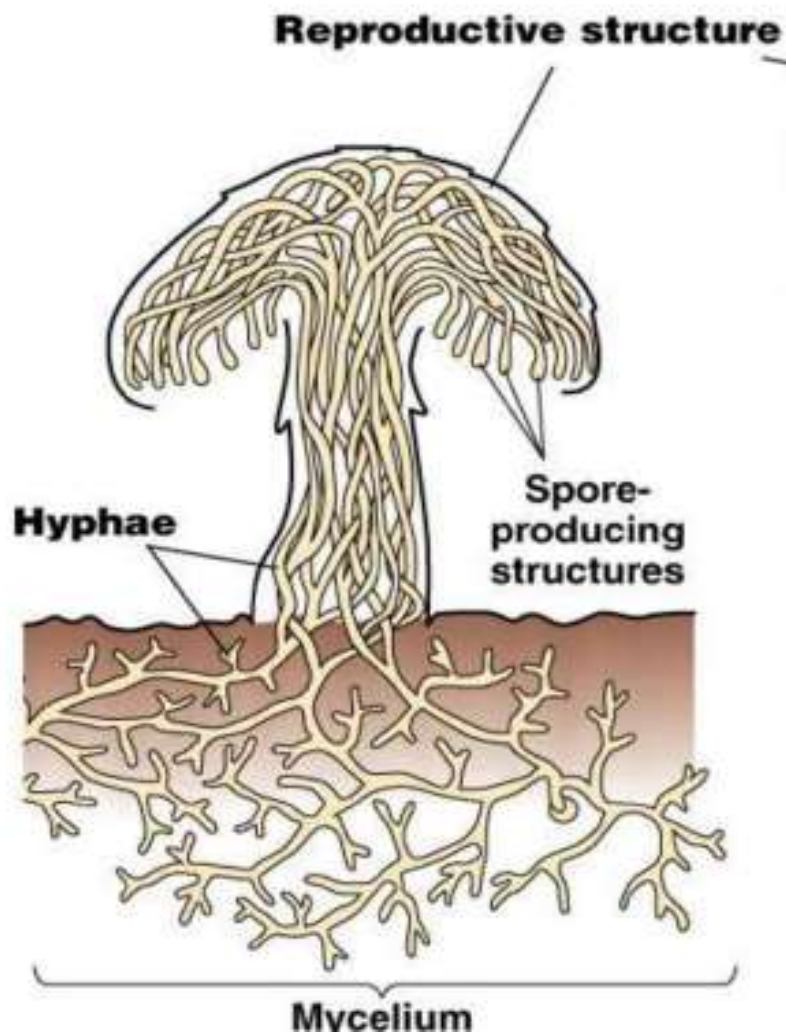


(a) septate hypha



(b) nonseptate hypha

- The **mycelium forms tissue like aggregates** called the **plektenchyma**, in certain stages, often during **transition to the sexual or asexual reproduction phase.**





# Classification of fungi

**Depending on cell morphology, fungi can be divided into 4 classes:**

- I. Moulds
- II. Yeasts
- III. Yeast like fungi and
- IV. Dimorphic fungi

**Based on their sexual spore formation fungi are divided into 4 classes:**

- I. Phycomycetes
- II. Ascomycetes
- III. Basidiomycetes
- IV. Fungi imperfecti

Depending on cell morphology

# 1. Moulds

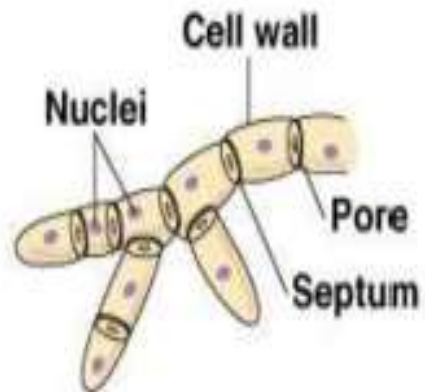
- Fungi which form mycelia are called moulds or filamentous fungi.
- Filaments of fungi are called hyphae.
- Diameter is 2-10 $\mu$ m .
- The cell walls contain chitin.
- Some hyphae may be divided by cross sections called septa
- Two types of hyphae: Septate and Non-septate
- **I. Septate:** septa divide the hyphae into distinct, uninucleate or multinucleate cell-like units.
- **II. Nonseptate/coenocytic:** does not contain septa and appear as long, continuous cells with many nuclei.
- **Example:** *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium notatum*



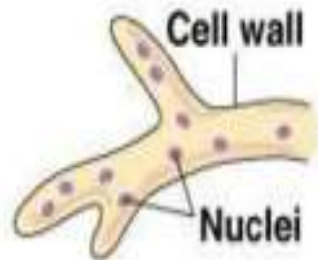
Septated



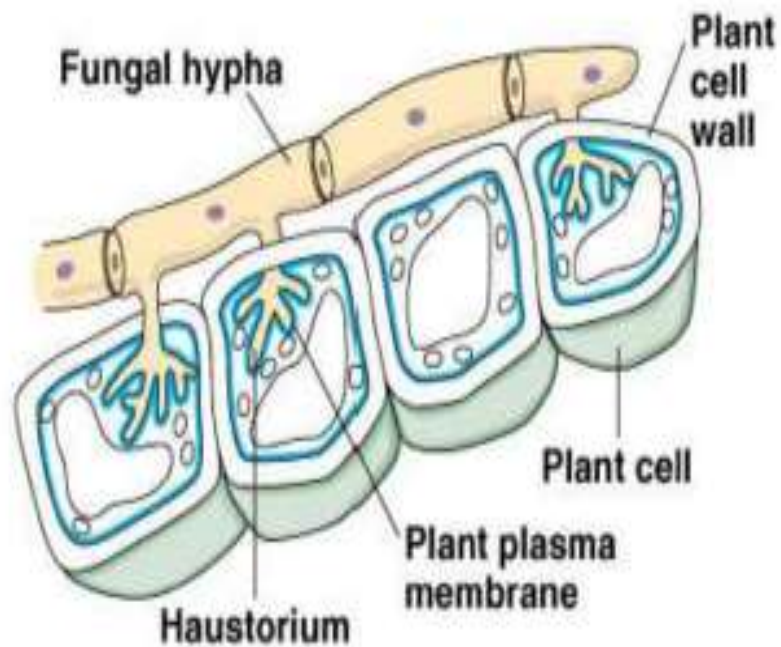
Nonseptated -coenocytic



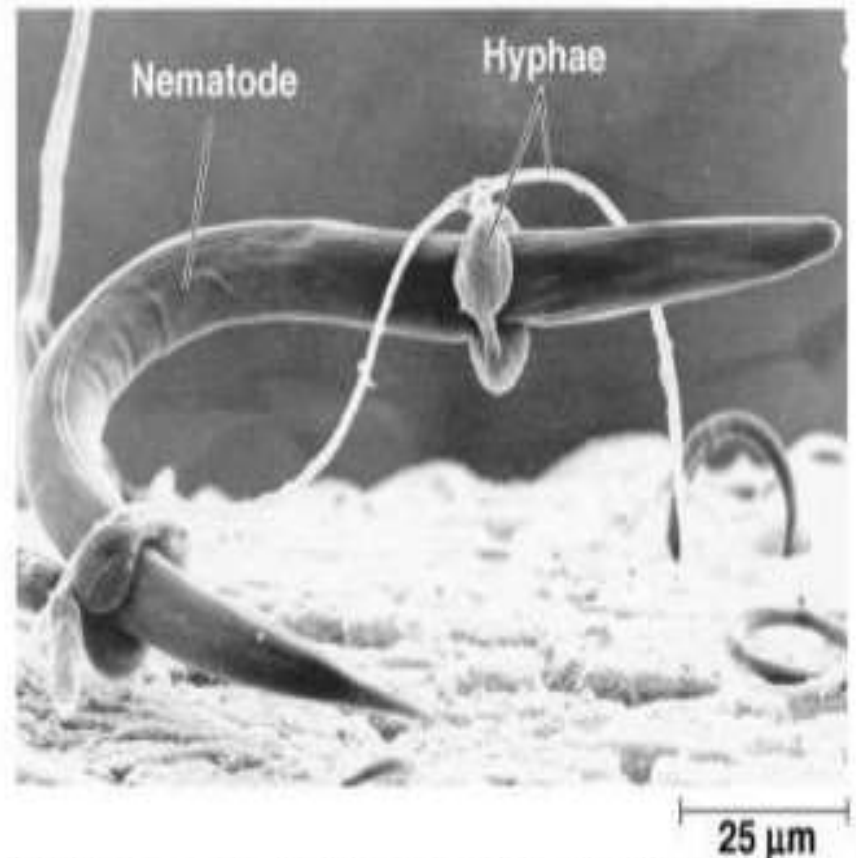
(a) Septate hypha



(b) Coenocytic hypha



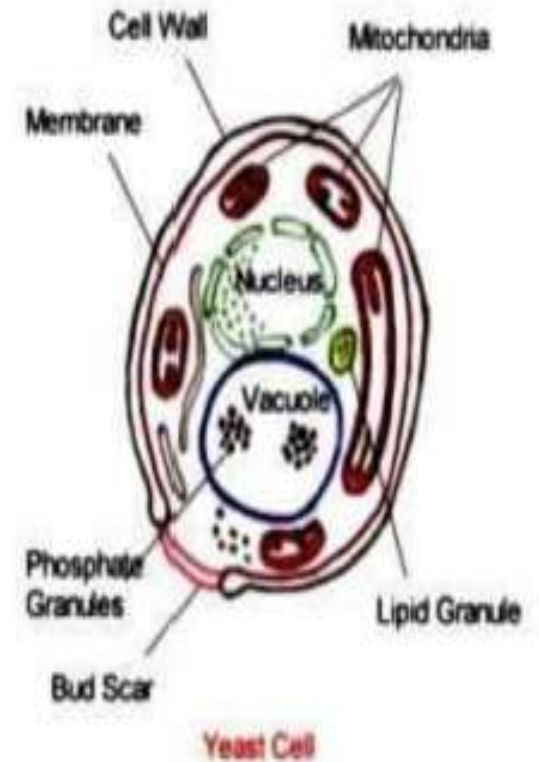
(c) Haustoria



(d) Hyphae adapted for trapping and killing prey

## 2. Yeasts

- Round, oval or elongated, unicellular fungi
- Reproduce by an asexual process called budding in which the cell develops a protuberance which enlarges and eventually separates from the parent cell
- On culture they form smooth, creamy colonies
- **Example:** *Saccharomyces cerevisiae*, *Cryptococcus neoformans*



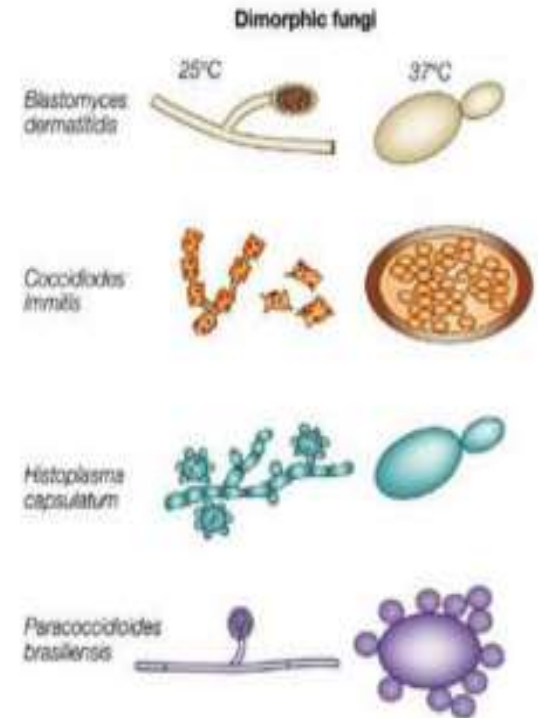
### 3. Yeast like fungi

- The bud remains attached to the mother cell and elongates, followed by repeated budding, forming chains of elongated chains known as pseudohyphae.
- **Example:** *Candida albicans*



## 4. Dimorphic fungi

- Mainly pathogenic species exhibit dimorphism i.e. 2 forms of growth
- Fungi can grow either as a mould or as a yeast
- Mould like forms produce vegetative and aerial mycellium and Yeast like forms reproduce by budding
- Dimorphism is temperature and CO<sub>2</sub> dependant.
- At 37°C, the fungus grows yeast like and at 25°C it shows mould like growth
- **Example:** *Mucor rouxii*, *Histoplasma capsulatum*



## Based on their sexual spore formation

### 1. Phycomycetes

- Fungi having non-septate hyphae, forms endogenous asexual spores (sporangiospores) contained within a sac like structures called sporangia.
- Also produce sexual spores known as oospores and zygospores.
- Example: *Mucor*, *Rhizopus*.



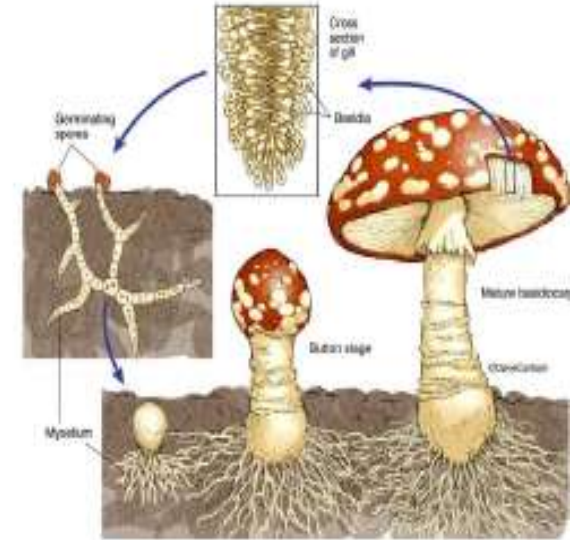
### 2. Ascomycetes

- Form sexual spores within a sac and are called ascospores.
- The sac is called as ascus.
- They form septate hyphae.
- Include both yeasts and filamentous fungi e.g. *Histoplasma*, *Candida* etc.



### 3. Basidiomycetes

- Reproduce sexually and form septate hyphae.
- These basidiospores are borne at the tip of the basidium
- **Example:** *Cryptococcus neoformans*



### 4. Fungi imperfecti

- Also called as Deuteromycetes or Hyphomycetes.
- Consist of group of fungi whose sexual phases have not been identified and they form septate hyphae and asexual conidia.
- Majority of the pathogenic moulds, yeasts, yeasts like fungi and dimorphic fungi.
- **Example:** *Trichophyton*, *Epidermophyton*



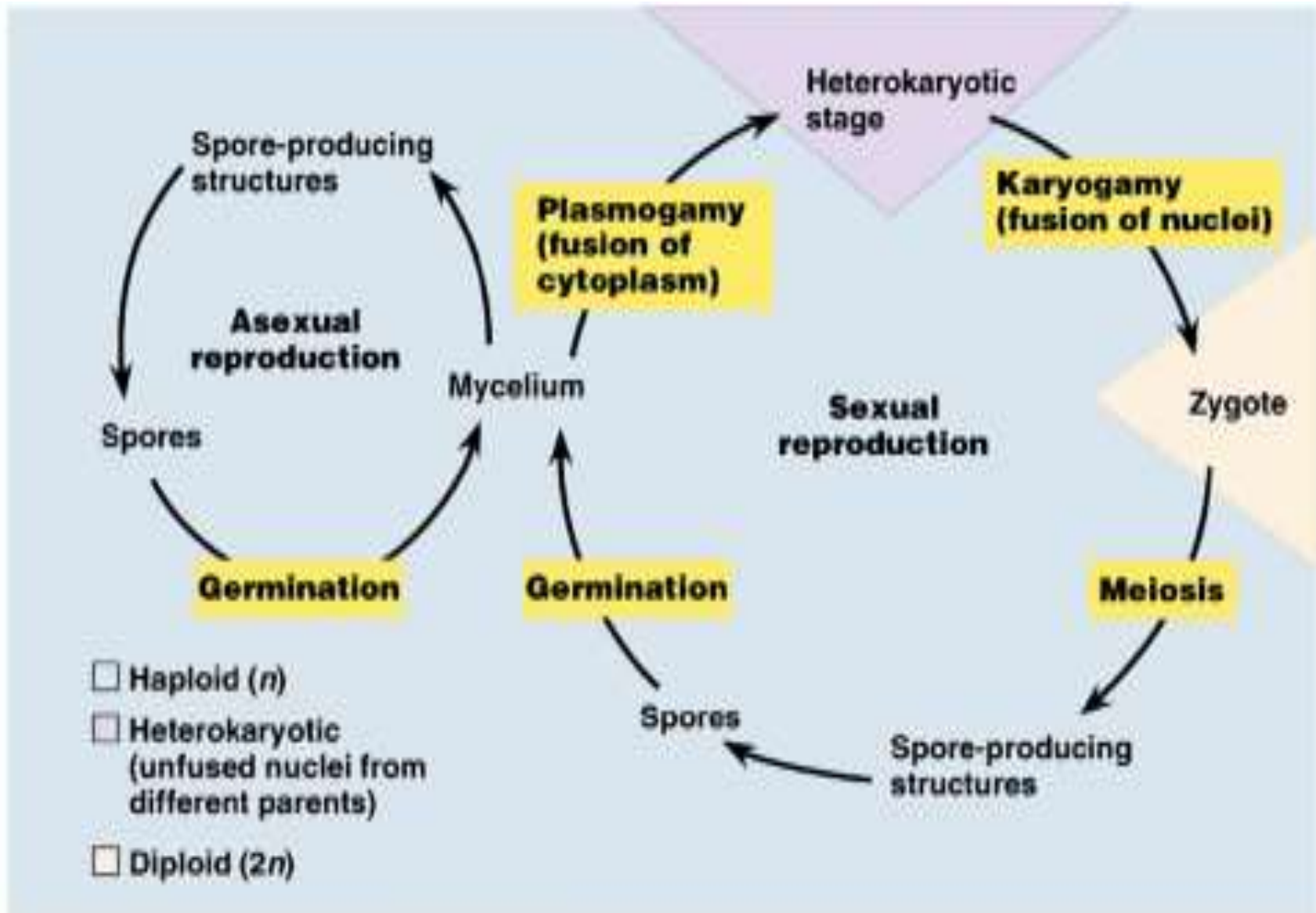


# Reproduction

In fungi reproduction may be **asexual or sexual**.

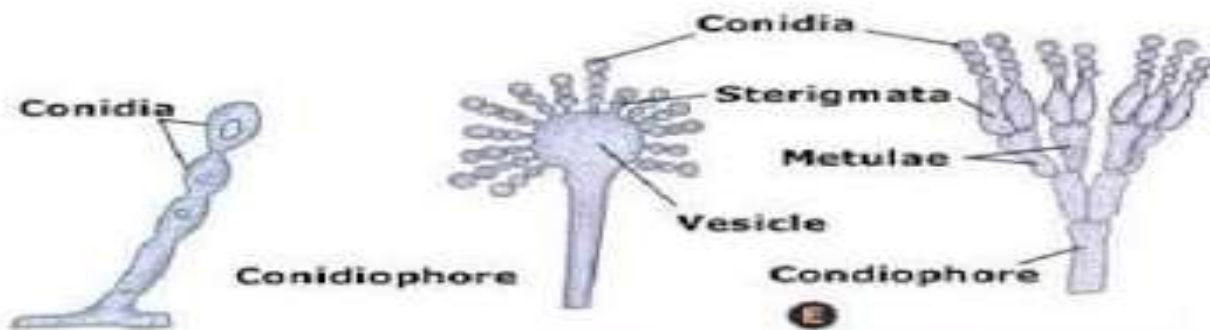
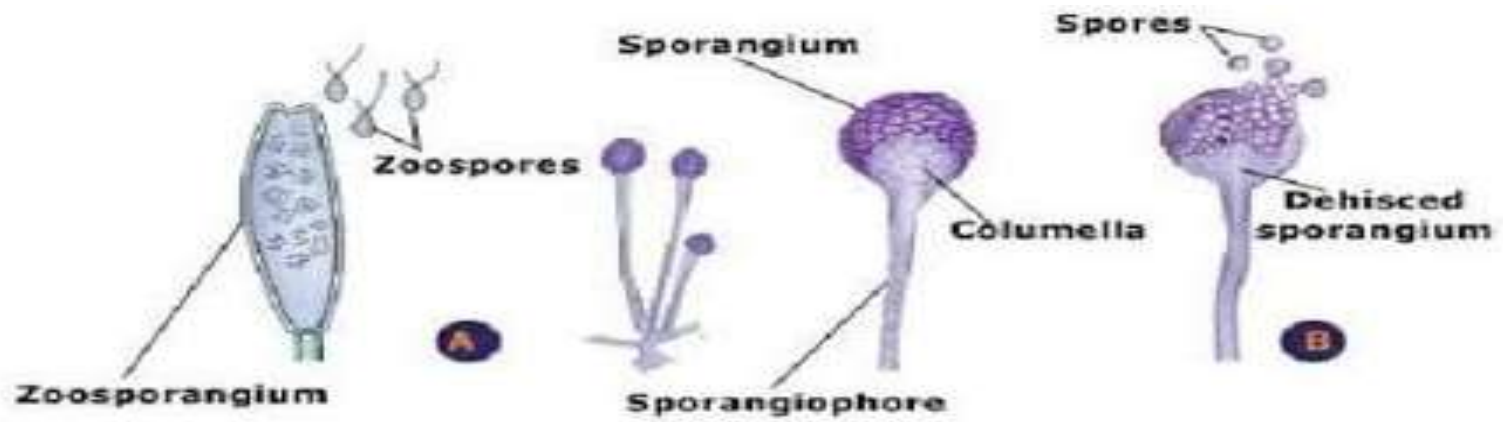
## **Asexual reproduction**

- Asexual reproduction also known as **somatic or vegetative reproduction**. It does not involve the sex cells or sex organs and the union of nuclei.
- **Asexual reproduction may occur by:**
  - Fission of somatic cells.
  - Budding of somatic cells or spores.
  - Fragmentation or disjoining of the hyphal cells. Spore formation.



There are many kinds of asexual spores:

- **Sporangiospores.**
- **Conidiospores or conidia(conidium).**
- **Oidia(oidium), arthrospores.**
- **Chlamydospores.**
- **Blastospores.**



A. Zoospores; B. Sporangiospores; C. Chlamydozoospores; D. Oidia; E. Conidia

## 1. Sporangiospores

- Single-celled spores.
- Formed at the end of hyphae within sacs **sporangia or sporangium.**
- Aplanospores: Non-motile sporangiospores.
- Zoospores- motile.
- Example- Mucor, Rhizopus.

## 2. Conidiospores or conidia(conidium)

- Microconidia is small and single-celled conidia.
- Macroconidia- large, multicellular conidia. They are formed at tip or side of the hyphae.
- Example– penicillium, aspergillus.

### **3. oidia(oidium), arthrospores**

- These spores are single-celled formed by fragmentation of hyphae cells.
- Example– In the milk mould *Endomyces lactis*.

### **4. Chlamydospores**

- These single-celled spores are surrounded by thick walls are highly resistant in adverse condition.
- These are formed by vegetative hypha cells.

### **5. Blastospores**

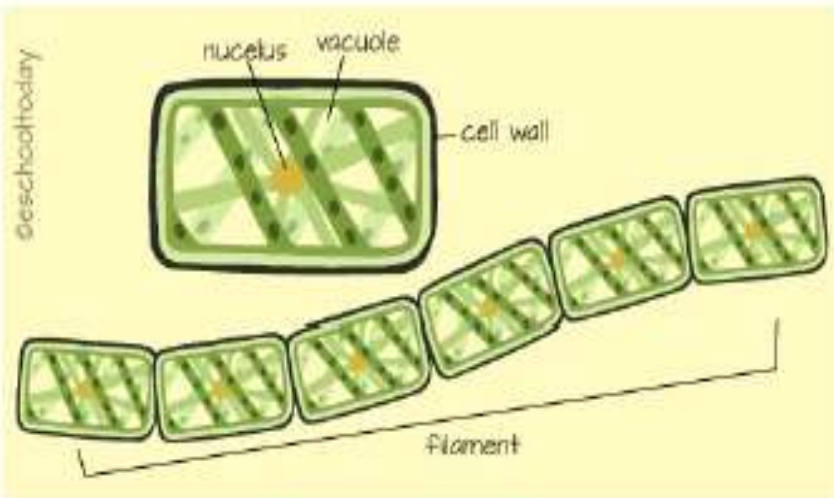
- Through budding spores are formed.



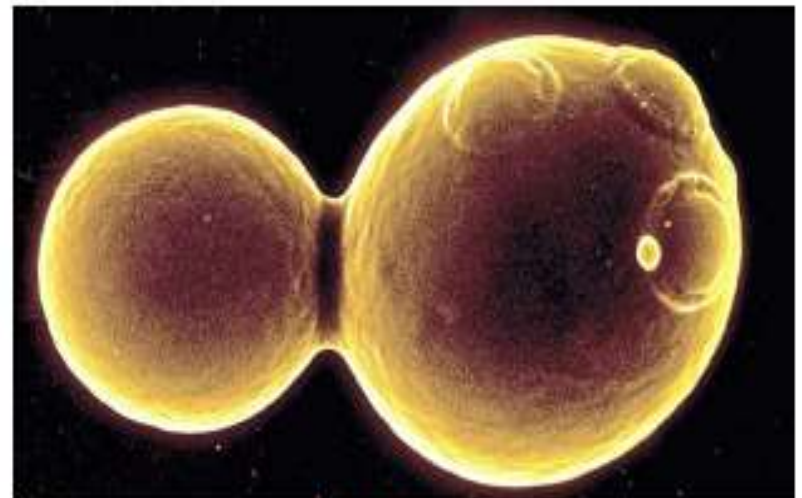
Sporangiophores



Conidia



Fragmentation



Budding

## Sexual reproduction

- It is carried out by **fusion of genetic materials** of two parent's cells.
- Sexual reproduction can be divided into three phases:
- **Plasmogamy**- Joining of two cells and fusion of their protoplast.
- **Karyogamy**- This involved fusion of two haploid nuclei.
- **Meiosis**- Reduction of chromosomes to the haploid number.
  - **Gametangia**- The sex organelles of fungi (if present).
  - **Antheridium**- Male gametangia.
  - **Oogonium**- Female gametangia.

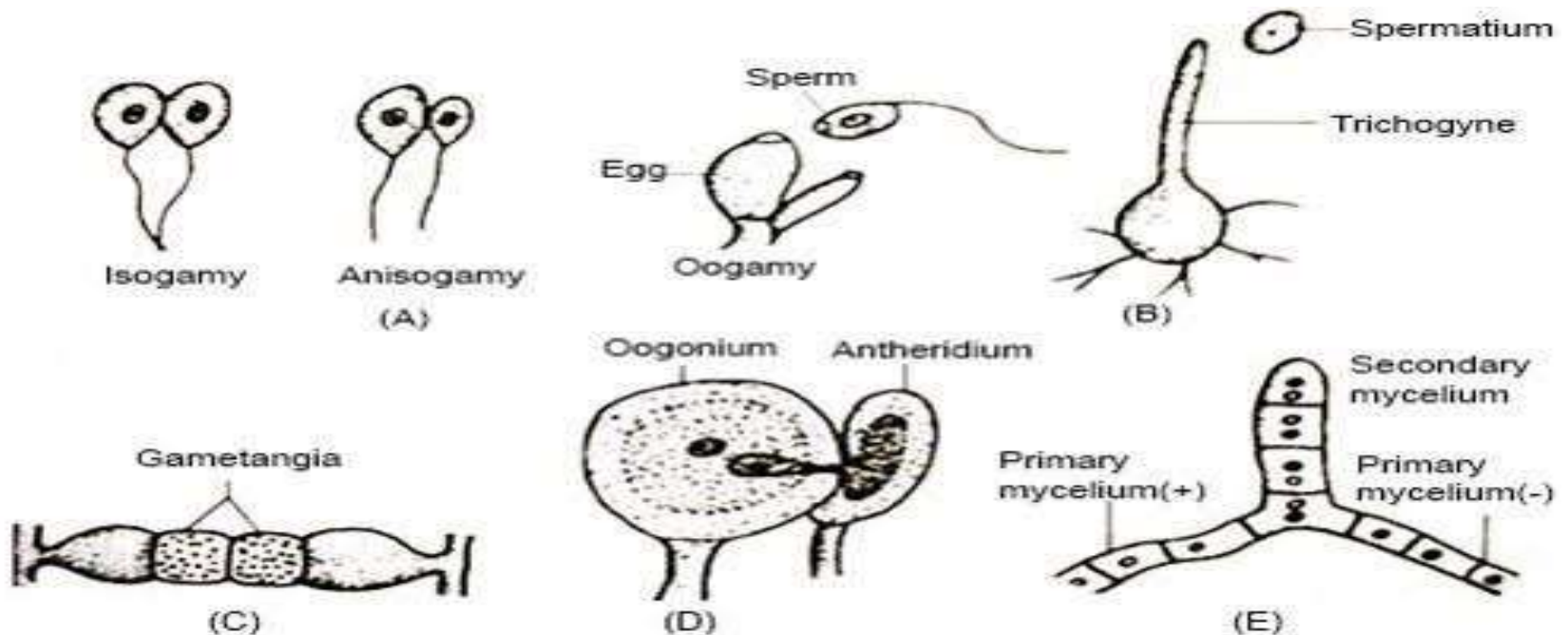


There are various types of sexual spores

- **Ascospores-** Single-celled produced in sac known as **Ascus**.
- **Basidiospores-** These spores are single celled and borne on a club- shaped structure known as **basidium** or **basidia**.
- **Zygosporos-** are **big in size, thick-walled** spores formed by **fusion of 2 sexually compatible hyphae or gametangia**.
- **Oospores-** formed within **Oogonium**, produced by **fertilization of oospheres** by male gametes.

## Fruiting bodies:

- Fruiting bodies are highly organized protective structure in which sexual and sexual spores may be surrounded.
- **Acervulus and pycnidium- asexual fruiting bodies.**
- **Perithecium and apothecium- sexual fruiting bodies.**



# Classification

Fungi are classified in four major divisions:

- Chytridiomycota
- Zygomycota
- Ascomycota
- Basidiomycota

## 1. Chytridiomycota

- It is commonly known as chytrids.
- Habitat- Aquatic or terrestrial.
- Ploidy- Diploid.
- Motile stage- Present.
- Asexual spores- Holocarpic.
- Pathogenic relationship- Obligate parasite.

## 2. Zygomycota

- These are known as zycomycetes.
- Habitat– Terrestrial.
- Ploidy– Monoploid
- Motile stage– Absent.
- Sexual spore– Zygosporangia.
- Asexual spore– Sporangiospores/Chlamydospores.
- Parasitic relationship– Facultative parasite.
- Example– Black bread mould *Rhizopus stolonifer*.

### **3. Ascomycota**

- Commonly known as Ascomycetes or sac fungi.
- Habitat– Terrestrial.
- Ploidy– Monoploid.
- Motile stage– Absent
- Sexual spore– Ascospores.
- Asexual spore– Conidia.
- Pathogenic relationship is facultative or obligate.

### **4. Basidiomycota**

- It is commonly Known as basidiomycetes or club – fungi.
- Habitat– Terrestrial.
- Ploidy– Monoploid, dikaryotic.
- Motile stage– Absent.
- Asexual spore– Arthrospores, oidia, conidia.
- Sexual spore– Basidiospores.
- Pathogenic relationship is facultative or obligate parasite.

# Introduction to Virus

- **Virology** (the study of viruses) – is such a significant part of microbiology
- A Virus is a **non-cellular particle** made up of **genetic material** and **protein** that can reproduce only in the living cells.
- A complete virus particle as it exists **outside the cell** called **virion** (or **viral particle**). Viruses **can infect** all types of cells: bacteria, protozoa, plants, animals, human.
- Viruses have a **host range**. That is, viruses infect **specific cells** or tissues of specific hosts, or specific bacteria, or specific plants.
- **Viral specificity** refers to the **specific kinds of cells** a **virus can infect**. It is regulated by the **specificities of attachment, penetration** and **replication** of the virus.

Surface layer:

Outer membrane

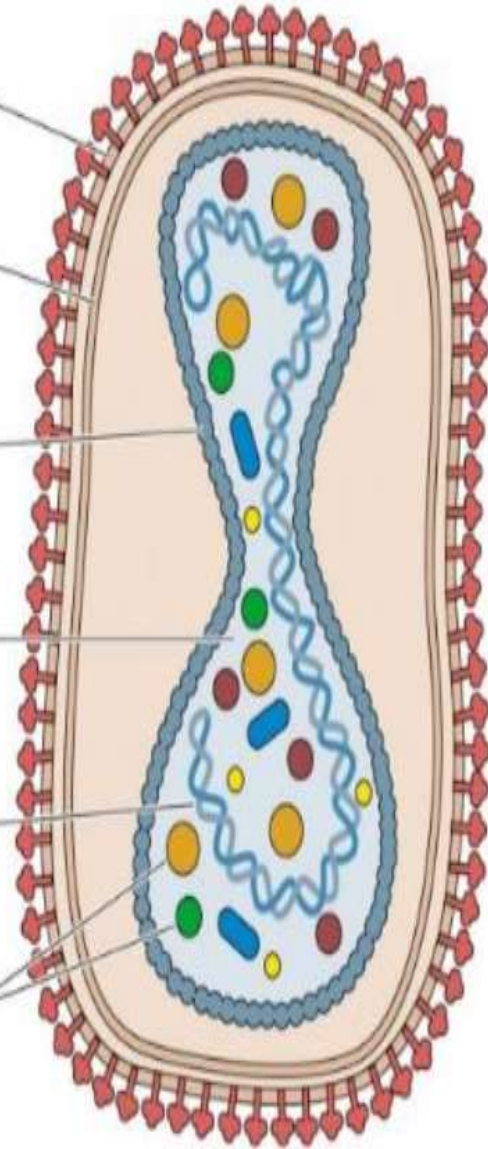
Inner membrane

Core wall

Core

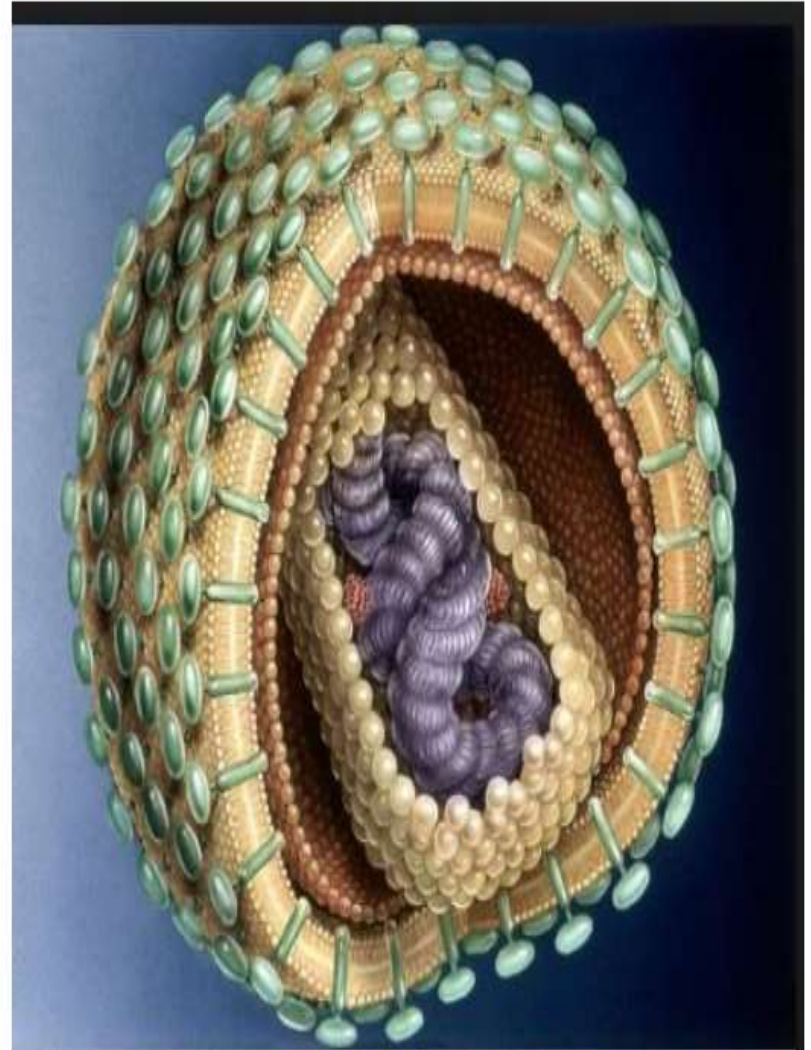
DNA genome

Virion enzymes



## Virus structure (HIV)

Virus  
structure



# Scheme of the structure of viruses

## Envelope viruses

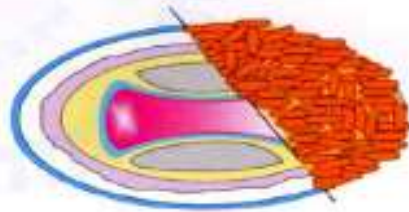
### Double-stranded DNA



Herpesviridae



Hepadnaviridae



Poxviridae

### Single-stranded RNA



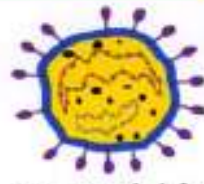
Coronaviridae



Paramyxoviridae



Bunyaviridae



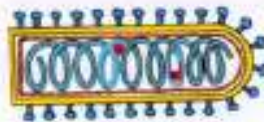
Arenaviridae



Orthomyxoviridae



Retroviridae



Rhabdoviridae



Togaviridae



Flaviviridae



Filoviridae

## Nonenvelope viruses

### Double-stranded DNA



Adenoviridae



Polyomaviridae  
Papillomaviridae

### Single-stranded DNA



Parvoviridae



Circinoviridae

### Double-stranded RNA



Reoviridae

### Single-stranded RNA



Picornaviridae



Caliciviridae



## Properties of viruses

- Viruses are not cells, **do not have nuclei** or **mitochondria** or **ribosomes** or other **cellular components**.
- Viruses **replicate** or **multiply**. Viruses **do not grow**.
- Viruses replicate or multiply only **within living cells**.
- Viruses are **obligate intracellular parasites**.
- The term virus was coined by **Pasteur**, and is from the **Latin word for poison**.

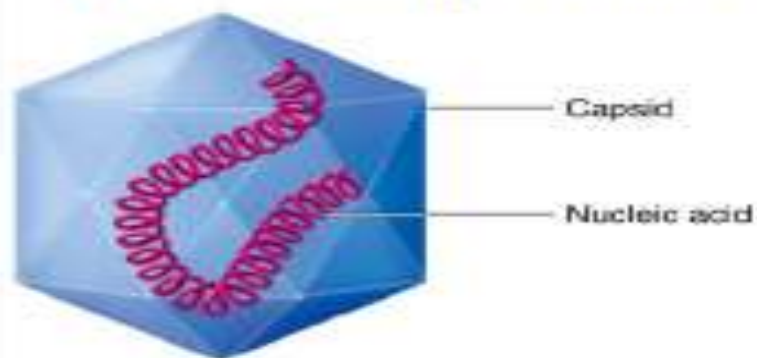
## Components of viruses -

- A **virion** is an **infectious** virus particle - not all virus particles are infectious.
- Viruses are composed of a **nucleic acid, RNA or DNA never both.**
- All viruses have a **protein coat or shell** that surrounds and **protects the nucleic acid core.**
- Some viruses have a **lipid envelope** or membrane surrounding a **nucleo capsid core.**
- The source of the envelope is from the membranes of the host cell.
- Some viruses package enzymes - e.g. RNA dependent- RNA polymerase or other enzymes some do not package enzymes

## Structure

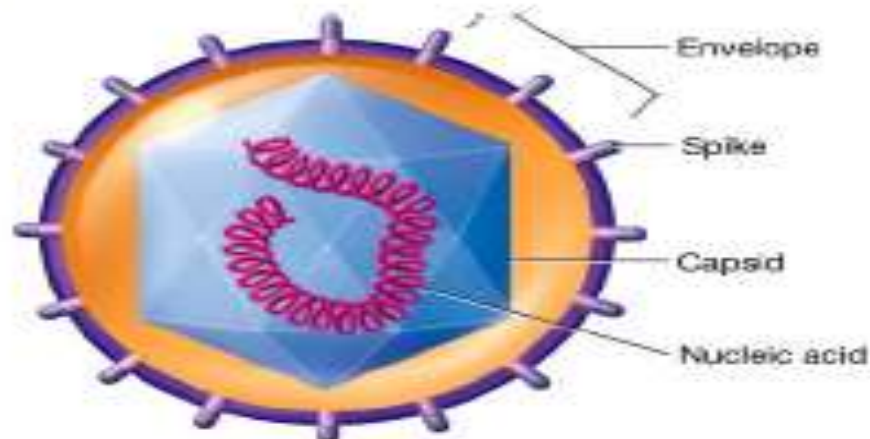
- Size and morphology
- Capsid
- Envelope
- Complex
- Nucleic acid

There are two major structures of viruses called the naked nucleocapsid virus and the enveloped virus



(a) Naked Nucleocapsid Virus

**NAKED**



(b) Enveloped Virus

**Enveloped**

## Capsid

- Protective outer shell that surrounds viral nucleic acid
- **Capsid spikes** - used for binding to cell surface proteins
- Composed of **capsomer** subunits - collectively protect the nucleic acid from the environment

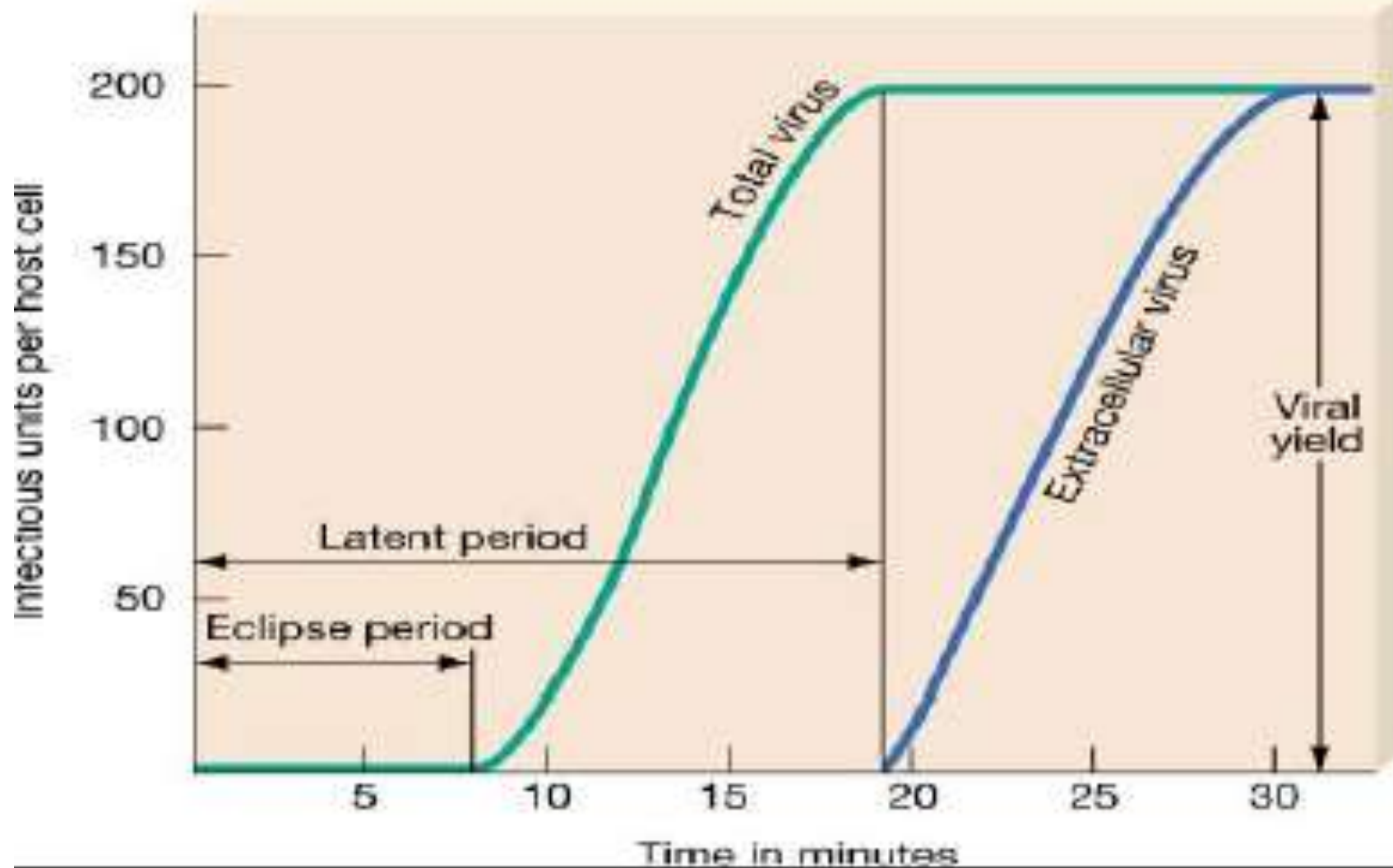
## Envelope

- **Lipid and proteins** - basically a modified version of our membranes
- **Envelope spikes** - bind to cell surface proteins
- During release of animal viruses, a part of the host membrane is taken

## Nucleic acid

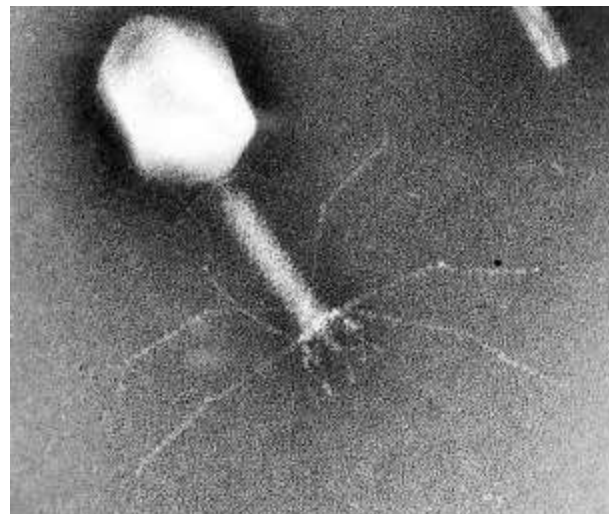
- Viruses contain either **DNA or RNA**
- Possess only the genes to invade and regulate the metabolic activity of host cells
- Ex. Hepatitis B (4 genes) and herpesviruses (100 genes)
- No viral metabolic genes, as the virus uses the host's metabolic resources

## Growth curve for a bacteriophage

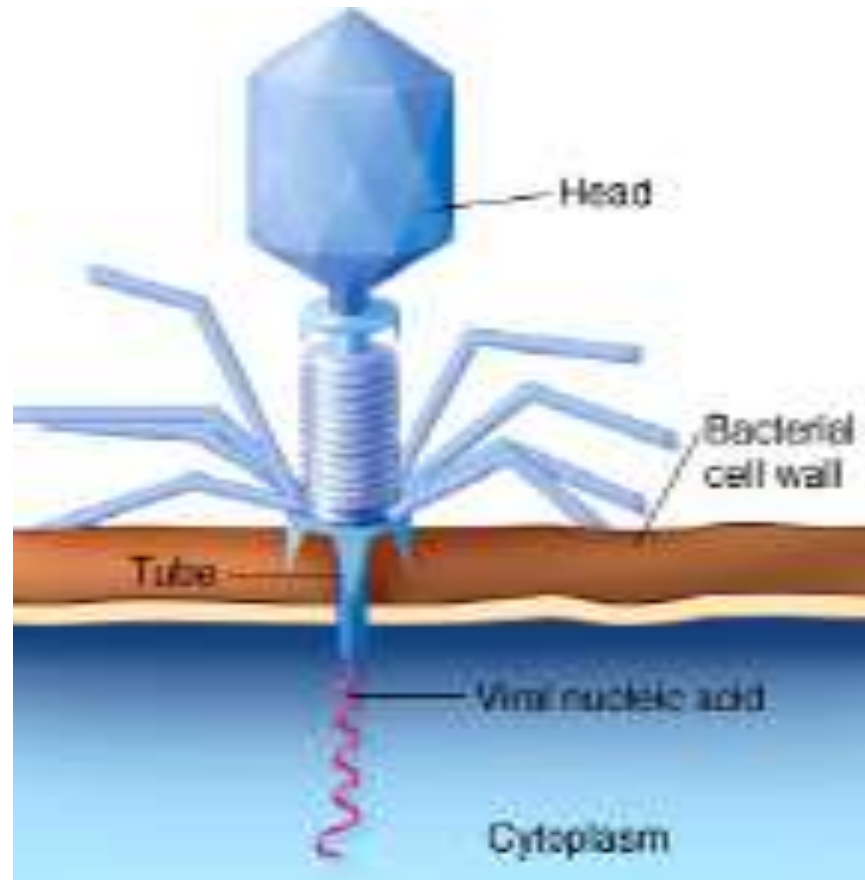


# Bacteriophages

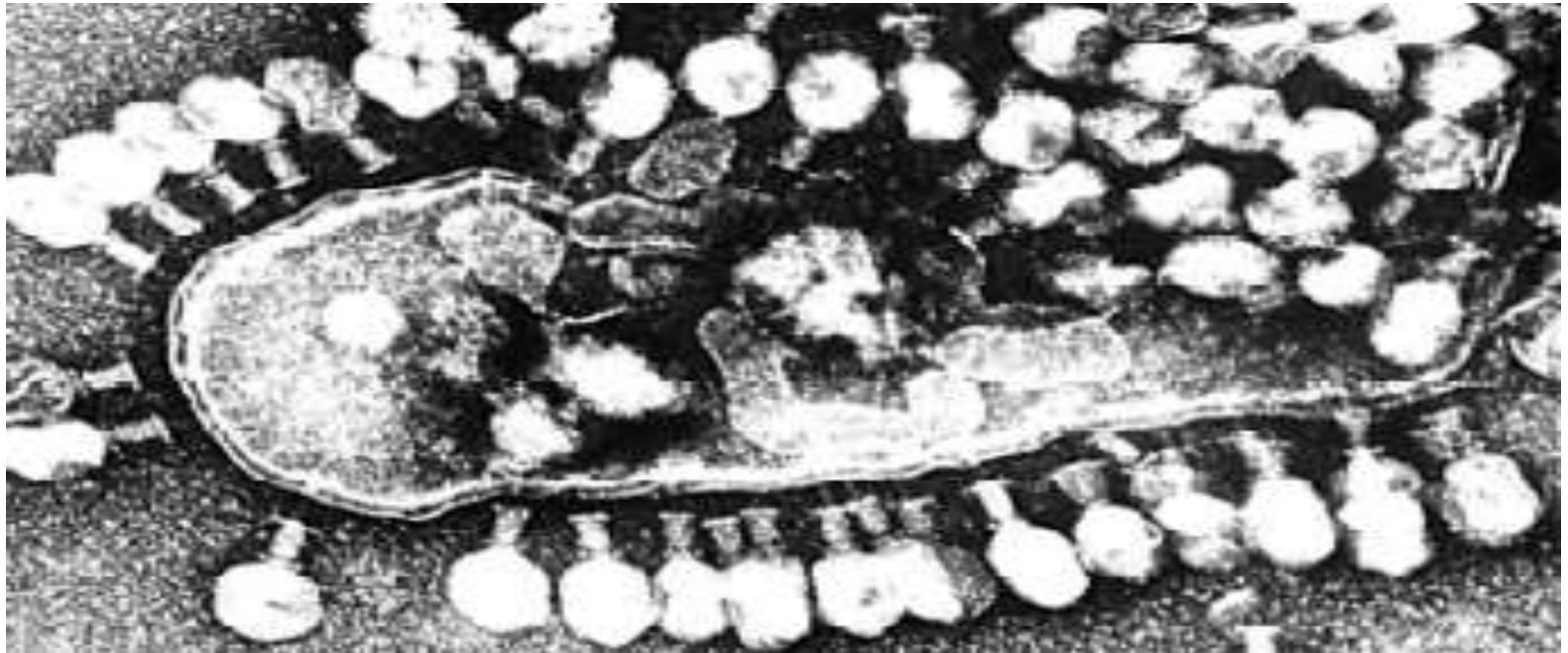
- **Bacteriophage**
  - Polyhedral head
  - Helical tail
  - Fibers for attachment
- Are considered either **LYTIC** or **TEMPERATE**
- Are often associated with virulence genes in bacteria
  - EX. - diphtheria toxin in *Clostridium diphtheriae* - also Bo-Tox from *C. botulinum*



**T-even bacteriophage penetrate the host cell by specifically binding and injecting their DNA into the host cell.**



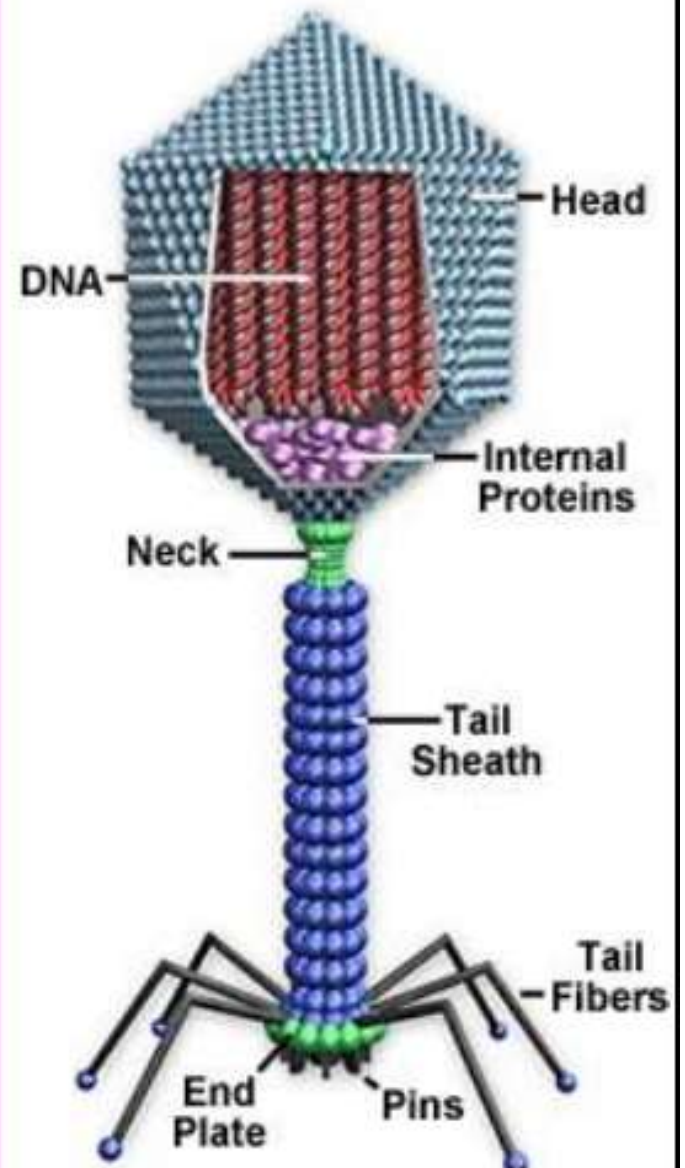
- After replication, bacteriophage release lysozyme, weaken/destroy/rupture cell and release numerous virions.





## Structure of Bacteriophages

1. **Size:** Most phages range in size from 24-200 nm in length.
2. **Head or Capsid:** Some are icosahedral others are filamentous. The head is composed of proteins. Inside the head is found the nucleic acid. The head acts as the protective covering for the nucleic acid.
3. **Tail:** Many but not all phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. In the more complex phages the tail is surrounded by a contractile sheath which contacts during infection of the bacterium. At the end of the tail the more complex phages have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell.



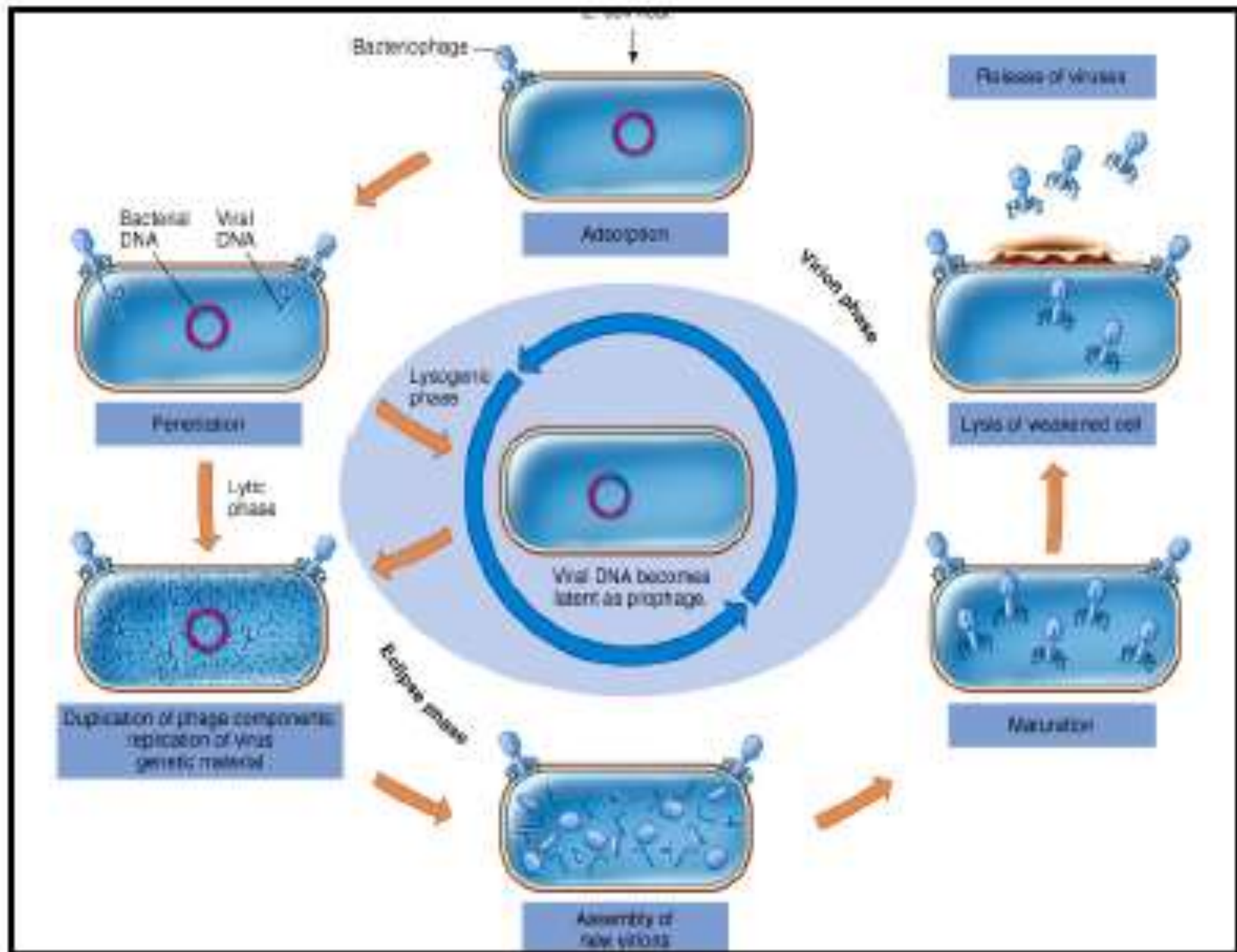
All bacteriophages have a *lytic or infectious cycle*, in which the virus, incapable of replicating itself, injects its genetic material into a bacterium. By pirating its host's enzymes and protein-building capacities, the virus can reproduce and repackage, making about 100 new copies before it bursts from and destroys the bacteria.

Some bacteriophages, however, behave differently when they infect a bacterium. The injected genetic material instead integrates itself into its host DNA, passively replicating with it to be inherited by bacterial daughter cells. In about 1 in 100,000 of these *lysogenic cells*, the viral DNA spontaneously activates and starts a new lytic cycle.

# There are two main ways that viruses reproduce or multiply

1. Lytic Cycle – the virus enters the cell, replicates itself hundreds of times, and then bursts out of the cell, destroying it.

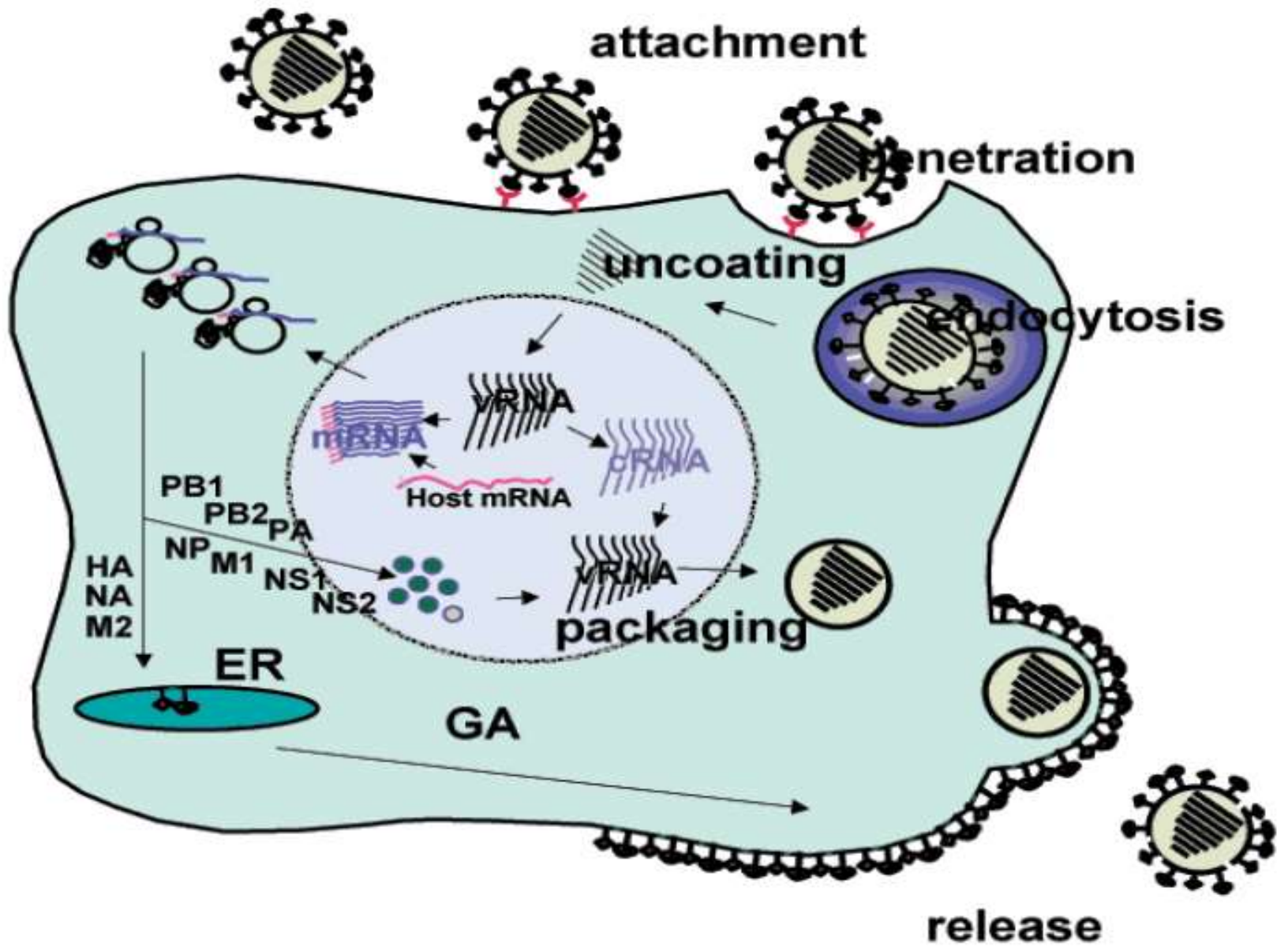
2. Lysogenic Cycle – the virus DNA integrates with the host DNA and the host's cell helps create more virus DNA. An environmental change may cause the virus to enter the Lytic Cycle.



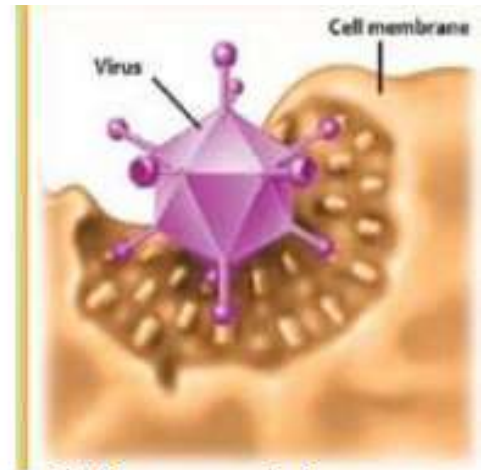
# Viral Replication - Lytic Cycle

**Lytic** - the virus may be "lytic" (assembling new viral particles from the host's biomolecules) and eventually lysing the host to release the newly-assembled viral population. Lytic stages include:

- **Adsorption** - virus attaches to host's cellular membrane
- **Penetration** - virus injects genetic material through membrane
- **Uncoating** - viral coat digested releasing viral genetic material
- **Synthesis** - virus commences cellular take-over and assembly
- **Release** - newly-made viral particles released, cell lysed.



**1. Attachment (Adsorption)** *This is the first step in viral replication.*



- Specific molecules on the surface of the virions attach specific receptors on the plasma membrane of cell. Specific-binding proteins of the virus interact with specific receptors on the target cell surface. Many kinds of virus receptors are now known. Adsorption occurs to specific cellular receptors. Some receptors are glycoproteins, others are phospholipids or glycolipids.

## 1. Attachment (Adsorption)

- Some viruses have a very narrow host range, meaning that they can only infect one or a small number of cell types. For example, HIV infects only human T cells, because the surface protein interacts with CD4 receptors on the surface of the T cell.
- Some complex viruses have broad host ranges, meaning that they can infect a large number of different cell types, for example, Influenzavirus and Herpesviruses.
- The expression of receptors on the surface of cells largely determines the TROPISM of viruses. Tropism is the type of cell in which viruses are able to replicate.





### 3. Uncoating

- **Uncoating** is the step in which viral enzymes degrade the virus capsid; that then exposes the viral genome to permit multiplication of the virus.

### 4. Strategies of Viral Replication, Gene expression (biosynthesis):

- synthesis of viral mRNA (transcription), synthesis of viral proteins (translation), and genome replication.
- The viruses must use host cellular machinery to replicate and make functional and structural proteins. Strategies for genomic expression for different taxonomic group of viruses are different.
- All virulent viruses shut off cellular protein synthesis and disaggregate cellular polyribosomes, favoring a shift to viral synthesis.

Host Cell Cytoplasm

Cell membrane

Receptors

Spikes

**1 Adsorption.** The virus attaches to its host cell by specific binding of its spikes to cell receptors.

**2 Penetration.** The virus is engulfed into a vesicle and its envelope is  
**3 Uncoated,** thereby freeing the viral RNA into the cell cytoplasm.



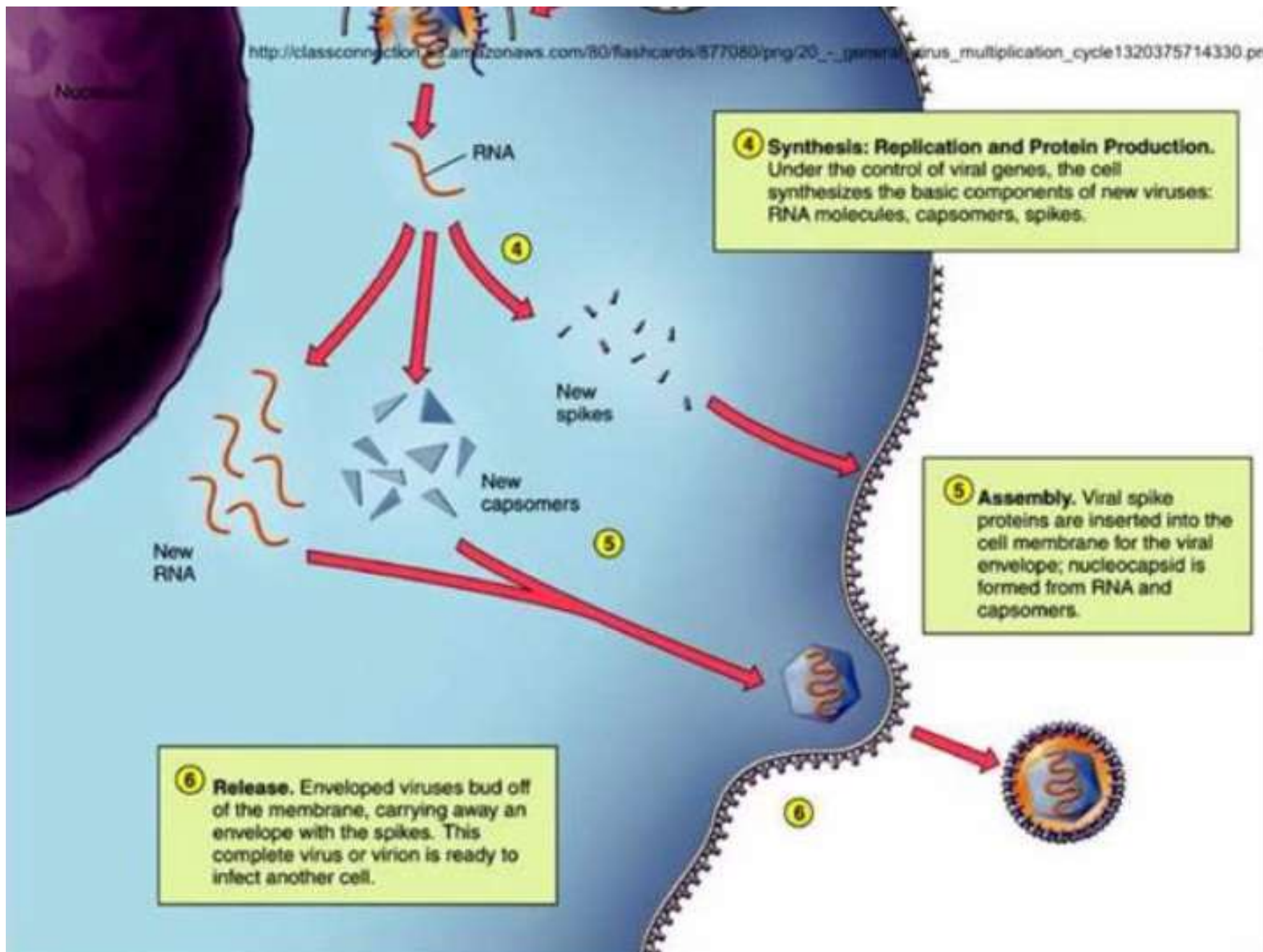
**4 Synthesis: Replication and Protein Production.** Under the control of viral genes, the cell synthesizes the basic components of new viruses: RNA molecules, capsomers, spikes.



Nucleus

## 5. Virion assembly (maturation)

- The process assembly of the new components into virions.
- The process of virion assembly involves bringing together newly formed viral nucleic acid and the structural proteins to form the nucleocapsid of the virus. There are basically two strategies that viruses employ.
- For example, for many nonenveloped viruses, the viral capsid is partially assembled, the newly synthesized capsid proteins associate together into a capsid-like structure. The viral genome is then inserted into the capsid to form a nucleocapsid.



**4 Synthesis: Replication and Protein Production.** Under the control of viral genes, the cell synthesizes the basic components of new viruses: RNA molecules, capsomers, spikes.

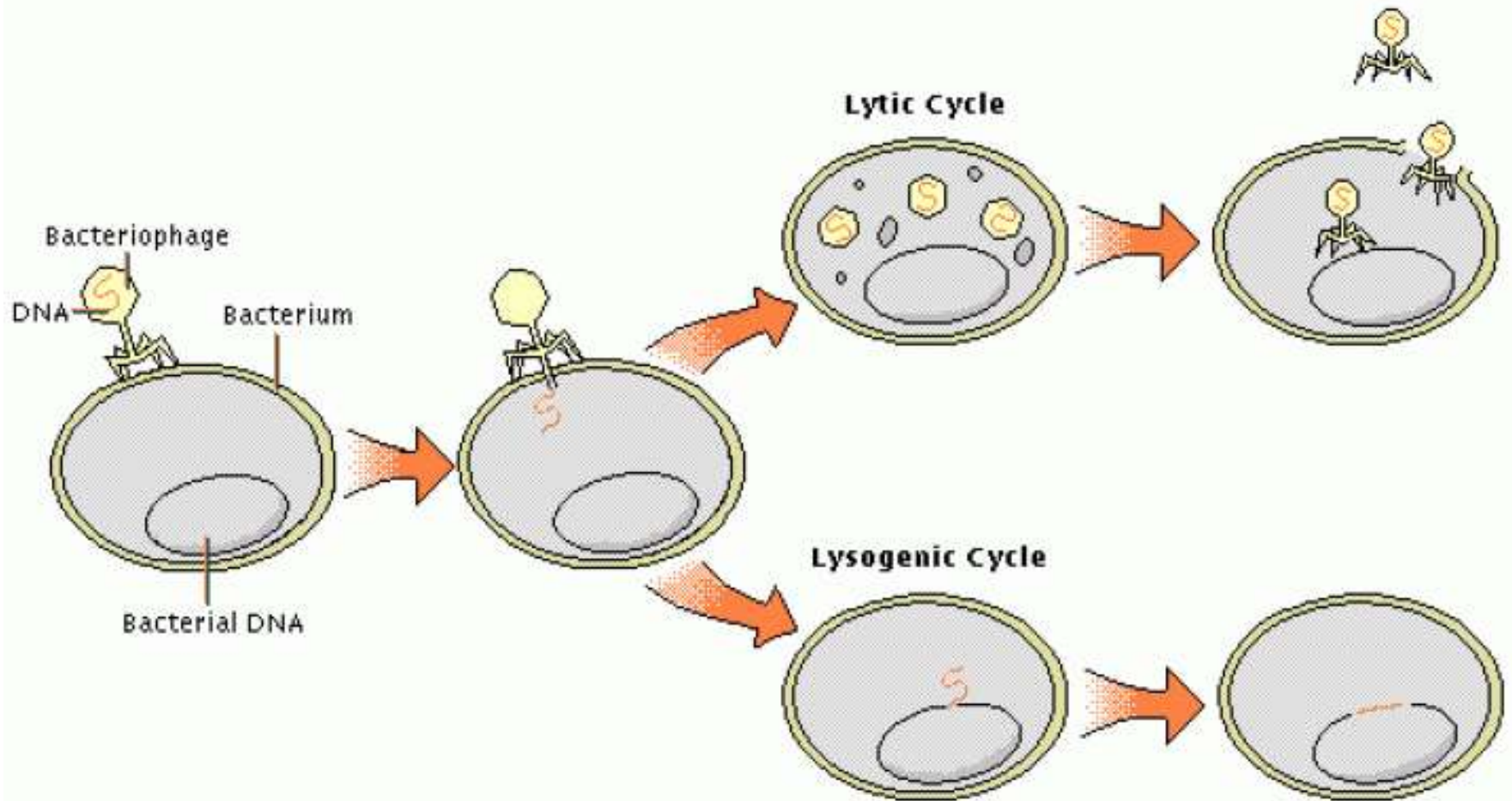
**5 Assembly.** Viral spike proteins are inserted into the cell membrane for the viral envelope; nucleocapsid is formed from RNA and capsomers.

**6 Release.** Enveloped viruses bud off of the membrane, carrying away an envelope with the spikes. This complete virus or virion is ready to infect another cell.

# 6.Release

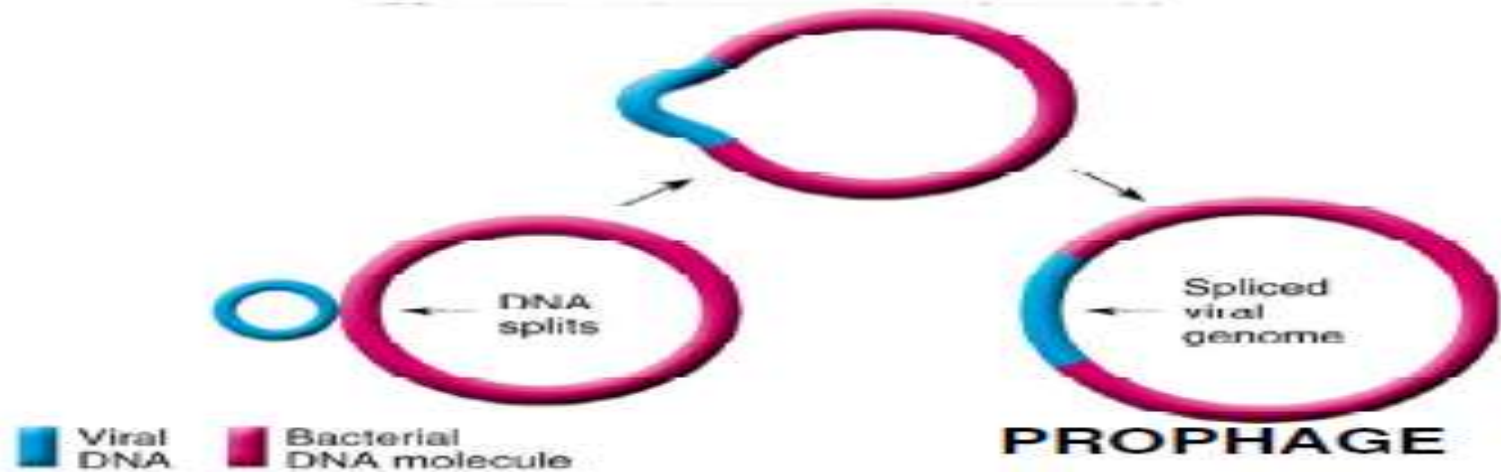
- There are basically two mechanisms for the release of mature Virions from the infected cell: Cytopathogenic or non-Cytopathogenic.
- Most non-enveloped viruses accumulate within the cytoplasm or nucleus. Release occurs only when the cell lysis or until the cell slowly degenerates and dies.
- Most enveloped viruses are released by budding from the plasma membrane. It's a process which can occur over a prolonged period without much damage to the cell.
- Viruses can replicate tens of thousands of times within only a few hours.

# Lytic and Lysogenic Cycle of Bacteriophages



- Lytic cycle: Phage causes lysis and death of host cell.
- **Lysogenic cycle: Prophage DNA incorporated in host DNA.**

## Temperate phage - formation of a prophage and **LYSOGENY**



**Temperate phages** can cause disease

For example, *Corynebacterium diphtheriae* and *Clostridium botulinum* contain **prophages** that have genes which encode for toxins

Without these prophages, they **DO NOT** produce the toxin – without toxin, no disease

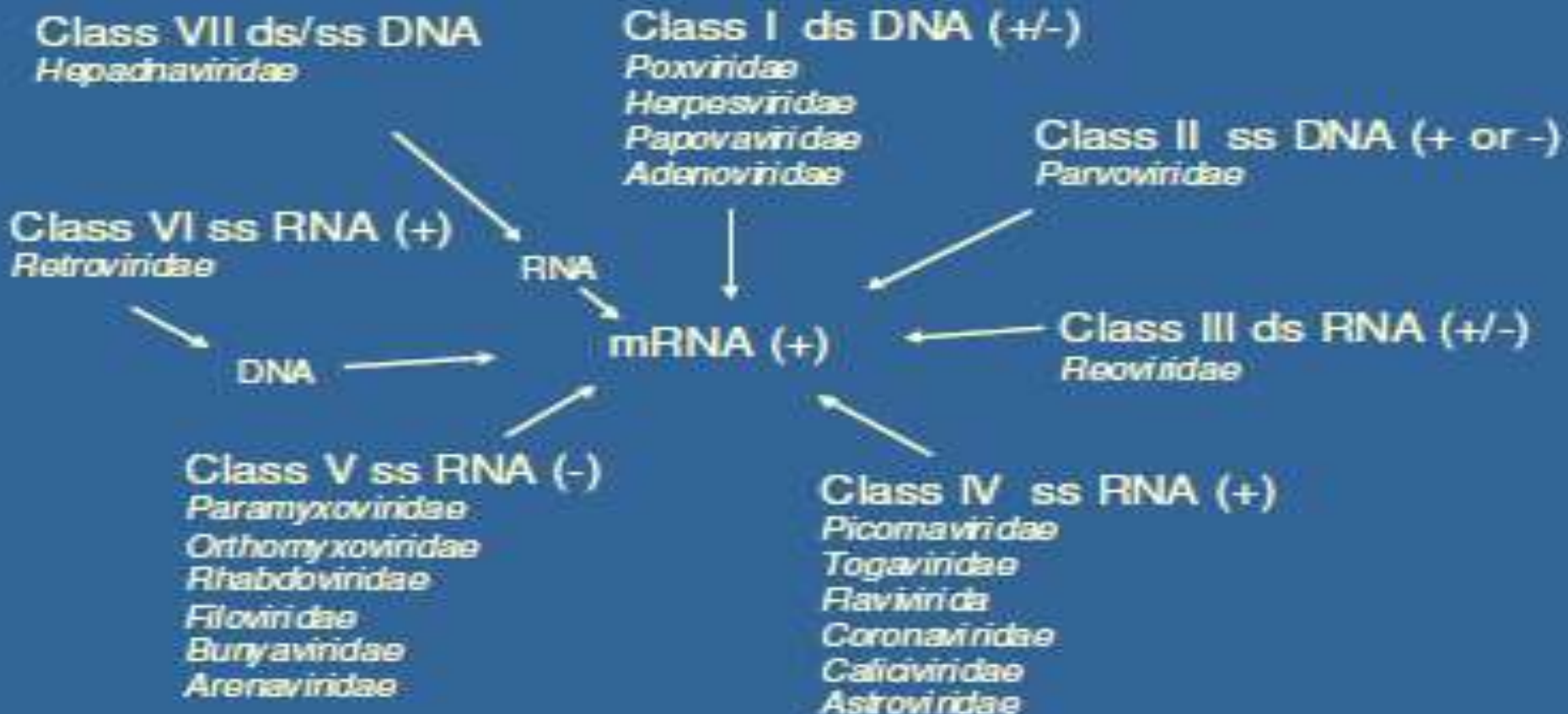
Thus, they are examples of bacteria and viruses interacting to cause medically relevant disease



## Classification

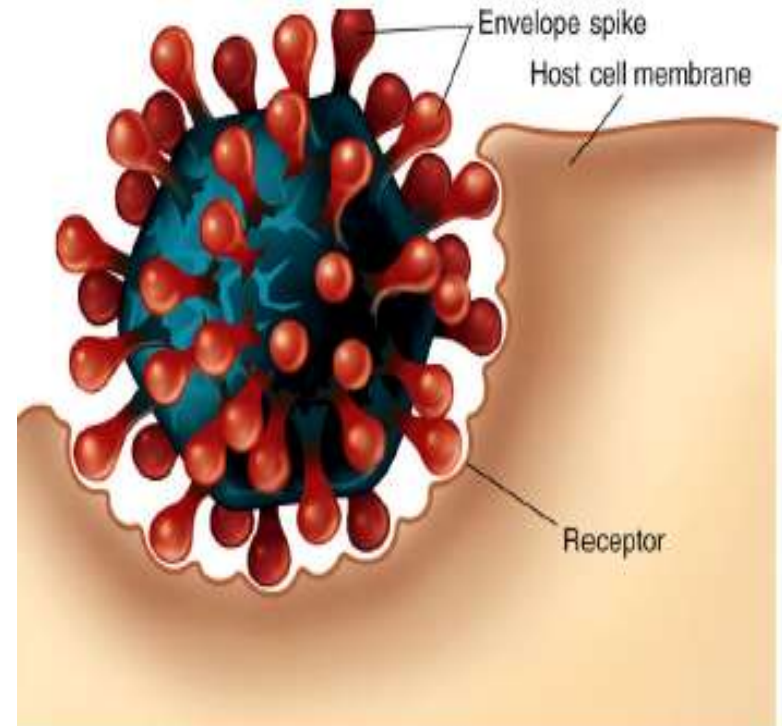
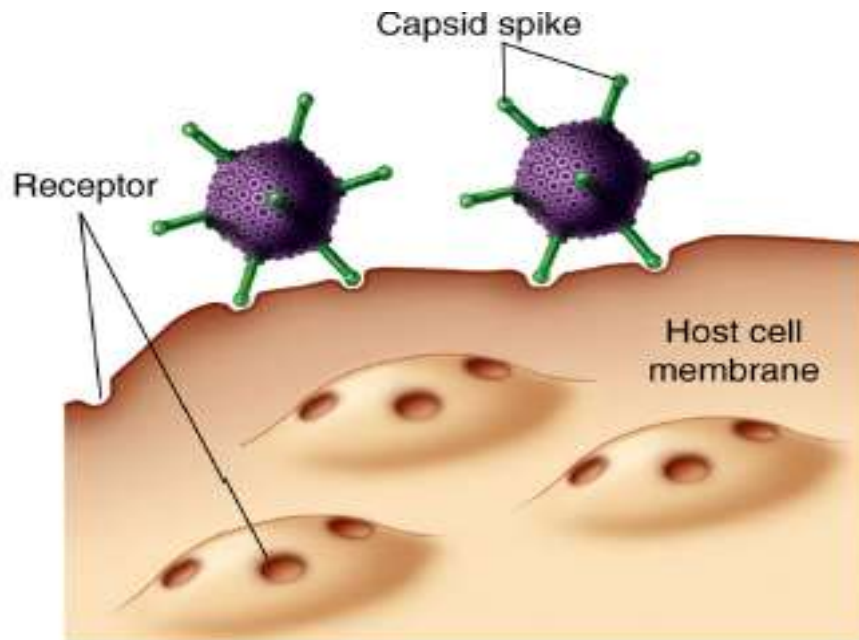
- Structure
- Chemical composition
- Genetic makeup
- Host relationship
- Type of disease
- **Baltimore Classification of Viruses**

### Baltimore Classification of Animal Viruses

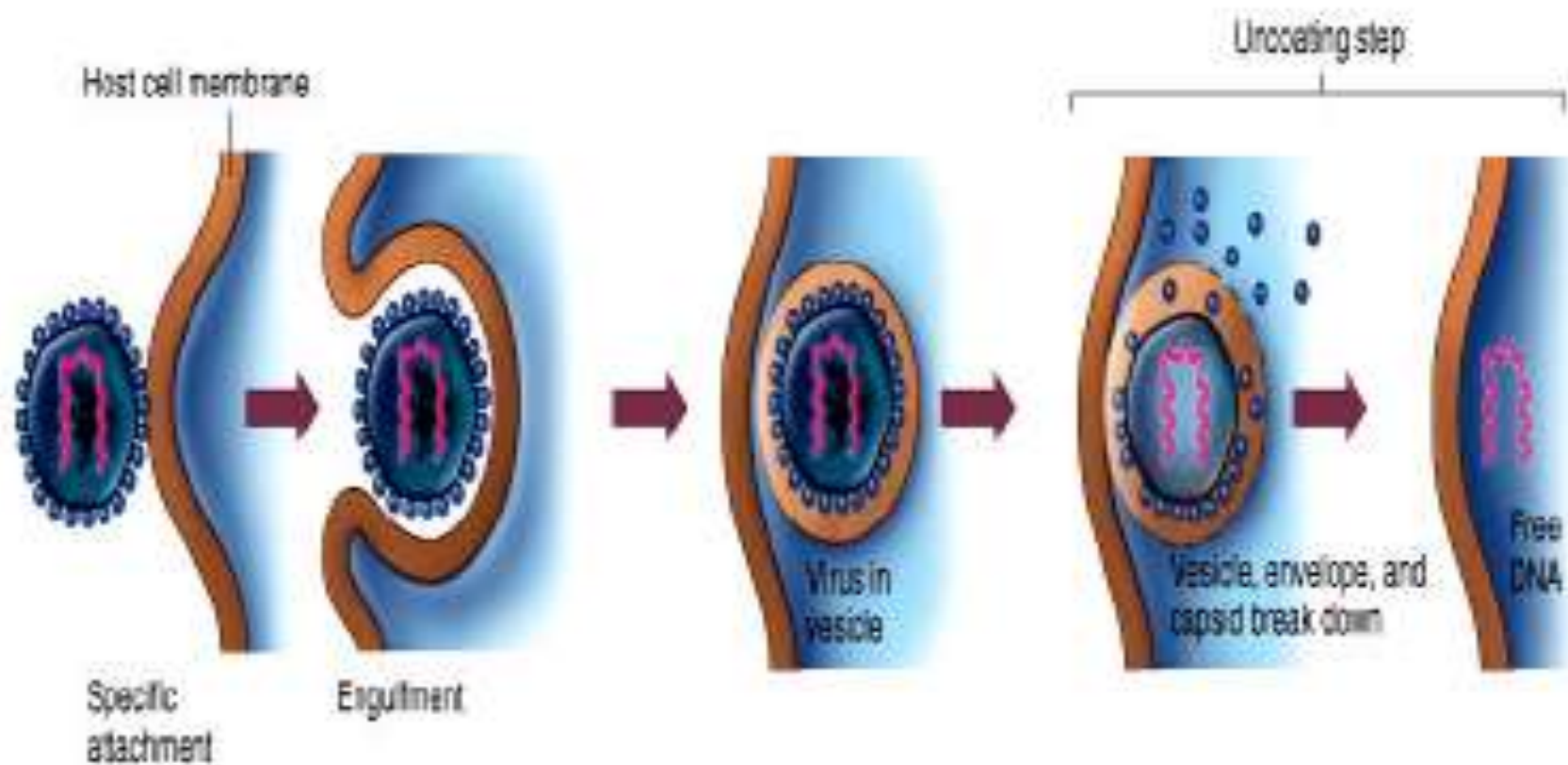


# General Steps in Viral Multiplication

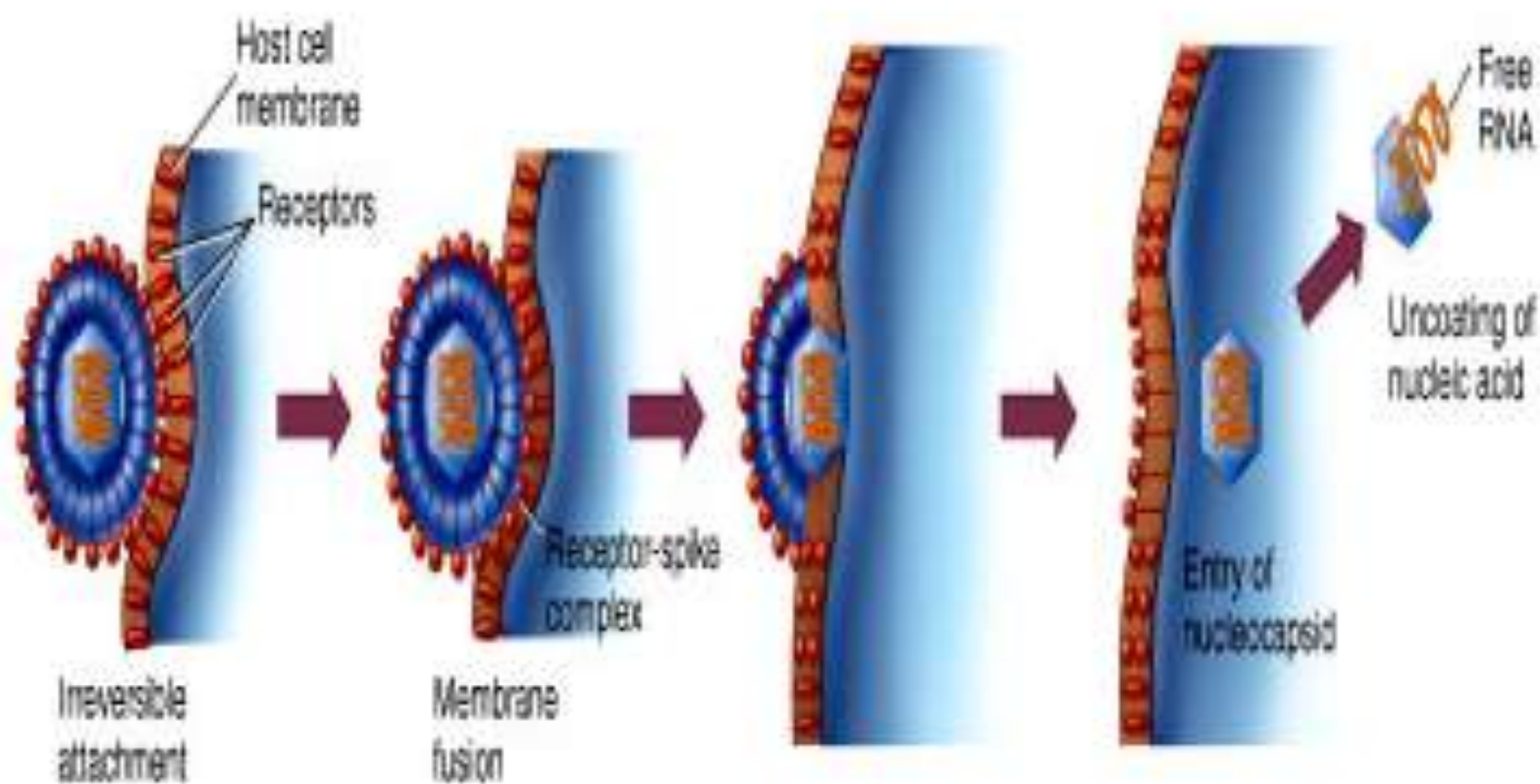
- Adsorption
- Penetration
- Uncoating
- Synthesis
- Assembly
- Release



# Endocytosis



# Membrane Fusion



# Virus replication

## Viral Replication

- Viruses require living cells for reproduction. The cell that may be infected named host cell or target cell. Viruses are obligate intracellular parasites that are dependent on cellular energy production and cellular machinery for synthesis of their components.
- Viruses have unique replication strategies – disjunctive mode of reproduction. This mode consists of separable synthesis of viral components in a host cell and spontaneous macromolecular interaction for their maturation.

- There are permissive and nonpermissive cells in human organism. The **permissive** cell has a suitable cell receptors and intracellular requirements are sufficient for supporting the replication of a particular virus. A productive viral infection proceeds in permissive cells with form new virion.
- Virus replication is not supported in the **nonpermissive cell because it does not** have specific receptors. The virus cannot replicate itself in nonpermissive cell.

- Viral life cycles in cells is terms **viral reproduction (replication)**. **Our knowledge of** viral replication is now very detailed and is expanding rapidly. Every viral family has a different strategy of replication. Process of viral replication may be short – 4-12 hours or very long – for all the organism life. An understanding of viral replication provides a basis for understanding pathogenesis, immunity, chemotherapy, and role of viruses in cancer.

## **Cultivation and Replication**

- ***In vivo* methods**
  - **Laboratory animals**
  - **Embryonic bird tissues**
- ***In vitro* methods**
  - **Cell or tissue culture**

### **Cultivation of animal viruses –**

**It is possible to study viruses in animals, but due to the complexity of the animal, expense of animals and the political environment relative to the use of animals, alternatives have been developed.**

**Chick embryos in eggs - influenza**

**Persistent infection – transformation/cancer**



# CULTIVATION OF VIRUSES:

***1. Laboratory animals***

***2. Embryonated eggs***

***3. Cell culture***

- » Primary cell cultures
- » Diploid cell strains
- » Continuous cell lines

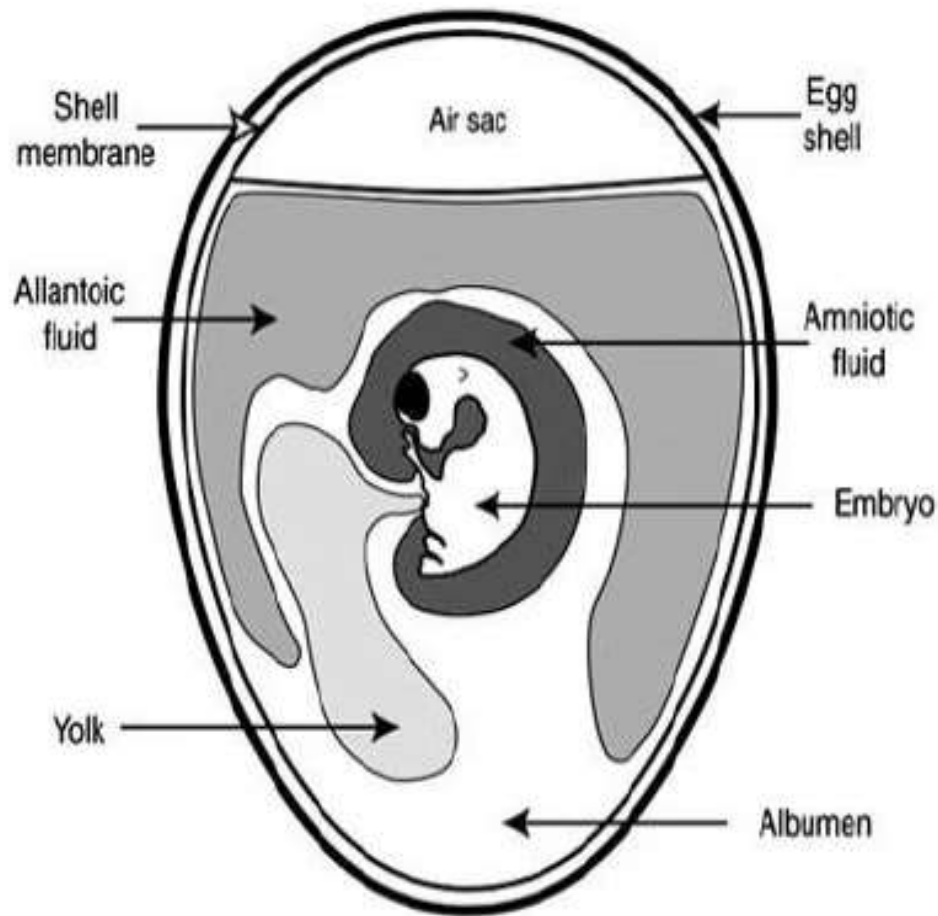


Laboratory animals are used for primary isolation of certain viruses and for studies of the pathogenesis of viral diseases.

They are inoculated by intracerebral or intranasal routes. Specimens may also be inoculated by intraperitoneal and subcutaneous routes.



# Growth of virus on embryonated eggs



This method is used for the cultivation of more than 30 species of viruses. The material containing the virus is inoculated into the amnion, allantois, yolk sac and others. After inoculation of the chick embryo, it is incubated and examined daily for virus growth.

The virus is inoculated into the allantois of the embryonated egg



*Microbiologist is inoculating 10-day of embryonated chicken eggs. CDC.*

