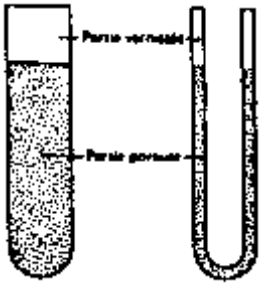
Virology Course

I. Introduction and Historical Background

By the end of the 1880s, thanks to the work of Louis Pasteur and Robert Koch, the germ theory of infectious diseases was established: for each infectious disease, a specific microorganism could be identified. This organism:

1. is visible under the microscope,
2. can be cultured on a suitable nutrient medium,
3. is retained by the Chamberland filter.

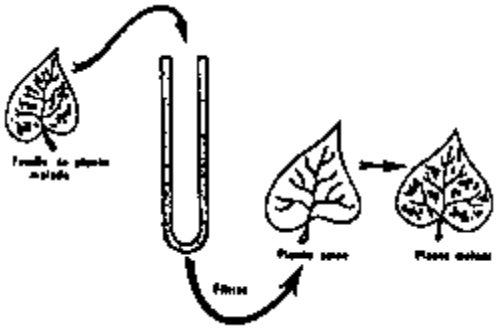


Charles Chamberland, an assistant to Pasteur, was seeking to improve the bacteriological quality of water supplied to Paris. In 1884, he invented a porcelain filter (still called the "Chamberland filter") that retains all bacteria.

The Chamberland Filter: Discovery of Viruses

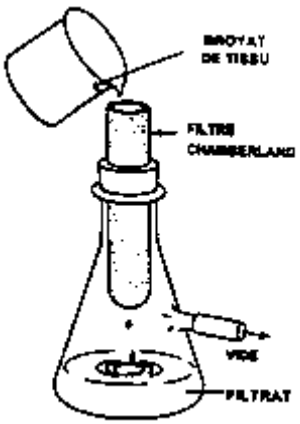
Thanks to the Chamberland filter, the existence of particular infectious agents was suspected.

As early as 1881, Pasteur showed that the infectious agent responsible for rabies is invisible under a microscope and cannot be isolated on artificial culture media. He "cultivated" the agent by inoculating brain material from a rabid dog into the brain of a trepanned rabbit.



In 1892, **Ivanovski** discovered that a disease affecting tobacco plants (tobacco mosaic disease) could be transmitted through sap passed through a Chamberland filter. He thought the agent was a toxin.

Beijerinck, continuing the experiment, demonstrated that the causal agent is not a toxin but a new type of infectious agent that replicates inside host cells. He named it a *"**contagium vivum fluidum**"* – an ultrafilterable agent (**1898**).



When microbiologists fail to isolate the agent responsible for a presumed infectious disease, they mash the lesions from infected plants or animals and filter the suspension using the Chamberland filter. If the filtered liquid, when injected into a healthy host of the same species, reproduces the disease, a **virus** is suspected.

Soon, viral etiology was established for many diseases:

- Tobacco mosaic (plants) – 1898
- Foot-and-mouth disease (animals) – 1898
- Yellow fever (humans, monkeys, mosquitoes) – 1903
- Poliomyelitis (humans) – 1909
- Cancer in chickens (transmissible via filtered tumor extract) – 1911
- Bacterial lysis (microbes) – 1915

Note:
(1) Work by Karl Landsteiner, better known for blood groups and synthetic antigens.
(2) Peyton Rous' work on chicken sarcoma – awarded the Nobel Prize in 1966.

All living cells can be targeted by specific viruses. Viruses are classified into three main groups:

- Plant viruses
- Animal viruses
- Bacterial viruses (bacteriophages)

Unlike bacteria, viruses:

1. cannot be seen under a light microscope,
2. cannot be cultured on nutrient media,
3. pass through the Chamberland filter.

Key discoveries:

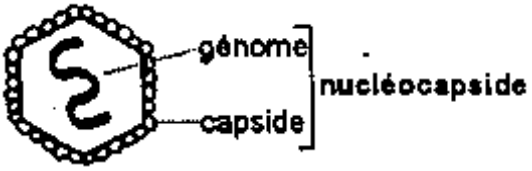
- 1935: Stanley purified the Tobacco Mosaic Virus (TMV)
- 1941: X-ray crystallography revealed that viruses are made of identical subunits
- 1955: Fraenkel-Conrat reconstituted infectious virus from purified protein and RNA
- 1980s: Discovery of HIV by Gallo and Montagnier
- 1977: Official eradication of smallpox by WHO, based on Jenner's vaccine

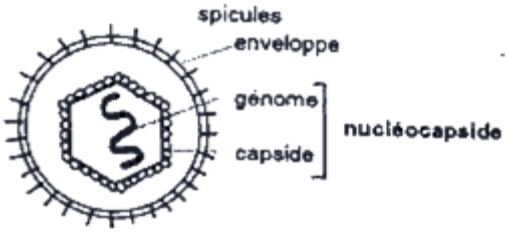
II. Definition of a Virus

A virus is an **absolute obligate intracellular parasite**, which can only replicate inside a host cell by hijacking its cellular machinery.

Viruses are small biological particles (20–300 nm) composed of:

- a **genome** (DNA or RNA),
- a protein coat (**capsid**),
- and optionally, a lipid **envelope**.





The term "**virus**" refers to the infectious agent in all stages of the viral cycle, inside or outside a host cell.

The **virion** is the complete, infectious viral particle, composed of:

1. a nucleic acid genome (RNA or DNA),
2. a protective protein shell (capsid),
   → Together, they form the **nucleocapsid**, defining a **naked virus**.

Some virions have: 3. a lipid envelope surrounding the nucleocapsid → **enveloped viruses**

Thus, viruses can be **naked** or **enveloped**.

III. Structure and Classification of Viruses

A. Structural Elements, Architecture, and Assembly

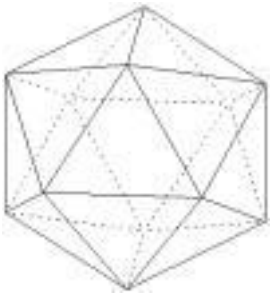
1. The Capsid

The **capsid** is formed by the assembly of small, identical viral proteins encoded by the viral genome.

Functions of the capsid:

- Protects the genome when virions are outside the host.
- In naked viruses, the capsid contains viral determinants that bind specifically to cellular receptors.

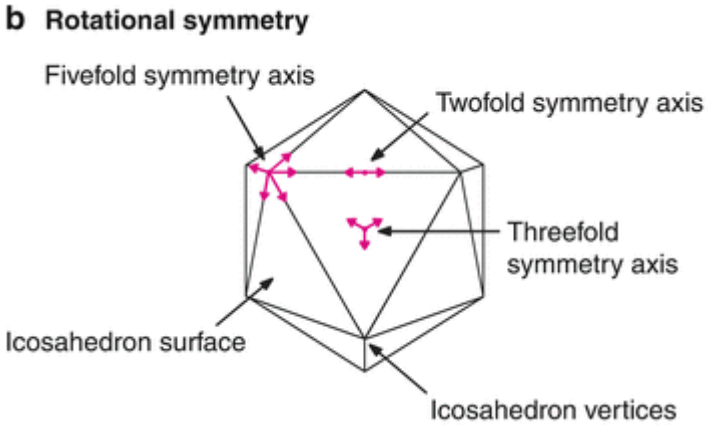
There are **two main structural types of capsids**:



- **Icosahedral (cubic) symmetry**
- **Helical symmetry**

a. Icosahedral (Cubic) Structure:

- Based on a regular icosahedron: 20 triangular faces, 12 vertices, and 30 edges.
- Has three types of symmetry axes:
  - 15 twofold axes (through edge centers)
  - 10 threefold axes (through face centers)
  - 6 fivefold axes (through vertices)

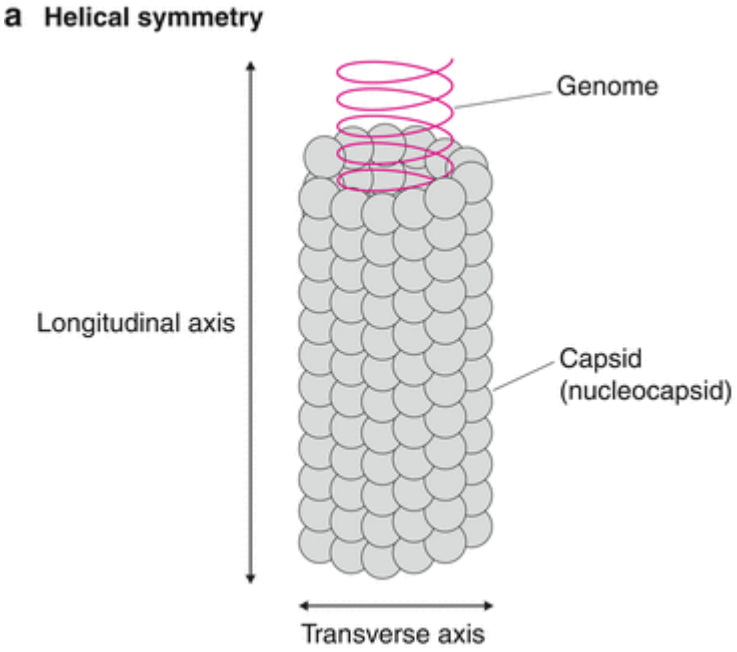


Capsid composition:

- Contains the coiled nucleic acid (no tight association between genome and proteins)
- Made of regularly arranged **capsomers**, themselves composed of structural units
  - **Hexons** (6-unit capsomers): hexagonal prisms on edges and faces
  - **Pentons** (5-unit capsomers): pentagonal prisms on the vertices

b. Helical Symmetry:

- **Example: Tobacco Mosaic Virus (TMV)** (naked nucleocapsid)
  - Cylindrical rod: 300 nm long, 17 nm in diameter
  - Contains a single RNA molecule wound like a spring (6400 nucleotides; 3 x 10^6 Da)
  - Capsid made of identical protein units forming a continuous spiral ribbon
- **Example: Influenza Virus**
  - Enveloped nucleocapsid with helical structure



- Dimensions: 800 nm long, 9 nm diameter (flexible, coiled)
- RNA genome in 9 subgenomic segments
- Envelope: 110 nm in diameter, spherical appearance with **spikes (spicules)** (8–10 nm), lipid origin with cellular elements

2. The Envelope

Some viruses (e.g., HIV) have a **lipid envelope** (also called **peplos**) surrounding the nucleocapsid.

Functions of the envelope:

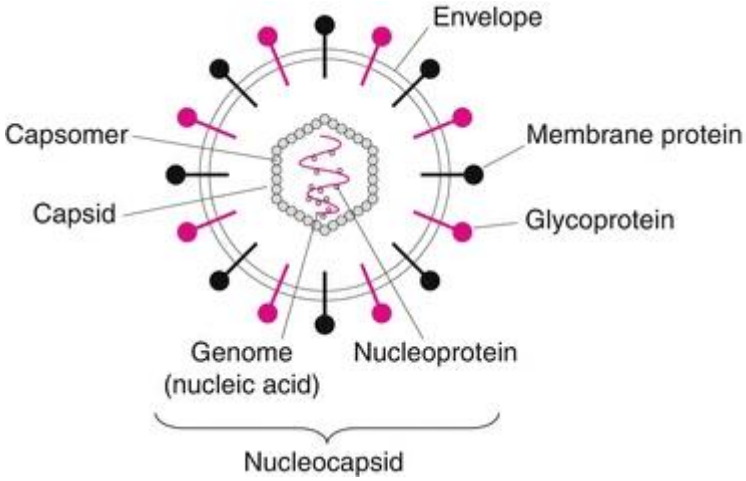
- Contains **glycoproteins (spicules)** that bind to specific cellular receptors
- Can **fuse** with the host membrane to release the nucleocapsid into the cytoplasm

Key points:

- The envelope makes the virus **fragile**
- Sensitive to heat, detergents, solvents like ether
- Loss of the envelope disables infectivity

General rule:

- Naked viruses are more resistant than enveloped viruses
  Exceptions: Poxviruses and Hepadnaviruses (enveloped but resistant)



3. The Genome

A virus contains **only one type of nucleic acid**, either:

- **DNA**, or
- **RNA** (never both)

The nucleic acid codes for:

- **Structural proteins** (capsid and envelope)
- **Enzymes**
- **Regulatory proteins** (bind promoters to regulate viral gene expression)

DNA Viruses:

- Usually **double-stranded**
- Most are **linear**, some **circular** (e.g., Papovaviruses)

RNA Viruses:

- Mostly **single-stranded**
- Exception: **Reoviruses** (double-stranded)
- Usually **linear and continuous**

- Exception: **Myxovirus influenzae** → segmented RNA (9 fragments)

Some viruses also carry internal proteins:

- Form a **core (nucleoid)** with the genome
- Include **enzymes**, such as **transcriptase** to convert viral RNA into mRNA (e.g., Myxoviruses)

B. Virus Classification

Viruses can be classified by several criteria:

1. Symptoms they cause
2. Epidemiological characteristics
3. Cytological changes they induce
4. **Structural features** (used from 1941 to 1962)

Lwoff-Horne-Tournier (LHT) Classification System

Based on 4 main criteria:

1. **Nature of genetic material:** RNA (R) or DNA (D)
2. **Capsid symmetry:** Cubic (C) or Helical (H)
3. **Presence of envelope:** Enveloped (E) or Naked (N)
4. **Virion morphology:**
    - Number of capsomers (cubic)
    - Helix diameter (helical)

Examples:

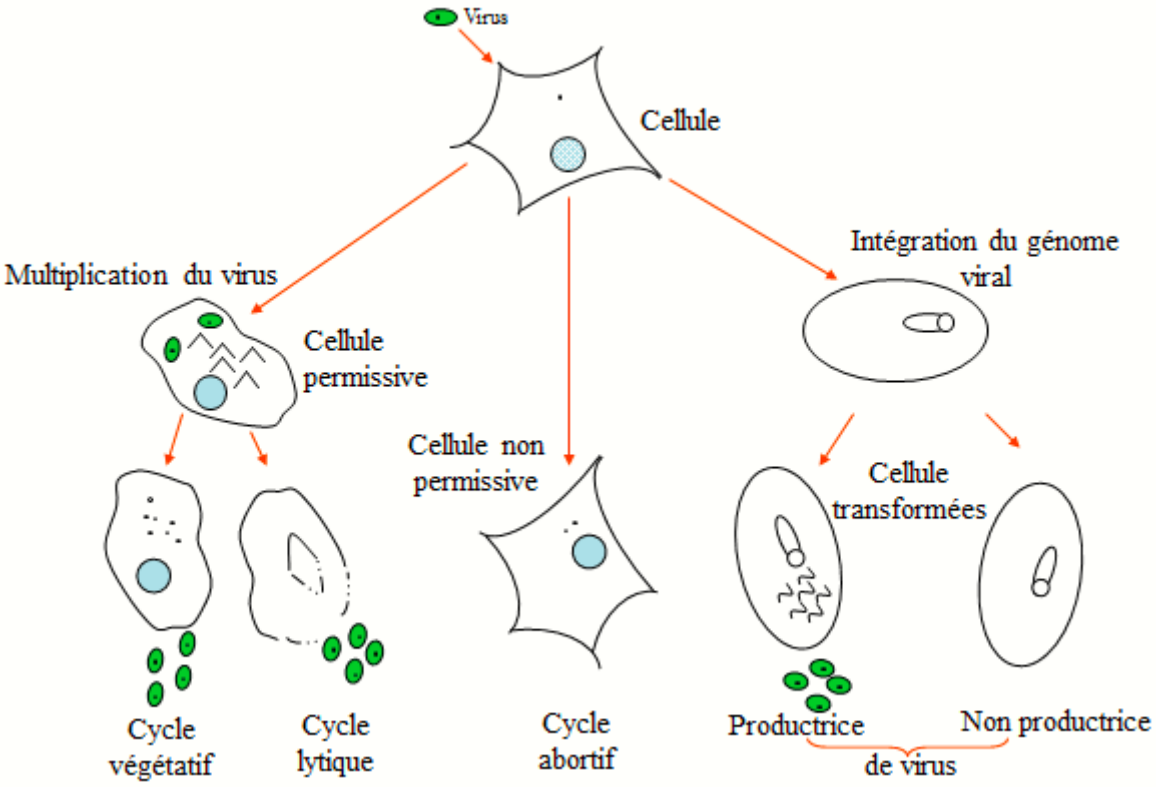
Genome	Symmetry	Envelope	Group	Diameter/Capsomers	Virus Examples
R	H	N	Plant viruses	17 nm	Tobacco Mosaic Virus (TMV)
R	H	E	Orthomyxoviridae	9–18 nm	Influenza A/B, Measles, Rabies
R	C	N	Picornaviridae	32 capsomers	Poliovirus, Coxsackie, Reovirus
D	C	N	Papovaviridae	72/252 capsomers	Papillomavirus, Adenovirus
D	C	E	Herpesviridae	162 capsomers	Herpes, Varicella, EBV
D	H	E	Poxviridae	9–10 nm	Smallpox, Vaccinia

IV. Virus–Cell Interactions

A. Viruses and Animal Cells

When a virus infects a cell, various outcomes can result depending on the nature of the virus and the host cell. Not all infections lead to viral replication — some are abortive or latent.

1. Types of Virus–Cell Interactions



a. Productive Interaction (Lytic or Vegetative Cycle):

- The virus successfully replicates inside the cell.
- In many cases, this ends with **cell lysis** and death → **lytic cycle**.
- In some, virions are released without lysis → **vegetative cycle**.
- The cell is **permissive** when it allows full viral replication.

b. Abortive Interaction:

- The virus enters the cell but **cannot replicate**.
- The cell is **non-permissive** and cannot support viral replication.

c. Integrative Interaction (Latency / Oncogenesis):

- The viral genome persists in the cell:
  - Either as a **free episome** (e.g., plasmid)
  - Or **integrated into the host genome** as a **provirus**
- Expression of some viral genes may cause:
  - **Transformation** of the cell → uncontrolled growth, immortality
  - These are **oncogenic viruses**

2. Virus–Virus Interactions

A **defective virus** lacks one or more essential functions for replication or persistence.

- It requires the presence of a **helper virus** to replicate.
- Example: **Hepatitis D virus (HDV)** (discovered in 1977)
  - RNA genome coding for only one capsid protein (delta antigen)

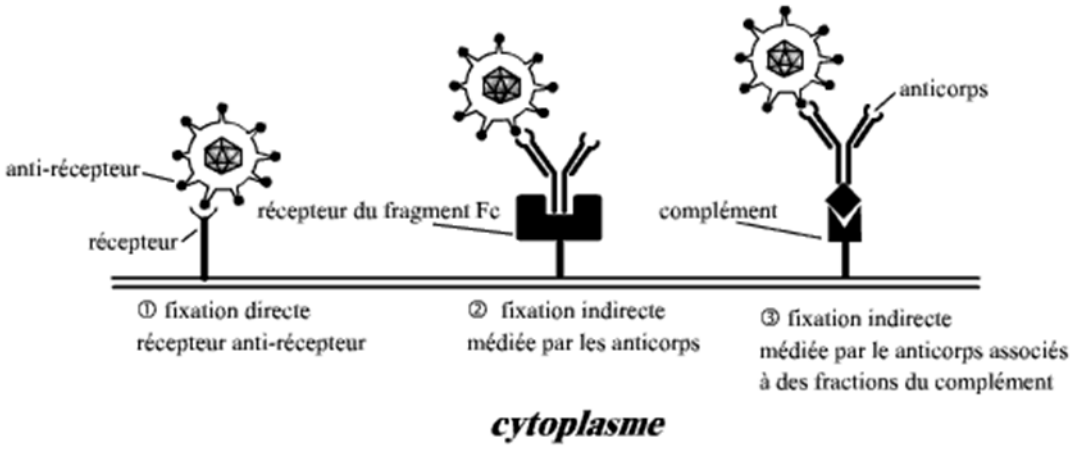
- Smallest known human virus
- Requires **Hepatitis B virus (HBV)** to provide the envelope

3. Viral Replication Cycle in a Permissive Cell

All viruses, regardless of type, follow these general steps:

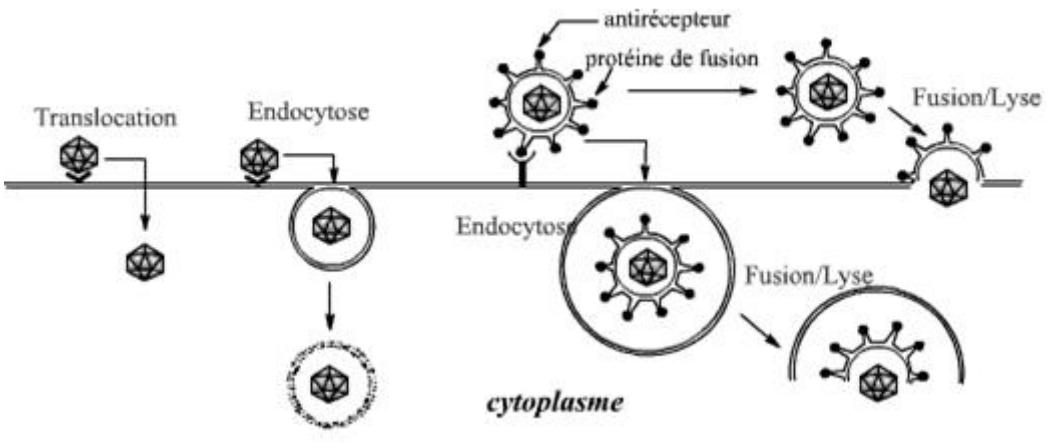
Step 1: Adsorption (Attachment)

- Virus binds to a specific **cellular receptor**
  - Receptors can be: blood group antigens, HLA, glycoproteins...
- Binding may be:
  - **Direct** (virus binds receptor)
  - **Indirect** (via antibodies or complement fragments)



Step 2: Penetration
Three main mechanisms:

- **Translocation**: virus crosses membrane directly
- **Endocytosis**: virus is engulfed into vesicles (naked or enveloped)
- **Fusion** (enveloped viruses): viral envelope fuses with the cell membrane, releasing the capsid into the cytoplasm



Step 3: Uncoating (Decapsidation)

- Viral genome is released from the capsid
- Happens in the **cytoplasm** or **nuclear pores**
- Can be:
  - **Passive** (cell enzymes degrade the capsid)
  - **Active** (virus uses its own decapsidase enzymes)

Step 4: Synthesis of Viral Components

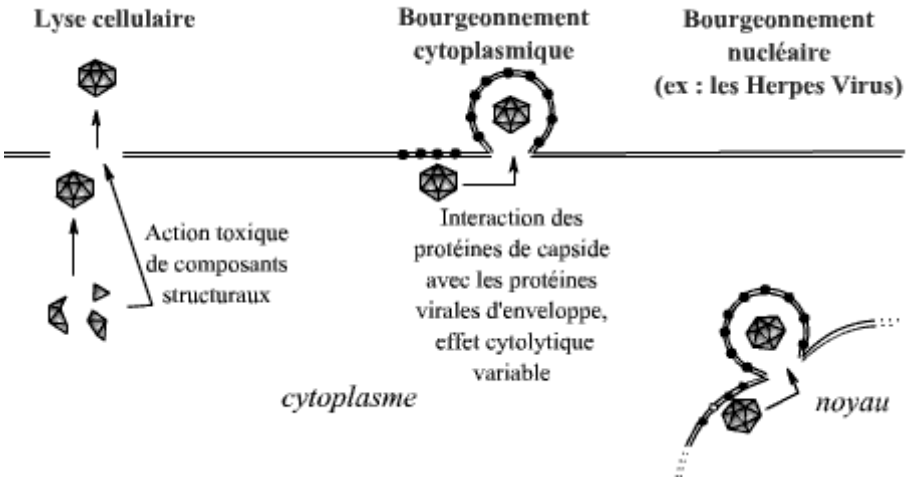
- **Replication of the genome**
- **Synthesis of proteins**: structural, enzymatic, regulatory

Step 5: Assembly and Maturation

- Viral proteins and genomes are assembled into **new virions**

Step 6: Release of Virions

- **Lysis** of the host cell (common in naked viruses)
- **Budding through the cytoplasmic membrane** (enveloped viruses)
- **Budding through the nuclear membrane** (e.g., herpesviruses)



Differences Between DNA and RNA Virus Replication

a) RNA Viruses

- **Most are single-stranded**
- **Positive-sense RNA (+RNA)** can be **directly translated** into proteins
- **Negative-sense RNA (-RNA)** must be **transcribed** into +RNA by a **viral RNA polymerase**

Model: Poliovirus (+RNA, Icosahedral symmetry)

1. **Adsorption and Penetration**
2. **Replication and Protein Synthesis**:
   - Viral RNA acts as **mRNA**, translated into:

        - RNA polymerase (replicase)
        - Host protein synthesis inhibitors
        - Capsid proteins
    - The genome is also **template** for –RNA synthesis
    - –RNA serves as template for more +RNA
3. **Assembly and Release**
    - New virions accumulate in the cytoplasm
    - Cell lysis releases them

Model: Myxovirus (Influenza Virus, –RNA, Helical, Enveloped)

- Brings its own **transcriptase** to transcribe –RNA into mRNA
- **Synthesis occurs in two locations**:
    - **Nucleus**: viral RNA, capsid proteins
    - **Cytoplasm**: glycoproteins like **hemagglutinin**
- Assembly occurs near the membrane → **budding**

b) DNA Viruses

- Replicate mainly in the **nucleus** (except Poxviruses)

Model: Adenovirus (DNA, Cubic symmetry)

- DNA transcribed into **early mRNA** → enzymes, T antigen
- **Late mRNA** → structural proteins
- Proteins translated in cytoplasm → transported to nucleus
- DNA replication uses host or viral polymerases

Model: Poxvirus (DNA, Helical symmetry)

- Entire replication occurs in the **cytoplasm**
- DNA transcribed into early and late mRNA
    - Early → enzymes
    - Late → structural proteins

V. Viruses and Bacteria: Bacteriophages

1. Definition

A **bacteriophage** (or **phage**) is a virus that infects and replicates within **bacteria**.

2. Structure of Bacteriophages

Phages are similar in architecture to other viruses:

- **Capsid** made of protein subunits, which protects the nucleic acid (DNA or RNA)
- Some have **complex structures**: head (capsid), tail, tail fibers

Example model: Bacteriophage T4 (DNA phage)

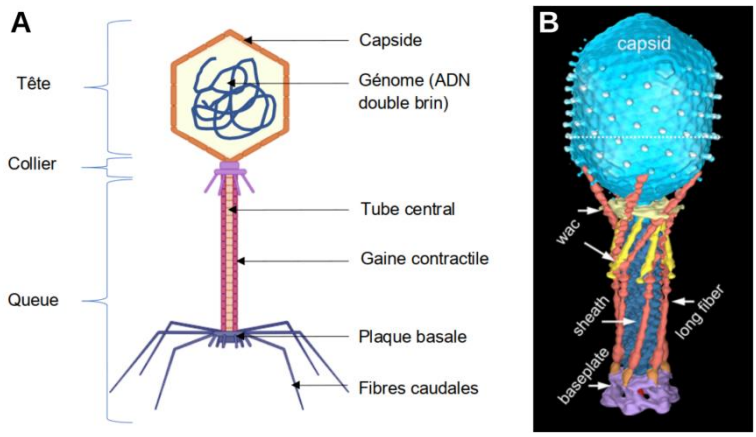


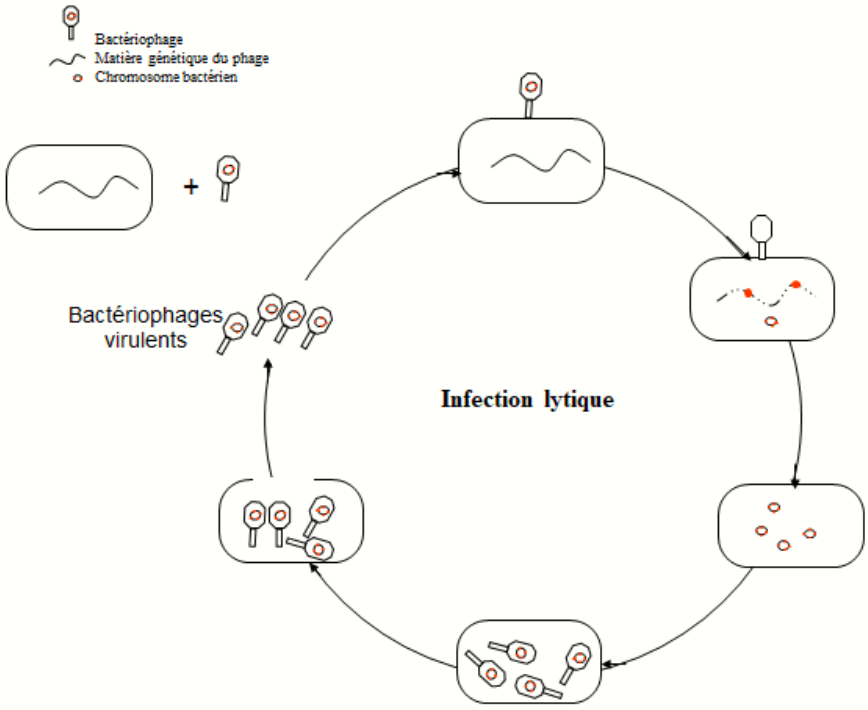
Figure - Le bactériophage T4

3. Lytic Infection and Virulent Phages – Multiplication Cycle

Phage infection in bacterial cells can occur in two major ways:

a) Lytic Cycle (Virulent phages)

- Phage attaches to bacterial surface
- Injects its **DNA** into the bacterial cytoplasm
- Bacterial **DNA is degraded**
- **Viral DNA replicates** and **proteins are synthesized**
- New virions are assembled
- The host cell **lyses**, releasing the new phages



Steps:

1. **Adsorption and Penetration**
   - Attachment to cell wall

- Enzymatic digestion of wall
- Tail sheath contracts
- DNA injection

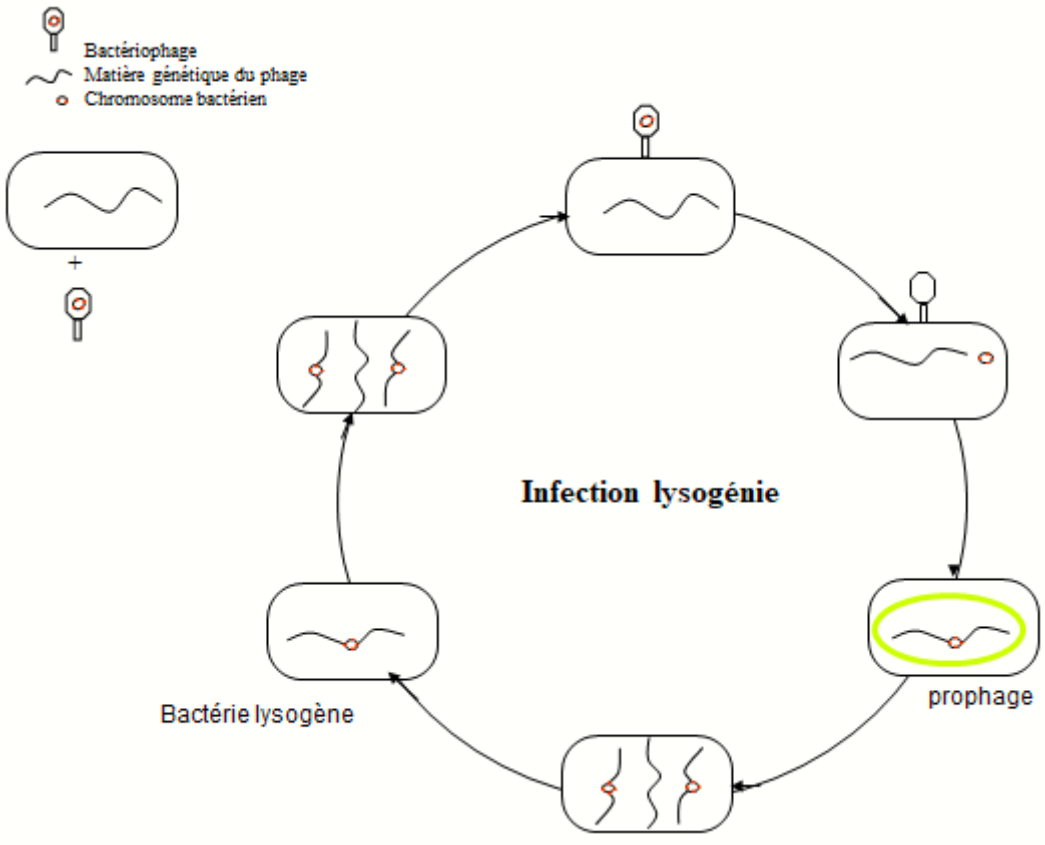
2. **Replication and Protein Synthesis**
   - Host metabolism shuts down
   - Viral early mRNA and enzymes produced
   - Viral DNA replication
   - Capsid and tail proteins synthesized
3. **Maturation and Release**
   - Virions assembled
   - Cell bursts (lysis) → **100s of new phages released**

b) Lysogenic Cycle (Temperate Phages)

In some bacterial cells, the injected phage **DNA does not replicate immediately**.

Instead:

- It integrates into the bacterial genome → **prophage**
- It is **replicated along with the bacterial chromosome**
- The infected bacteria are called **lysogenic bacteria**
- Under certain conditions, the prophage can be reactivated → enter **lytic cycle**



VI. Viroids and Prions – Simplest Infectious Agents

1. Viroids

- Discovered in **1978**

- Composed **only of RNA**, no protein coat
- Circular, single-stranded RNA (~300 nucleotides)
- Do not encode any proteins
- Replicate inside **plant cells**
- Disrupt normal RNA processing
- Found only in **plants** (e.g., potatoes, tomatoes)

2. Prions

- Discovered in **1982**
- "Prion" = *proteinaceous infectious particle*
- Responsible for **spongiform encephalopathies**, including:
  - Mad cow disease (BSE)
  - Creutzfeldt–Jakob disease (humans)

Characteristics:

- **Resistant to nucleases, UV, and boiling**
- **Do not contain nucleic acids** (no RNA/DNA)
- Composed solely of **abnormal proteins**
- Derived from a **normal cellular protein** (PrPc) that becomes misfolded (PrPres)

The misfolded protein:

- Converts normal proteins into more abnormal forms
- Aggregates in lysosomes
- Causes **neuronal death**, forming holes → brain becomes **sponge-like**

Stanley Prusiner received the **1997 Nobel Prize** for discovering the prion.