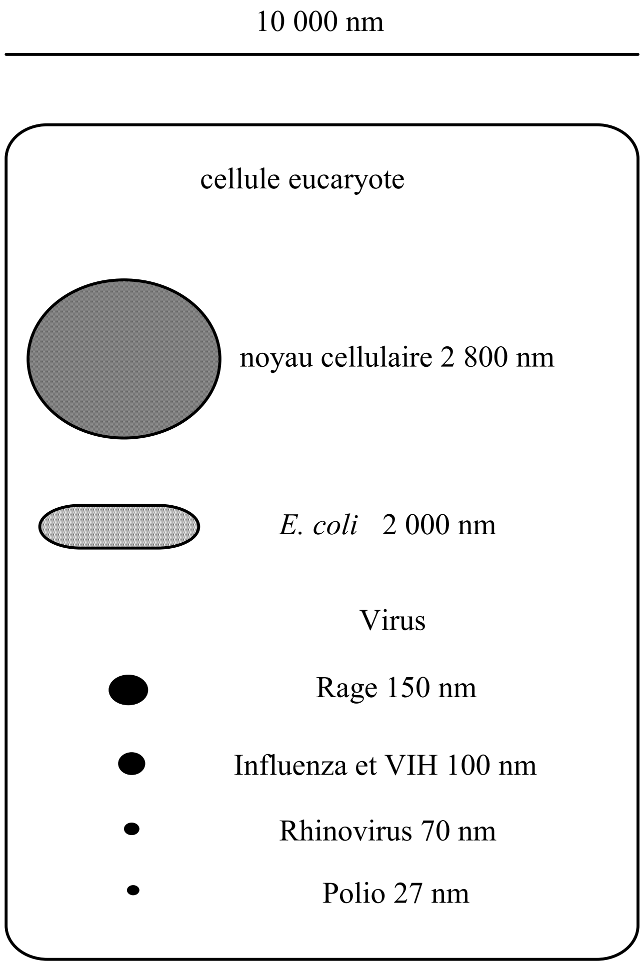
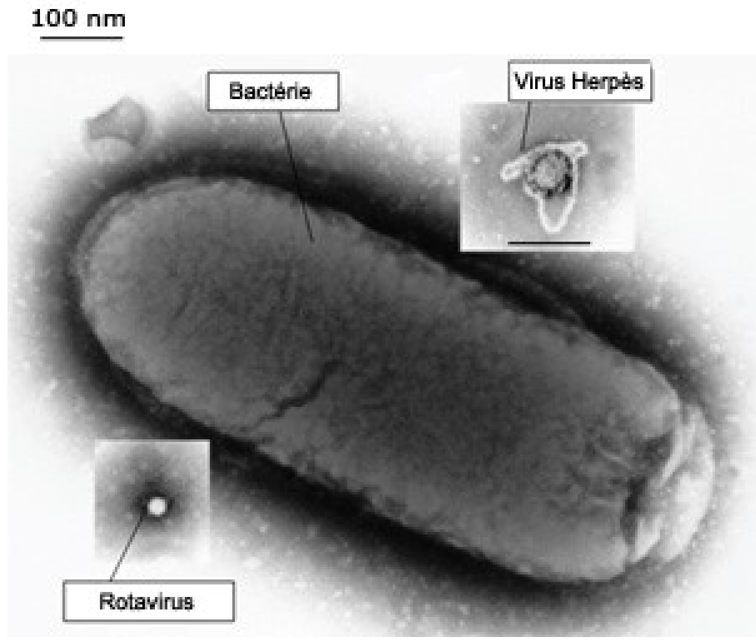
# Chaper VI:
# Bacterial and viral genetics

# Bacterial and viral genetics

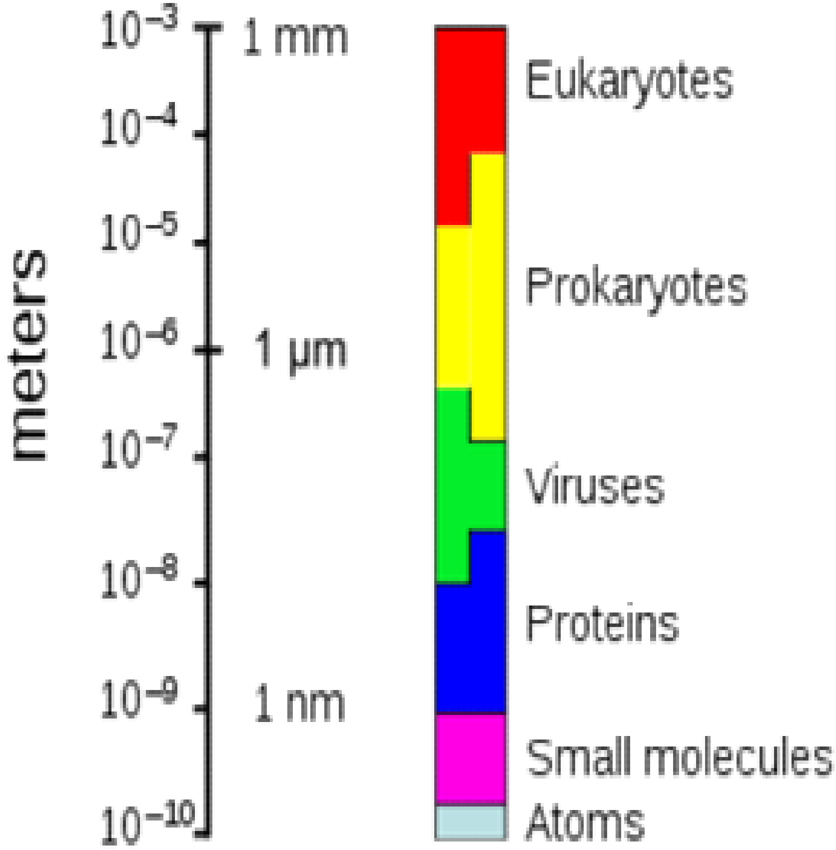



Note: Giant viruses that are the size of bacteria such as mimivirus have recently been discovered.

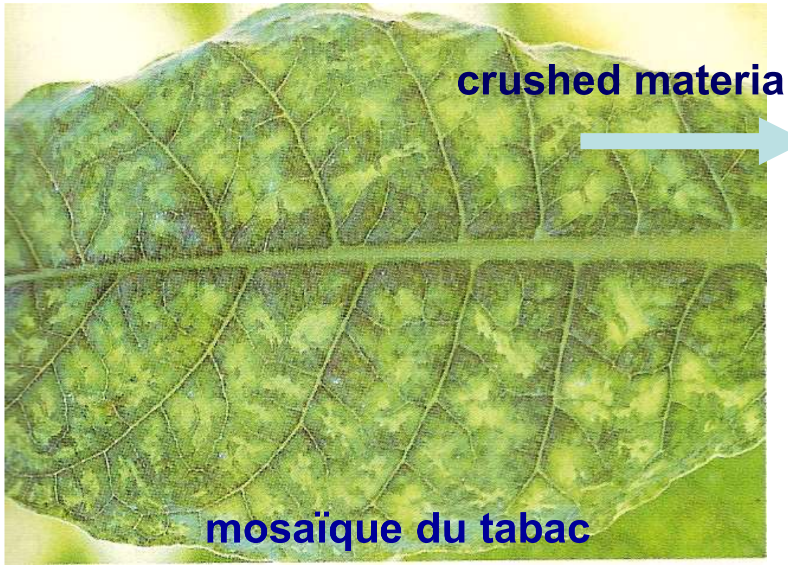
# Microorganism size

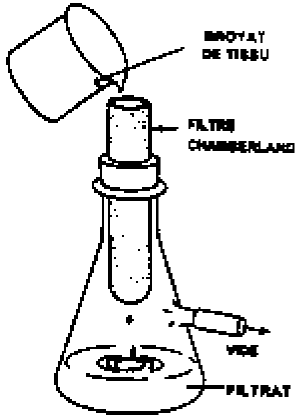
**The sizes presented by prokaryotes, compared to those of other organisms and biomolecules**



# I. Viral genetics

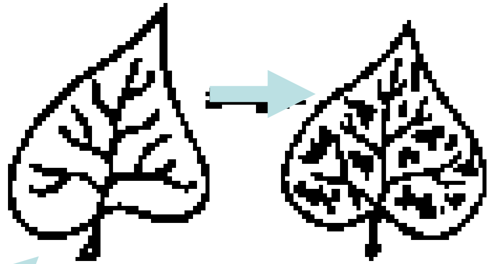
# Viruses



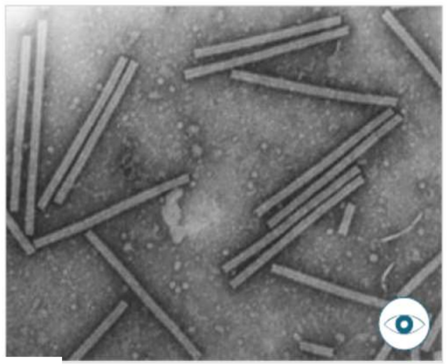

filtration

Leaf gets sick



Putting the filtrate in contact with a healthy leaf

Tobacco mosaic is caused by an unknown agent that passes through bacterial filters.
The agent is therefore smaller than 1 µm and is different from a bacterium



# I. Viral genetics

- **1. Structure and replication of viruses:**
  - Virion size between 10 and 400 nm in diameter
  - Very simple organization
  - Obligatory intracellular parasite
    - Inability to replicate autonomously
    - Nucleic acid genome (DNA or RNA)
    - Inability to synthesize proteins
    - Inability to generate energy
    - Insensitive to antibacterial agents (antibiotics)

# I. Viral genetics

- **1.1. Virus genome:**
  - DNA Viruses :
    - Single-stranded DNA
    - Double-stranded DNe
  - RNA Viruses :
    - Single-stranded RNA (polarity + or -)
    - Double-stranded RNA

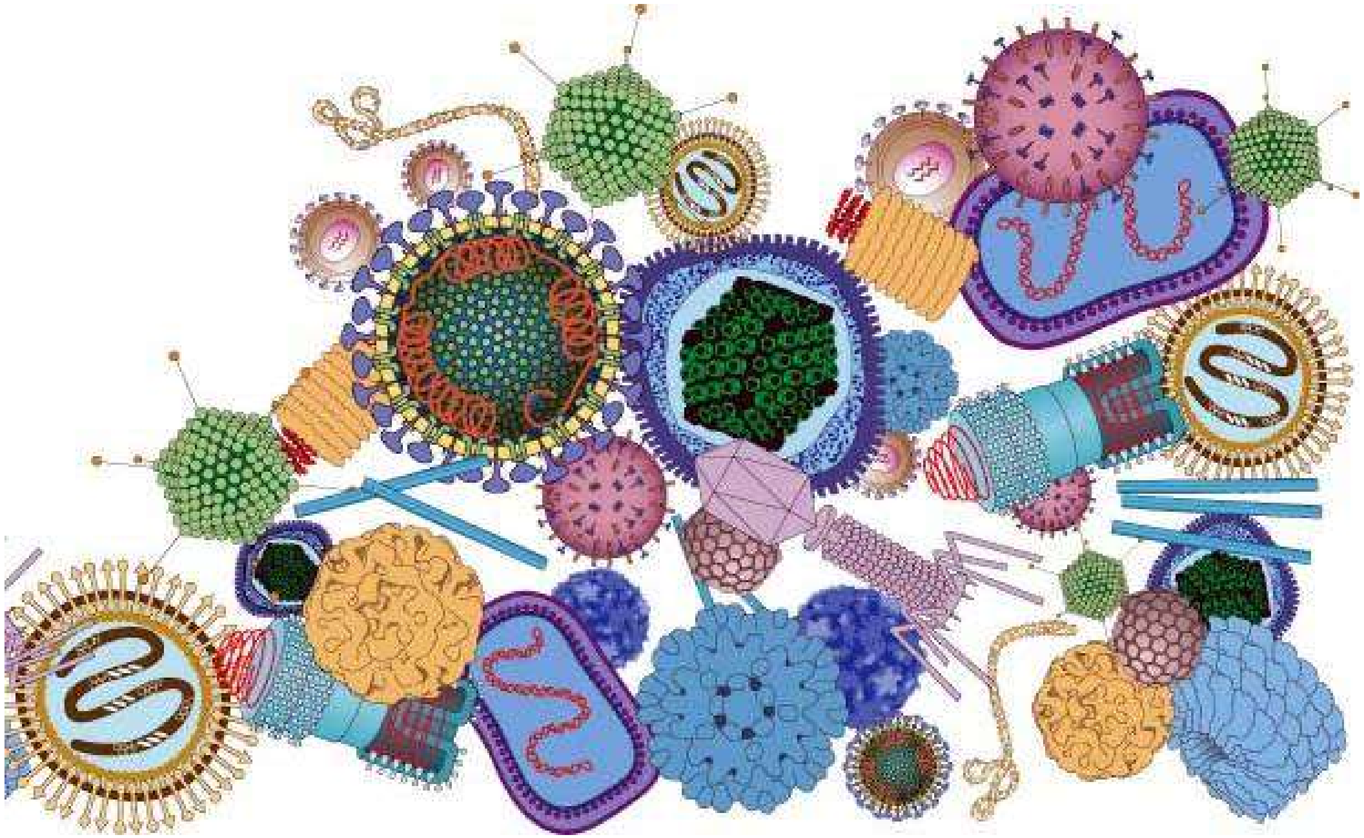
-Linear or Circular

-Segmented or non-segmented

- The viral genome most often contains a single nucleic acid molecule

# I. Viral genetics

- **1.2. Capsid and envelope:**

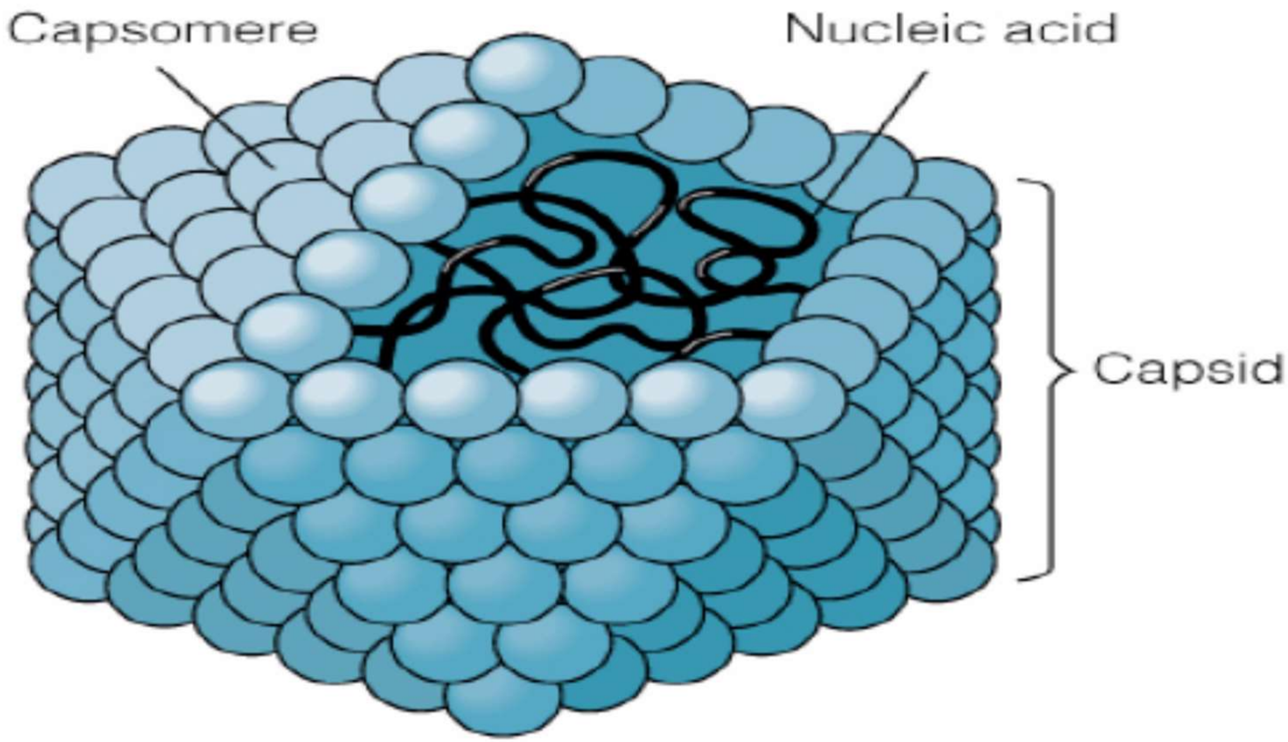


# I. Viral genetics

- **1.2. Capsid and envelope :**
  - **Capsid**: a shell that surrounds the viral nucleic acid. This capsid is formed by the assembly of repetitive protein subunits sometimes called **capsomeres**. The assembly formed by the capsid and the viral nucleic acid is called **nucleocapsid**.
  - In addition to the capsid and the viral nucleic acid, some viruses are surrounded by a lipid envelope (coat): we then speak of "**enveloped**" viruses. On the other hand, in the absence of an envelope, we speak of "**naked**" viruses.

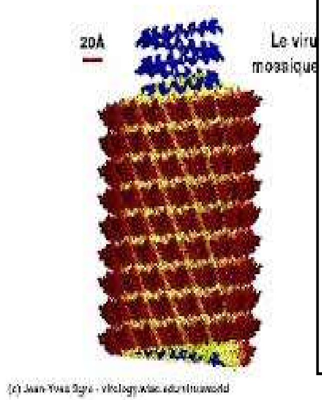
# I. Viral genetics

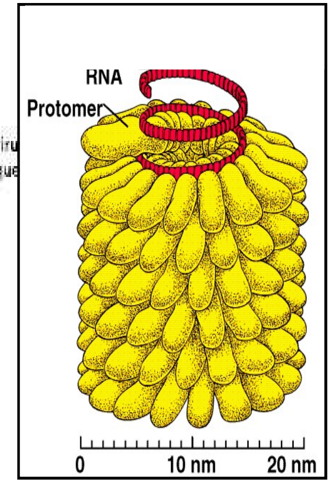
- **1.2. Capsid and envelope :**
  - Capsid structure

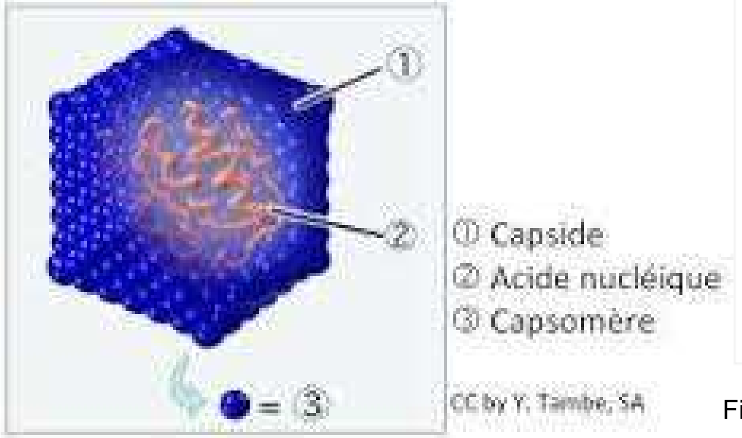


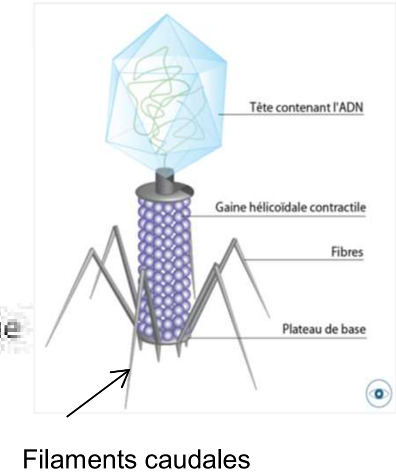
# I. Viral genetics

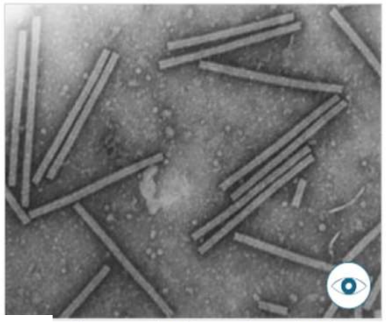
- **1.2. Capsid and envelope :**
  - Capsid structure



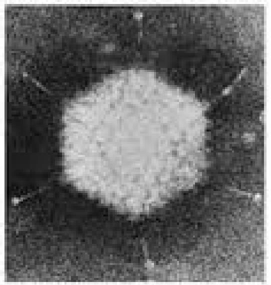



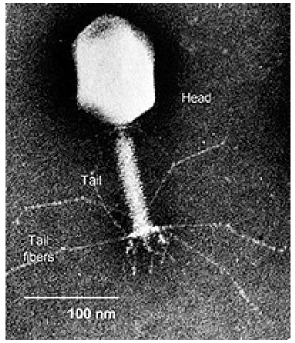



*Micrographie électronique du virus de la mosaïque du tabac*

Helical Structure



Icosahedral structure

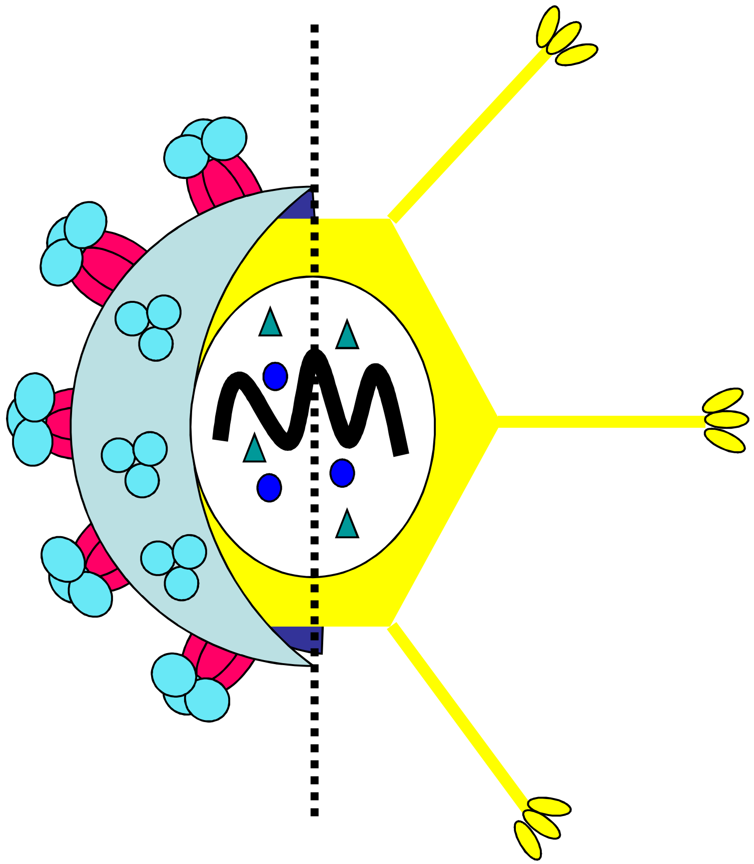



Complex structure

# I. Viral genetics

- **1.2. Capsid and envelope :**

Structure enveloppée



# I. Viral genetics

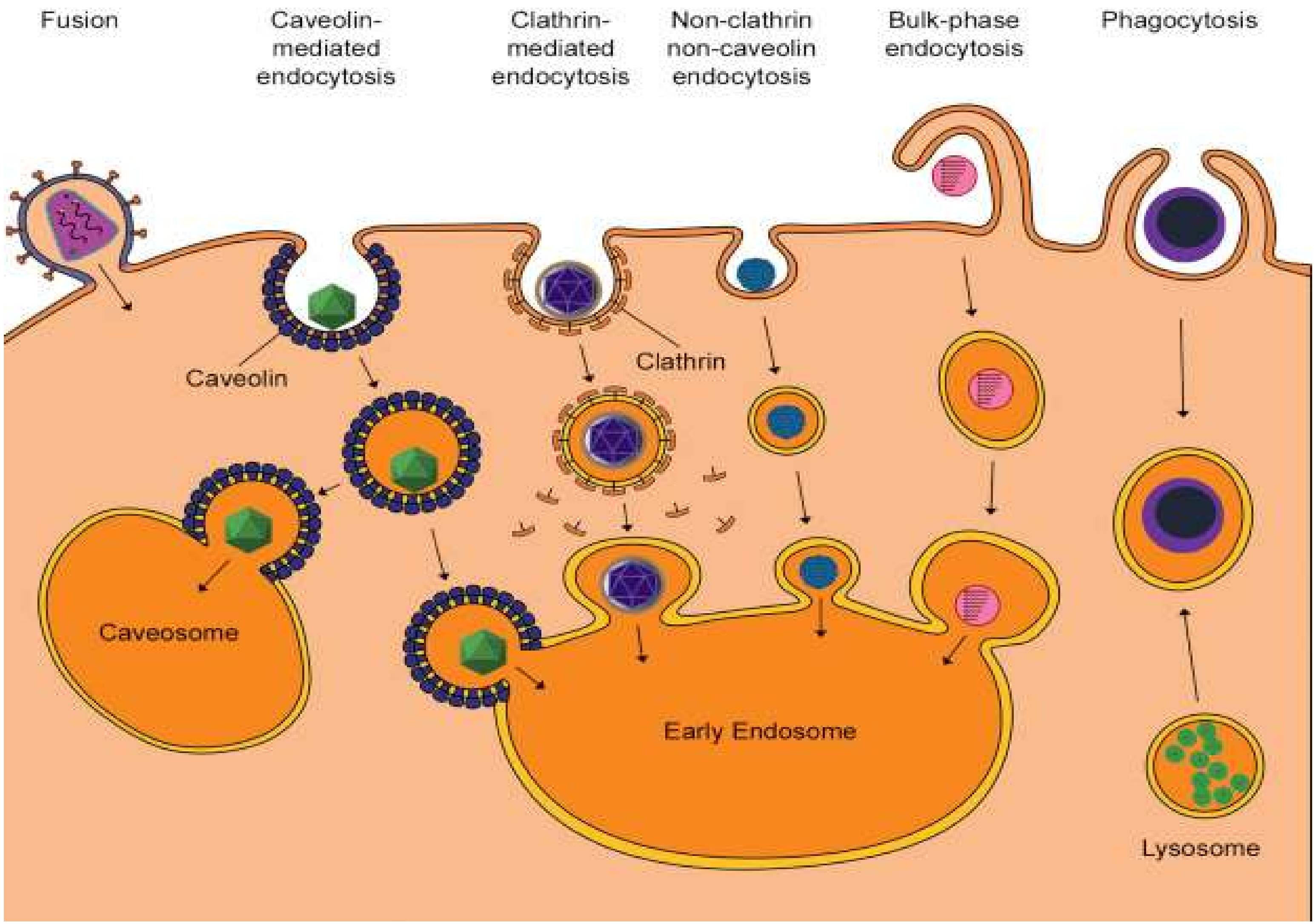
- **1.3. Virus replication:**
- The seven stages of virus replication are categorized as follows:
  - 1.Attachment
  - 2.Penetration
  - 3.Uncoating
  - 4.Replication
  - 5.Assembly
  - 6.Maturation
  - 7.Release

# I. Viral genetics

- **1.3. Virus replication:**
  - Attachment to the cytoplasmic membrane
  - 1) Injection of viral nucleic acid into the cell or 2) entry and decapsidation and release of viral nucleic acid
  - Use of cellular machinery (polymerases, ribosomes, etc. of the cell) for the benefit of the synthesis of viral constituents
  - Replication and release of new virions

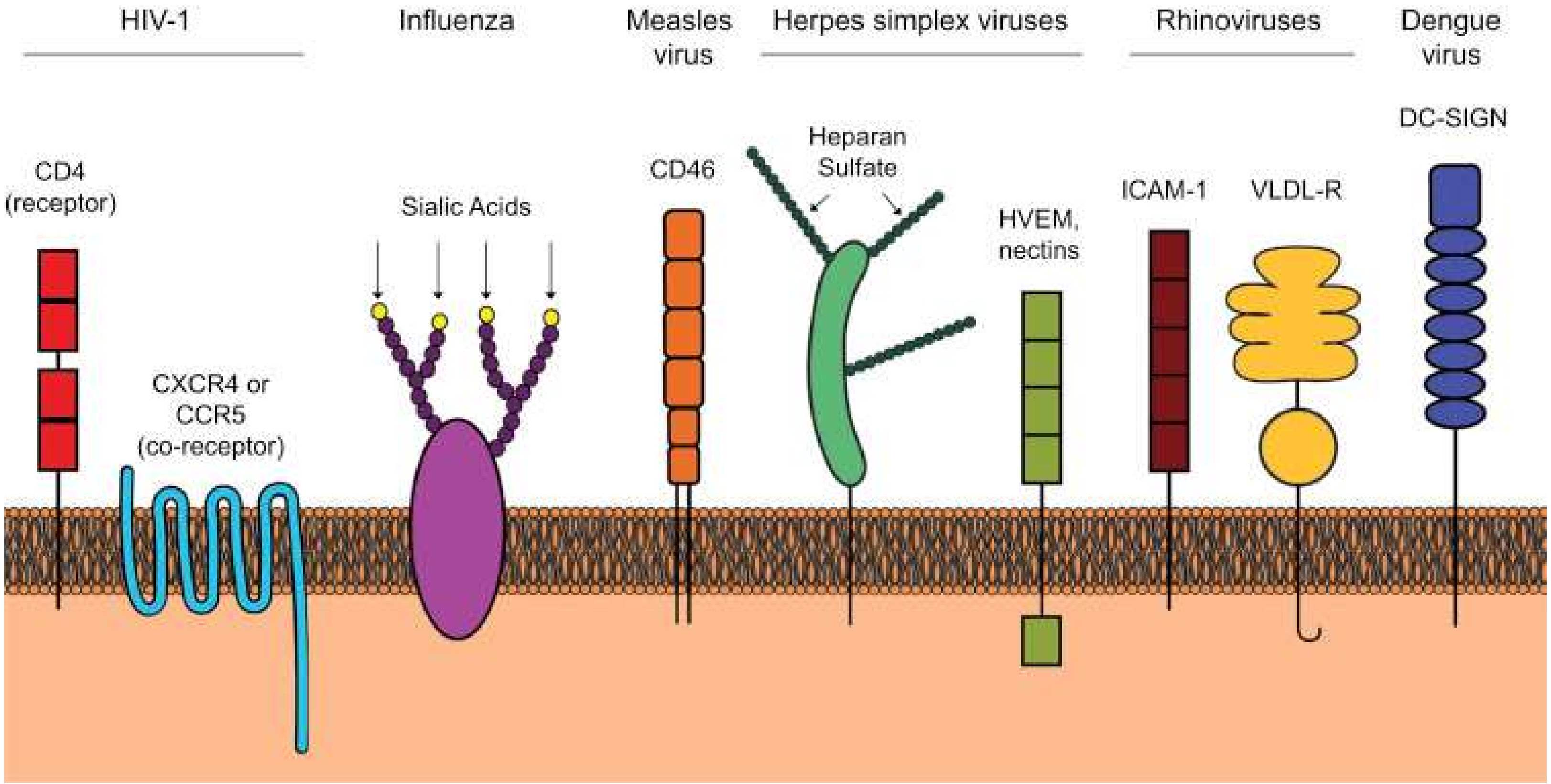
# I. Viral genetics

- **1.3. Viral penetration into the cell.:**



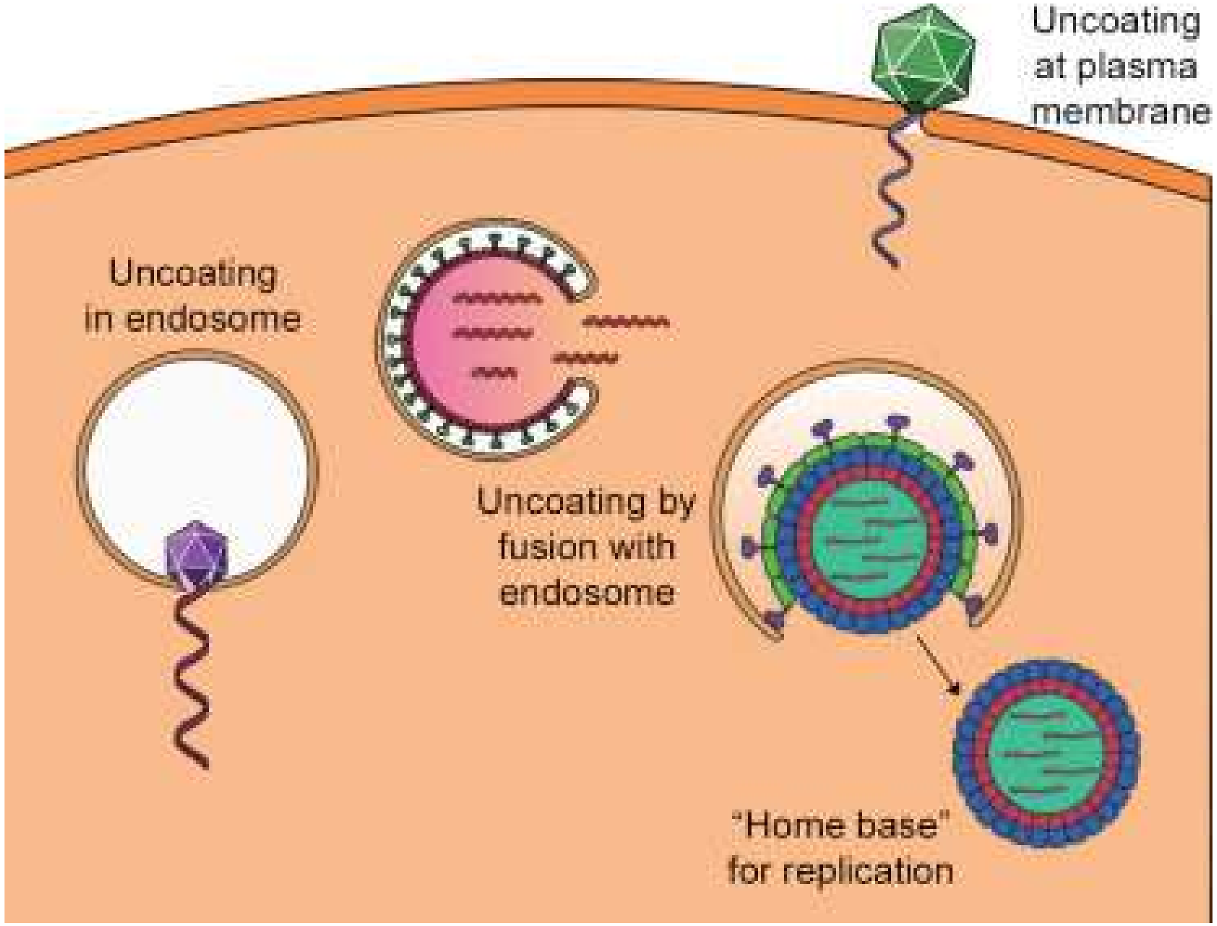
# I. Viral genetics

- **1.3. Different viruses use specific cell surface receptors for attachment:**



# I. Viral genetics

- **1.3. Uncoating of virion capsids:**

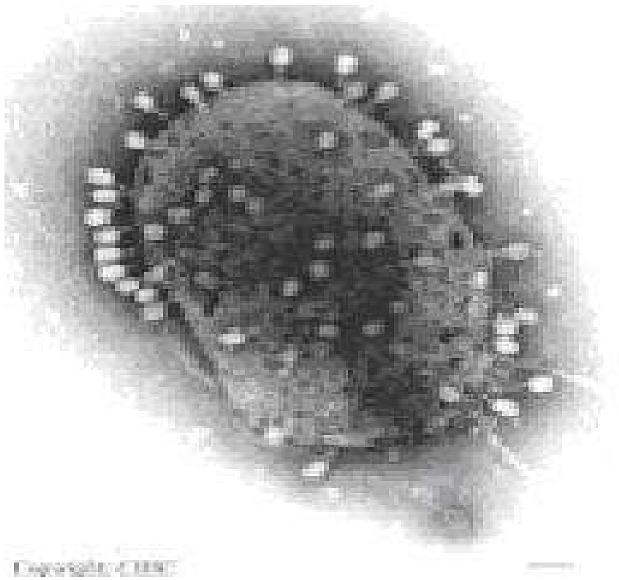


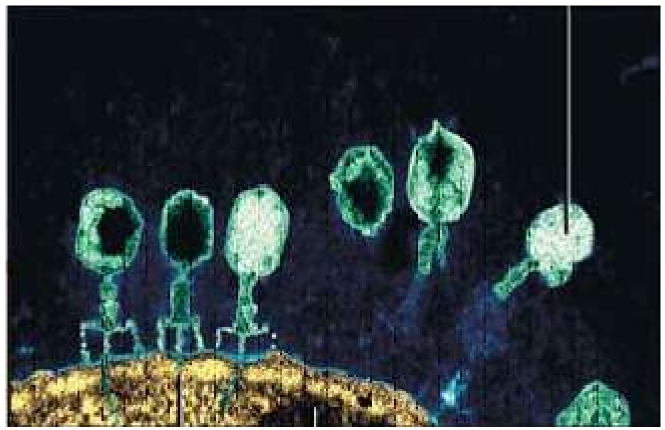
# I. Viral genetics

- **1.3. Virus replication:**
  - 1.3.1. Replication of Bacteriophages
  - 1.3.2. Replication of animal viruses
  - 1.3.2. Plant viruses

# I. Viral genetics

- 1.3.1 Replication of Bacteriophages :





*E. Coli* attacked by T4 phages

# I. Viral genetics

- 1.3.1 Replication of Bacteriophages :
  - a- The lytic cycle
  - b- The lysogenic cycle

# I. Viral genetics

- 1.3.1 Replication of Bacteriophages :
  - a- The lytic cycle : exp. T4 Phage

    **Lysis** = cell bursting

    5 steps:

    – Attachment: Binding sites must match receptor sites on the host bacterial cell

    – Penetration: Viral DNA is injected into the bacterial cell

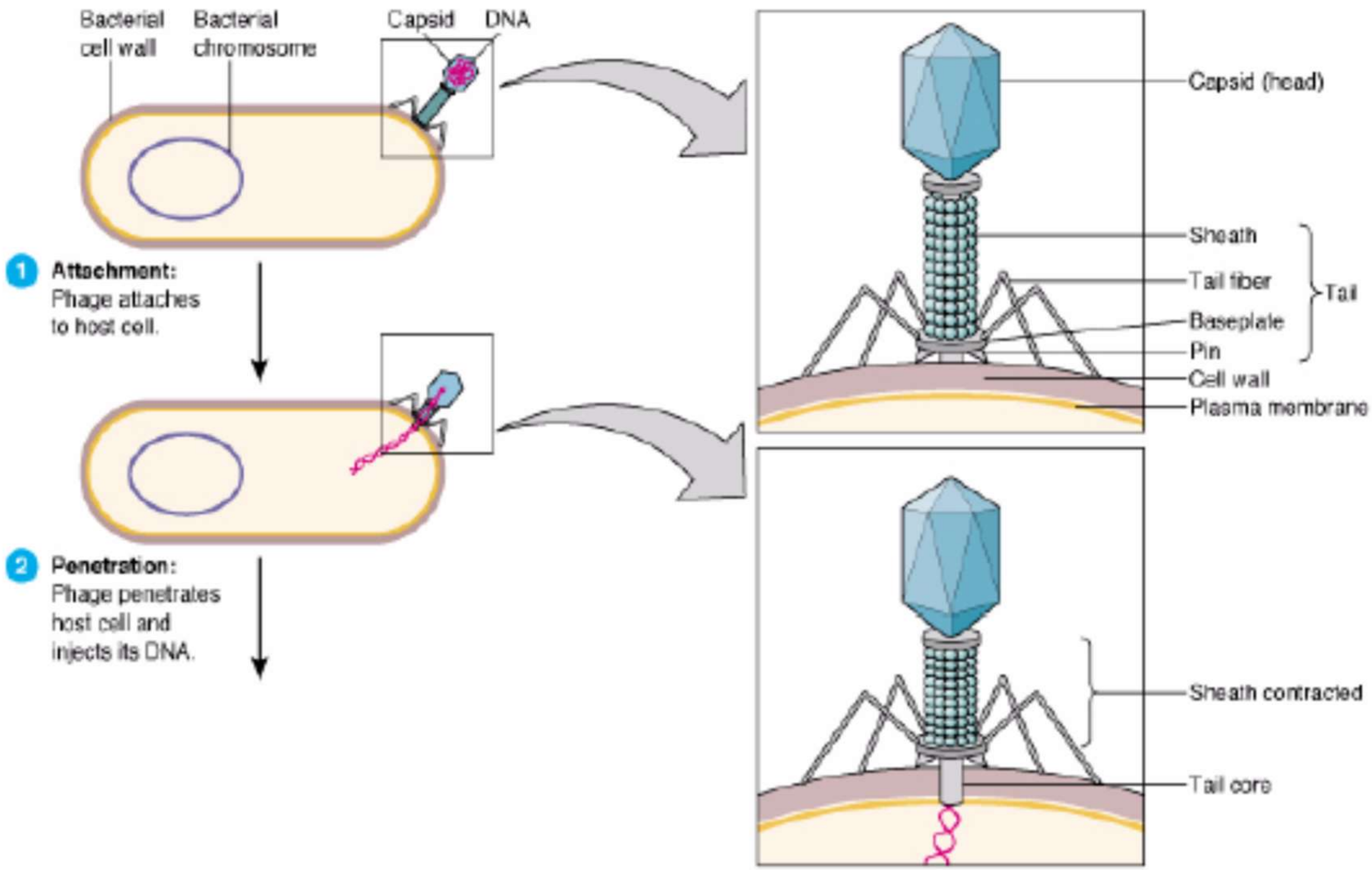
    – Biosynthesis: Virus uses enzymes and cellular machinery for replication, transcription, and translation

    – Maturation: Viral particles are assembled

    – Release of new viral particles: Lysis occurs

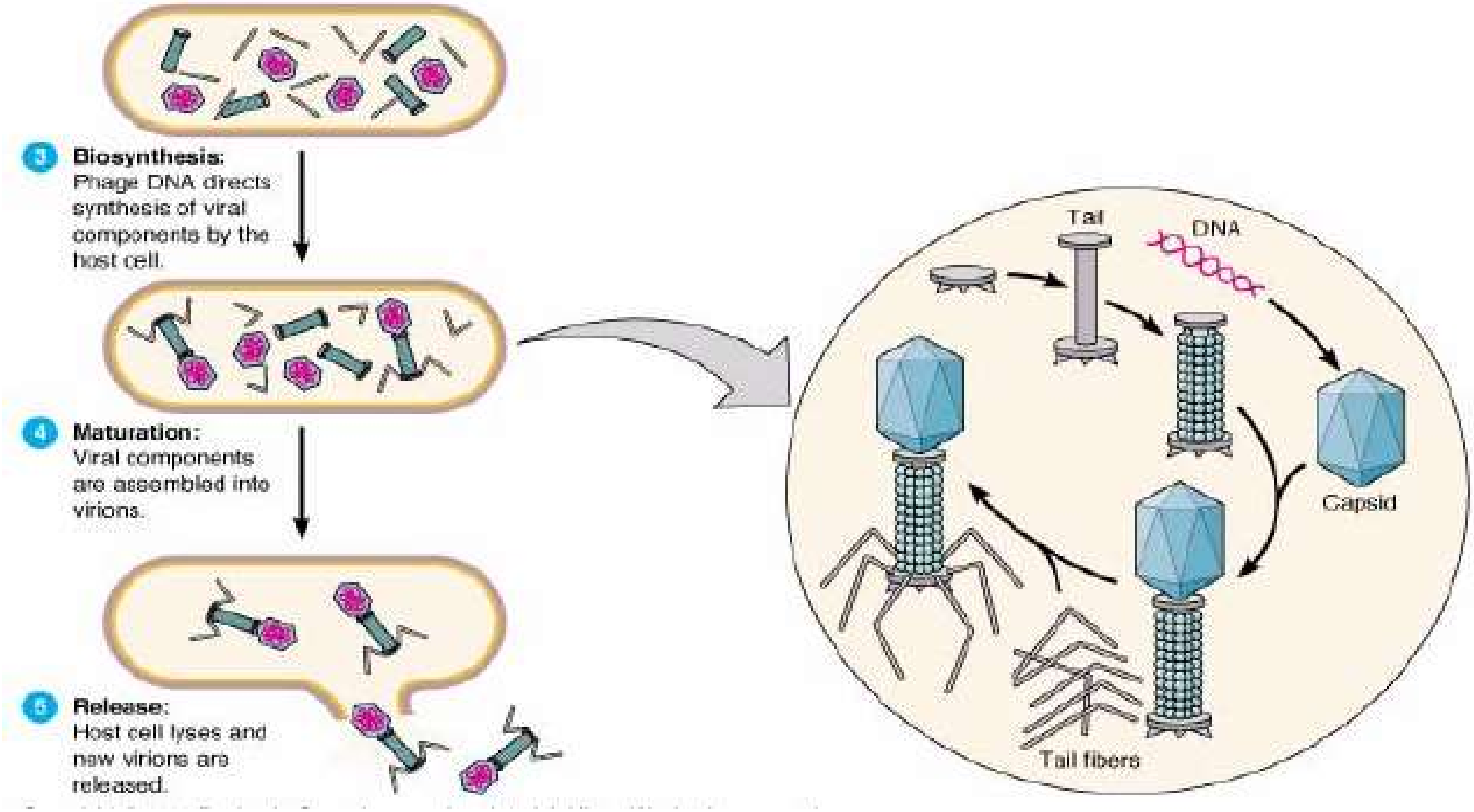
# I. Viral genetics

- 1.3.1 Replication of Bacteriophages :
  - a- The lytic cycle : exp. T4 Phage



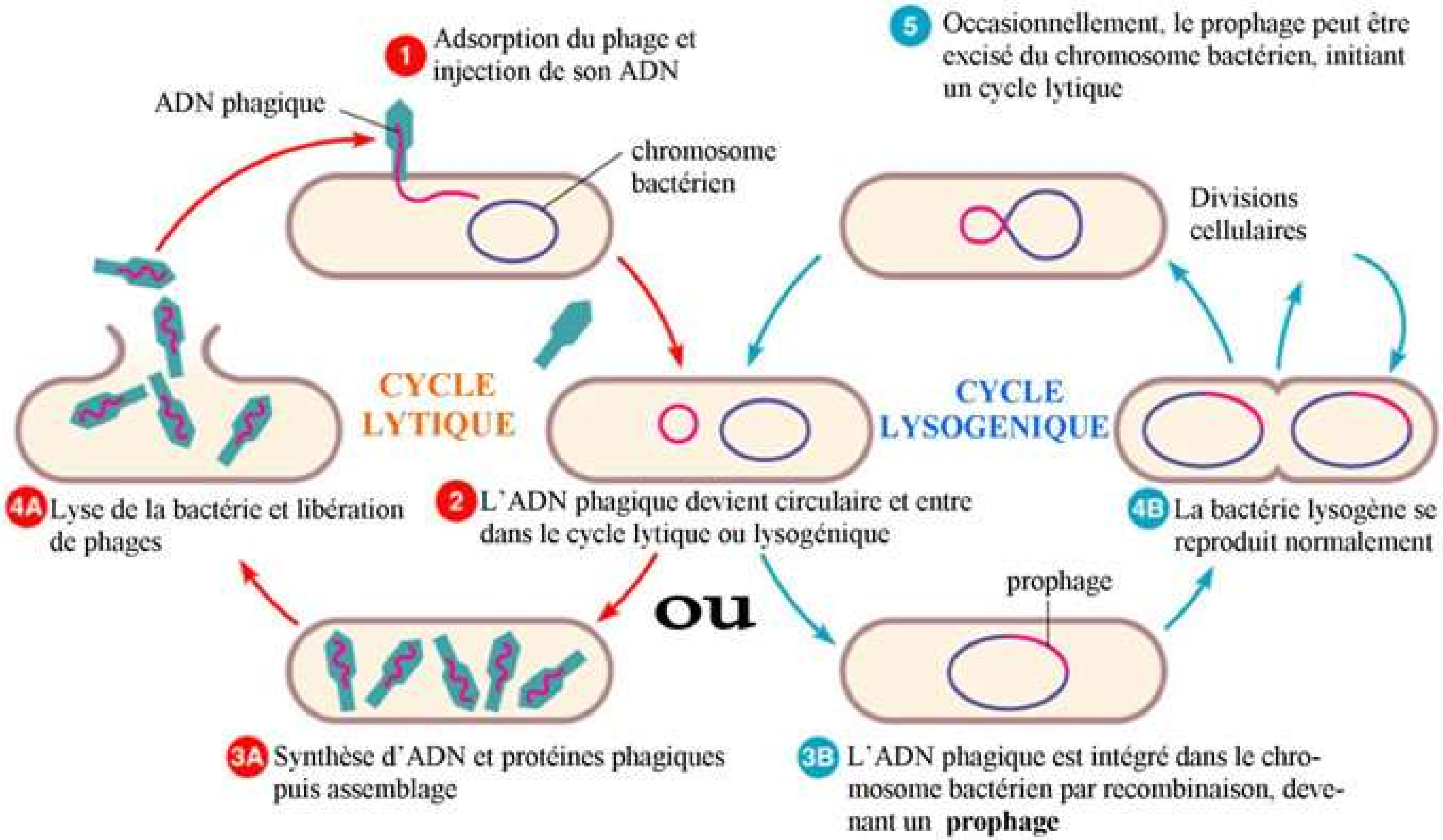
# I. Viral genetics

- 1.3.1 Replication of Bacteriophages :
  - a- The lytic cycle : exp. T4 Phage



# I. Viral genetics

- 1.3.1 Replication of Bacteriophages :
  - b- Lysogenic cycle : exp. **ƛ phage**
    - Does not destroy the cell
    - But if induction → Lytic cycle



# I. Viral genetics

- **1.3.2. Animal viruses:**
  - Viruses that cause disease in humans and other animals
  - Obligate intracellular parasites
  - Can multiply only by infecting a host cell

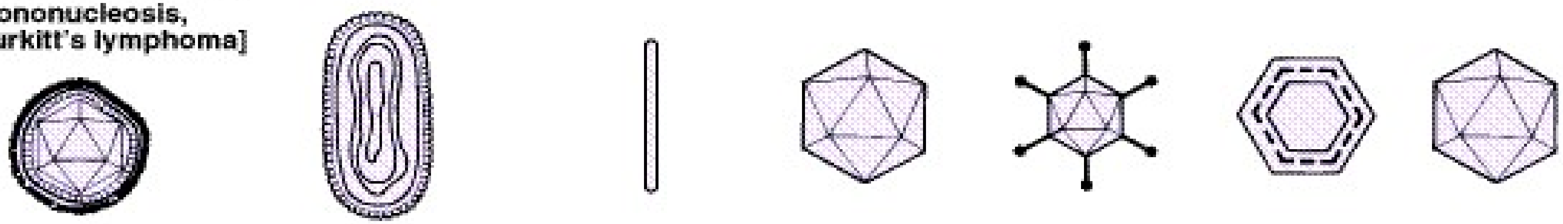
# Replication of animal viruses

## 1.3.2.1. Classification of animal viruses

- The International Committee on Virus Nomenclature has since 1976 classified viruses according to the following criteria :
    - The type of nucleic acid: DNA or RNA (if RNA, positive (+) or negative (-) polarity).
    - The symmetry of the capsid (icosahedral or helical).
    - The presence or absence of envelopes (peplos).
    - The number of capsomeres.

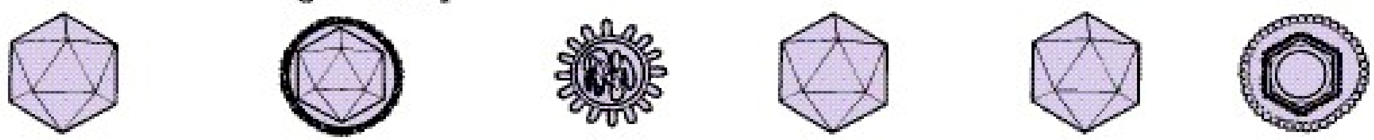
# Taxonomy of DNA Animal Viruses

| Strandedness | Double | Double | Double | Double | Double | Double | Single |
|---|---|---|---|---|---|---|---|
| Enveloped or naked | Enveloped | Enveloped | Enveloped | Naked | Naked | Naked | Naked |
| Capsid symmetry | Icosahedral | Complex | Cylindrical | Icosahedral | Icosahedral | Icosahedral | Icosahedral |
| Size (nm) | 180–200 (enveloped) 100–110 (capsid) | 200–260 x 250–290 (enveloped) | 40–110 x 200–400 (enveloped) 35–40 x 200–350 (capsid) | 45–55 | 60–90 | 130–180 | 22 |
| Site of capsid assembly | Nucleus | Cytoplasm | Nucleus | Nucleus | Nucleus | Cytoplasm | Nucleus |
| Virus family | *Herpesviridae* | *Poxviridae* | *Baculoviridae* | *Papovaviridae* | *Adenoviridae* | *Iridoviridae* | *Parvoviridae* |
| Re-presentative genera [Host, disease] | Herpes simplex virus, Type 1 [Humans: fever blisters, respiratory infections, encephalitis] Type 2 [Humans: genital infections]; Varicella-zoster [Humans: chickenpox]; Epstein-Barr virus [Humans: infectious mononucleosis, Burkitt's lymphoma] | Orthopoxvirus [Humans: smallpox] [Humans, cattle: cowpox]; Avipoxvirus [Chickens: fowlpox] | Nuclear polyhedrosis virus; Granulosis virus [Insects] | Papillomavirus [Humans, rabbits: benign tumors (warts)]; Polyomavirus [Humans, mice: tumors in mice] | Mastadenovirus [Humans: respiratory infections]; Aviadenovirus [Birds] | Iridovirus [Arthropods]; African swine fever virus | Parvovirus [Humans, canines: gastroenteritis] |



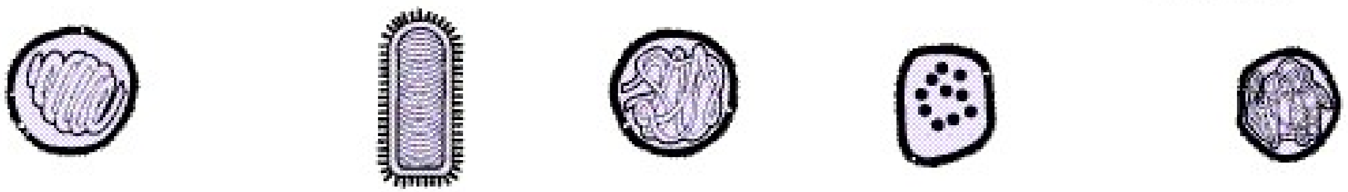
# Taxonomy of RNA Animal Viruses

| Strandedness | Double | Single | | | | |
|---|---|---|---|---|---|---|
| Single-strand sense | | Positive | Positive | Positive | Positive | Positive<br>DNA intermediate during replication |
| Enveloped or naked | Naked | Enveloped | Enveloped | Naked | Naked | Enveloped |
| Capsid symmetry | Icosahedral | Icosahedral | Helical | Icosahedral | Icosahedral | Icosahedral |
| Size (nm) | 75–80 | 40–75 (enveloped)<br>25–35 (capsid) | 80–160 (enveloped)<br>14–16<br>(capsid diameter) | 22–30 | 35–40 | 80–100<br>(enveloped) |
| Site of capsid assembly | Cytoplasm | Cytoplasm | Cytoplasm | Cytoplasm | Cytoplasm | Cytoplasm |
| Virus family | *Reoviridae* | *Togaviridae* | *Coronaviridae* | *Picornaviridae* | *Calciviridae* | *Retroviridae* |
| Represent-ative genera and groups [Host, disease] | Orbivirus [Humans: encephalitis]<br>Rotavirus [Humans: diarrhea]<br>Cypovirus [Insects: cytoplasmic polyhedrosis virus | Alphavirus [Humans: encephalitis]<br>Flavivirus [Humans: yellow fever, dengue]<br>Rubivirus [Humans: rubella]<br>Pestivirus [Pigs: hog cholera] | Infectious bronchitis virus [Humans: upper respiratory infection] | Enterovirus [Humans: polio]<br>Rhinovirus [Humans: common cold]<br>Hepatovirus [Humans: hepatitis A] | Vesicular exanthema of swine<br>Norwalk virus<br>Hepatitis E virus | Oncornavirus C [Birds, mice: sarcomas, leukemias]<br>Human T-cell leukemia virus<br>Human immunodeficiency virus |



# Taxonomy of RNA Animal Viruses

| | | | | | |
|---|---|---|---|---|---|
| Strandedness | Single | | | | |
| Single-strand sense | Negative | | | | |
| Enveloped or naked | Enveloped | Enveloped | Enveloped | Enveloped | Enveloped |
| Capsid symmetry | Helical | Helical | Helical | ? | Helical |
| Size (nm) | 80–120 (enveloped) 9 (capsid diam.) | 70–80 x 130–240 (bullet-shaped) | 90–100 (enveloped) | 50–300 | 125–250 (enveloped) 18 (capsid diam.) |
| Site of capsid assembly | Cytoplasm | Cytoplasm | Cytoplasm | Cytoplasm | Cytoplasm |
| Virus family | *Orthomyxoviridae* | *Rhabdoviridae* | *Bunyaviridae* | *Arenaviridae* | *Paramyxoviridae* |
| Representative genera and groups [Host, disease] | Influenza virus [Humans, swine] | Lyssavirus [Warm-blooded animals: rabies] | California encephalits virus [Humans] | Lassa virus [Humans: hemorrhagic fever] | Paramyxovirus [Humans: colds, respiratory infections, mumps] Pneumovirus [Humans: pneumonia, common cold] Morbillivirus [Humans: measles] |

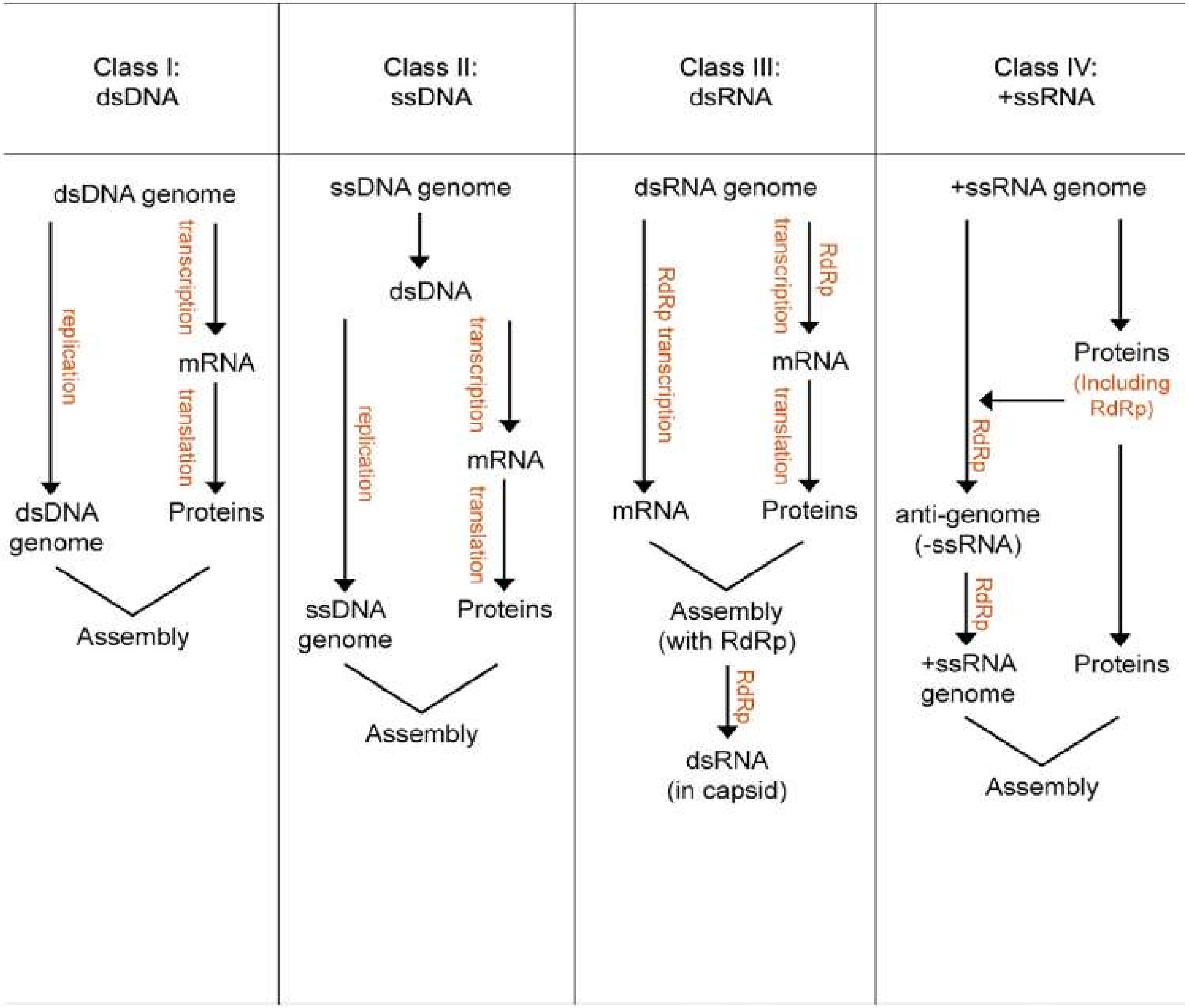


# Replication of animal viruses

- 1.3.2.1. Classification of animal viruses by **genome** type:

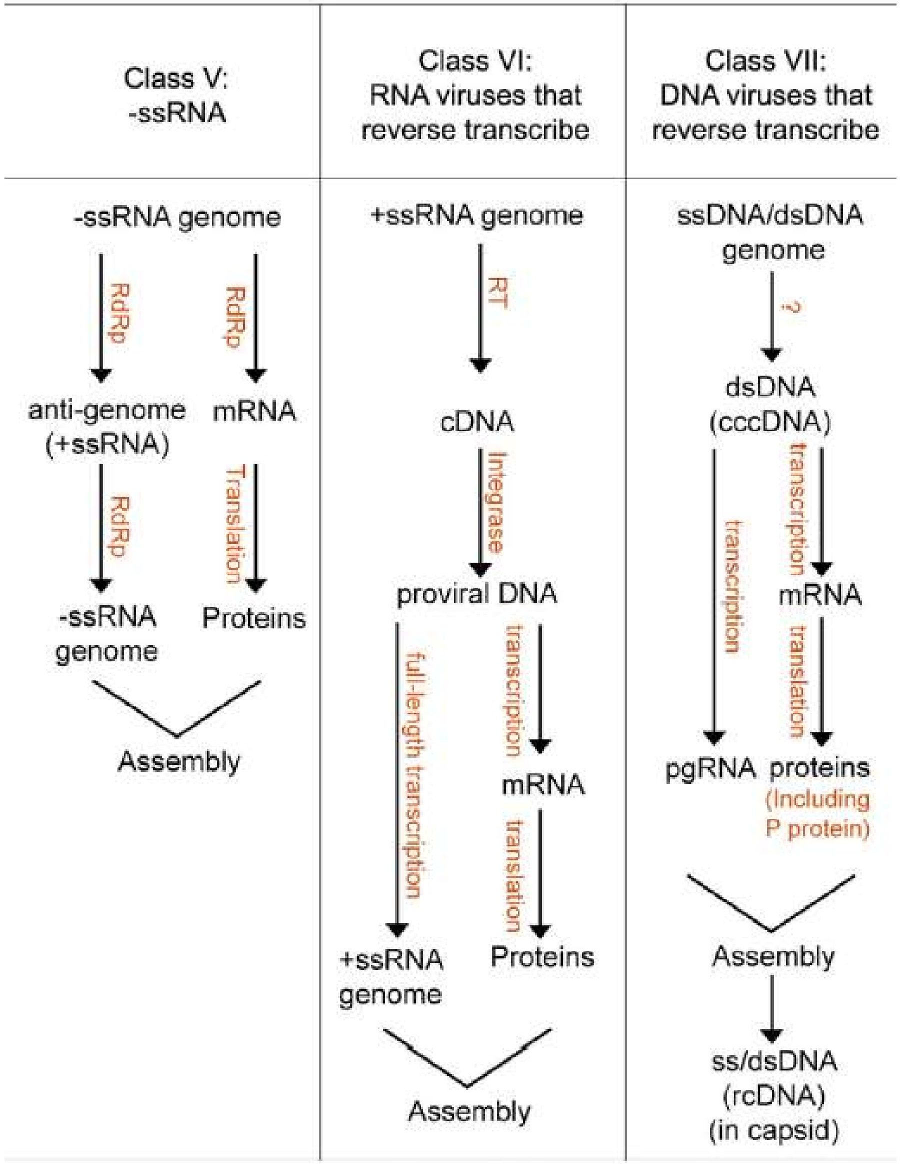
- Class 1.Double-stranded DNA viruses
- Class 2.Single-stranded DNA viruses
- Class 3.Double-stranded RNA viruses
- Class 4.Positive-sense RNA viruses
- Class 5.Negative-sense RNA viruses
- Class 6.RNA viruses that reverse transcribe
- Class 7.DNA viruses that reverse transcribe

# Replication of animal viruses




RdRp : RNA-dependent RNA polymerase

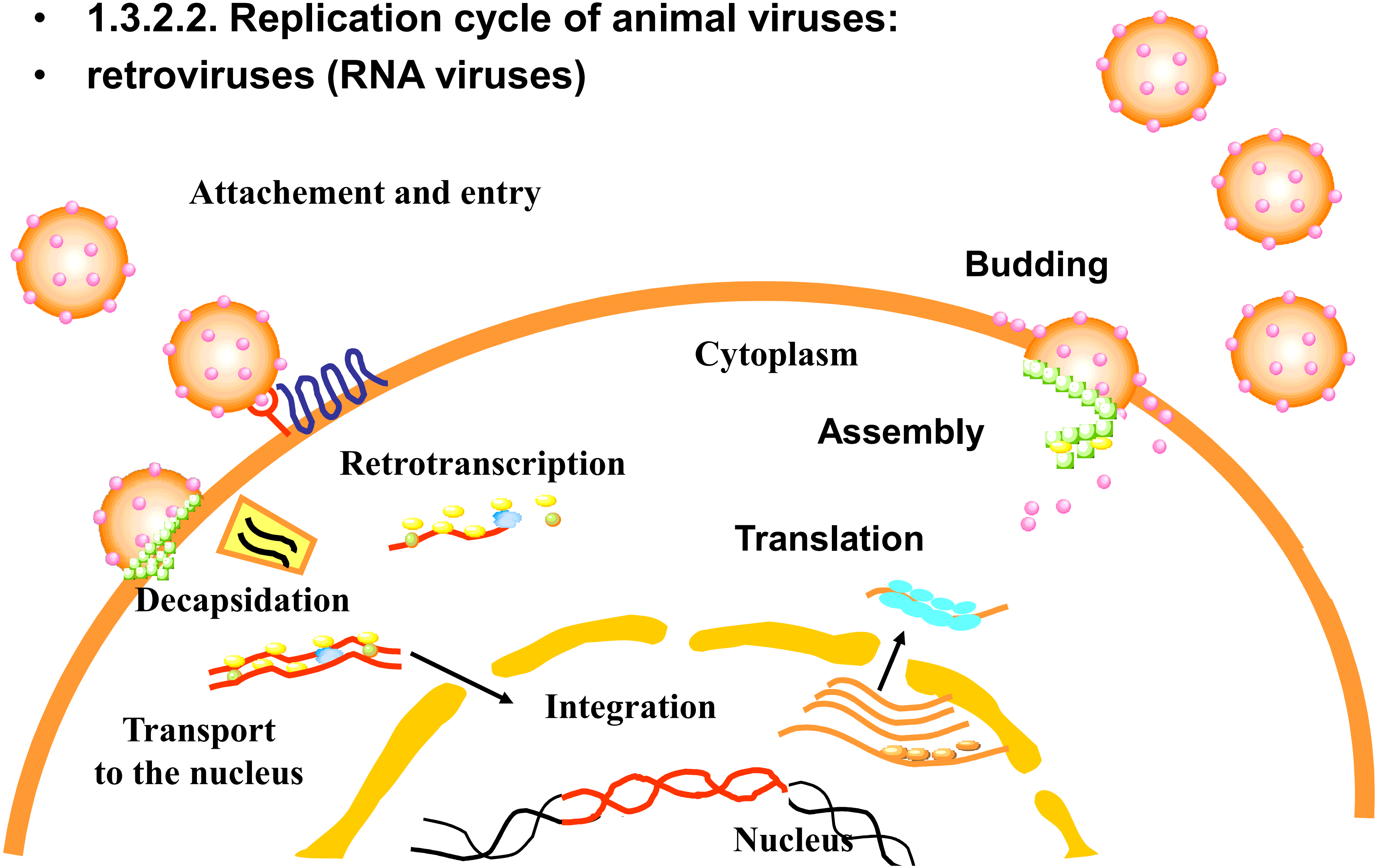
# Replication of animal viruses




**covalently closed circular DNA** (cccDNA)
**relaxed circular DNA** (rcDNA)
pregenomic RNA (pgRNA)

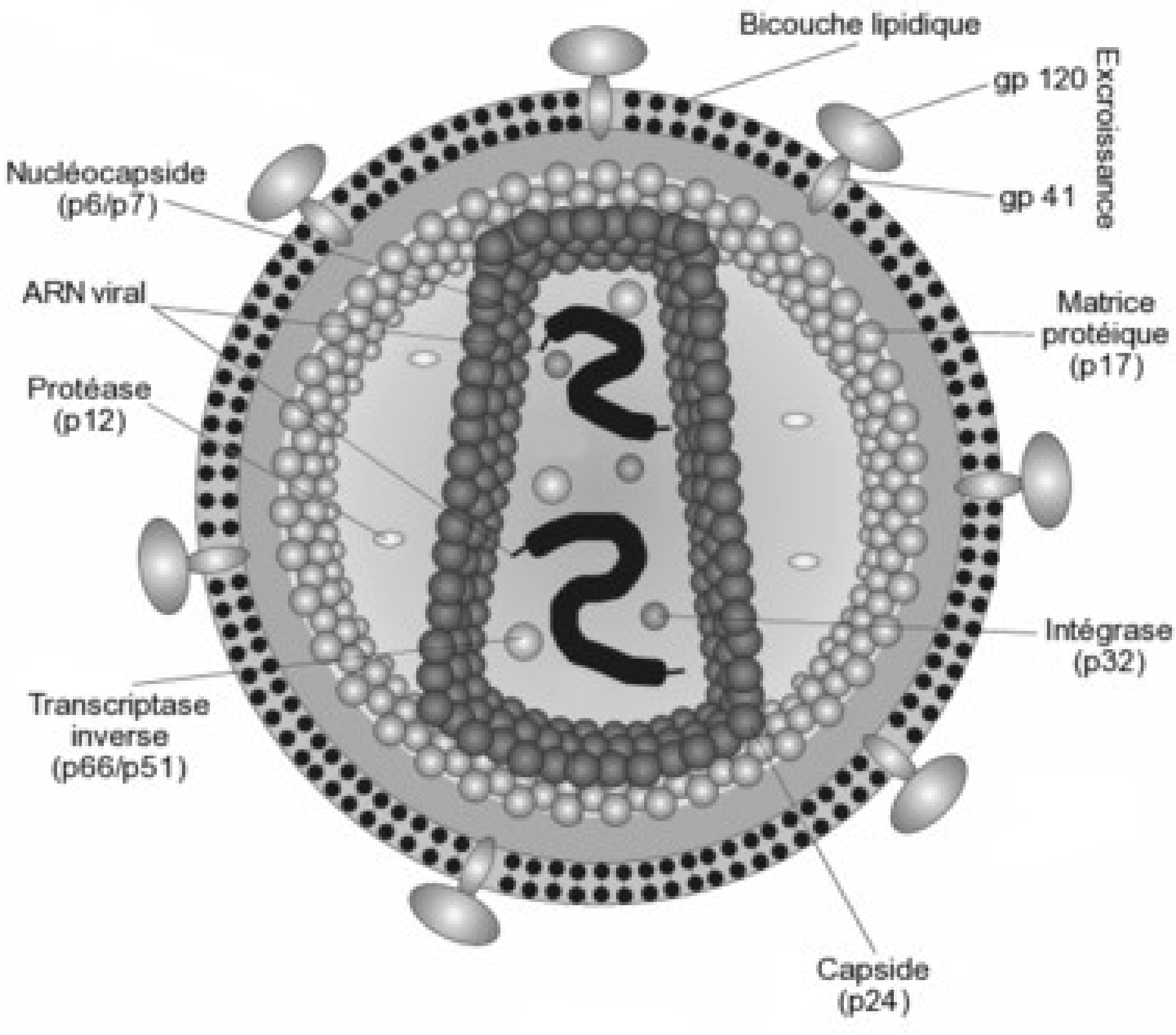
# Replication of animal viruses

- 1.3.2.2. Replication cycle of animal viruses:
- retroviruses (RNA viruses)



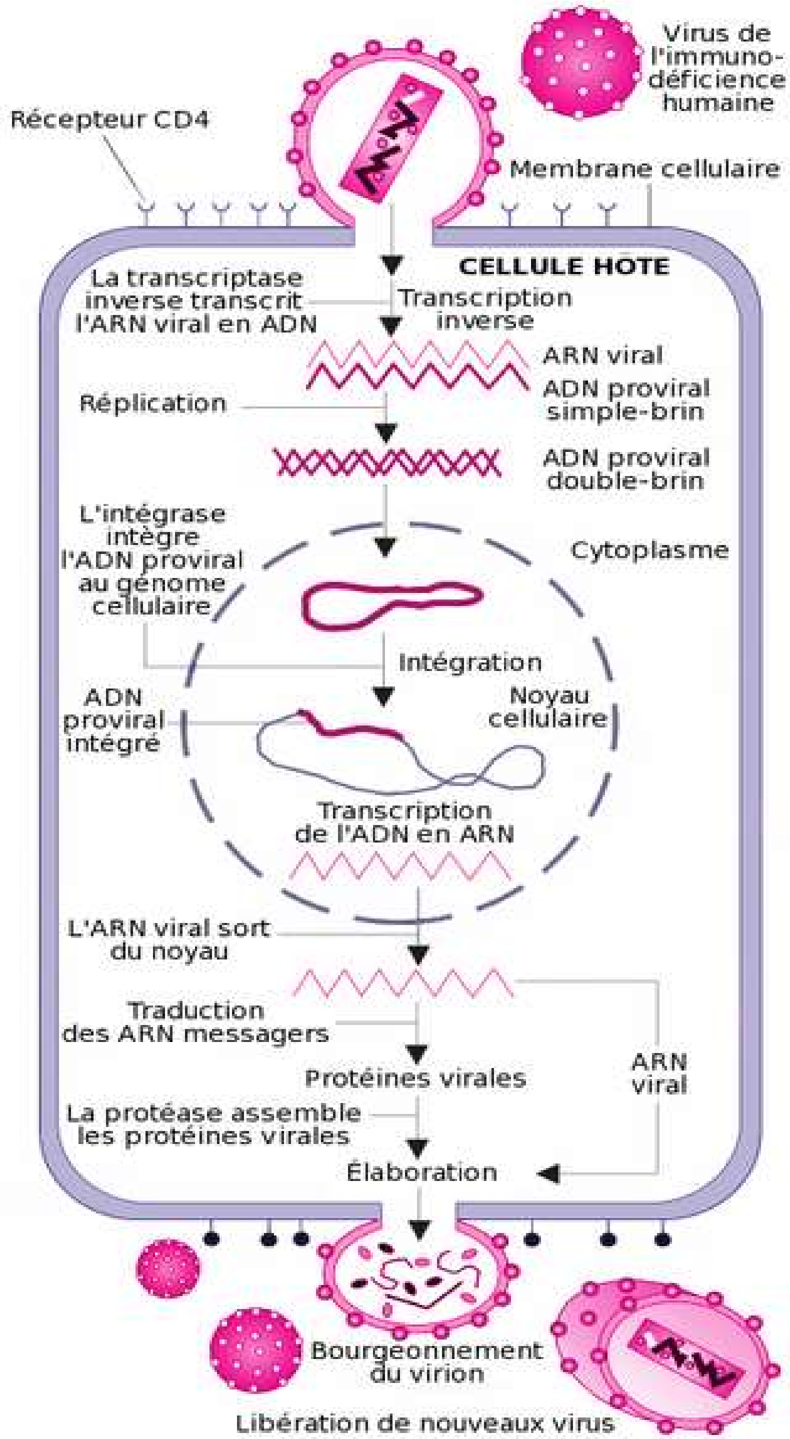
**Replication of RNA viruses** :

Exp: **HIV**



# Replication of RNA viruses :

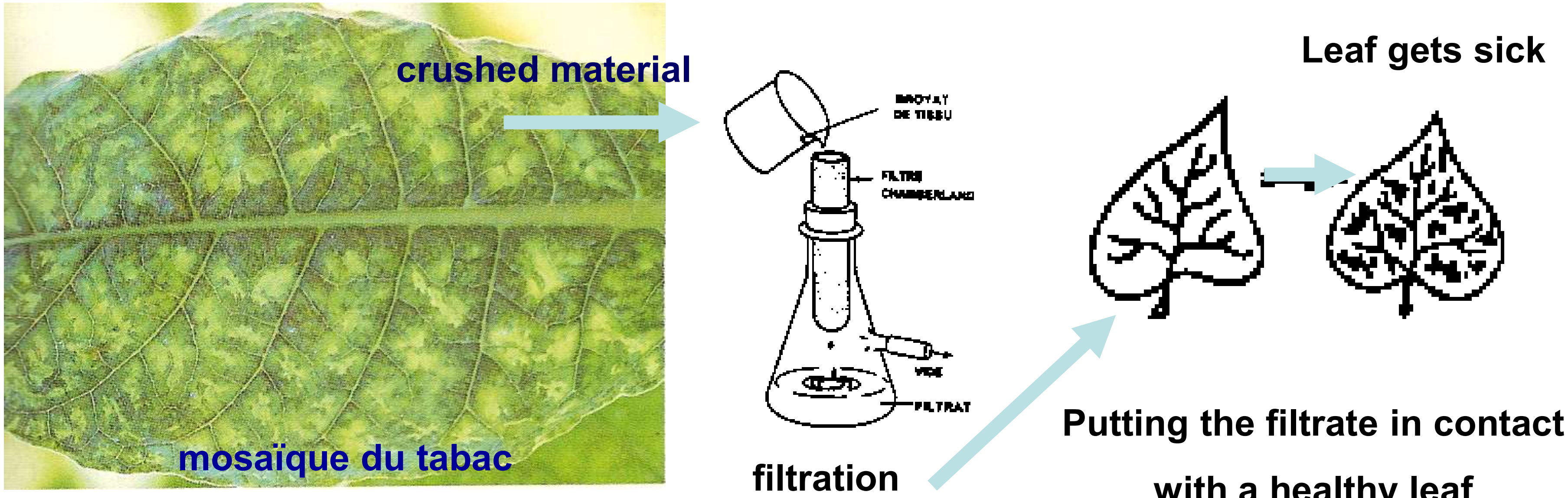
Exp: **HIV**



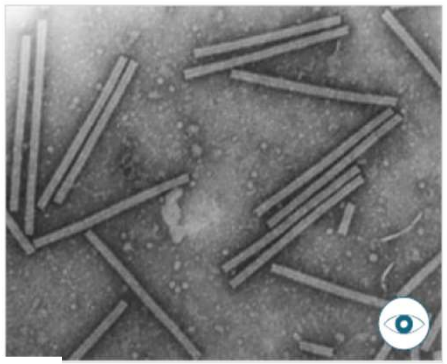
# I. Viral genetics

- **1.3. Plant viruses:**

# Viruses

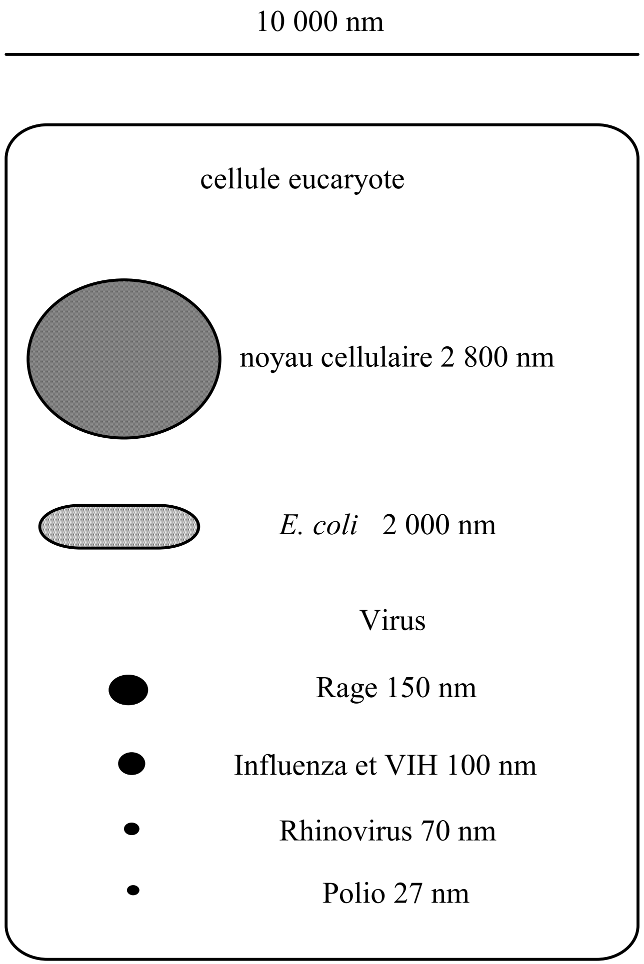


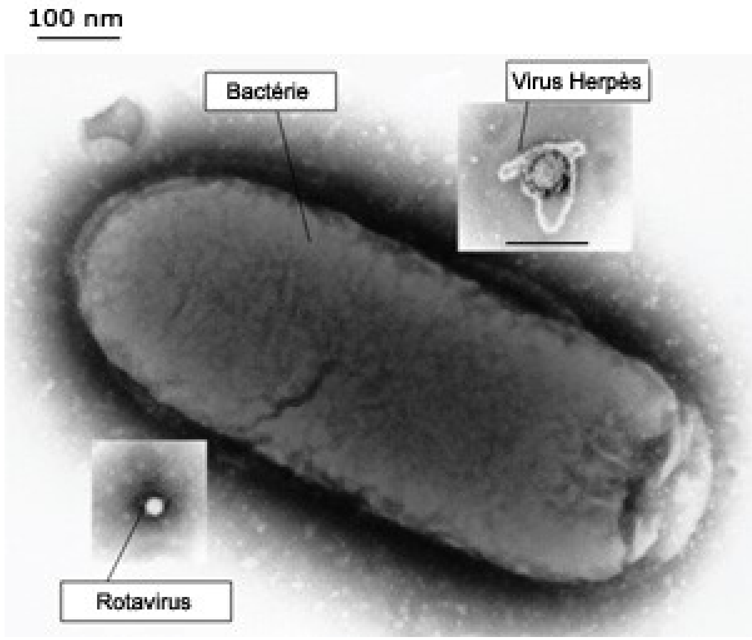

Tobacco mosaic is caused by an unknown agent that passes through bacterial filters.
The agent is therefore smaller than 1 µm and is different from a bacterium



# II. Bacterial genetics

# II. Bacterial genetics



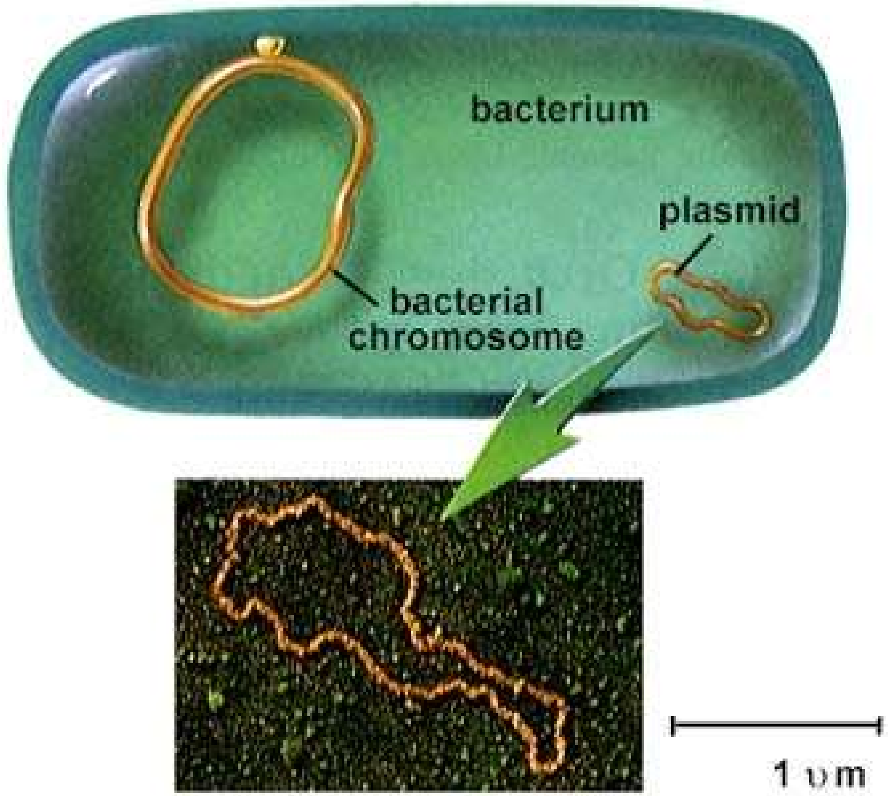




Prokaryotes: absence of nuclear membrane

**Note: 1 nm = $10^{-9}$ m**

# II. Bacterial genetics

1. Bacterial Genome : **chromosome and plasmids**



# II. Bacterial genetics

## 1. Bacterial Genome : **chromosome**

- **1. Length:** $10^5$ to $10^7$ base pairs. The genome length of *E. coli* = 1.6 mm.
- **2. Haploid genome**: only 1 copy of each chromosome per bacterium
- 
- **3. Structural organization:** The bacterial chromosome is often circular and present in the **nucleoid**
- **4. Information in the genome:**
  - Contain much fewer genes than eukaryotic genomes
  - Almost the entire bacterial genome is coding!

# II. Bacterial genetics

1. Bacterial Genome : **chromosome**

- In *E.Coli* , the genome is composed almost exclusively of genes
  - 4.6 Megabases / 3000 Genes

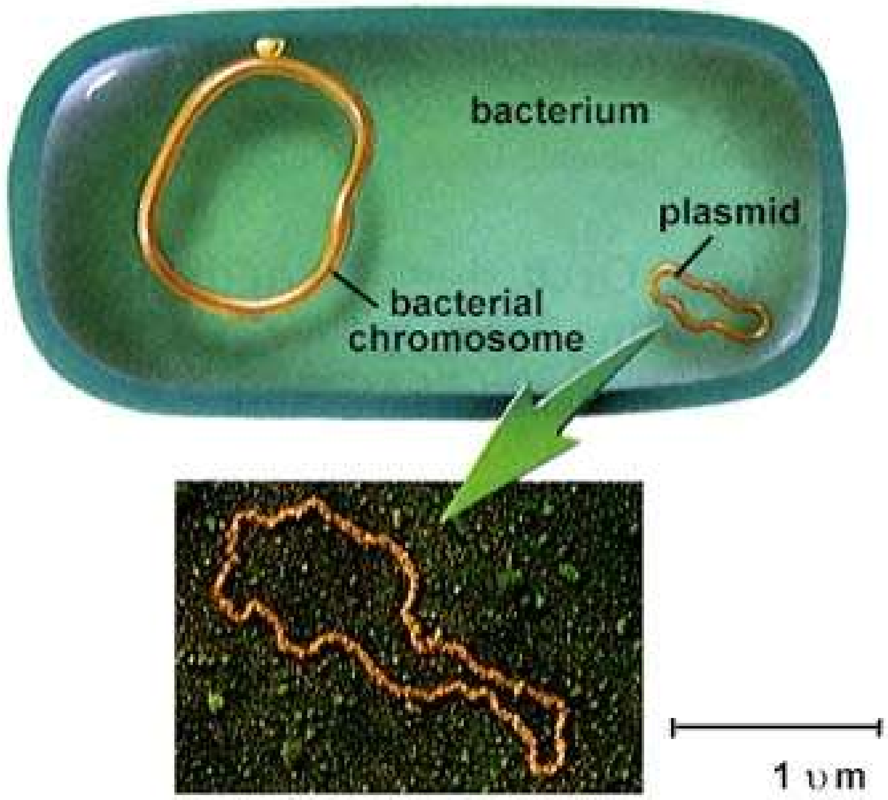
- In humans
  - 3200 Megabases / 25000 Genes

- Almost the entire bacterial genome is coding!

# II. Bacterial genetics

## 1. Bacterial Genome : **plasmids**

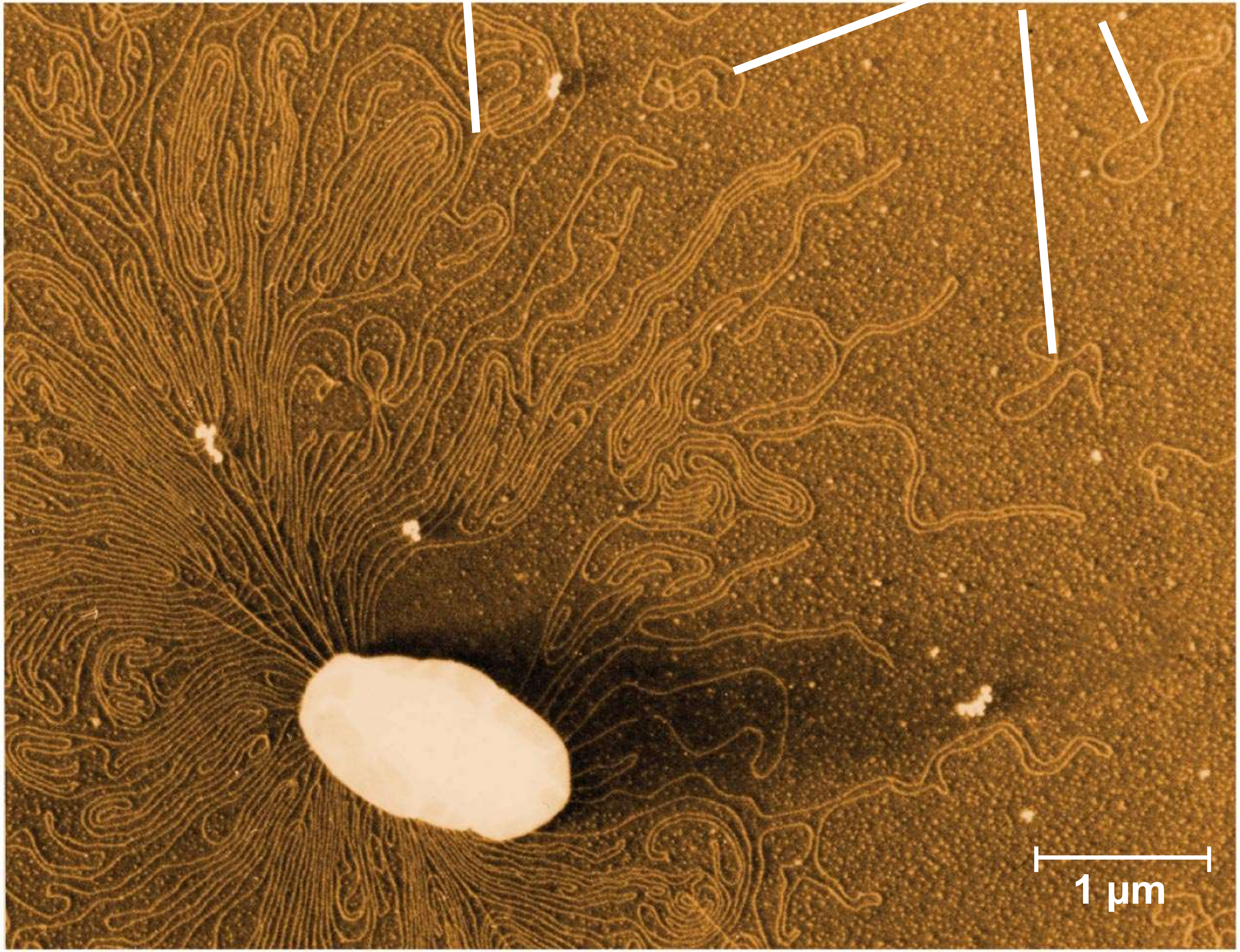
- **Double-stranded DNA fragments**
- **Circular**
- **Intracytoplasmic**
- **Self-replicating**
- **Carry “survival” genes**
  - **Adaptation to the environment**
  - **Antibiotic resistance +++**



**Haploid Circulaire Chromosome**

**Plasmids**

**anneaux d'ADN plus petits**



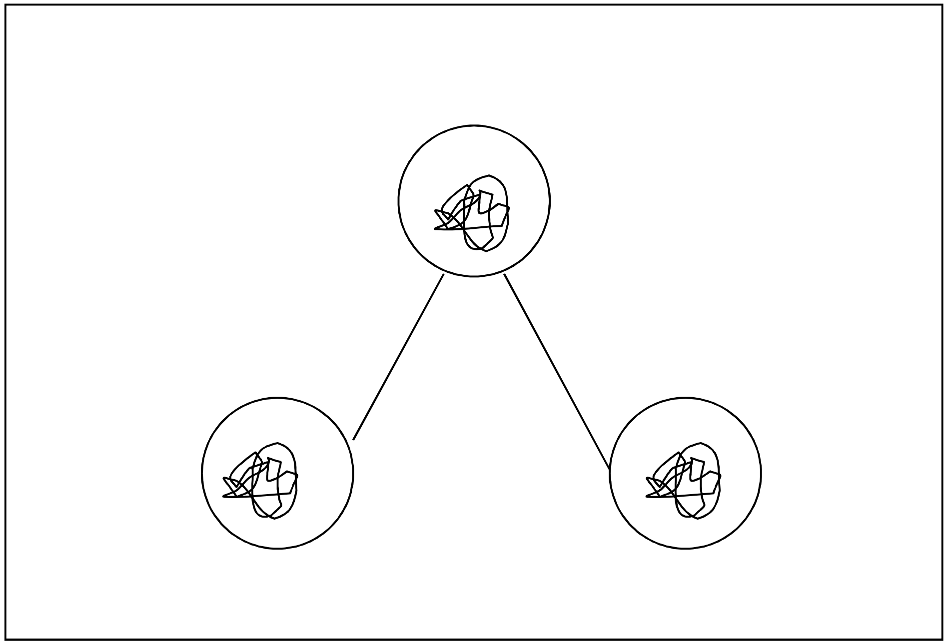
# II. Bacterial genetics

## 2. Replication of bacteries:

Division by **scissiparity** (bianary fission or fissiparity)

-replication of DNA from a single origin and continues along the chromosome

-no mitosis



≈ 20 min

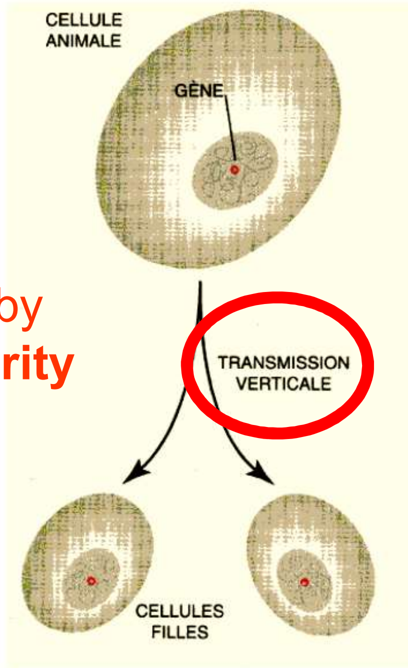
(17 min-33 h)

# II. Bacterial genetics

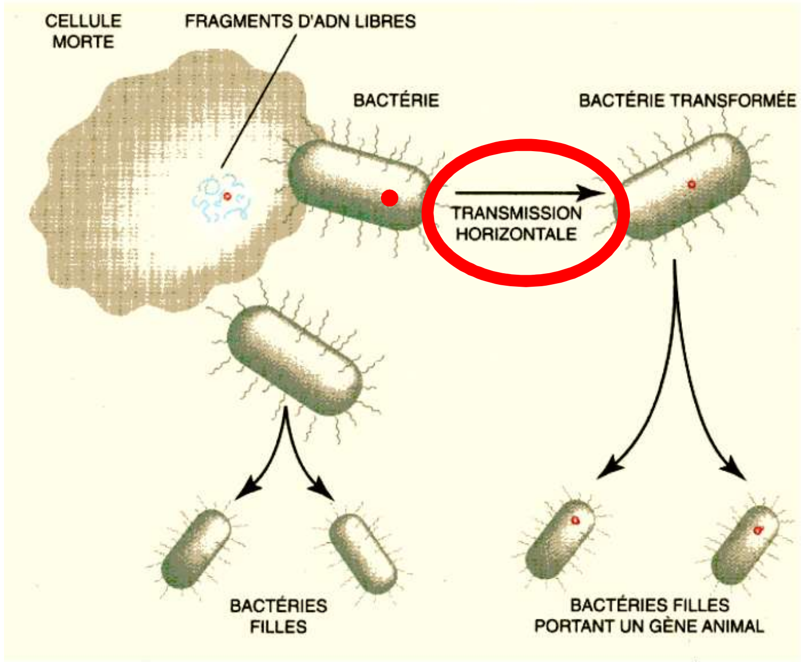
## 3. Genetic recombination and gene transfer in bacteria:

In nature, there are two ways for microorganisms to transfer genetic heritage:

Division by **scissiparity**



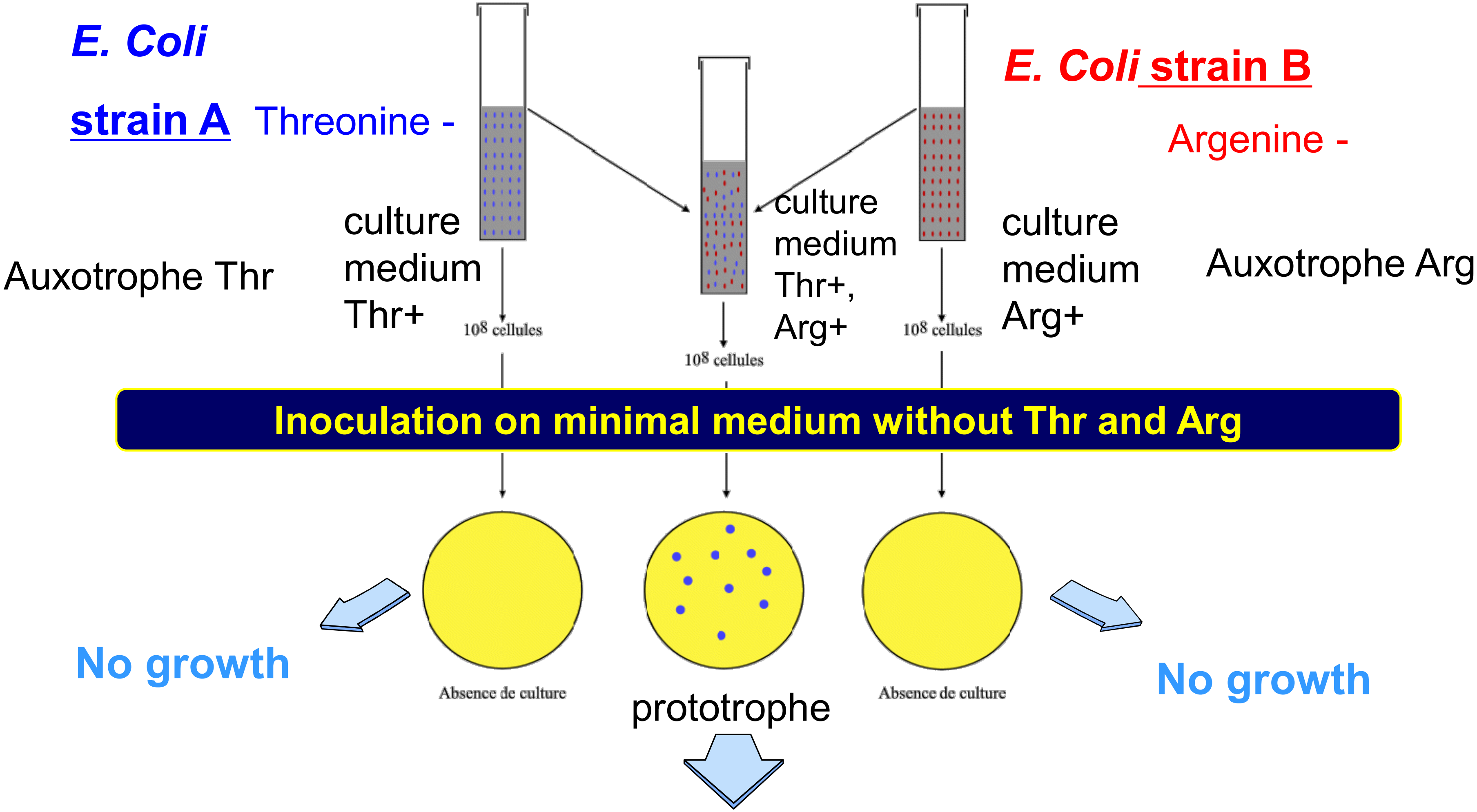

**Vertical gene transfer**




**Horizontal gene transfer**

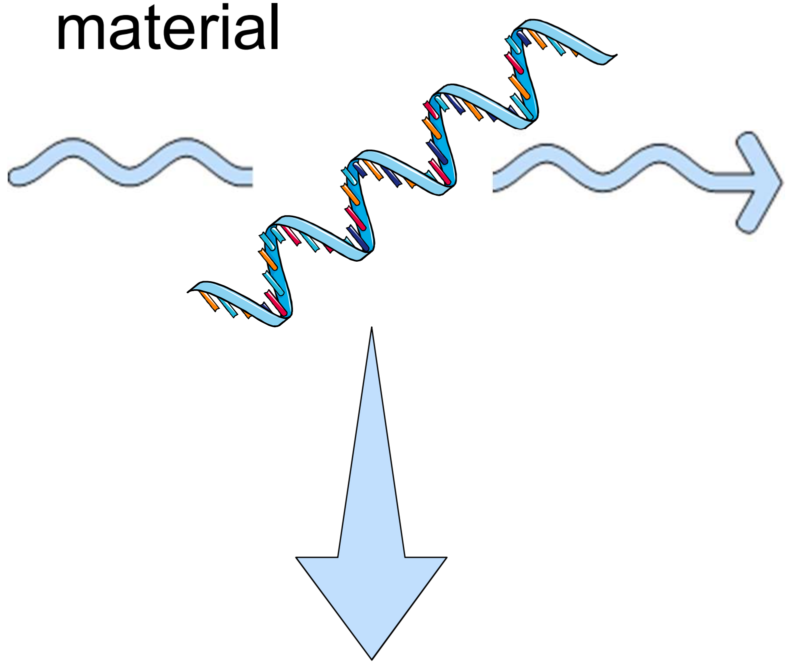
- **the "vertical" transfer of genes that occurs between a "parent" and its offspring.**
- **the "horizontal" transfer of genes that occurs between two distinct organisms.**

# 3. Genetic recombination and gene transfer in bacteria:




**Growth not due to spontaneous mutation**
**There was a transfer of genetic material between the two strains and recombination between the parental genes**

- **Gene transfer :**




There are: 3 major mechanisms

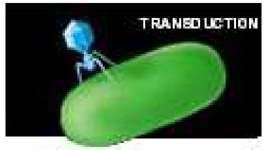
of DNA exchange between donor bacteria and recipient bacteria

# II. Bacterial genetics

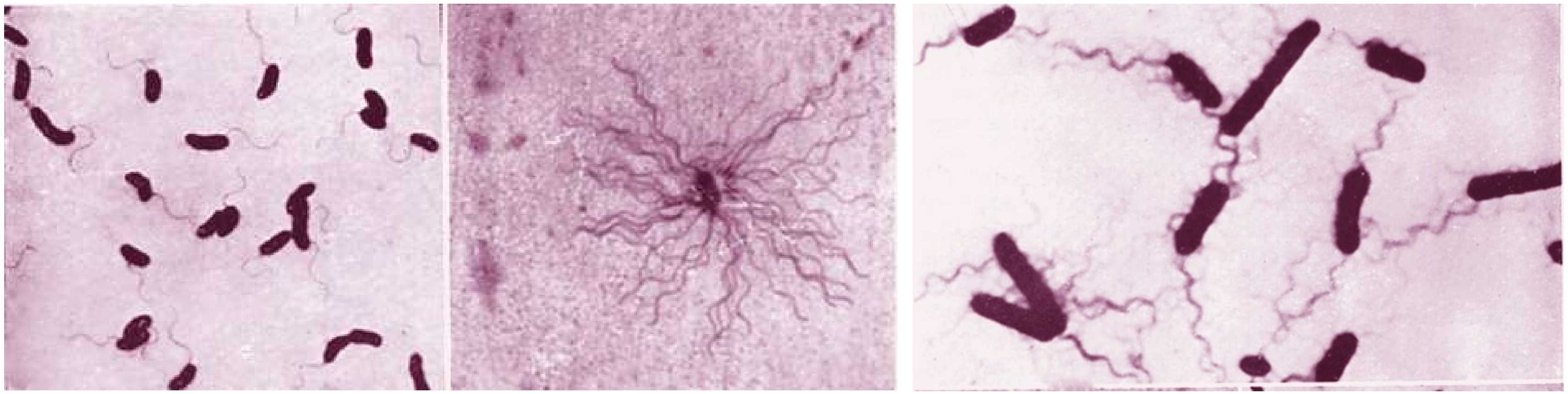
3. Genetic recombination and gene transfer in bacteria :

**1. TRANSFORMATION**


**2. TRANSDUCTION**




**3. L CONJUGAISON**

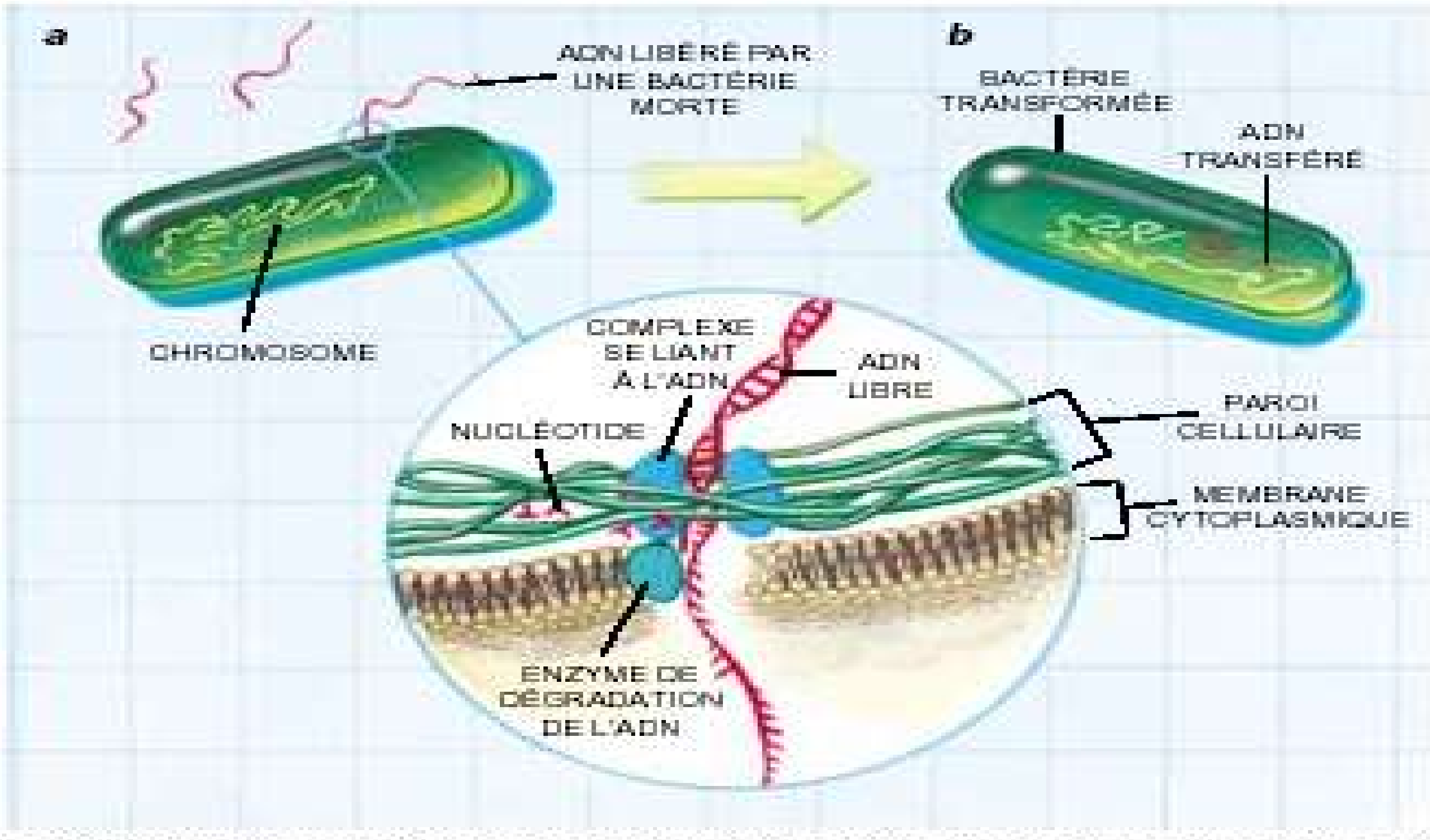
# II. Bacterial genetics

3. Genetic recombination and gene transfer in bacteria:

## 1. TRANSFORMATION

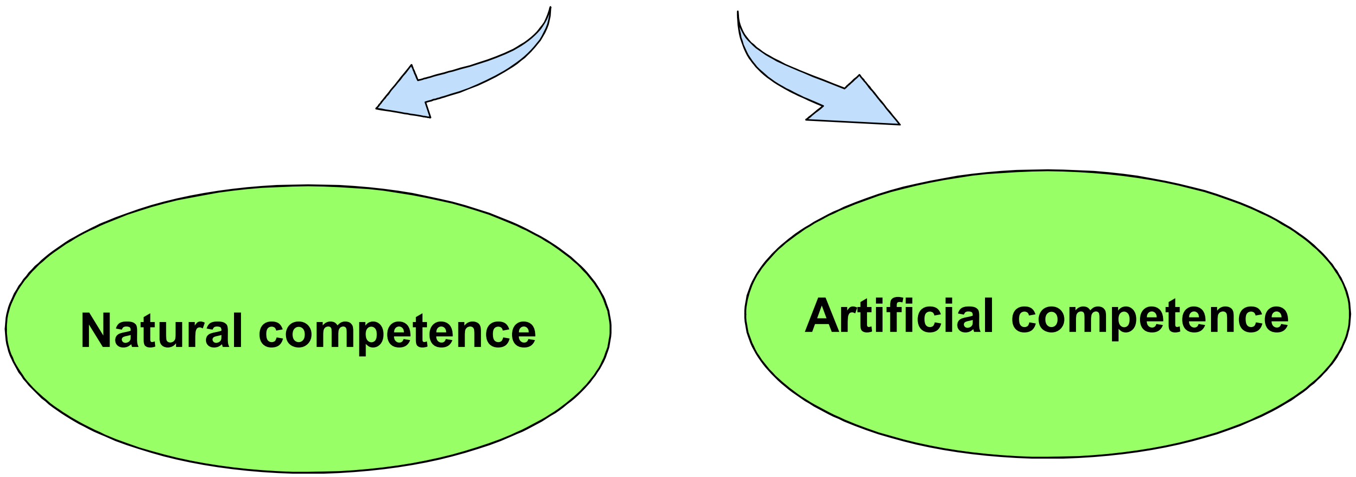

# 1. TRANSFORMATION


Genetic transfer in which a free, naked, double-stranded DNA fragment is taken up by a competent receptor bacterium before being eventually integrated into the chromosome



# 1. TRANSFORMATION

DNA can only enter if cells are competent.



# 1. <u>TRANSFORMATION</u>

## Natural competence

**Naturally competent bacteria**

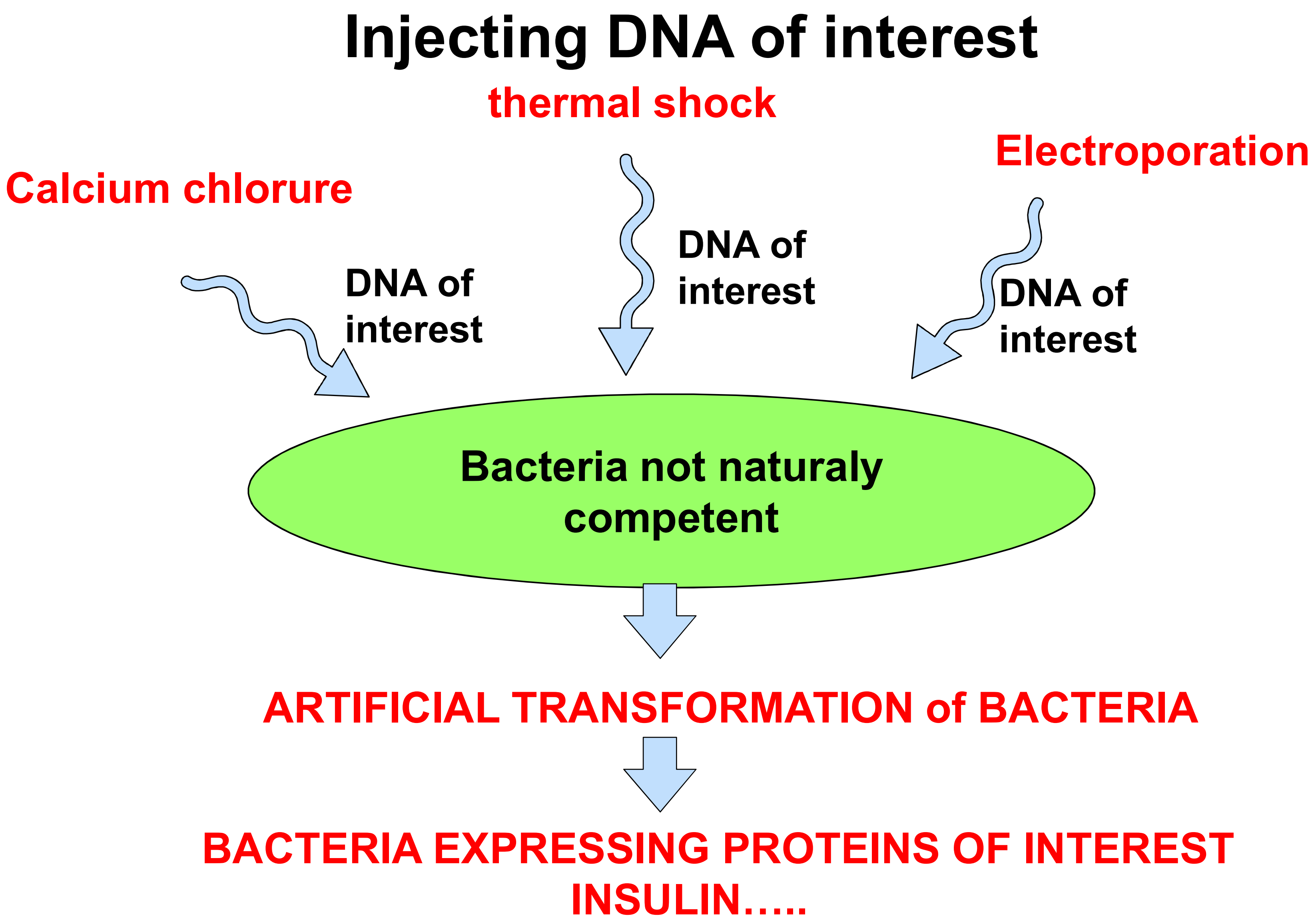
*Bacillus subtilis*, *streptococcus* spp, *Haemophilus influenzae*, *Neisseria* spp

They have the ability to capture free DNA present in the environment

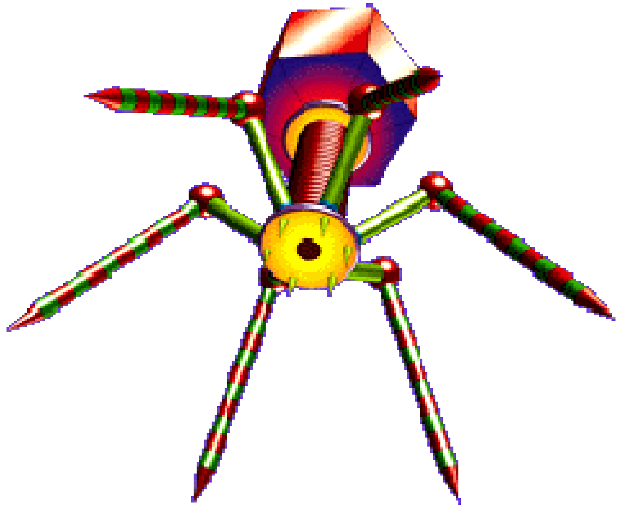
⇨ **<u>The general conditions of competence are as follows:</u>**

- presence on the surface of proteins specialized in the absorption of DNA from the surrounding solution
- These proteins only recognize and carry out the transfer of DNA from closely matched bacterial species

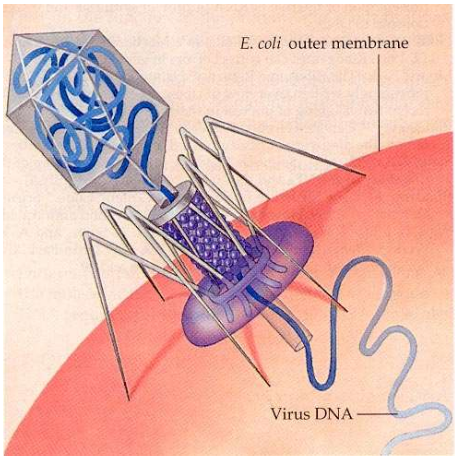
# Artificial competence

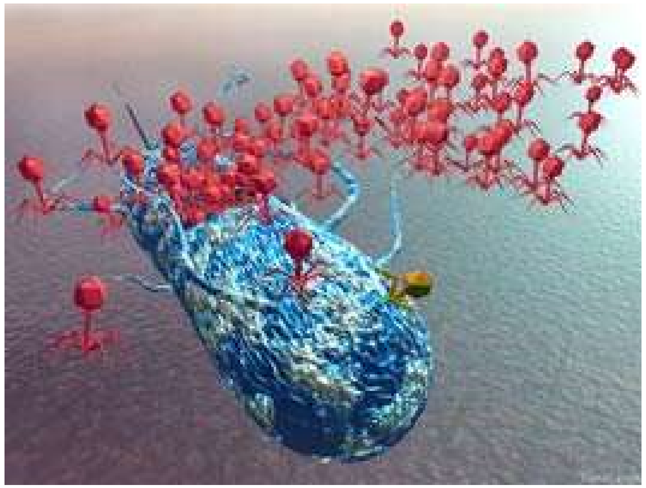


# 2. TRANSDUCTION



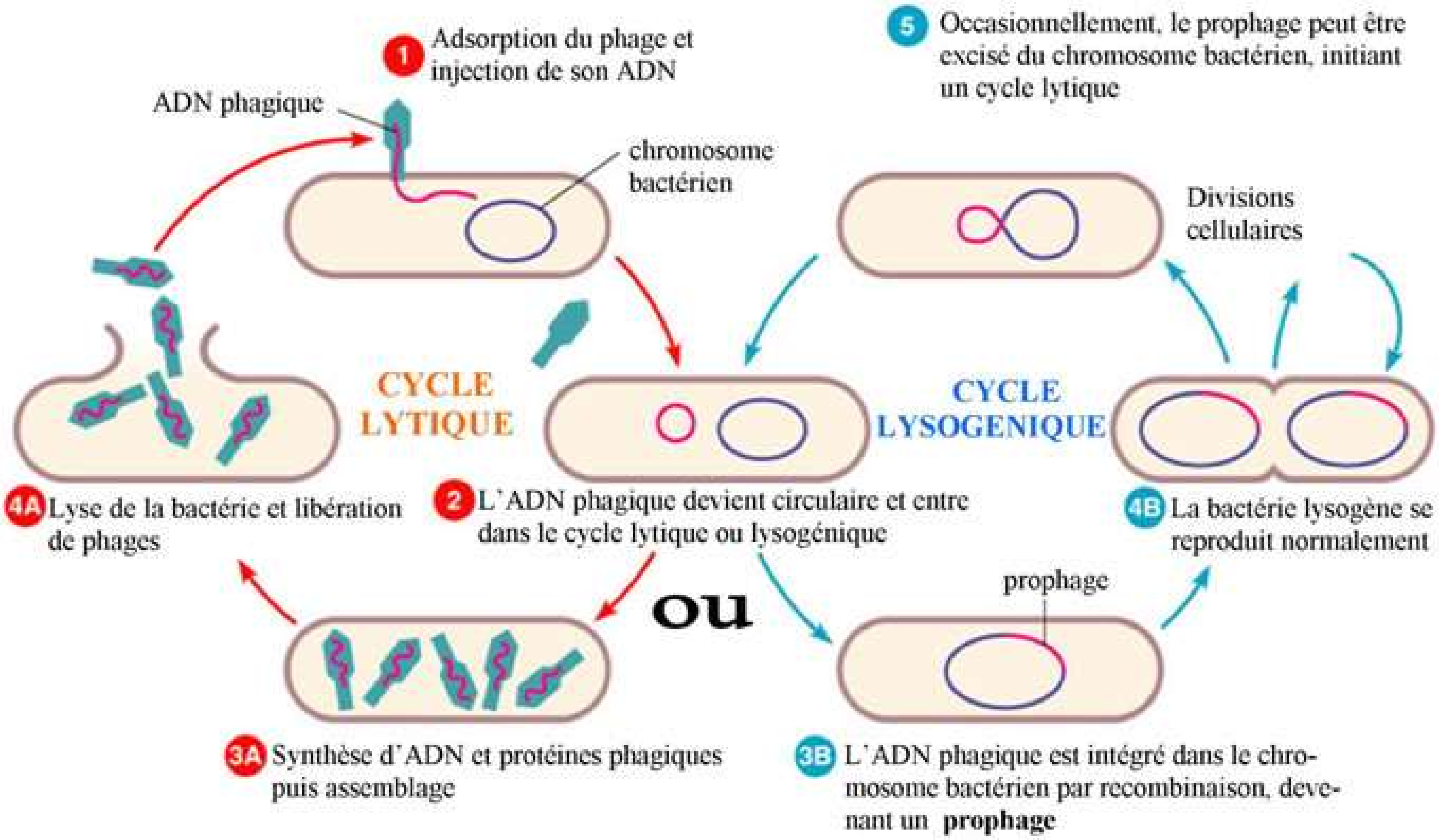
Genetic transfer of a DNA fragment from one bacterium to another carried out by bacteriophages called Vectors



# Reminder

– Lytic and Lysogenic cycle : exp. **ƛ phage**



# 2. TRANSDUCTION

- Transduction results from an encapsidation error:

- During virion assembly, a fragment of bacterial genome is encapsidated in place of viral DNA.

- The phage becomes defective (transducer), it is released during lysis of the bacteria and can inject bacterial DNA into another bacteria.

-According to bacteriophages, transduction is a phenomenon :

**generalized**

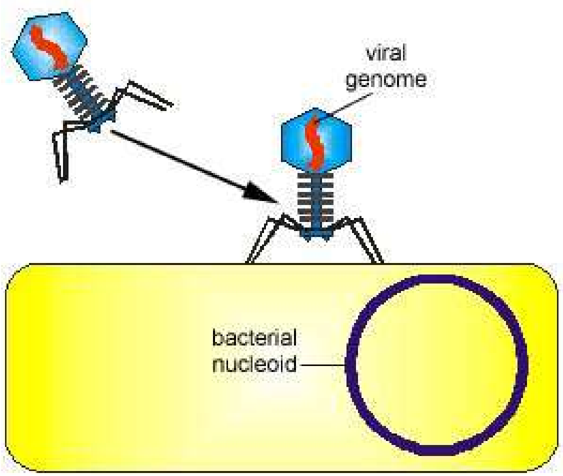
any bacterial gene is capable of being transferred to a recipient bacterium

**localized (specialized or restricted)**

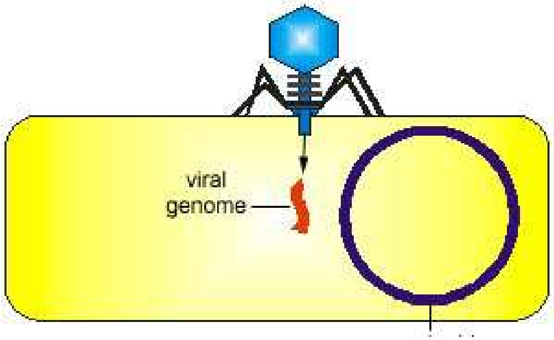
the transfer only concerns a few bacterial genes, depending on the bacteriophage entry site

# 2.1. Generalized transduction

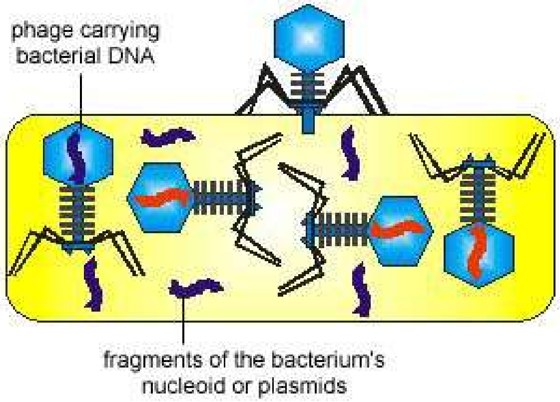
# Seven Steps in Generalized Transduction




**1.** A lytic bacteriophage adsorbs to a sensitive bacterium.

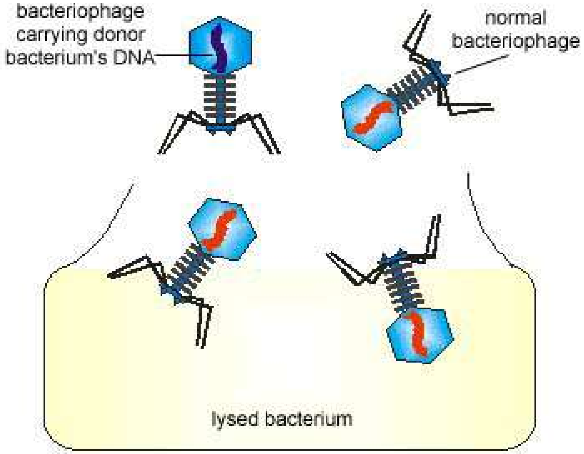



2. The bacteriophage genome enters the bacterium. The genome directs the bacterium's metabolic machinery to make bacteriophage components and enzymes

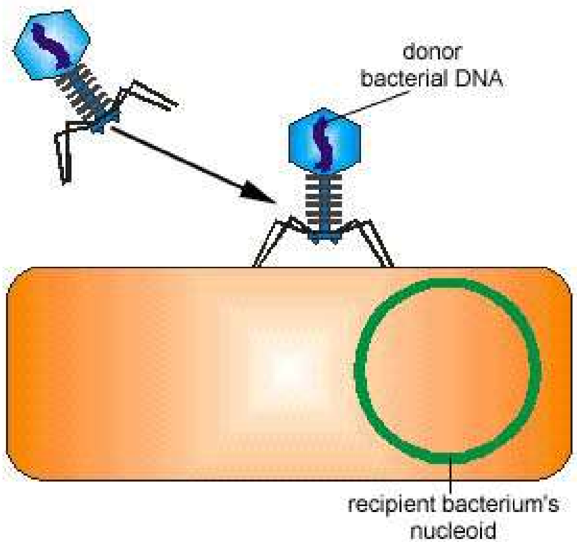



**3.** Occasionally, a bacteriophage capsid mistakenly assembles a fragment of this donor bacterium instead of a phage genome.

# Seven Steps in Generalized Transduction

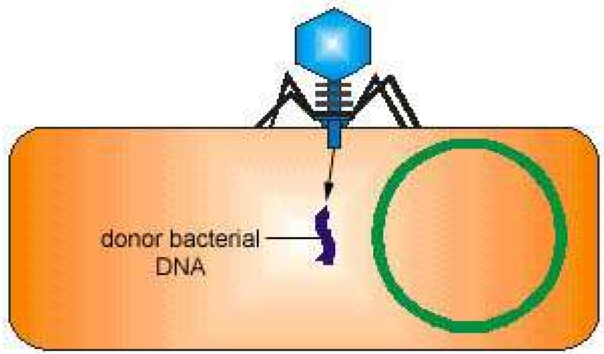



4. Bacteriophages are released.

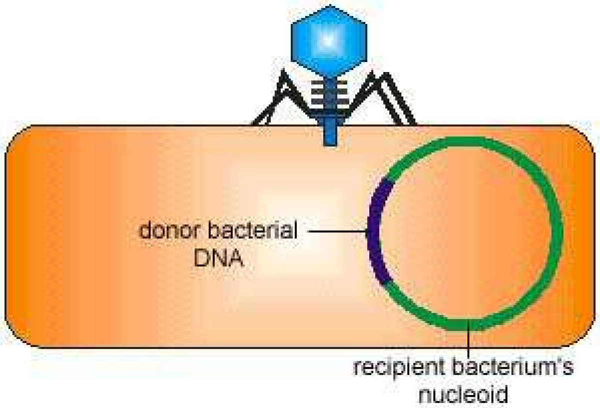



5The bacteriophage carrying the DNA of the donor bacteria adsorbs to a recipient bacteria.

# Seven Steps in Generalized Transduction



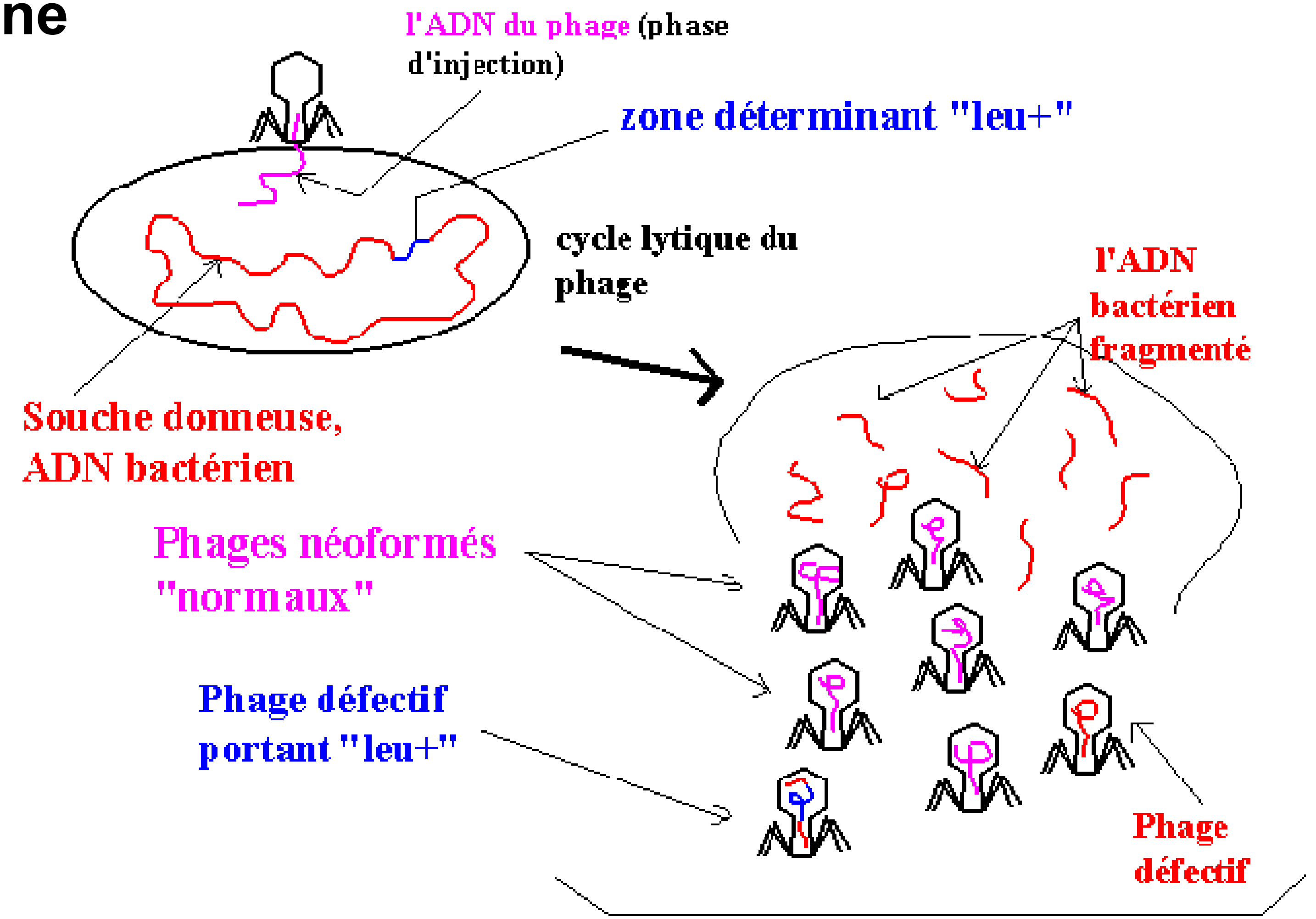

6. The bacteriophage inserts the DNA of the donor bacteria that it carries into the recipient bacteria..




7. The DNA of the donor bacteria is exchanged for part of the recipient's DNA by **crossing-over** (homologous recombination)

# 2.1. Generalized transduction

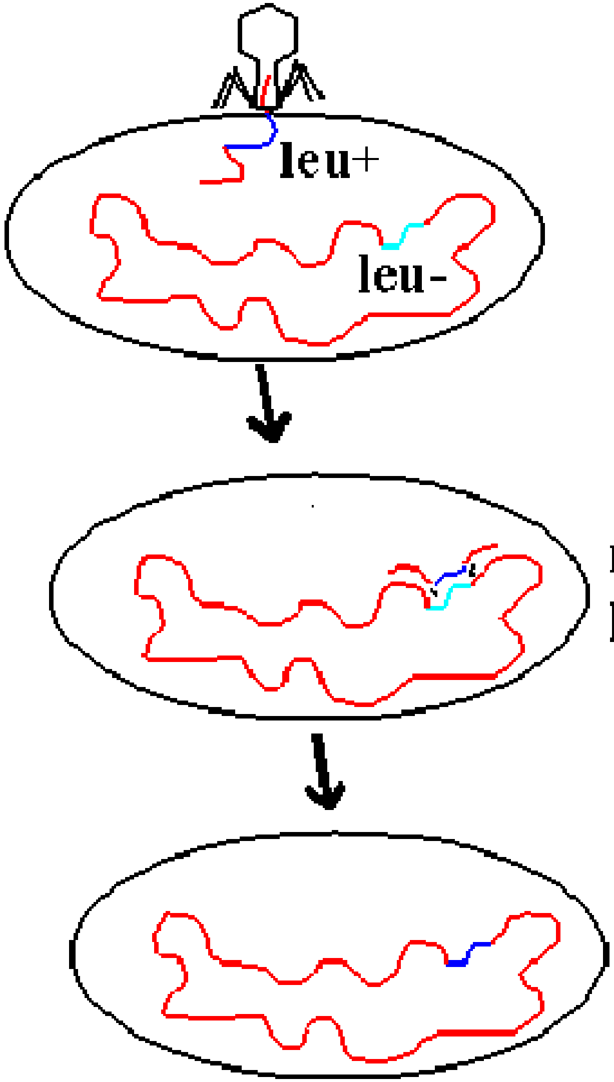
Exp.: **Leu Gene**




**Le lysat phagique est formé d'une très grande majorité de phages "normaux" et d'une petite minorité de phages défectifs potentiellement transductants.**

# 2.1. Generalized transduction

Exp.: **Leu Gene**

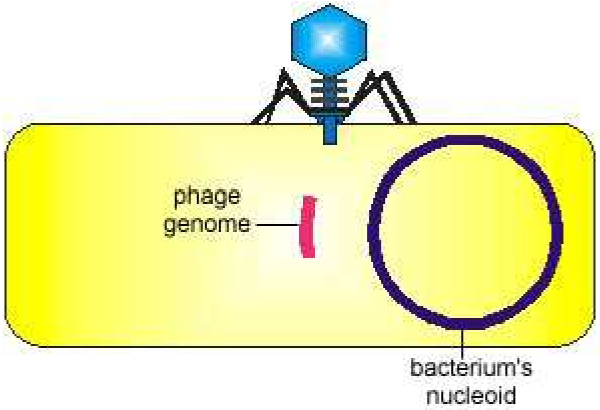



**recombinaison homologue**

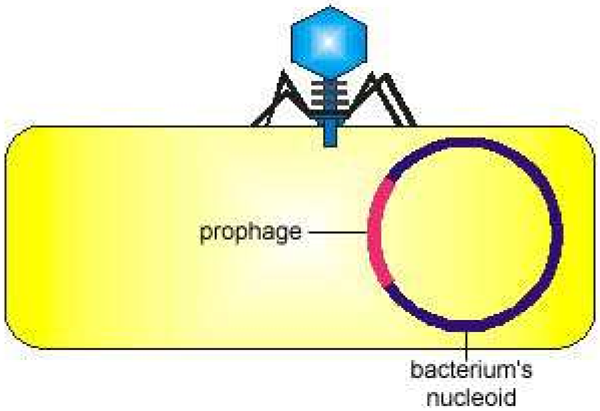
**cellule bactérienne transduite, devenue leu+. Elle cultive sur milieu sans leucine avec une descendance leu+.**

## 2.1. Localized transduction

# Six steps in localized transduction

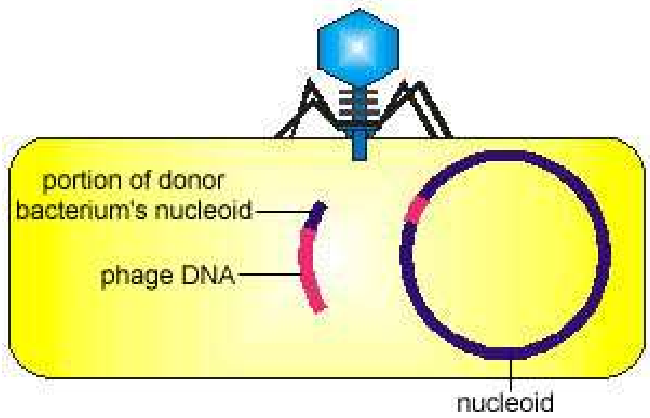



**1.** A temperate bacteriophage absorbs a susceptible bacterium and injects its genome.

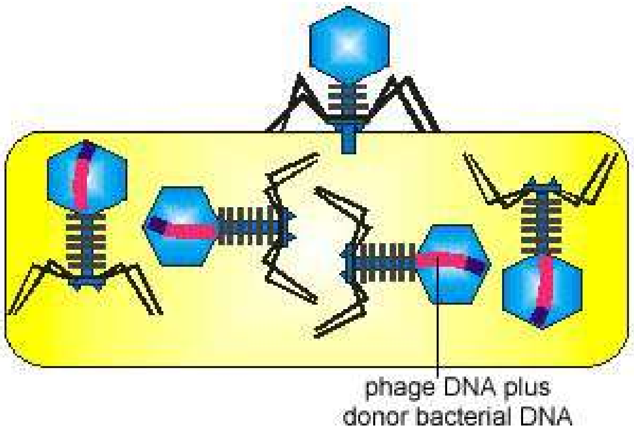



**2.** The bacteriophage inserts its genome into the genome of the bacteria and becomes a prophage.

# Six steps in localized transduction

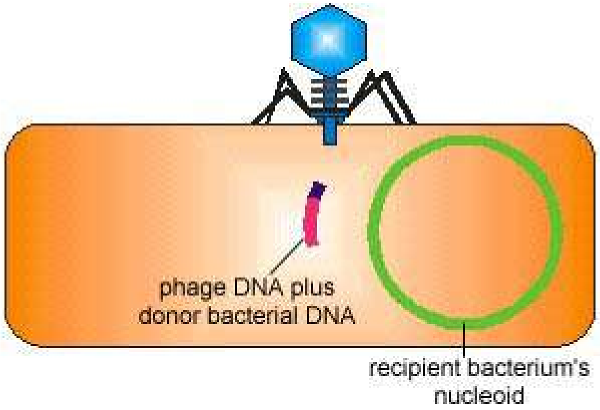



3. Occasionally during spontaneous induction, a small piece of the donor bacteria's DNA is taken as part of the phage genome in place of some of the phage DNA that remains in the bacteria's genome.

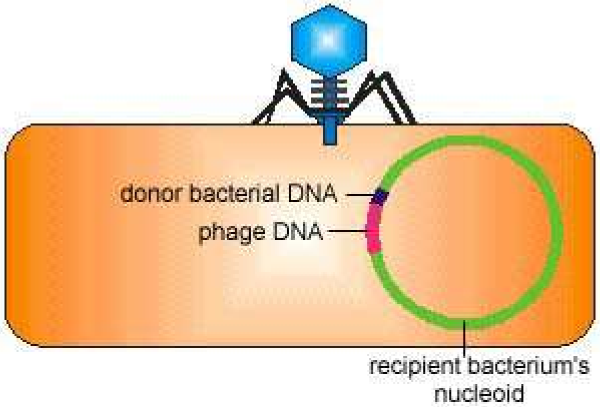



4. As the bacteriophage replicates, the segment of bacterial DNA replicates as part of the phage genome. Each phage now carries this segment of bacterial DNA.

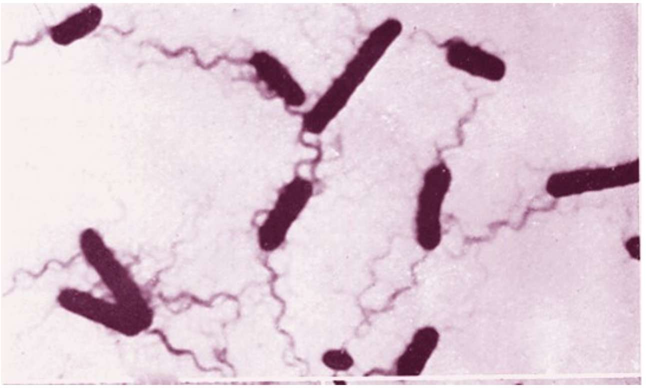
## Six steps in localized transduction



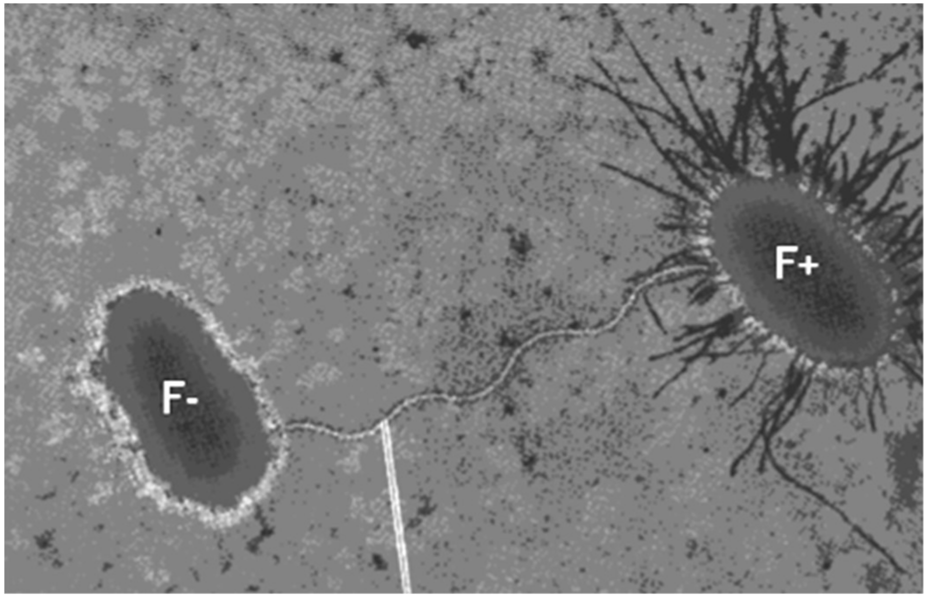

5. The bacteriophage absorbs a recipient bacterium and injects its genome.



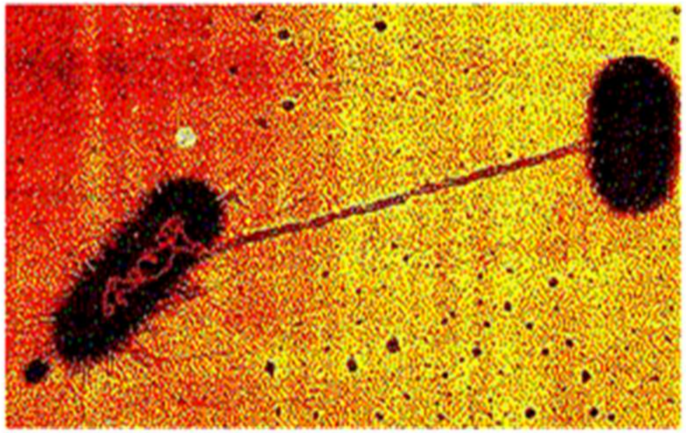

6. The genome of the bacteriophage that has integrated into the genome of the recipient bacterium carries a segment of DNA from the donor bacterium



# 3. CONJUGAISON

# 3. LA CONJUGAISON

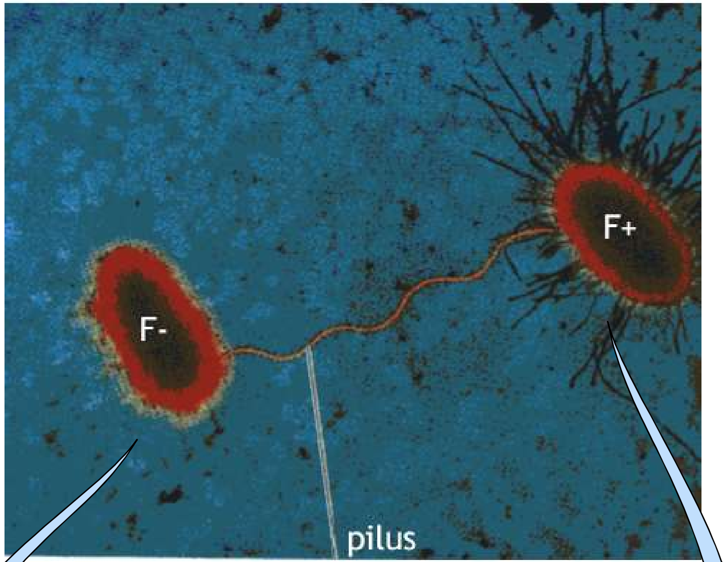
-Unidirectional genetic transfer of a fragment of chromosomal or plasmid DNA by direct contact between two bacteria

- Bacterial equivalent of sexual reproduction

- A donor = male and a recipient = female

# 3. CONJUGAISON

gene receptor = female bacteria




gene donor = male bacteria

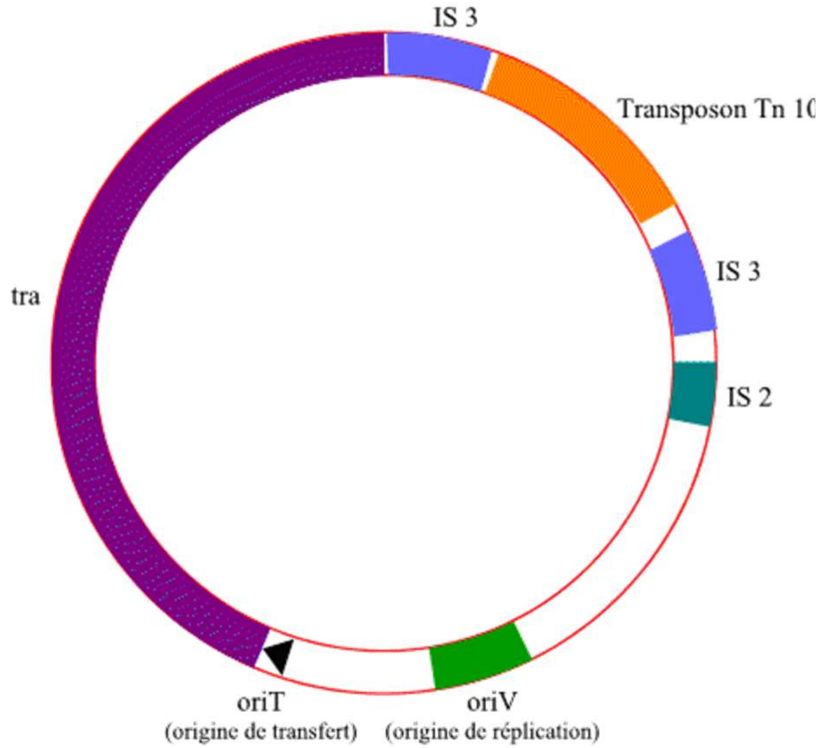
gene-receiving bacteria = F- because they lack the F factor

gene-donating bacteria = F+ have an F factor or fertility factor

# F factor

The F factor = large conjugative plasmid (94,500 bp) which controls:

-its own replication,
-its number of copies,
-the distribution of copies in the daughter cells
-its transfer




**<u>Composition:</u>**

**- 13 genes encode the synthesis of sexual pili = mooring cables**

**-2 genes encode surface exclusion proteins that prevent the attachment of sexual pili and therefore the pairing of two F+ bacteria.**

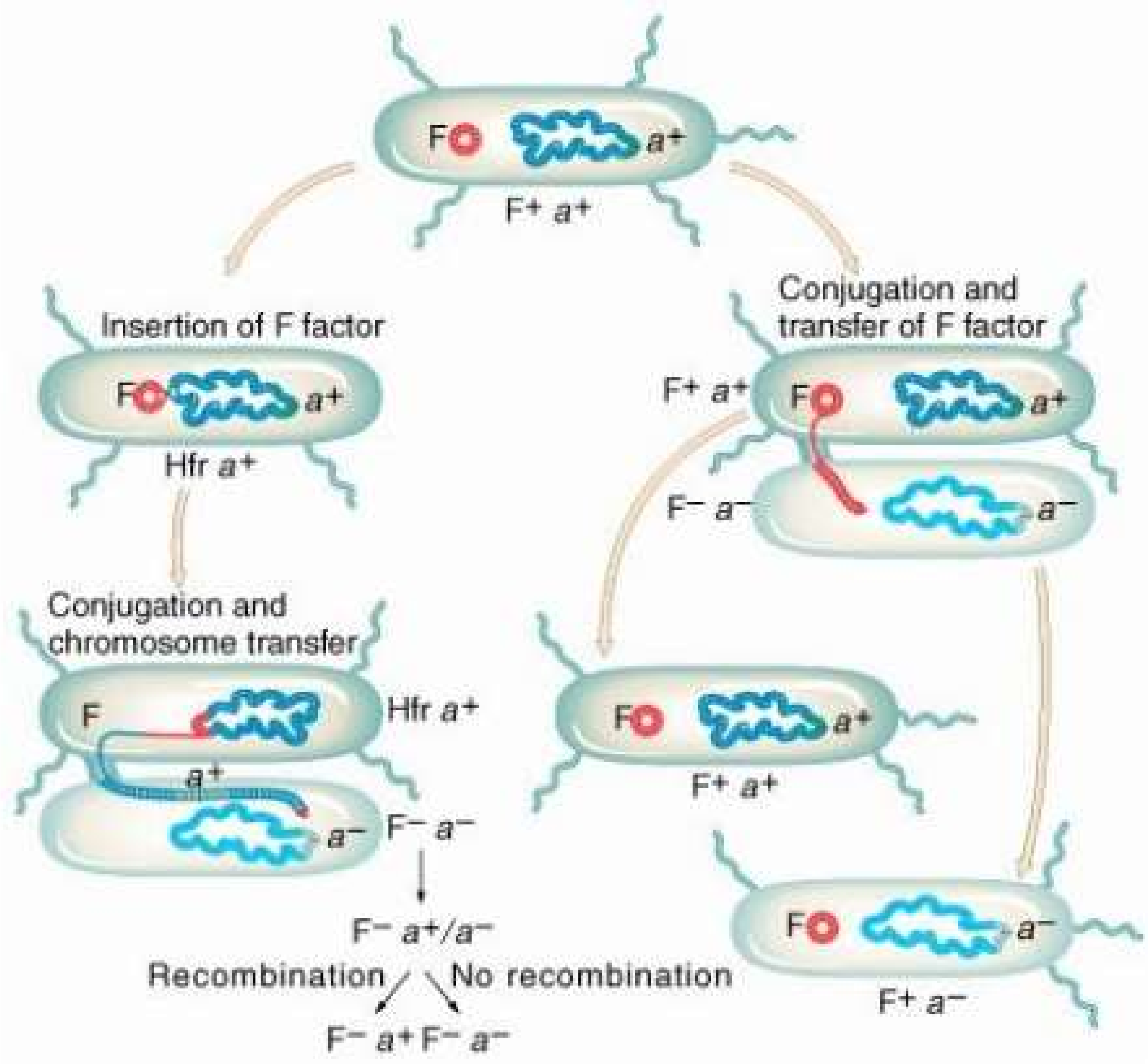
**-5 genes allow the synthesis and transfer of DNA.**

**-3 genes are regulatory genes**
**-A transfer origin**
**-A replication origin**

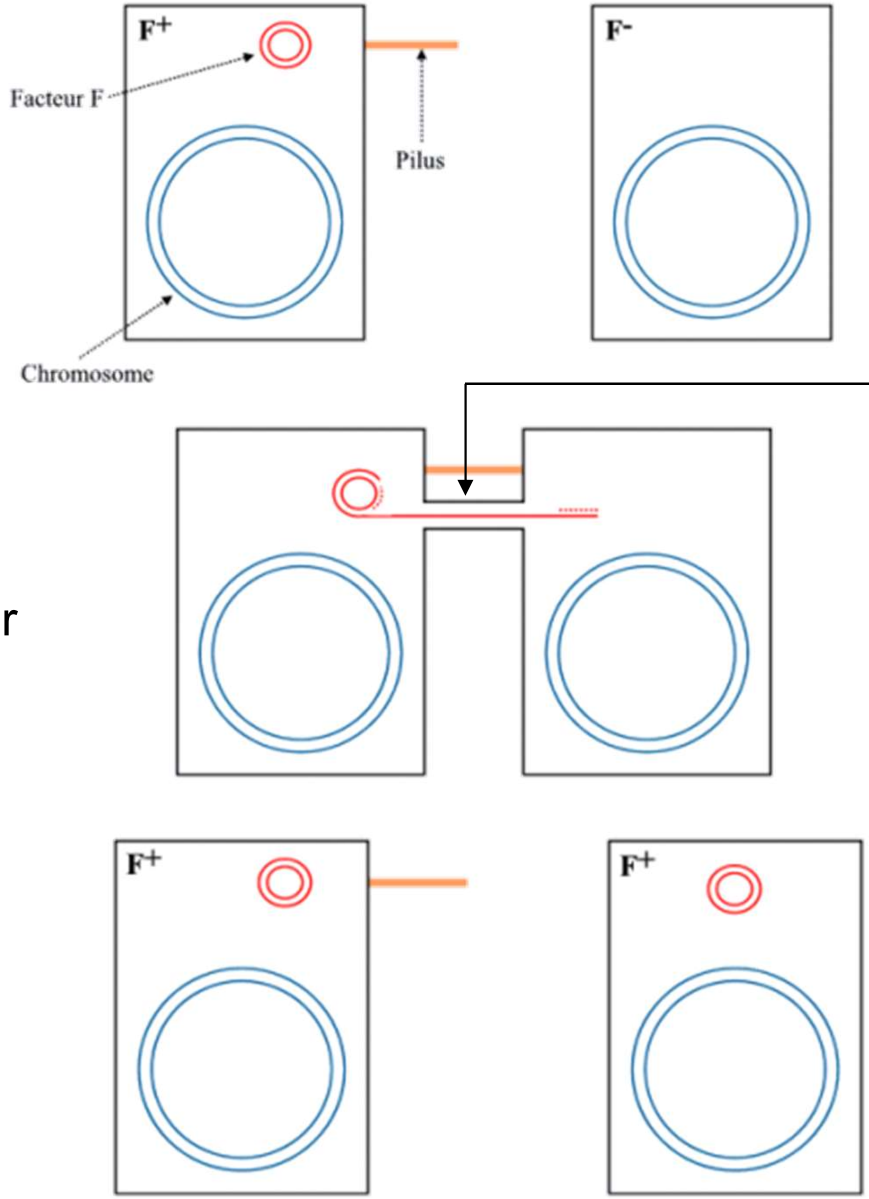
# 3. Conjugaison

1- Conjugation between F+ and F- bacteria

2- Conjugation between Hfr and F- bacteria



# 1. Conjugation between F+ and F- bacteria




Free F factor
in donor cell

F+/F- union bysexual
pili

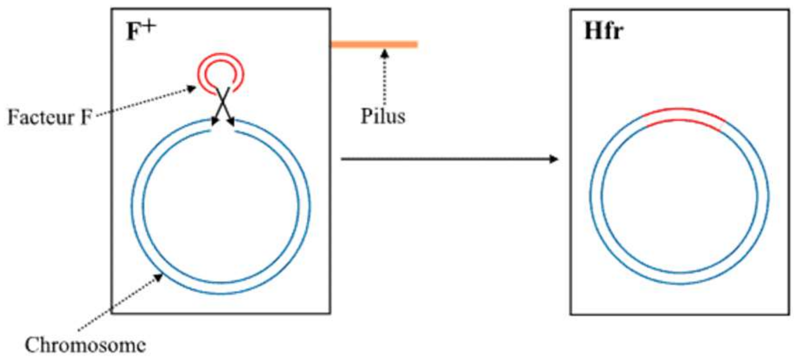
Creation of a
cytoplasmic bridge
Allowing the transfer
of DNA

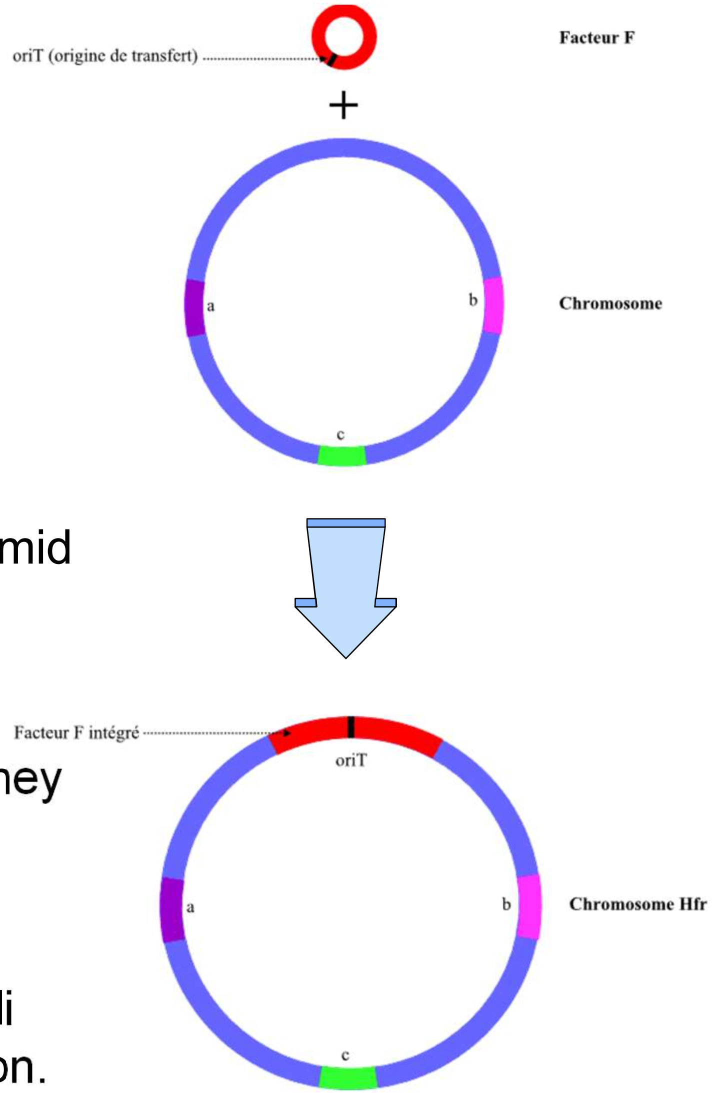
DNA transfer from
the transfer origin
(ori T) and
according to the
rolling circle model

The F factor
persists in the
donor bacteria
which remains F+
(male)

The recipient
bacteria has
acquired a copy
of the F factor
and becomes F+
(male).

# 2. Conjugation between Hfr and F- bacteria




## Formation of Hfr bacteria

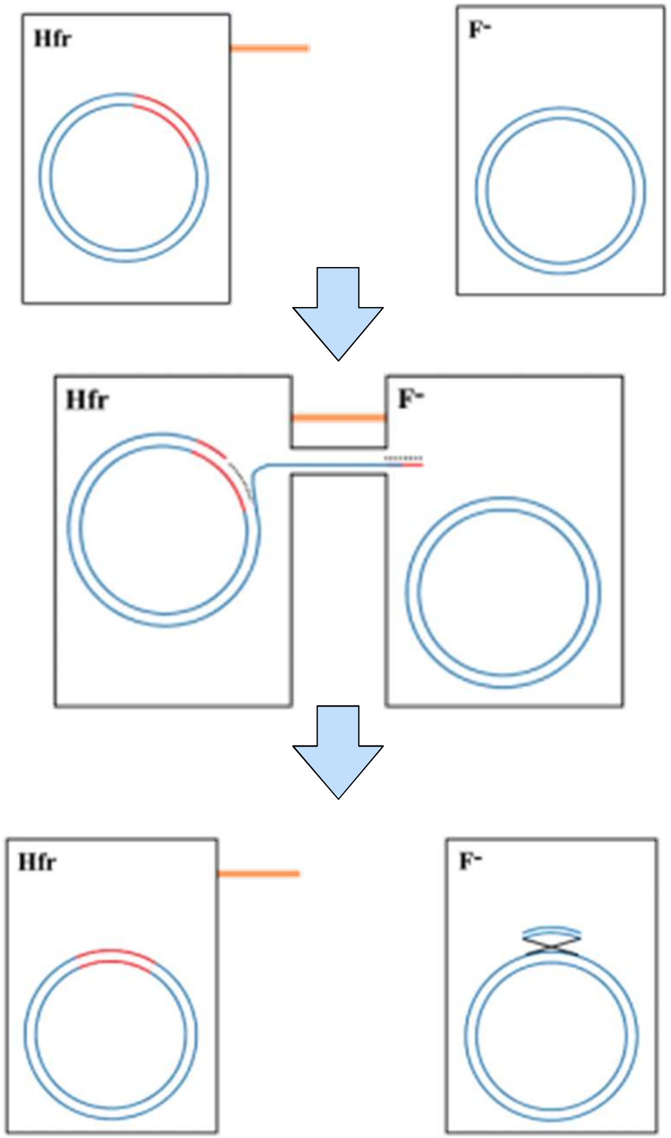
Hfr bacteria are derived from F+ bacteria, the F plasmid is no longer autonomous, it is integrated into the bacterial chromosome,

Hfr for High Frequency of Recombination because they are able to transfer chromosomal markers with a frequency 1000 times greater.

The F factor and the chromosome of Escherichia coli contain insertion sequences capable of recombination.

The integration of the F factor into the chromosome can occur at different locations and in different orientations.

# 2- Conjugation between Hfr and F- bacteria




Single-stranded DNA replication and transfer according to the “rolling circle” model

Linear transfer of one of the DNA strands starting from the transfer origin

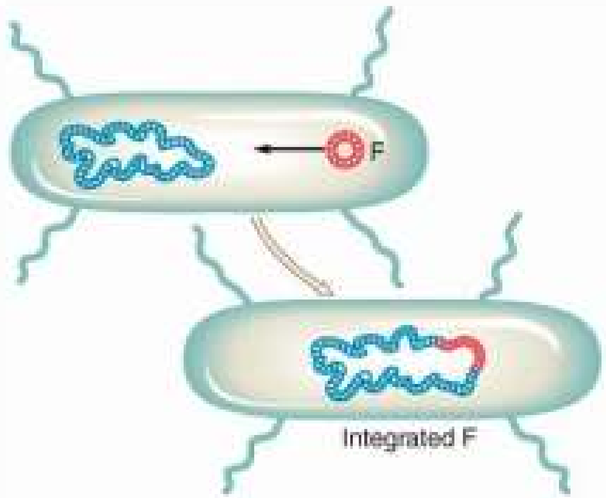
Uninterrupted transfer time 120 minutes

But frequent transfer interruptions

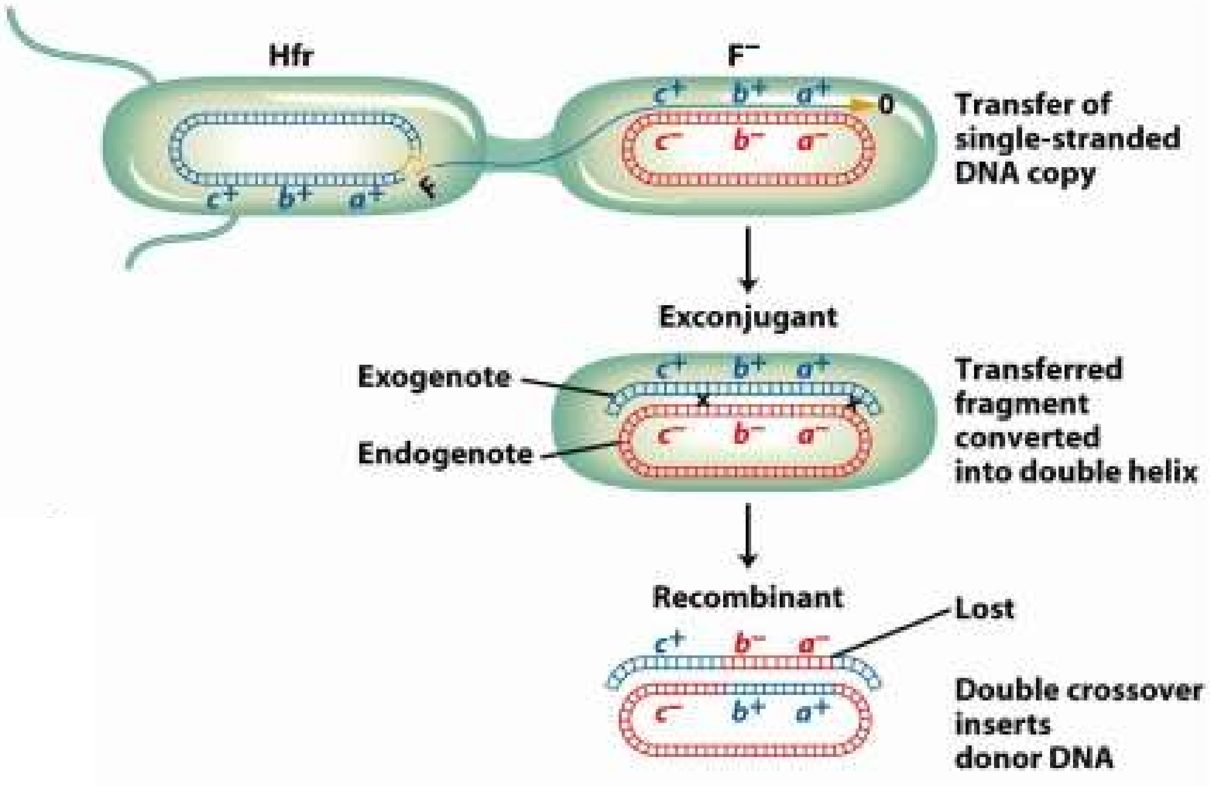
Incorporation of genes by "crossing over" (homologous recombination between two DNA molecules) into the chromosomes of the recipient bacteria

The recipient cell becomes temporarily and partially diploid

# 2- Conjugation between Hfr and F- bacteria




F is integrated into the host chromosome
(a)

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