General Microbiology/ 2nd year

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Bacterial Genetics

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plasmid

- A **plasmid** is a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently.
- They are most commonly found in bacteria as small circular, double-stranded DNA molecules
- Plasmids almost always carry at least one gene.
- Many of the genes carried by a plasmid are beneficial for the cells
- Some of these genes encode traits for
    - antibiotic resistance
    - resistance to heavy metal
    - virulence factors that enable a bacterium to colonize a host and overcome its defences
    - specific metabolic functions that allow the bacterium to utilize a particular nutrient including the ability to degrade toxic organic compounds.
    - Can also provide bacteria with the ability to fix nitrogen.

Factors affecting bacterial genes

Normal gene GCCGAATTTCACGGCCATTGCCATTAG

Mutations

Acquiring of new genetic materials

- ✓Transformation
- ✓ Transduction
- ✓ Conjugation

Abnormal gene GCCGAATTT **GCT** GGCCATTGCCATTAG **AAGGC…**

Factors affecting bacterial genes

GCCGAATTT **GCT** GGCCATTGCCATTAG

Mutations

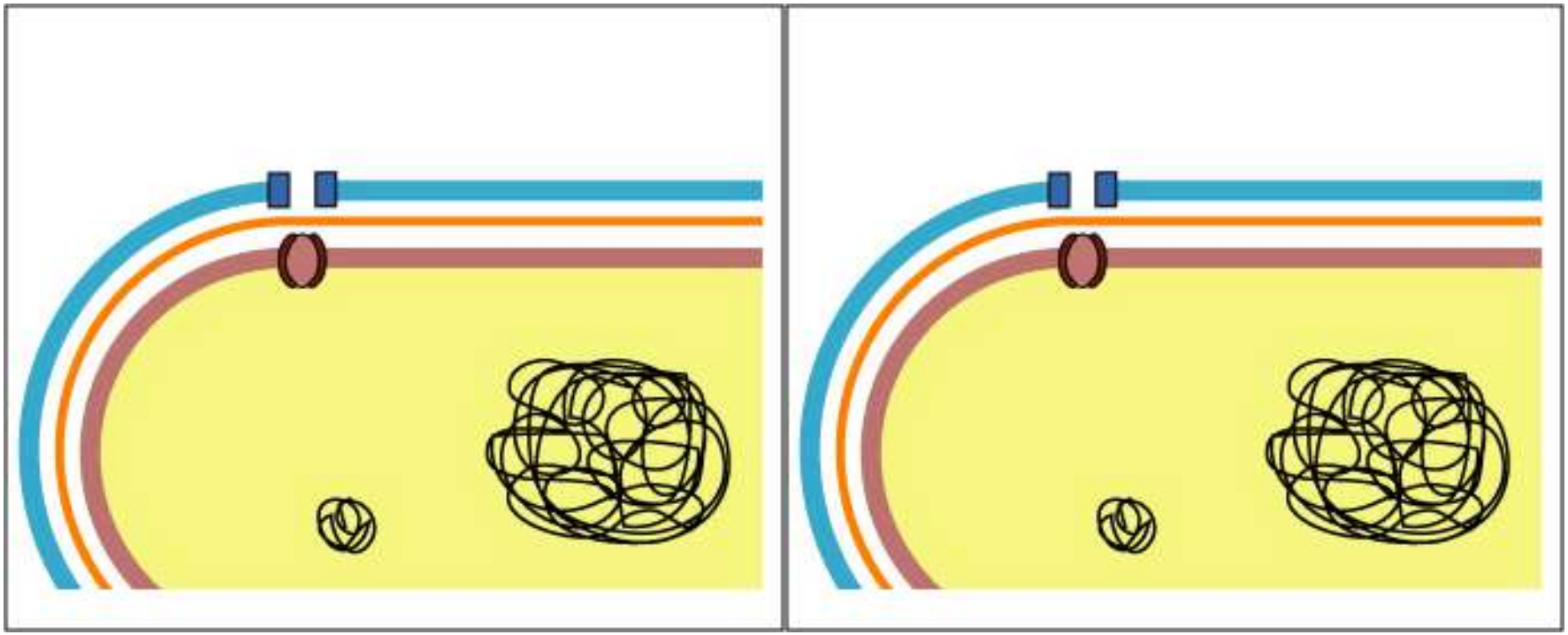
a. Natural mutations
   - Beneficial mutations
   - Harmful (lethal) mutations
   - Silent mutations

b. Induced mutations

Factors affecting bacterial genes

Example on Beneficial mutations

- Mutations enable the bacterium to survive exposure to various antibiotics. Such mutations may affect enzymatic activities , regulatory, or transport systems.



- Mutations enable bacteria to survive temporary exposure to high temperatures or starvation

Factors affecting bacterial genes

Acquiring of new genetic materials: Bacteria developed different ways to exchange genetic material

- **Transformation -** Bacteria incorporate DNA from their environment into their genome (i.e., the Griffith experiment).
- **Transduction -** Movement of DNA between bacteria by viruses.
- **Conjugation -** The direct transfer of DNA by bacteria usually via plasmids.

Factors affecting bacterial genes

Transformation

- The uptake by the bacterium of naked DNA
- Types:

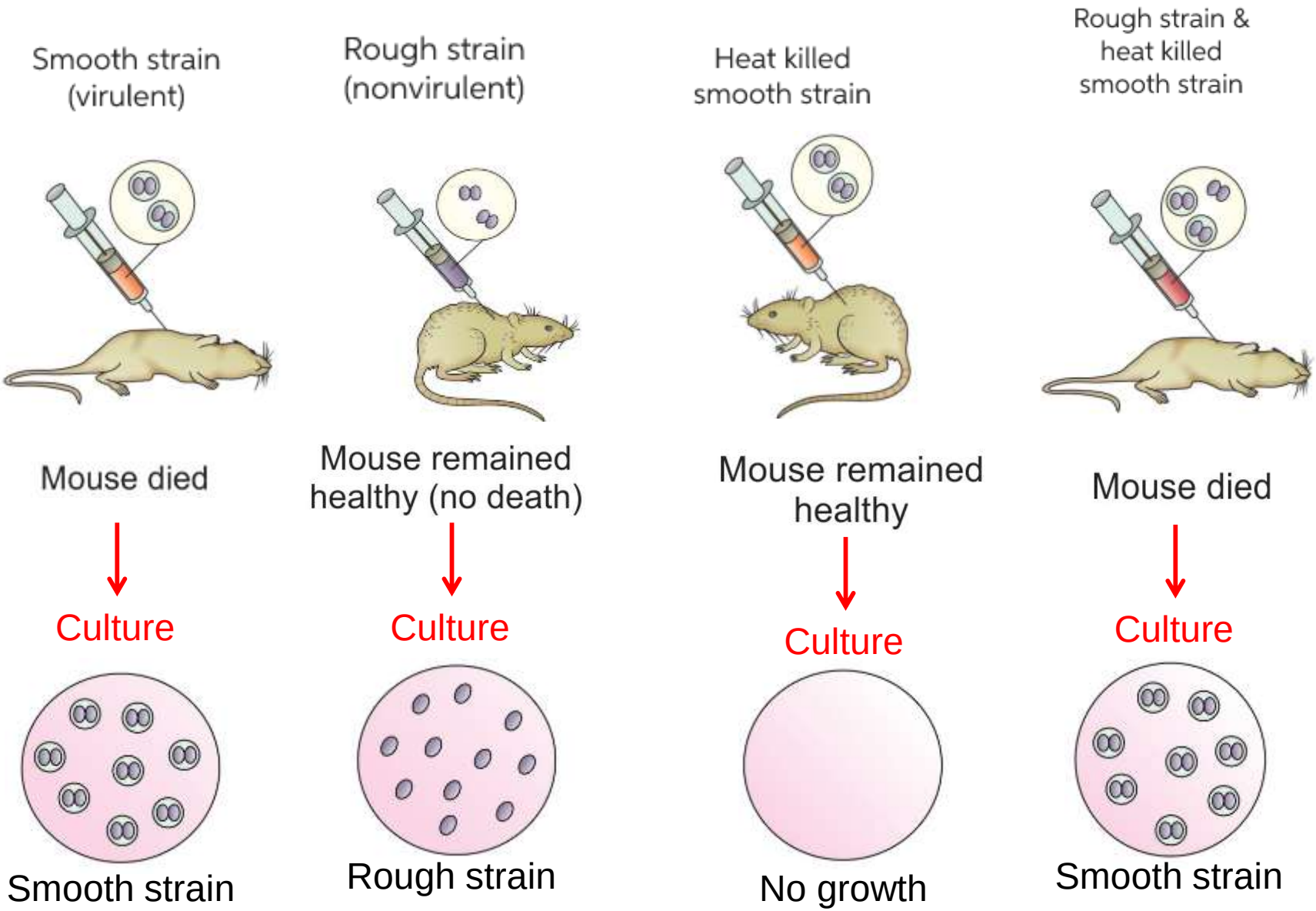
1. **Natural**: The uptake by the bacterium of naked DNA from the environment. Some bacteria have membrane proteins specialized for this function.
2. **Artificial**: uptake of DNA by a process called recombination or cloning.

Transformation

- Transformation is a method of genetic recombination in which a naked DNA from a donor bacteria is transferred to a competent recipient bacteria and incorporated into chromosome of the latter, *e.g. in Bacillus, Haemophilus, Neisseria, Pneumococcus.*
- Transformation occurs in nature.
- In Gram+ve bacteria the DNA is taken up as a single stranded molecule and the complementary strand is synthesized in the recipient.
- In Gram-ve bacteria double stranded DNA is transformed.
- **Significance:**
  - Transformation occurs in nature and it can lead to increased virulence.
  - It is widely used in recombinant DNA technology.

Natural Transformation

Griffith's Experiments with *Pneumococcus*



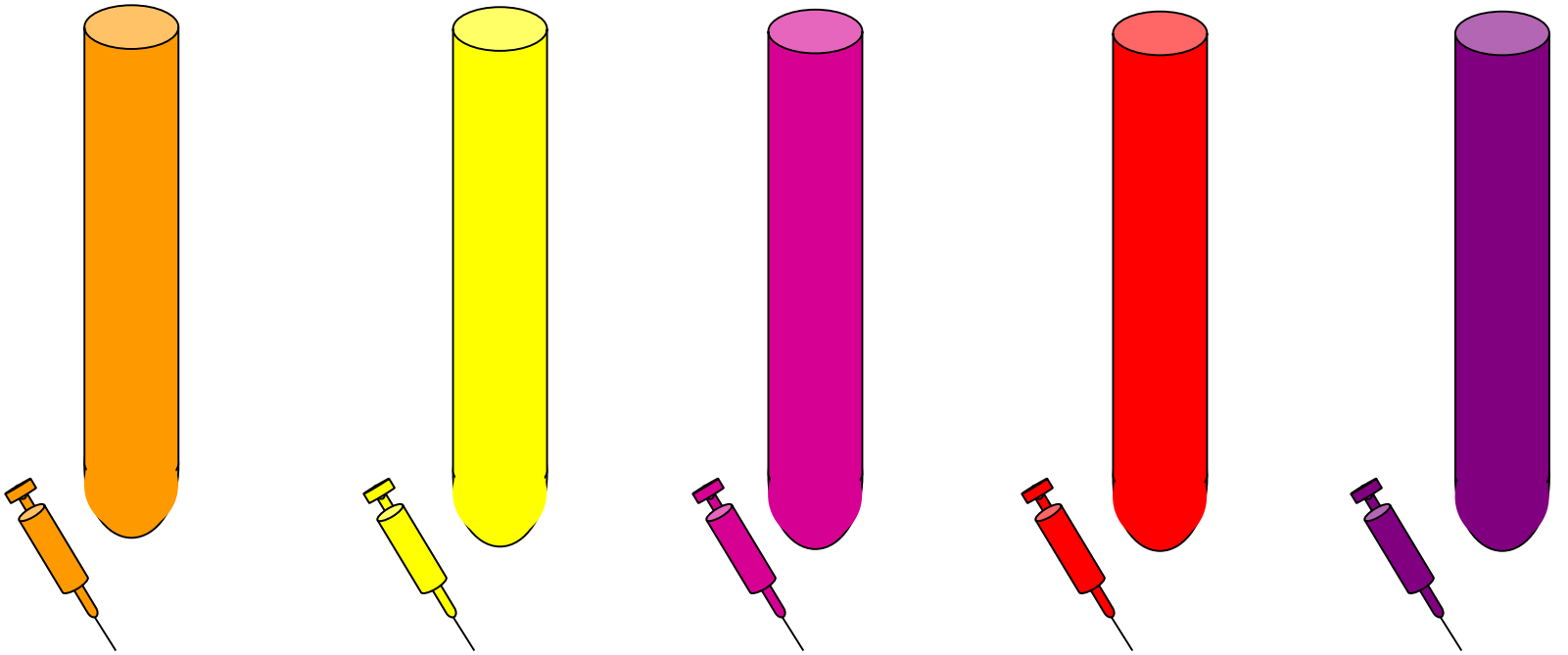
S-type Carbohydrates Destroyed (other components remained intact) + **Rough strain**

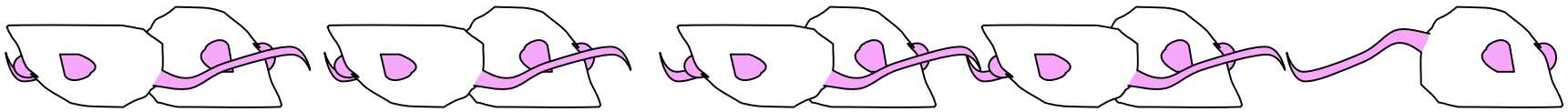
S-type Lipids Destroyed (other components remained intact) + **Rough strain**

S-type Proteins Destroyed (other components remained intact) + **Rough strain**

S-type RNA Destroyed (other components remained intact) + **Rough strain**

S-type DNA Destroyed (other components remained intact) + **Rough strain**



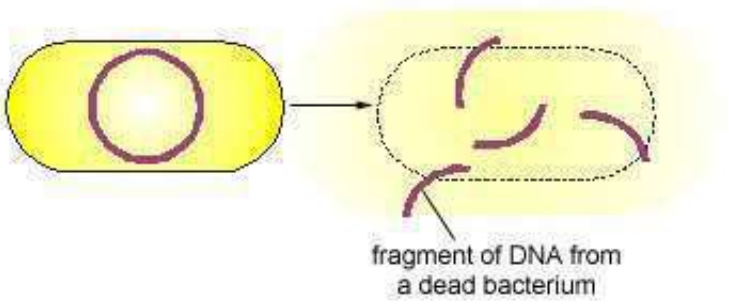


Conclusion:

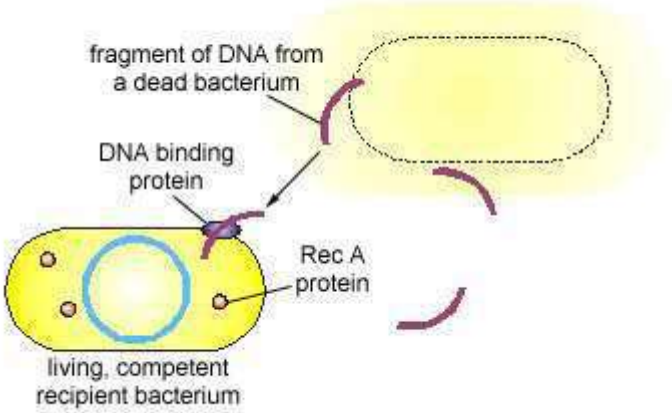
DNA was the transforming factor!

Mechanism of Transformation

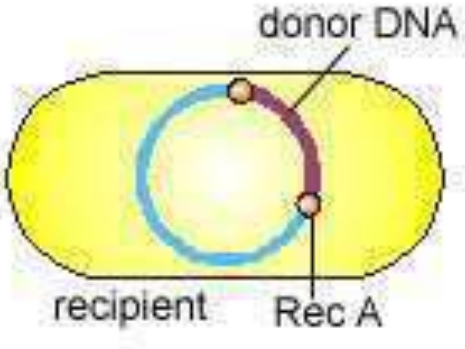
1. A donor bacterium dies and is degraded.



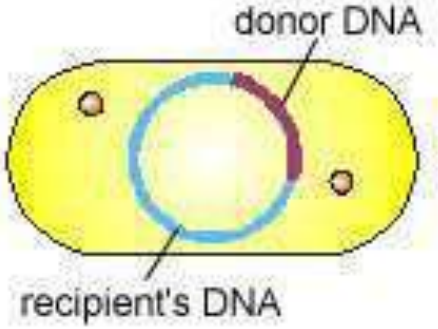
2. A fragment of DNA from the dead donor bacterium binds to DNA binding proteins on cell wall of a competent live recipient bacterium



3. The Rec A protein promotes genetic exchange between a fragment of the donor's DNA and the recipient's DNA.



4. Exchange is complete.

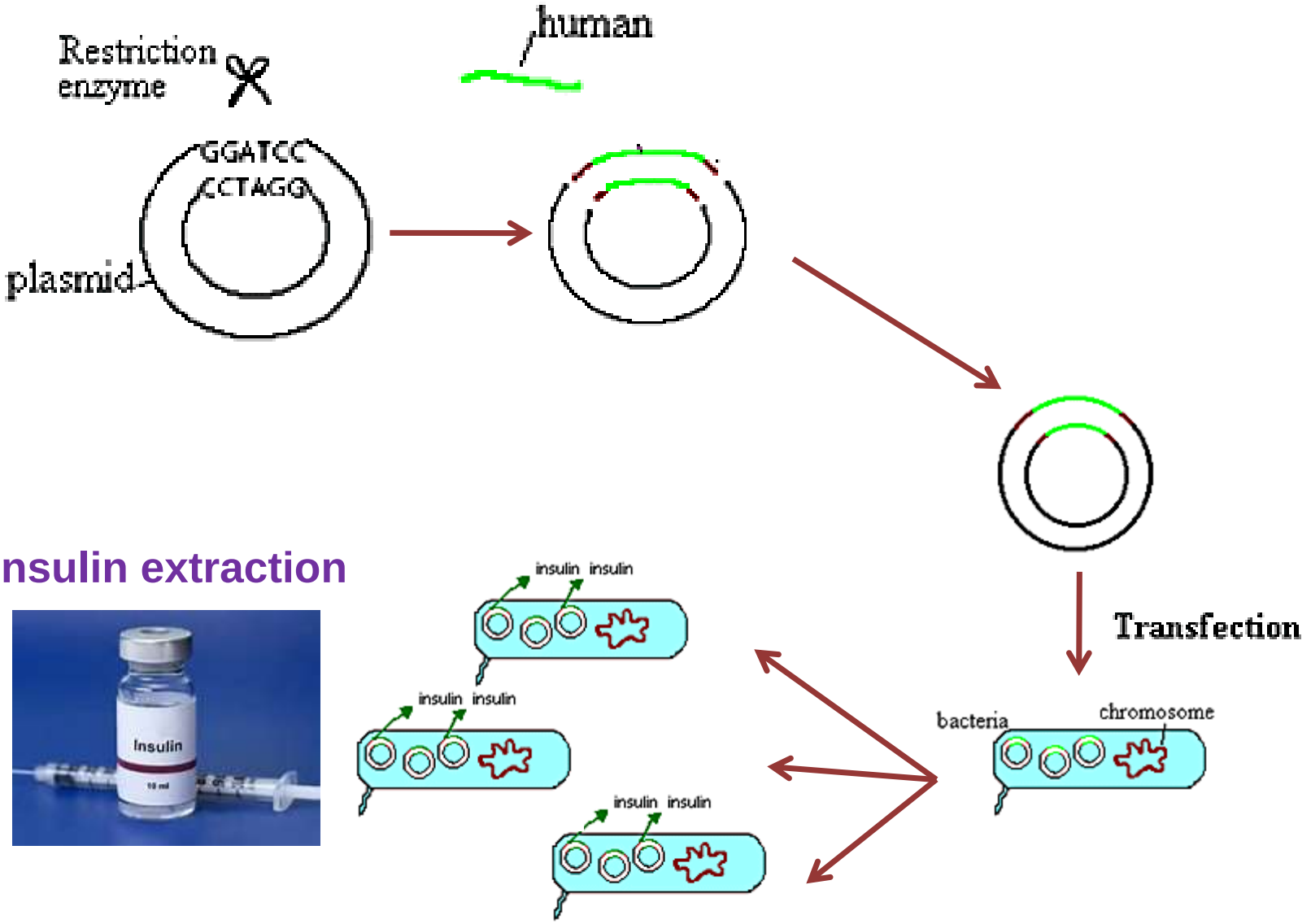


Mechanism of Transformation

- A bacterial cell dies or is degraded releasing its dsDNA molecule in environment.
- Nuclease enzymes cut the released DNA into fragments of usually about 20 genes long.
- The fragments bind to DNA binding proteins present on the surface of a competent recipient bacterium and subsequently translocated in the cytoplasm of recipient bacteria
- The DNA fragment from the donor is then exchanged for a piece of the recipient's DNA by means of Rec A proteins.

Artificial Transformation

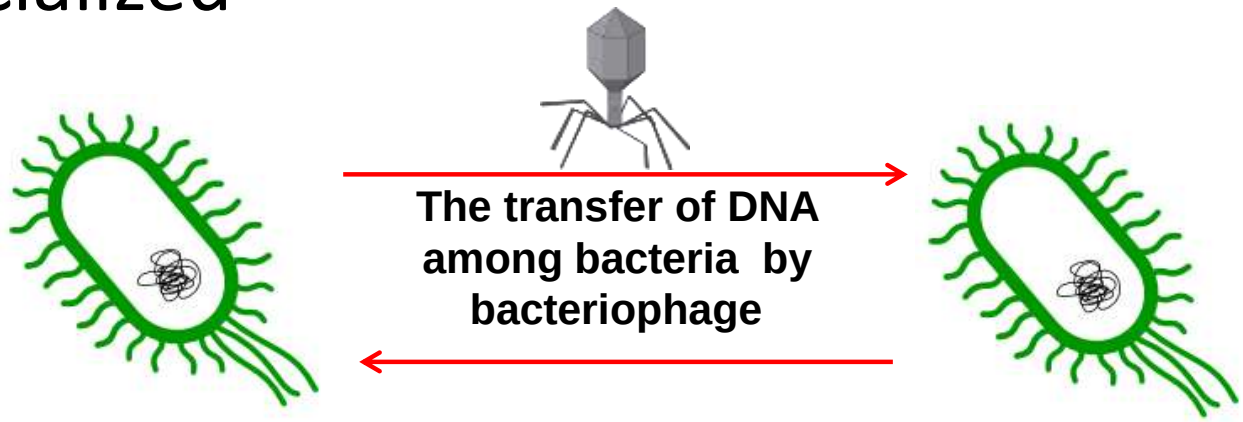
Artificial transformation: Cloning (Example, Insulin production)



Insulin extraction

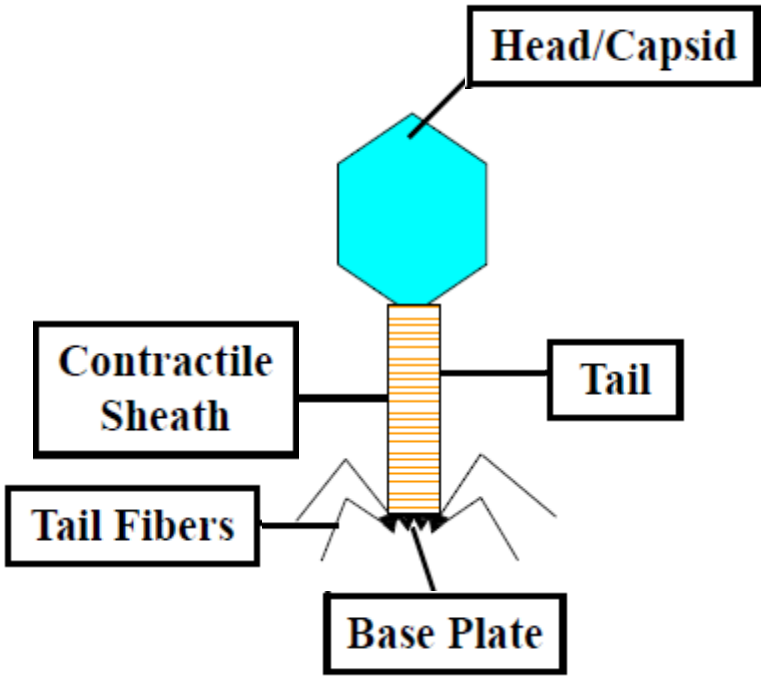
Transduction توصيل

- **Definition:** Gene transfer from a donor to a recipient bacteria through a bacteriophage
- **Bacteriophage (phage):** A virus that infects bacteria
- **Types of transduction**
  - Generalized
  - Specialized



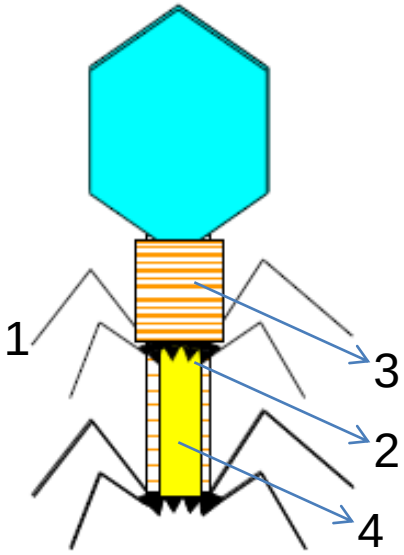
Phage Structure:

- Size (80 X 100 nm)
- Head or capsid
- Tail
  - contractile sheath,
  - base plate,
  - tail fibers.



Infection process of Host Cells:

1. Adsorption
   –Tail fibers
2. Irreversible attachment
   – Base plate
3. Sheath Contraction
4. Nucleic acid injection
5. DNA uptake



Types of Bacteriophage

1. **Virulent phage:** a phage that multiply within the host cell, lyse the cell and release progeny phage *(causes* lytic cycle)
2. **Temperate phage:** a phage that can either multiply via the lytic cycle or enter a quiescent integrated state in the bacterial cell (causes lysogenic cycle).

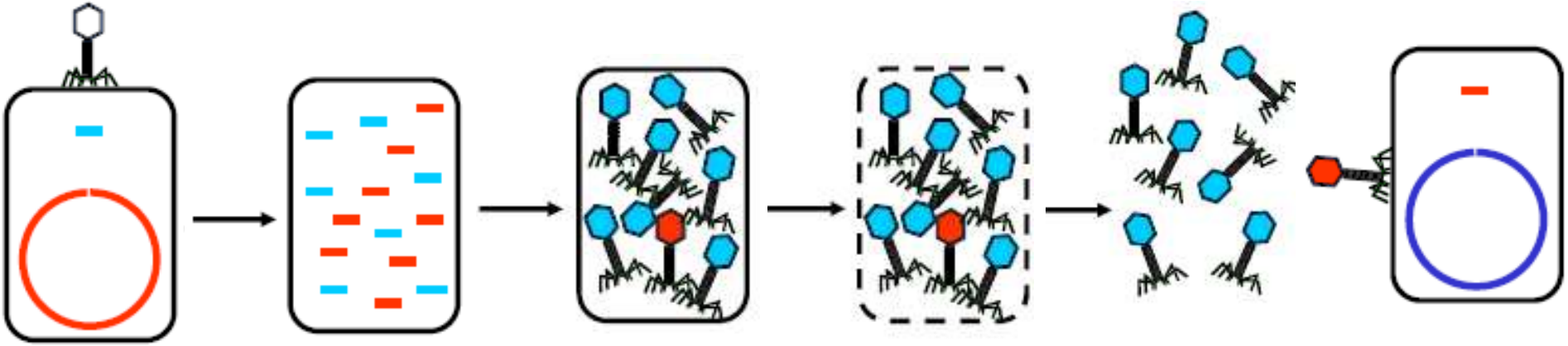
- Lysogenic cycle: expression of most phage genes repressed
- Prophage: Phage DNA in the quiescent integrated state
- Lysogen : Bacteria harboring a prophage

Generalized Transduction

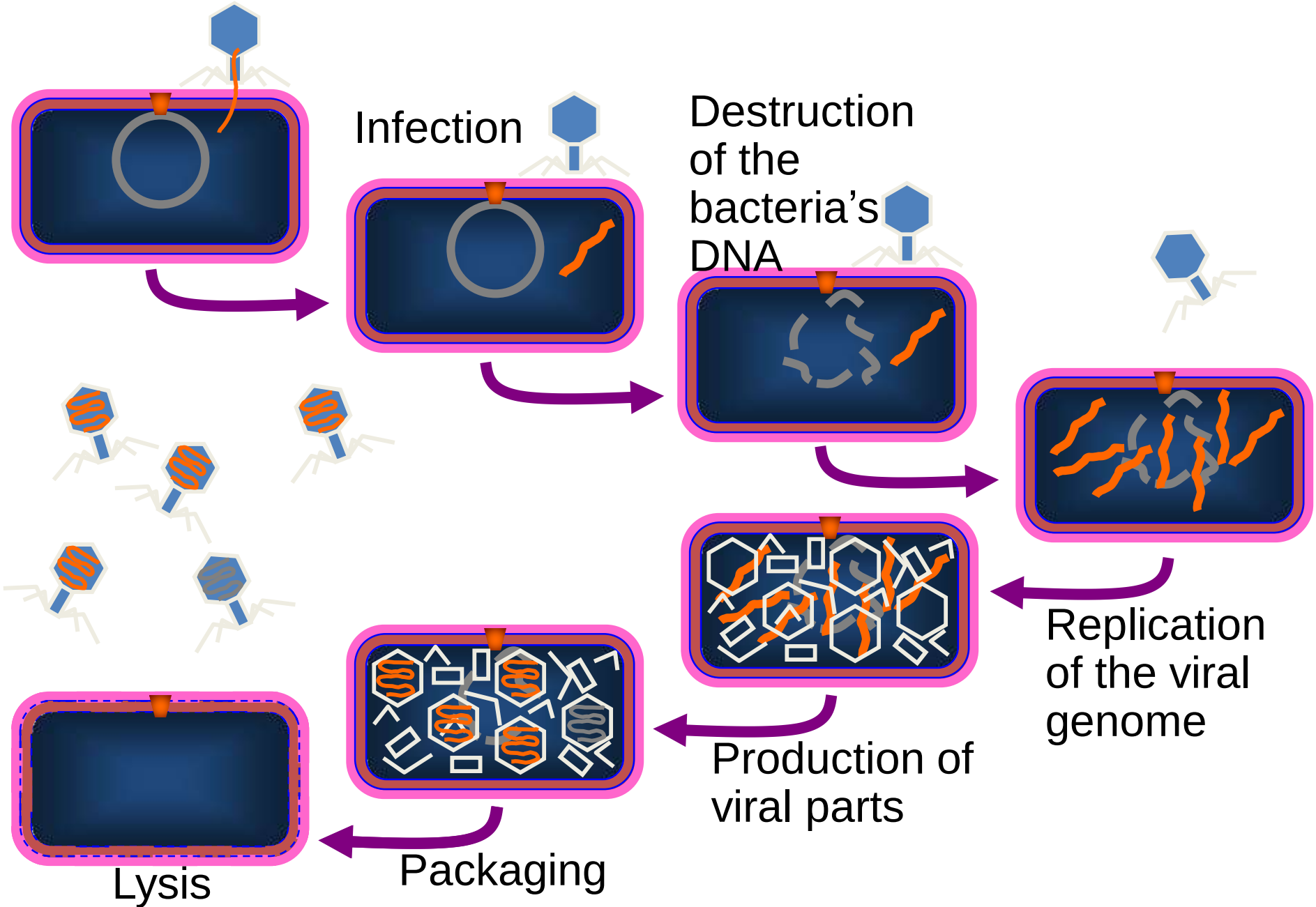
- **Generalized transduction can transfer any gene of donor bacteria to recipient bacteria**
- During the replication of a **lytic phage the capsid sometimes enclose** a small fragment of lysed bacterial DNA, instead of phage DNA, by a "head-full" mechanism. **This is a defective phage**
- Such a phage cannot lyse another bacterium because the DNA in the phage head does not have the genetic information to produce phage genome and proteins.
- On infection of another bacterium defective phage injects the fragment of donor bacterial DNA into the recipient bacteria, where it can be exchanged for a piece of the recipient's DNA, if their sequences are homologous.

Steps in Generalized Transduction

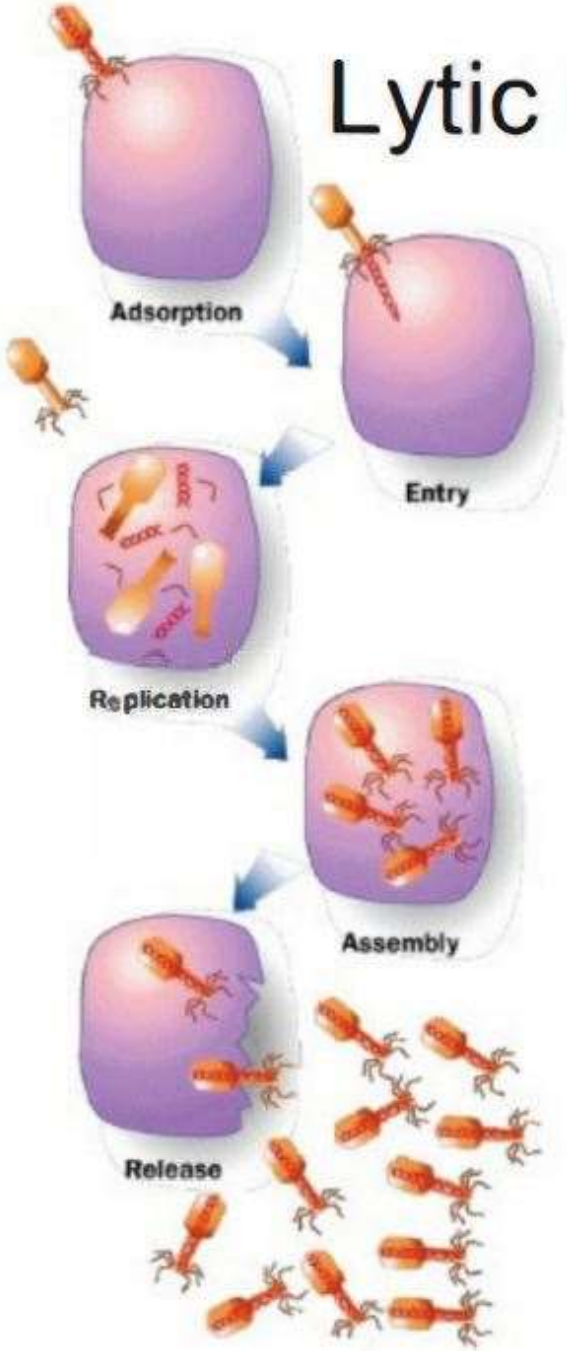
- Infection of Donor
- Phage replication and degradation of host DNA
- Assembly of phages particles and encapsidation of host DNA
- Release of phage
- Infection of recipient
- Homologous recombination



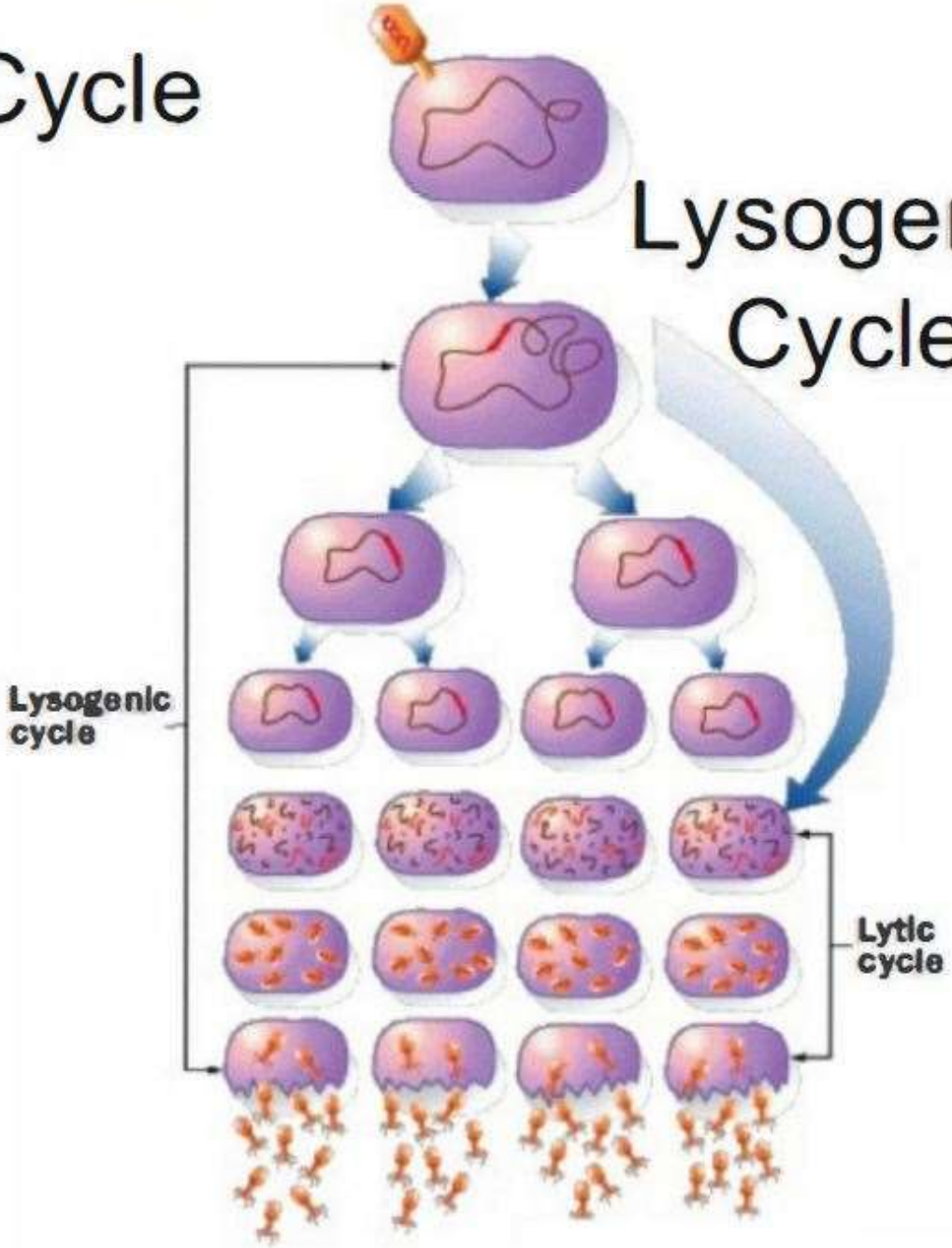
Generalized Transduction



Lytic Cycle



Lysogenic Cycle



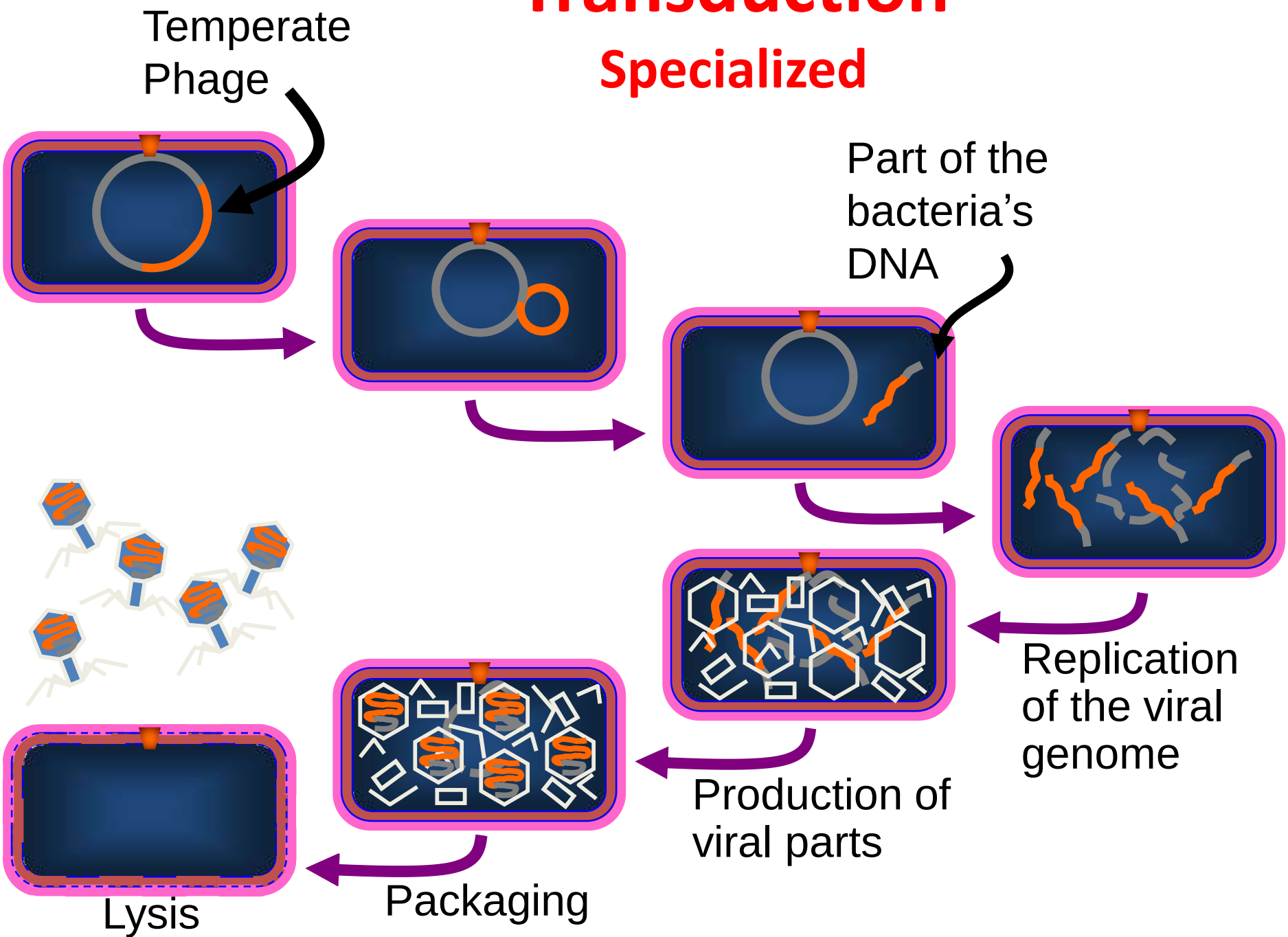
Specialized transduction

A transduction in which only certain donor genes can be transferred to the recipient

- Occur during the **lysogenic life cycle of a temperate phage.**
- During spontaneous induction of lysogeny, a small piece of bacterial DNA may sometimes be exchanged for a piece of phage genome.
- This piece of bacterial DNA replicates as a part of the phage genome and is incorporated into capsid of each phage progeny
- On infection of a recipient bacteria, the phage DNA containing donor bacterium genes are injected into the recipient bacterium where donor DNA fragment can be exchanged for a piece of the recipient's DNA, if their sequences are homologous
- Different phages may transfer different genes but an individual phage can only transfer certain genes.
- Lysogenic (phage) conversion occurs in nature and is the source of virulent strains of bacteria, e.g. toxin production in *Cl. botulinum, C. diptheriae, STEC, etc*

Transduction

Specialized



Transduction

Medical Importance: transfer of new toxin encoded gene to bacteria

Bacterium	Phage	Gene Product	Phenotype
Vibrio cholerae	CTX phage	cholerae toxin	cholera
Streptococcus pyogenes	T12	erythrogenic toxins	scarlet fever
Corynebacterium diphtheriae	corynephage beta	diphtheria toxin	diphtheria
Escherichia coli	lambda phage	shigalike toxin	hemorrhagic diarrhea
Clostridium botulinum	clostridial phages	botulinum toxin	botulism (food poisoning)

Conjugation

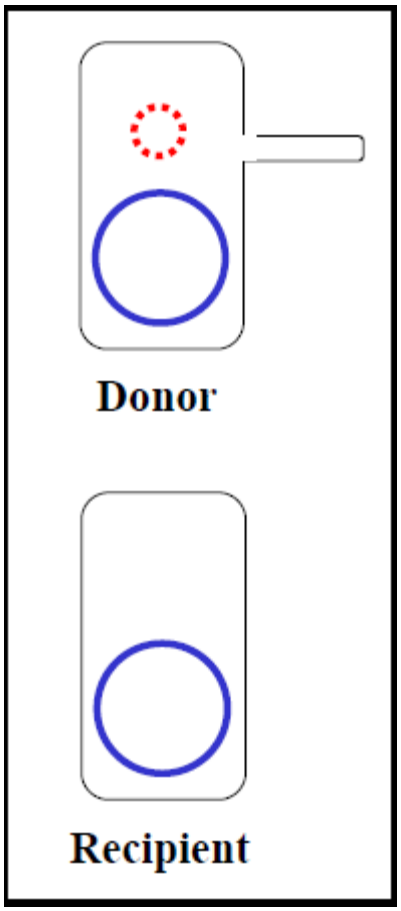
- Is the commonest process of sexual reproduction in bacteria.
- In conjugation two parental cells physically contact between two genetically different cells of the same or closely related species and transfer their genetic material through a small tube like projection called conjugation tube.
- The genetic material from one cell (donor or male) is transferred to other (recipient or female).

Conjugation

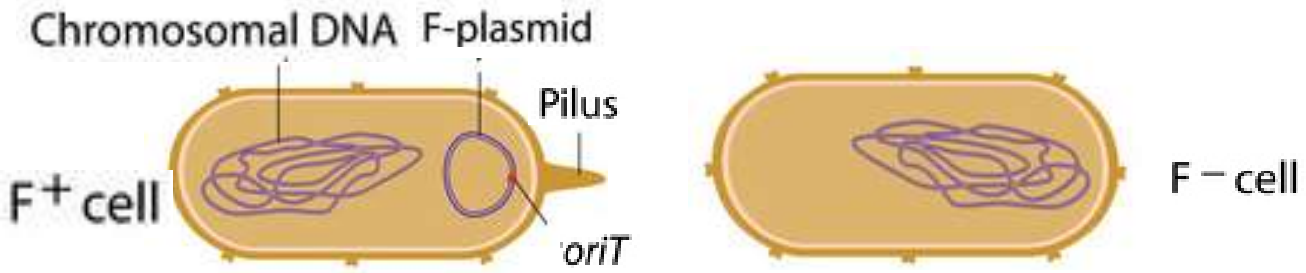
Definition: gene transfer from a donor to a recipient by direct physical contact between cells

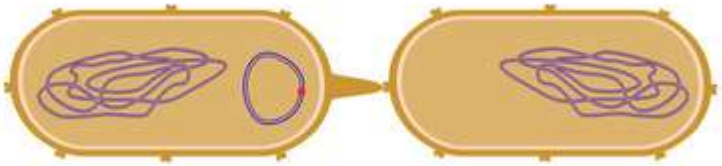
Mating types in bacteria

- Donor (Male/F+)
  - F factor (Fertility factor)
    - **F (sex) pilus**
- Recipient (Female/F-)
  - Lacks an F factor

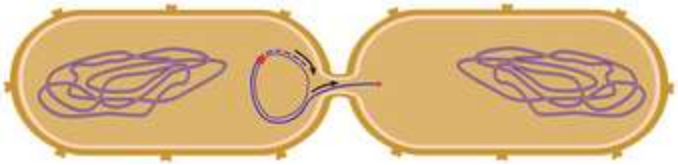


Conjugation

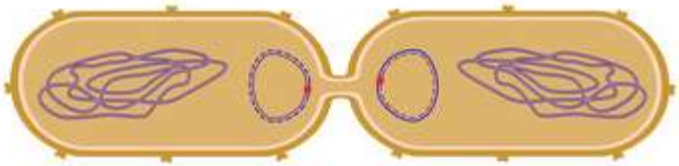




A conjugation tube forms between the donor cell and the recipient cell



A single strand from the plasmid DNA is transferred through the tube from the donor to the recipient.



A double stranded DNA is formed from the single strand in both the donor and the recipient cells.

Conjugation

Medically important factors transferred by conjugation

- Resistance (R) plasmids, which contain genes that provide resistance against antibiotics or poisons.

- colicinogenic (or *Col*) plasmids: determines the production of proteins called colicins (bacteriocins), which have antibiotic activity and can kill other bacteria

Transposons "Jumping Genes"

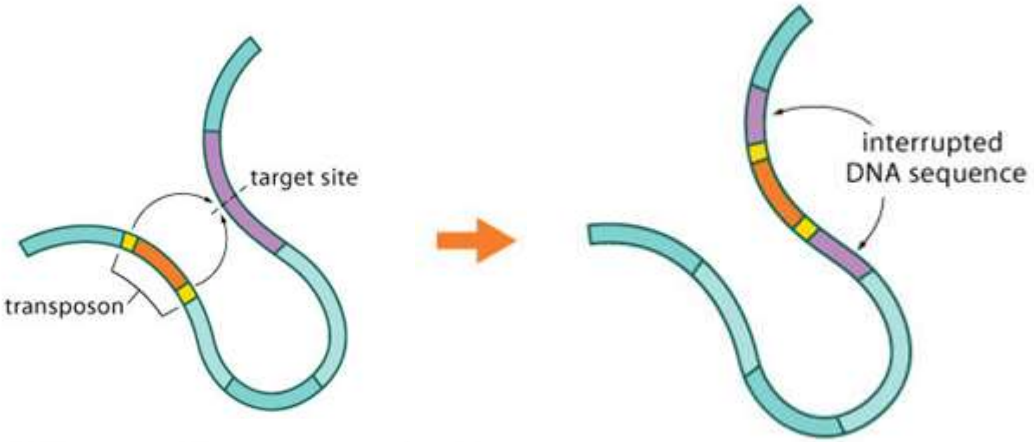
- Transposons – DNA elements that can hop (transpose) from one place in DNA to another
- Movement by a transposon is called transposition, catalyzed by enzymes called transposases
- Transposons usually encode their own transposases
- Transposons are known to exist in all organisms on earth

Significance

- Transposons can transfer from a plasmid to other plasmids or from a DNA chromosome to plasmid and vice versa that cause the transmission of antibiotic resistance genes in bacteria.
- In this way, genes can be transferred from one cell to another.

Transposons "Jumping Genes"

1. Cut-and-paste mechanism



2. Copy-and-paste mechanism