L5: Bacterial pathogenesis

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- **Pathogen**: microbe has ability to causing infection, some microbes are highly pathogenic, whereas others cause disease rarely (low pathogenic).
- **Virulence:** it is referred to degree of ability of microbe to produce disease. The virulence is determined by virulence factors such as capsule enzymes and toxins.
- **Infection**, in general, is presence and multiplication of microbe within human body, and cause infectious disease.
- **Characters of pathogen:**
- 1. Pathogenic organism should be able to enter the host body.
- 2. Pathogenic organism should be able to multiply in tissue.
- 3. Pathogenic organism should be able to damage the tissue.
- 4. They must be capable to resist the host defense.

Virulence factors of pathogen:

Some important virulence factors that have role in pathogenesis of bacteria:

- **1. Capsule:**
- • Which mediate adherence of pathogen to specific receptors on host cell surface.
- • prevent the phagocytes from adhering to bacteria, therefore act as antiphagocytic factor.
- **2. Pili:**
- • Which mediate adherence to specific sites on surface of host cell.
- **3. Enzymes:**
- • Several enzymes secreted by bacteria play role in pathogenesis.
- **4. Toxins**
- • The bacteria produce two types of toxins; exotoxin and endotoxin.

- **Sources of infection:**

1. **Human**: is common source of infection from patients or carriers.
   - Fomites are inanimate objects of patients that serve as source of organism.
2. **Animals**; are important source of organisms that infect human, the organisms can transmitted from infected animals to human and cause disease . The disease is called zoonosis.
3. **Soil;** may serves as source of infection for many pathogenic organisms or spores of some bacteria and fungi.
4. **Food**; contaminated food may be source of infection, eg. *Staphylococcal* food poisoning.
5. **Water**; many pathogenic MO such as *Vibrio cholerae*, hepatitis virus may be found in water.

- **Stages of bacterial pathogenesis:**

- **I-Transmission and portal of entry:**
- **A- Portals of entry (routes of infection)**: four important portals of entry
- 1. Respiratory tract.
- 2. Elementary tract.
- 3. Skin
- 4. Genital tract.

- **B-Mode of transmission**
- 1. **Inhalation**: the bacteria may be transmitted by inhalation of respiratory secretions of infected patients or by inhalation of contaminated dust with bacteria (from soil).
- 2. **Ingestion**: the bacteria can be transmitted by consumption of contaminated water or food.

- 3. **Skin contact, bite, wound**: The transmission may occur through skin contact or can be occurs by trauma. The transmission from animal to human can take place either directly from bite of reservoir animal host; or indirectly through the bite of insect vectors. Those bacteria may be introduced directly into blood stream or remain at site of infection.
- 4. **Blood transfusion**: some bacteria can be transmitted by blood transfusion. Injection of needles during intravenous drug use or indwelling catheter, have role in transmission.
- 5. **Sexual intercourse**: certain bacteria can be transmitted by sexual contact.
- **All above methods are called horizontal transmission.**
- 6. **Transplacental**; the infection of fetus can occur between mother and offspring across the placenta, at time of delivery, or during breast feeding. The transmission from mother to her fetus is called **Vertical transmission**.

II-Adherence and multiplication:

- Certain bacteria have specialized structures (adherence factors, such as pili and capsule) which allow them to adhere to specific sites on surface of human cell, thereby enhancing their ability to cause diseases. The bacteria can be multiplied by growth at site of adherence and form colonization, or spread to another tissues by invasion.
- **III- Invasion and damage of host tissues:**
- The invasion is ability of microorganism to spread into host tissues, some bacteria are non invasive, but multiply at site of adherence. Bacteria secrete several invasive factors that play role in pathogen

- **a. Enzymes:**
- 1. **Collaginase and hyaluronidase** ; which degrade collagen and hyaluronic acid ,thereby allowing the bacteria to spread through host tissues.
- 2. **Coagulase**; which accelerates the formation of fibrin clot from fibrinogen around bacteria to protect the bacteria from phagocytosis action.

fibrinogen-- **Coagulase** -- fibrin

- 3. **IgA protease**; which degrades this immunoglobulin; allowing the bacteria to adhere to mucous membrane.
- 4. **leucocidin**, which can destroy both neutrophile and macrophages.
- 5. **Hemolysin** which can destroy RBC
- 6. **Capsule;** prevent the phagocytes from adhering to bacteria therefore, capsule act as antiphagocytic factor.

- **b. Toxin production:**
- The bacteria produce two types of toxins, exotoxin and endotoxin.
- **Exotoxin** is produce by G+ ve and G- ve bacteria. In contrast,
- **endotoxin** which is produce by only G- ve bacteria. **Exotoxin** is protein, whereas **endotoxin** is lipid and carbohydrate.
- **Exotoxin** is good antigen and induce high titer of antibody (antitoxin), whereas **endotoxin** is weakly antigenic and not induce antitoxin. **exotoxin** is heat labile at 60C, when treated with formaldehyde (or acid or heat) can be converted in toxoid (the toxin is lost their toxicity but retain their antigenicity, therefore can be used as vaccine) whereas, **endotoxin** cannot be toxoid because the toxin is heat stable at more than 60C.

Table (4) the maine feature of exotoxin and endotoxin.

Comparison of Properties		
Property	**Exotoxin**	**Endotoxin**
Source	Certain species of gram-positive and gram-negative bacteria	Cell wall of gram-negative bacteria
Secreted from cell	Yes	No
Chemistry	Polypeptide	Lipopolysaccharide
Location of genes	Plasmid or bacteriophage	Bacterial chromosome
Toxicity	High (fatal dose on the order of 1 μg)	Low (fatal dose on the order of hundreds of micrograms)
Clinical effects	Various effects (see text)	Fever, shock
Mode of action	Various modes (see text)	Includes TNF and interleukin-1
Antigenicity	Induces high-titer antibodies called antitoxins	Poorly antigenic
Vaccines	Toxoids used as vaccines	No toxoids formed and no vaccine available
Heat stability	Destroyed rapidly at 60°C (except staphylococcal enterotoxin)	Stable at 100°C for 1 hour
Typical diseases	Tetanus, botulism, diphtheria	Meningococcemia, sepsis by gram-negative rods

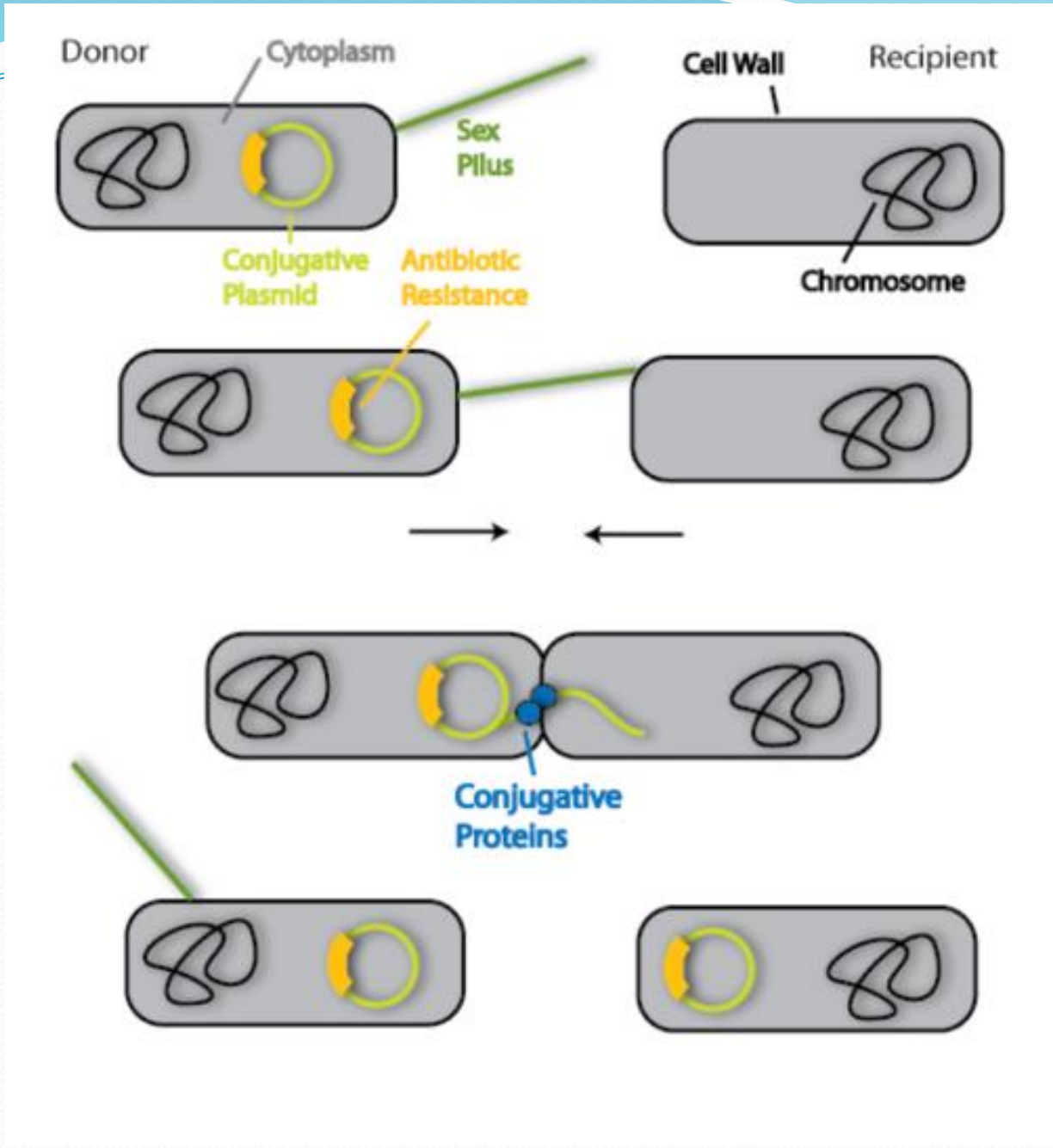
TNF = tumor necrosis factor.

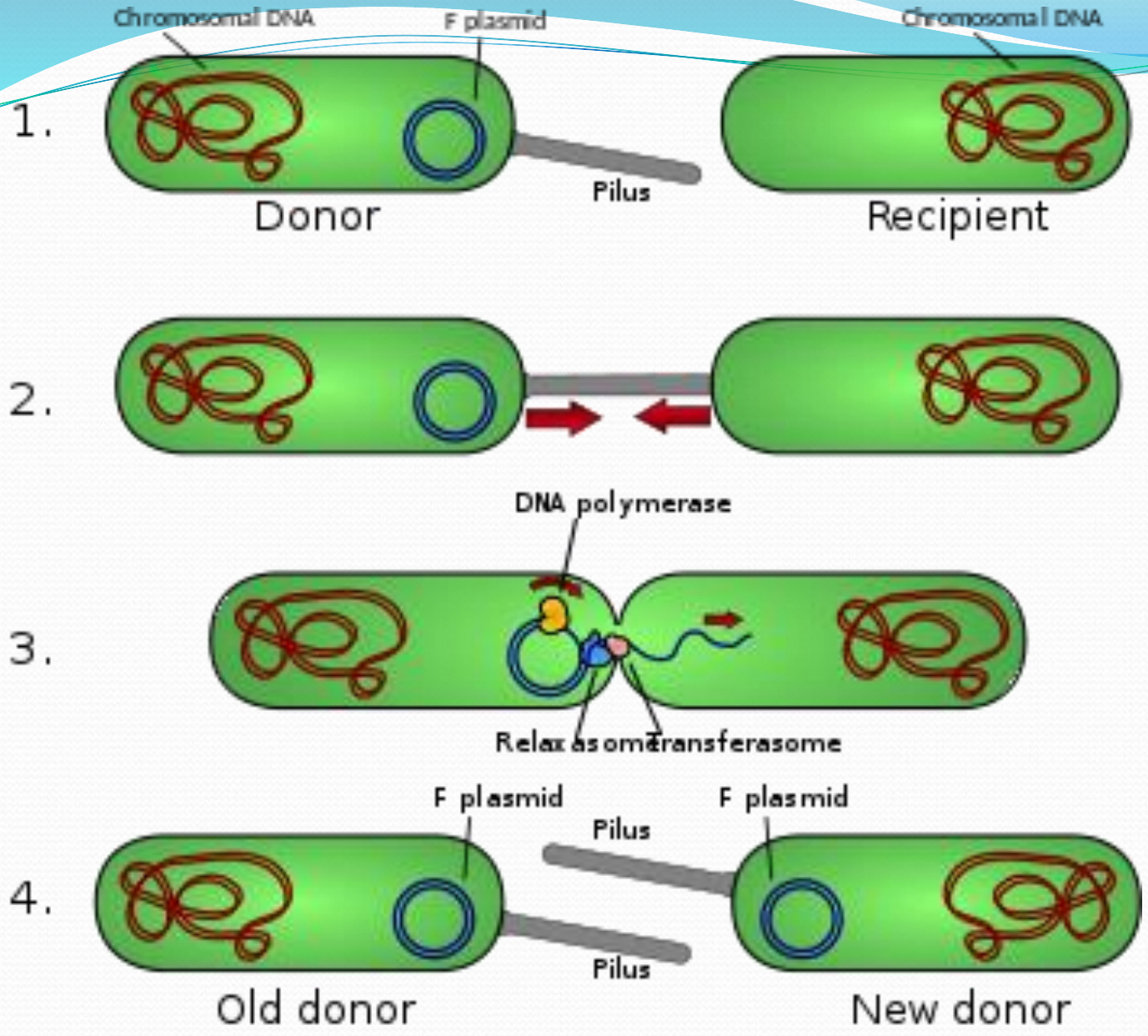
- **Genetic Transfer**
- Genetic transfer is the mechanism by which DNA is transferred from a donor to a recipient. Once donor DNA is inside the recipient. The result is a recombinant cell that has a genome different from either the donor or the recipient. Bacteria reproduce by the process of binary fission, the chromosome in the mother cell is replicated and a copy is allocated to each of the daughter cells. As a result, the two daughter cells are genetically identical.
- **Horizontal gene transfer (HGT)** refers to the transfer of genes between organisms in a manner other than traditional reproduction. Also termed lateral gene transfer (LGT), it contrasts with **vertical transfer**, the transmission of genes from the parental generation to offspring via sexual or asexual reproduction.

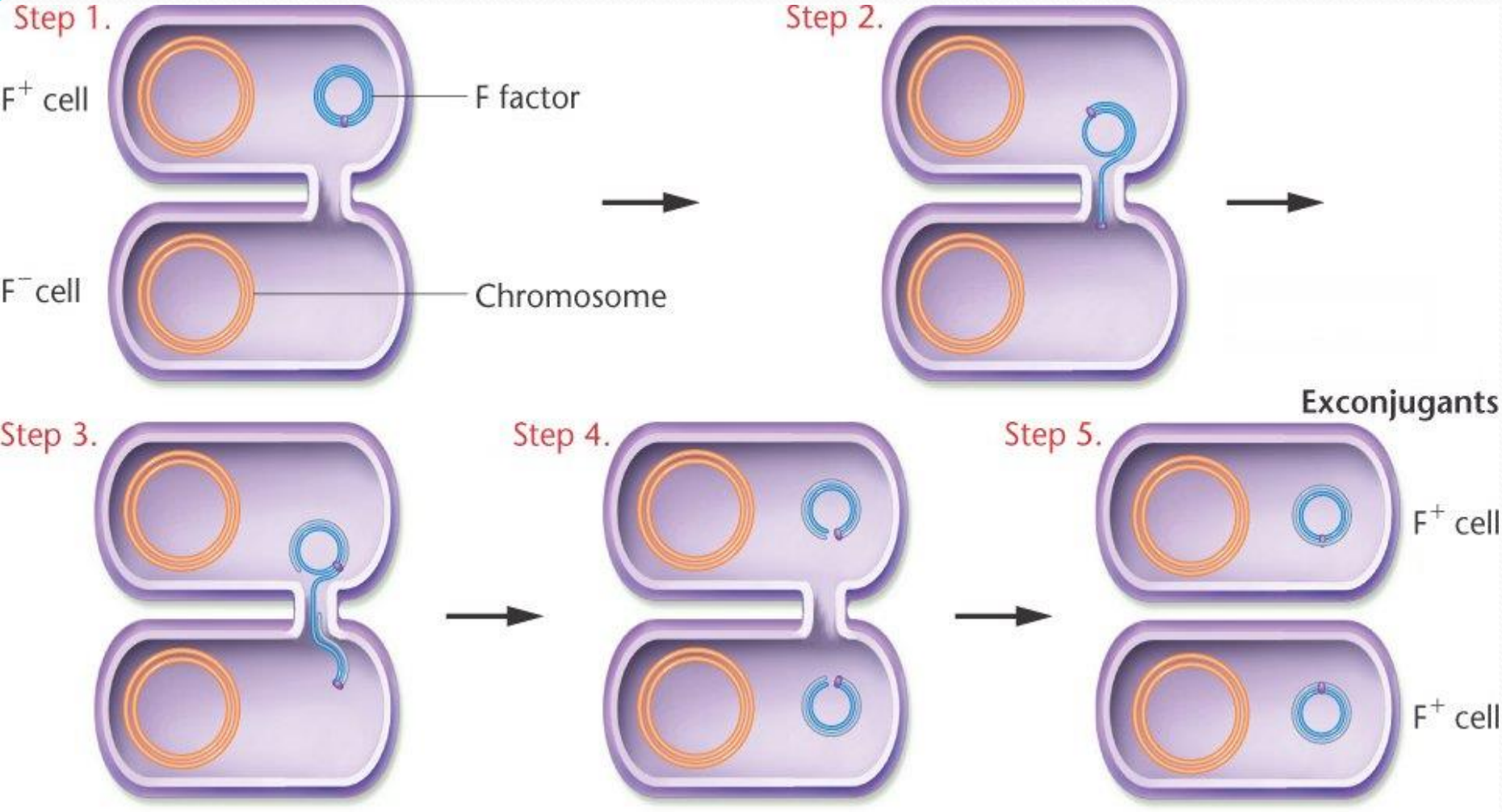
- Horizontal gene transfer is the primary reason for bacterial antibiotic resistance, and plays an important role in the evolution of bacteria that can degrade novel compounds such as human-created pesticides and in the evolution, maintenance, and transmission of virulence. This horizontal gene transfer often involves temperate bacteriophages and plasmids. Genes that are responsible for antibiotic resistance in one species of bacteria can be transferred to another species of bacteria through various mechanisms (e.g., via F-pilus), subsequently arming the antibiotic resistant genes' recipient against antibiotics, which is becoming a medical challenge to deal with. Horizontal gene transfer is common among bacteria, even among very distantly related ones. This process is thought to be a significant cause of increased drug resistance when one bacterial cell acquires resistance, and the resistance genes are transferred to other species. Transposition and horizontal gene transfer, along with strong natural selective forces have led to multi-drug resistant strains of S. aureus and many other pathogenic bacteria. Horizontal gene transfer also plays a role in the spread of virulence factors, such as exotoxins and exoenzymes, amongst bacteria. A prime example concerning the spread of exotoxins is the adaptive evolution of Shiga toxins in *E. coli* through horizontal gene transfer via transduction with Shigella . Strategies to combat certain bacterial infections by targeting these specific virulence factors and mobile genetic elements have been proposed. For example, horizontally transferred genetic elements play important roles in the virulence of *E. coli, Salmonella, Streptococcus and C. perfringens.*

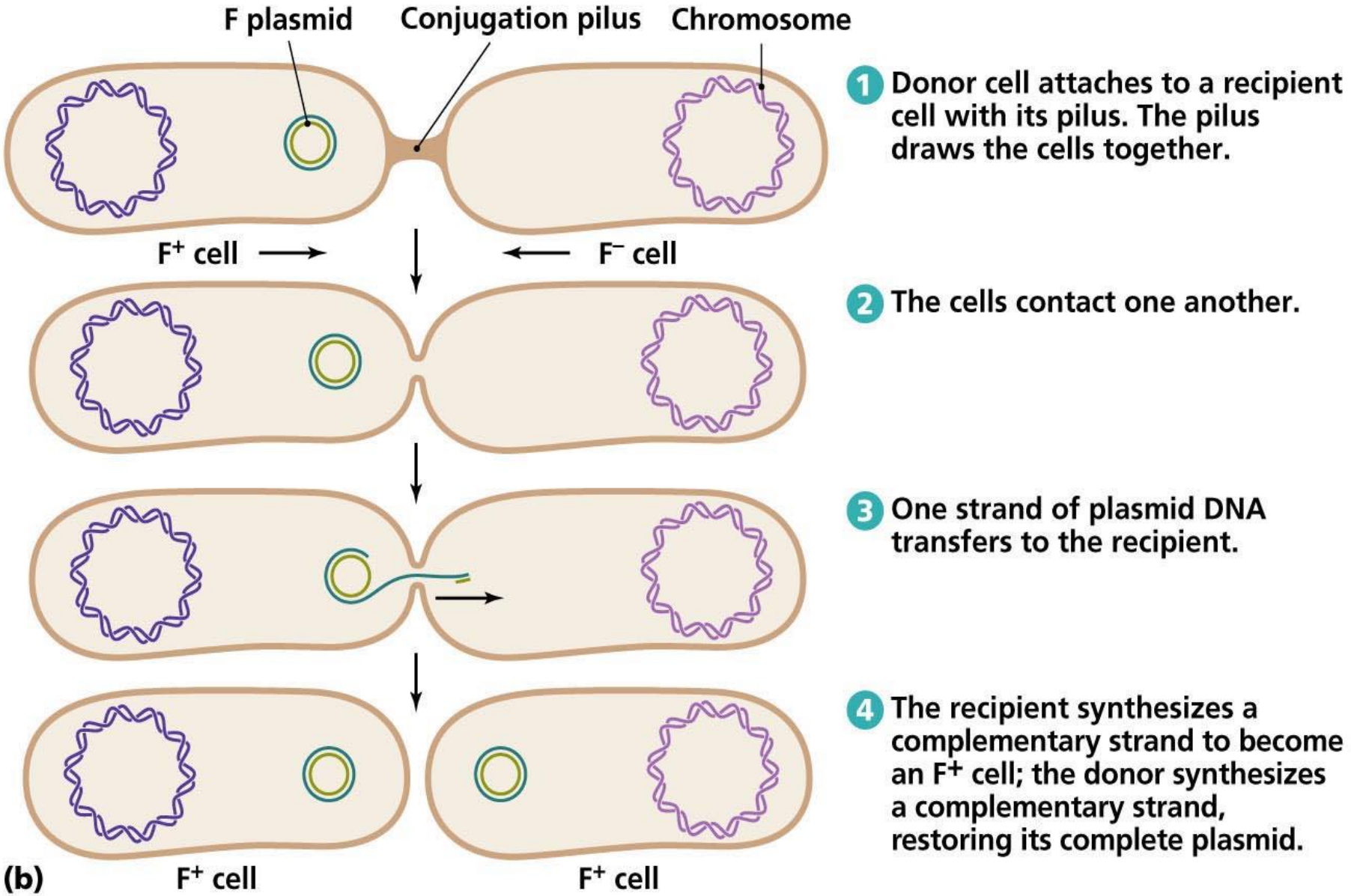
- **Mechanism**
- In bacteria genetic transfer can happen three ways; **Transformation, Transduction & conjugation**

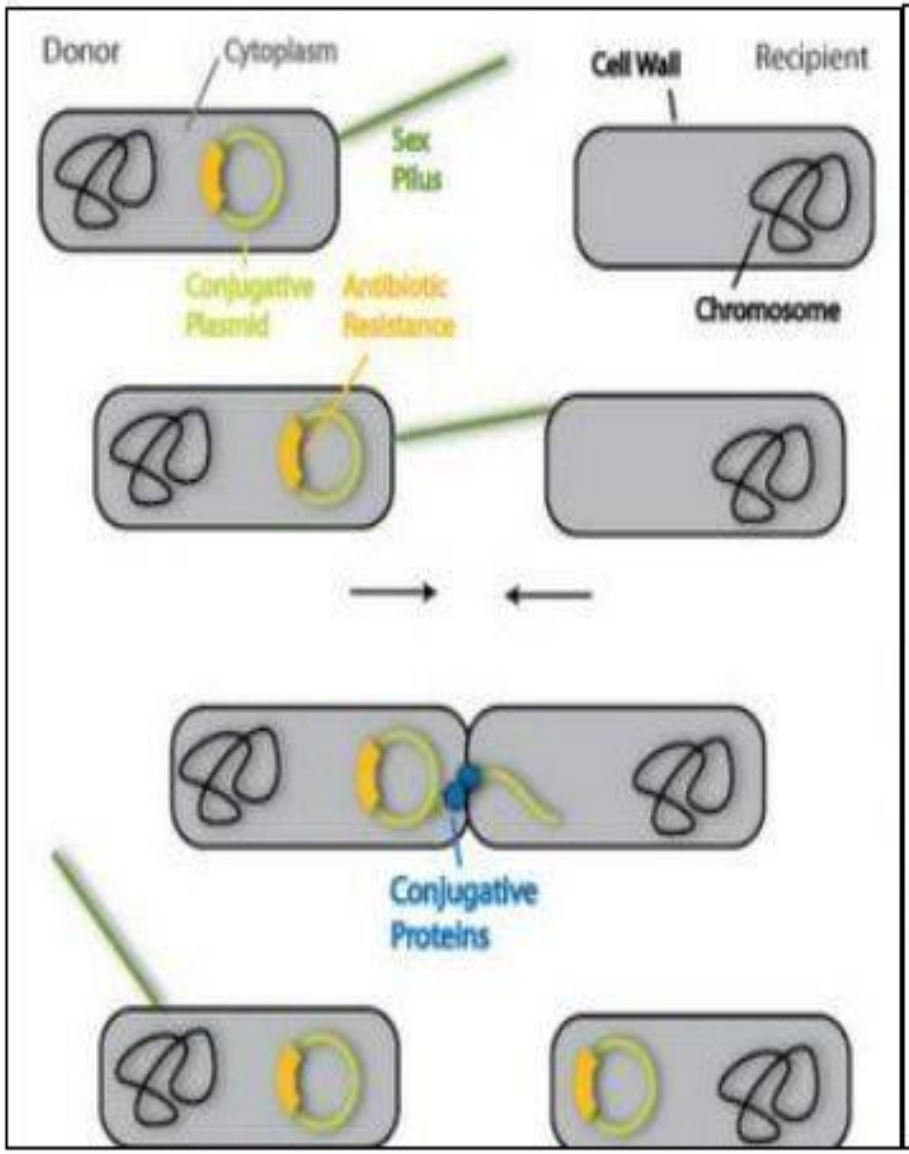
- **1-Bacterial conjugation**, a process that involves the transfer of DNA via a plasmid from a donor cell to a recombinant recipient cell during cell-to-cell contact (by Pilli). The F plasmid, facilities conjugation, this can give a bacterium new genes that may help it survive in a changing environment (for example plasmids can give the bacteria genes drug resistance). Remember that a recombination event must occur after transfer in order that the change in the genome be heritable (passed on to the next generation).

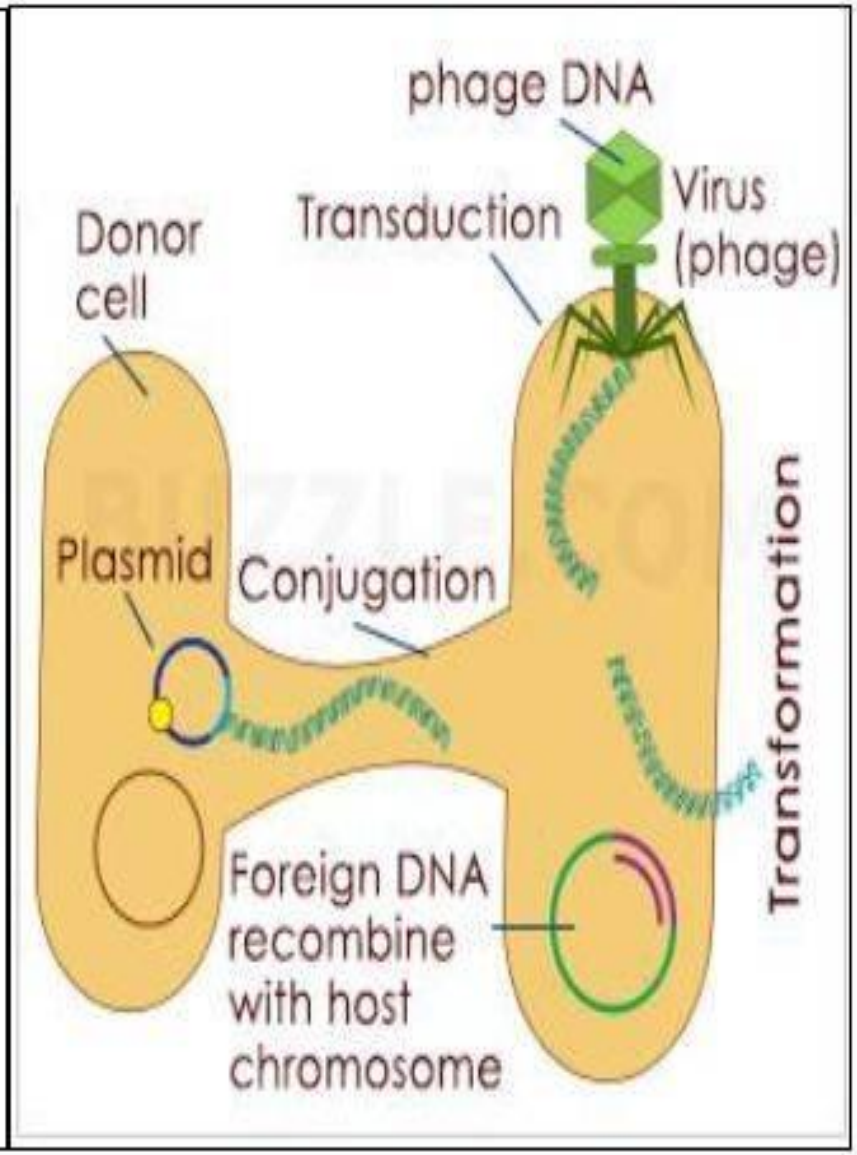

Donor
Cytoplasm
Cell Wall
Recipient
Sex
Pilus
Conjugative
Plasmid
Antibiotic
Resistance
Chromosome
Conjugative
Proteins


1.
Chromosomal DNA
F plasmid
Chromosomal DNA
Pilus
Donor
Recipient
2.
3.
DNA polymerase
Relaxasome
Transferasome
4.
F plasmid
Pilus
F plasmid
Pilus
Old donor
New donor

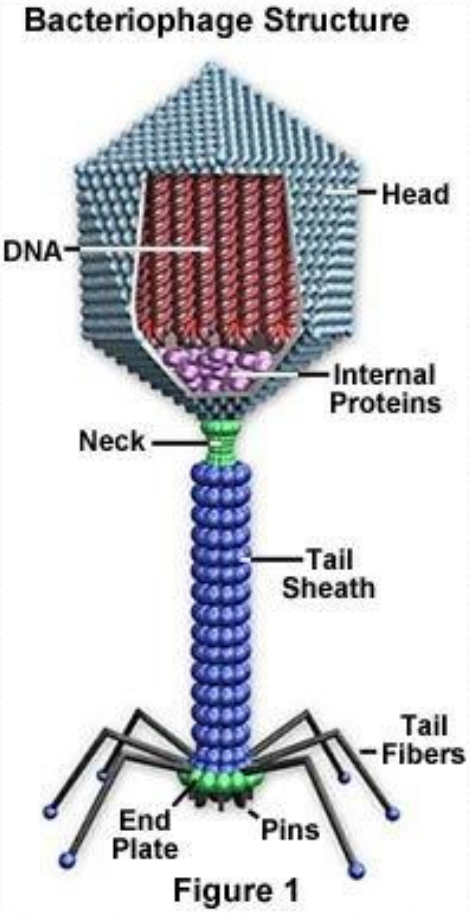

Step 1.
F+ cell
F factor
F− cell
Chromosome
Step 2.
Step 3.
Step 4.
Step 5.
Exconjugants
F+ cell
F+ cell

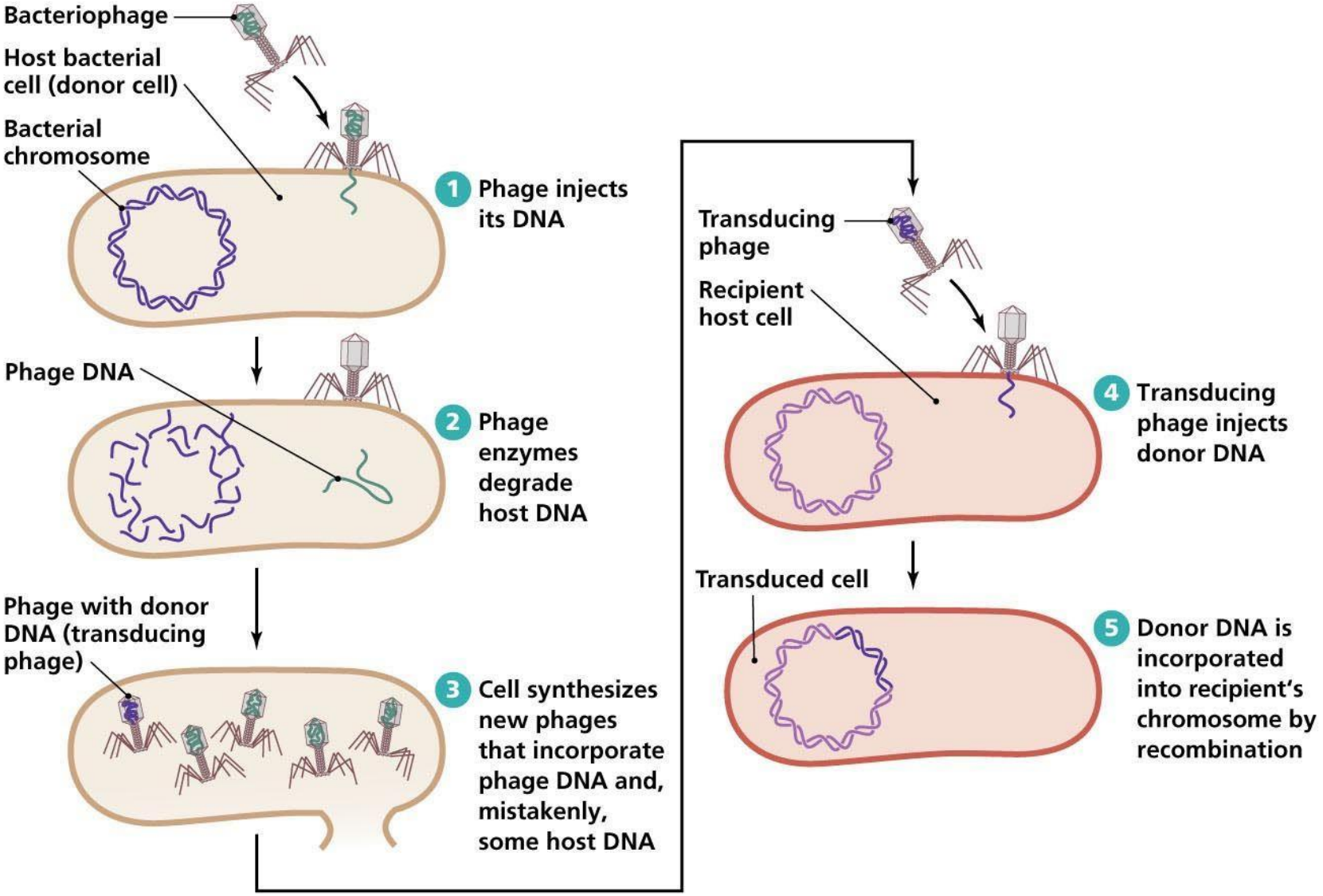


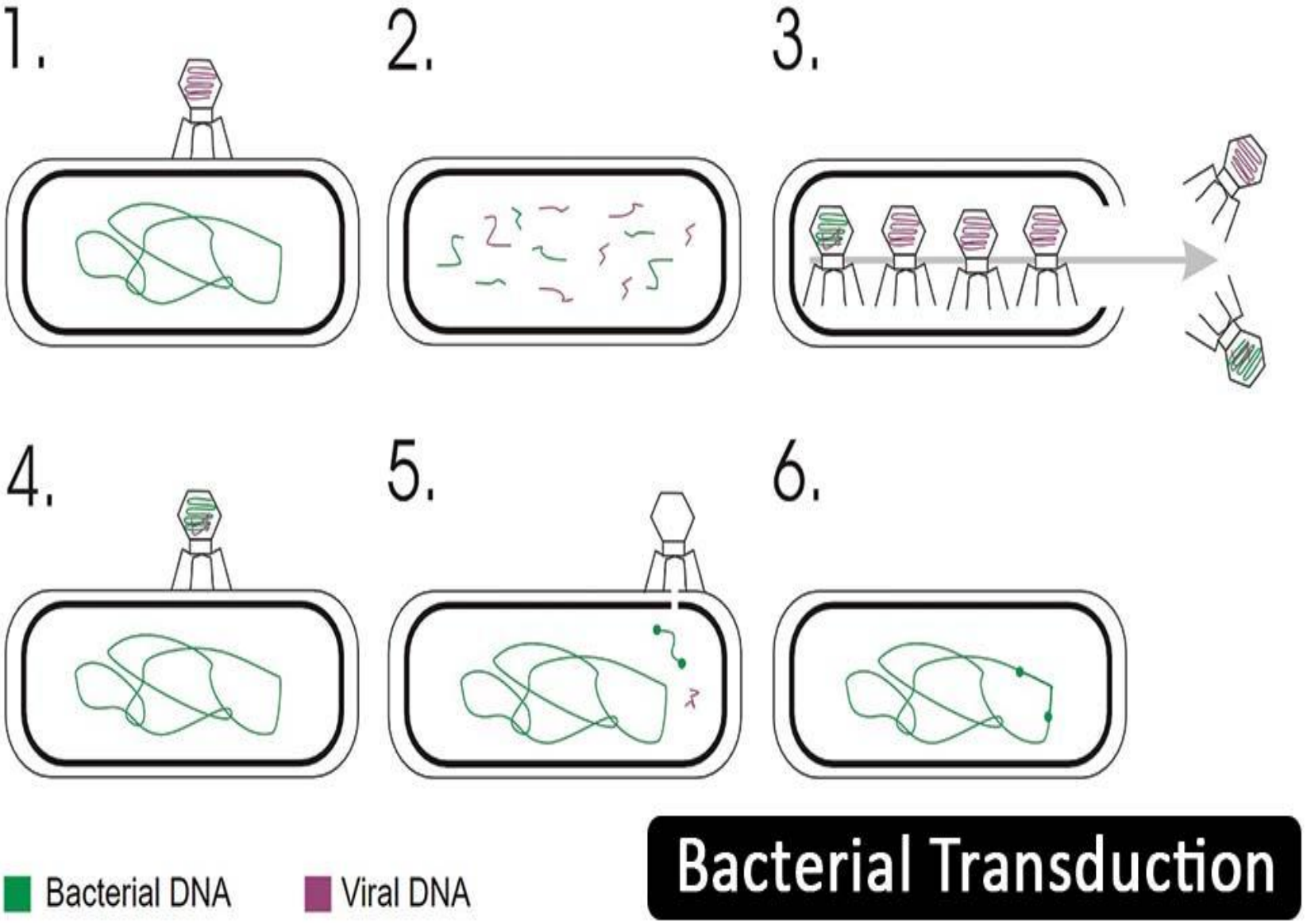

Donor
Cytoplasm
Cell Wall
Recipient
Sex Pilus
Conjugative Plasmid
Antibiotic Resistance
Chromosome
Conjugative Proteins

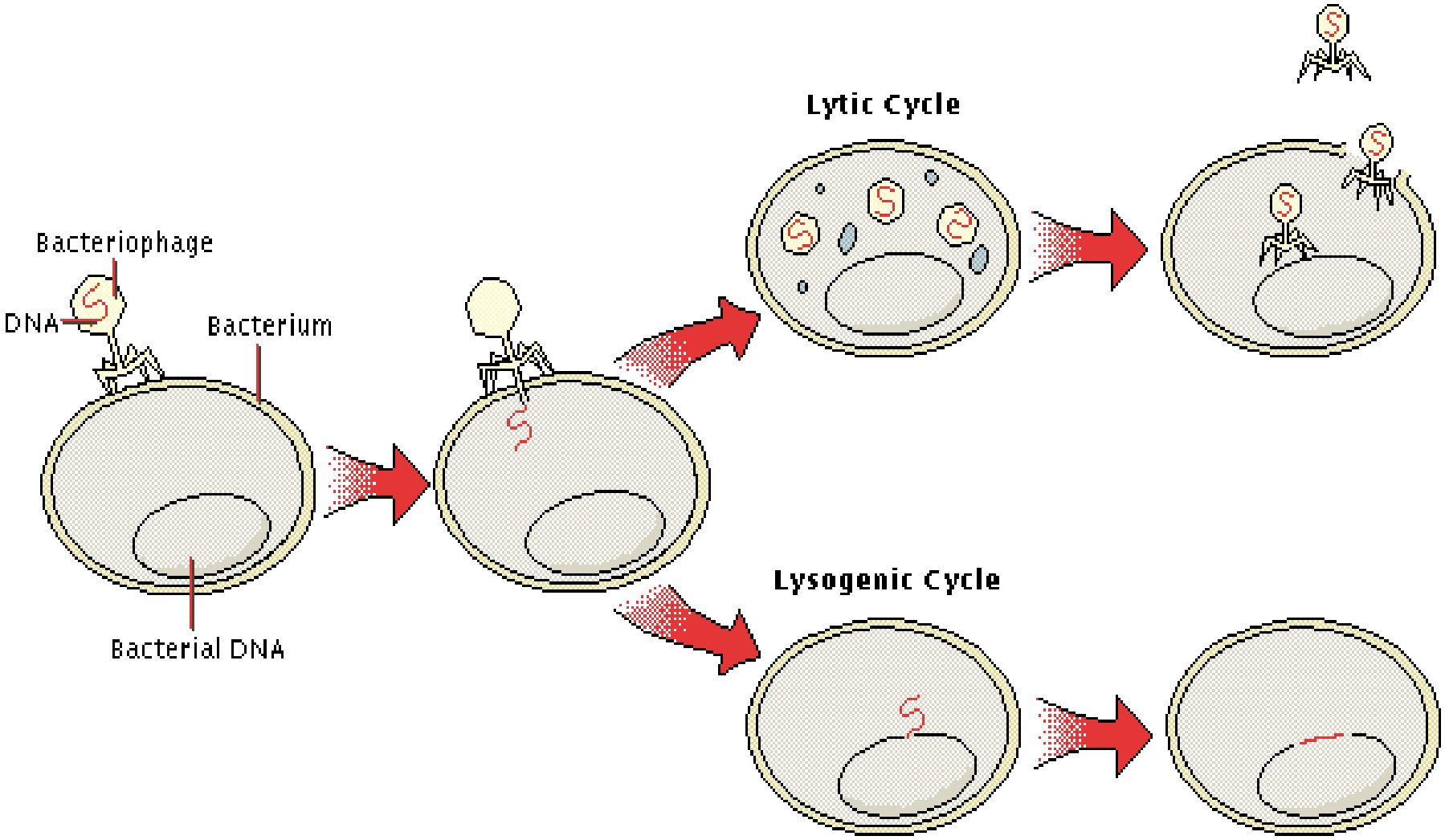

phage DNA
Virus (phage)
Transduction
Donor cell
Plasmid
Conjugation
Transformation
Foreign DNA recombine with host chromosome

- **2-Transduction**, the process in which bacterial DNA is moved from one bacterium to another by a virus (a bacteriophage, or lambda phage). Phages are obligatory intracellular parasites and must invade a host cell in order to reproduce. **There are two cycle of Transduction; lytic cycle and lysogenic cycle.** Phage lambda T4 multiplies by the lytic cycle which kills the host and lambda multiplies by the lysogenic cycle which does not cause the death of the host cell. In lysogeny, the phage DNA remains latent in the host until it breaks out in a lytic cycle. On the other hand, **Transduction can be generalized or specialized**. In generalized transduction a phage attaches to cell wall of bacterium and injects DNA, While In specialized transduction, only certain bacterial genes can be transferred.

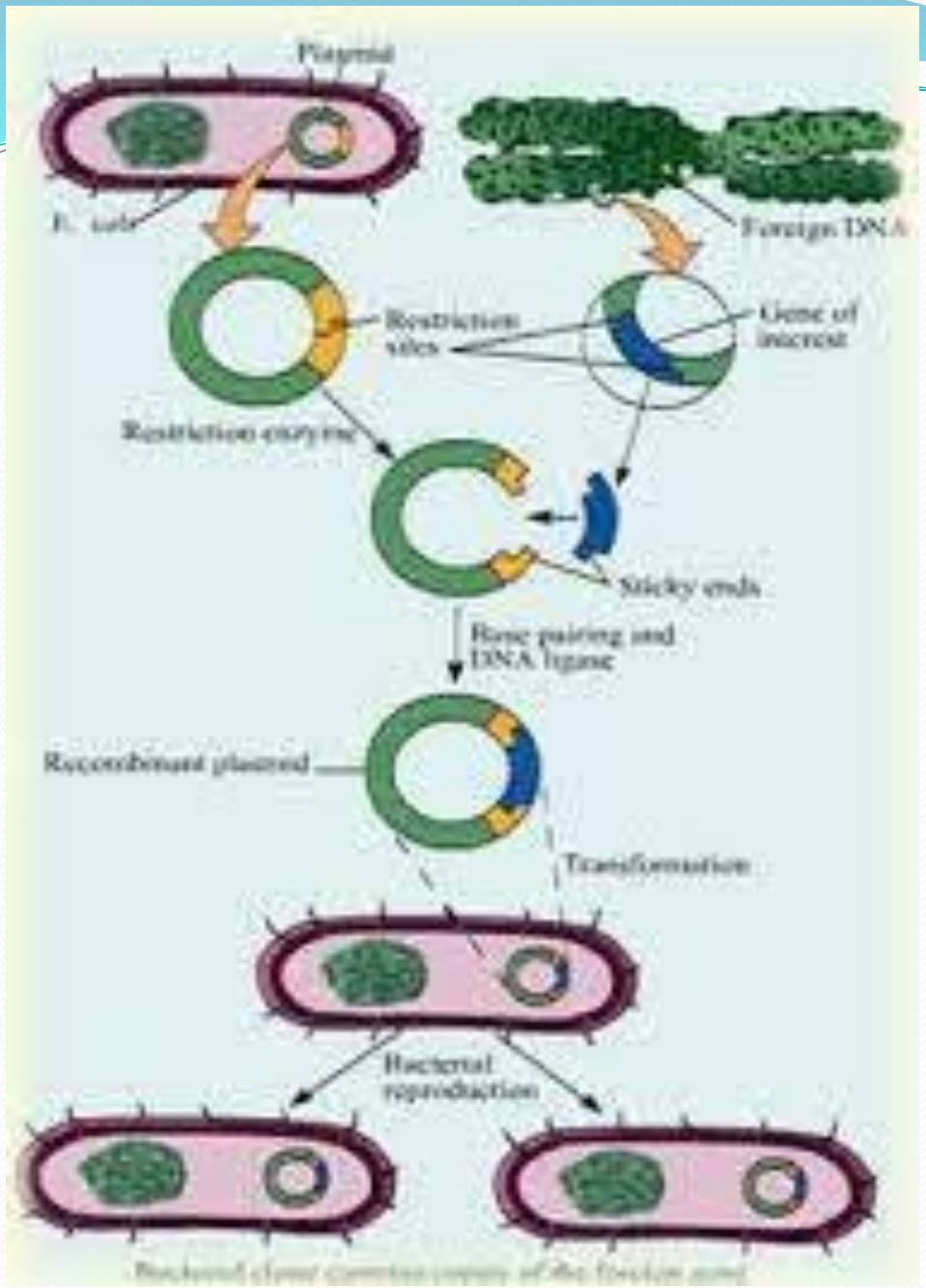

Bacteriophage Structure
Head
DNA
Internal Proteins
Neck
Tail Sheath
Tail Fibers
End Plate
Pins
Figure 1

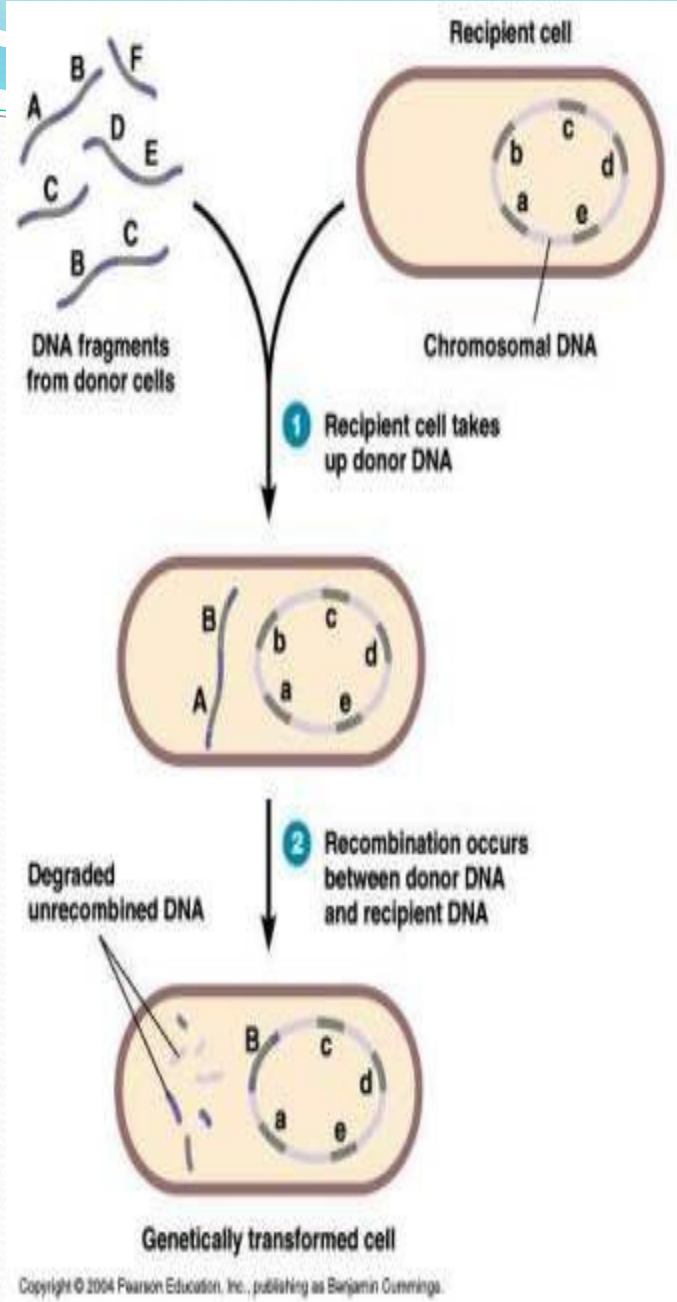



1.
2.
3.
4.
5.
6.
Bacterial DNA
Viral DNA
Bacterial Transduction


Bacteriophage
DNA
Bacterium
Bacterial DNA
Lytic Cycle
Lysogenic Cycle

- **3-Transformation**, the genetic alteration of a cell resulting from the introduction, uptake and expression of foreign genetic material (DNA or RNA). This process is relatively common in bacteria, but less so in eukaryotes. After death or cell lyses, some bacteria release their DNA into the environment. Other bacteria, generally of the same species, can come into contact with these fragments, take them up and incorporate them into their DNA by recombination. Any DNA that is not integrated into the chromosome will be degraded. The genetically transformed cell is called a recombinant cell because it has a different genetic makeup than the donor and the recipient. Recombination can give rise to genetic diversity in the population. Transformation is often used in laboratories to insert novel genes into bacteria for experiments or for industrial or medical applications. See also molecular biology and biotechnology.


Plasmid
Foreign DNA
Restriction sites
Gene of interest
Sticky ends
Base pairing and DNA ligase
Recombinant plasmid
Transformation
Bacterial reproduction


Recipient cell
DNA fragments from donor cells
Chromosomal DNA
1 Recipient cell takes up donor DNA
2 Recombination occurs between donor DNA and recipient DNA
Degraded unrecombined DNA
Genetically transformed cell
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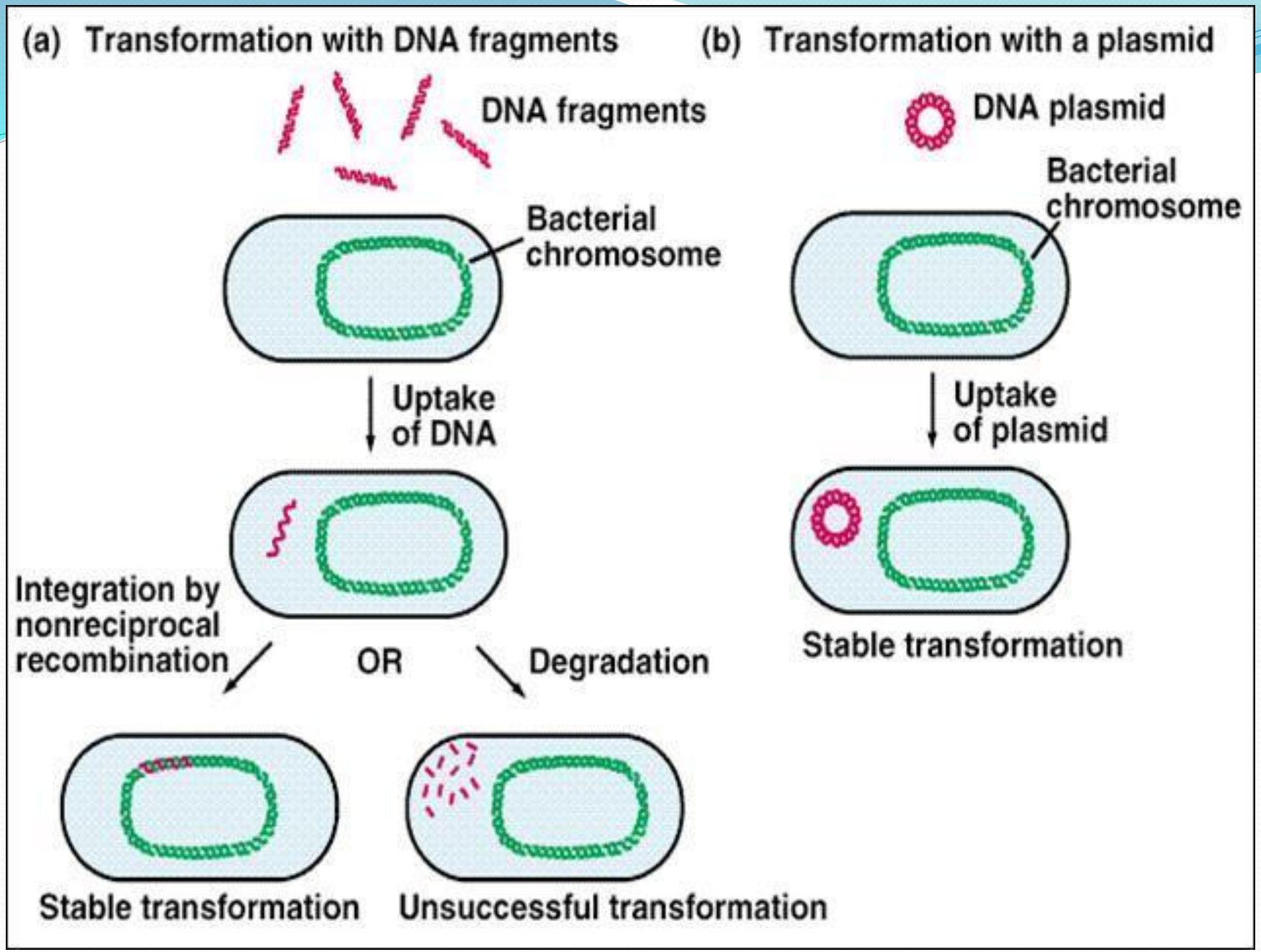
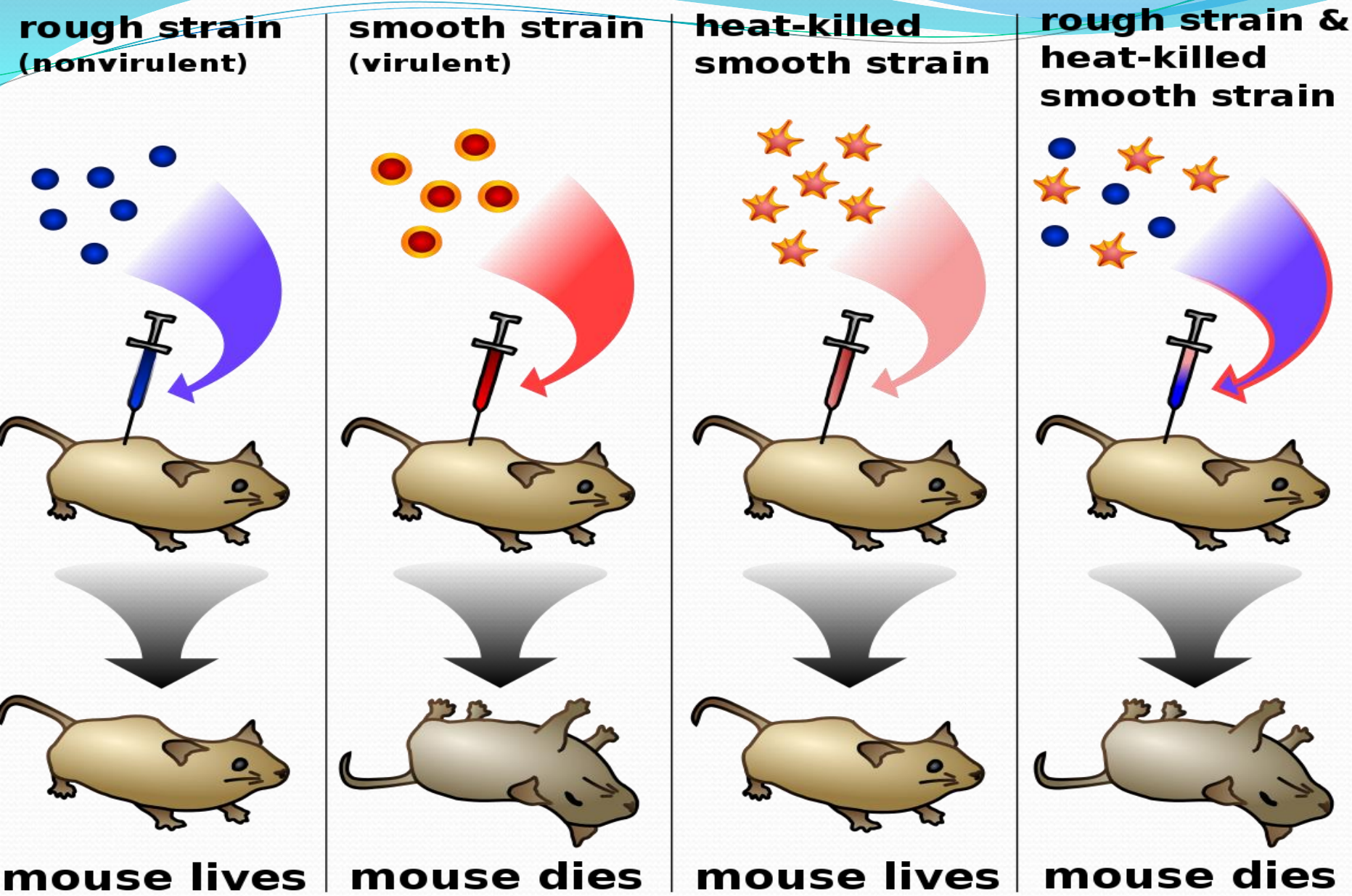

(a) Transformation with DNA fragments
(b) Transformation with a plasmid
DNA fragments
DNA plasmid
Bacterial chromosome
Bacterial chromosome
Uptake of DNA
Uptake of plasmid
Integration by nonreciprocal recombination
OR
Degradation
Stable transformation
Stable transformation
Unsuccessful transformation

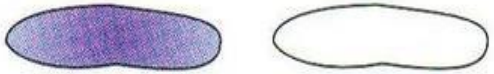
- **Griffith's Experiment**
- The transformation process was first demonstrated in 1928 by Frederick Griffith.
- Griffith experimented on Streptococcus pneumoniae, a bacteria that causes pneumonia in mammals.
- When he examined colonies of the bacteria on petri plates, he could tell that there were two different strains.
- The colonies of one strain appeared smooth. Later analysis revealed that this strain has a polysaccharide capsule and is virulent, that it, it causes pneumonia.
- The colonies of the other strain appeared rough. This strain has no capsules and is avirulent.
- When Griffith injected living encapsulated cells into a mouse, the mouse died of pneumonia and the colonies of encapsulated cells were isolated from the blood of the mouse.
- When living nonencapsulated cells were injected into a mouse, the mouse remained healthy and the colonies of nonencapsulated cells were isolated from the blood of the mouse.
- Griffith then heat killed the encapsulated cells and injected them into a mouse.
- The mouse remained healthy and no colonies were isolated.
- The encapsulated cells lost the ability to cause the disease.

- **Griffith's Experiment**
- However, a combination of heat-killed encapsulated cells and living nonencapsulated cells did cause pneumonia and colonies of living encapsulated cells were isolated from the mouse.
- How can a combination of these two strains cause pneumonia when either strand alone does not cause the disease. If you guessed the process of transformation you are right
- The living nonencapsulated cells came into contact with DNA fragments of the dead capsulated cells.
- The genes that code for their capsule entered some of the living cells and a crossing over event occurred.
- The recombinant cell now has the ability to form a capsule and cause pneumonia.
- All of the recombinant's offspring have the same ability.
- That is why the mouse developed pneumonia and died.


rough strain
(nonvirulent)
smooth strain
(virulent)
heat-killed
smooth strain
rough strain &
heat-killed
smooth strain
mouse lives
mouse dies
mouse lives
mouse dies

- **Transposon** (**jumping gene or Transposable Genetic Elements**) are pieces of DNA that can move from one location on the chromosome another, from plasmid to chromosome or vice versa or from one plasmid to another. Transposons is a mobile segment of DNA that can sometimes pick up a resistance gene and insert it into a plasmid or chromosome, thereby inducing horizontal gene transfer of antibiotic resistance.
- **-Plasmids** are small, circular pieces of DNA that are separate and replicate independently from the bacterial chromosome. Plasmids contain only a few genes that are usually not needed for growth and reproduction of the cell.

-Recombination: Genetic recombination refers to the exchange between two DNA molecules. It results in new combinations of genes on the chromosome.

Step	Microscopic Appearance of Cell	Chemical Reaction in Cell Wall (very magnified view)
1. Crystal violet		 Both cell walls affix the dye
2. Gram's iodine		 Dye crystals trapped in wall / No effect of iodine
3. Alcohol		 Crystals remain in cell wall / Cell wall partially dissolved, loses dye
4. Safranin (red dye)		 Red dye has no effect / Red dye stains the colorless cell

Gram (+) Gram (–)

Gram (+) Gram (–)