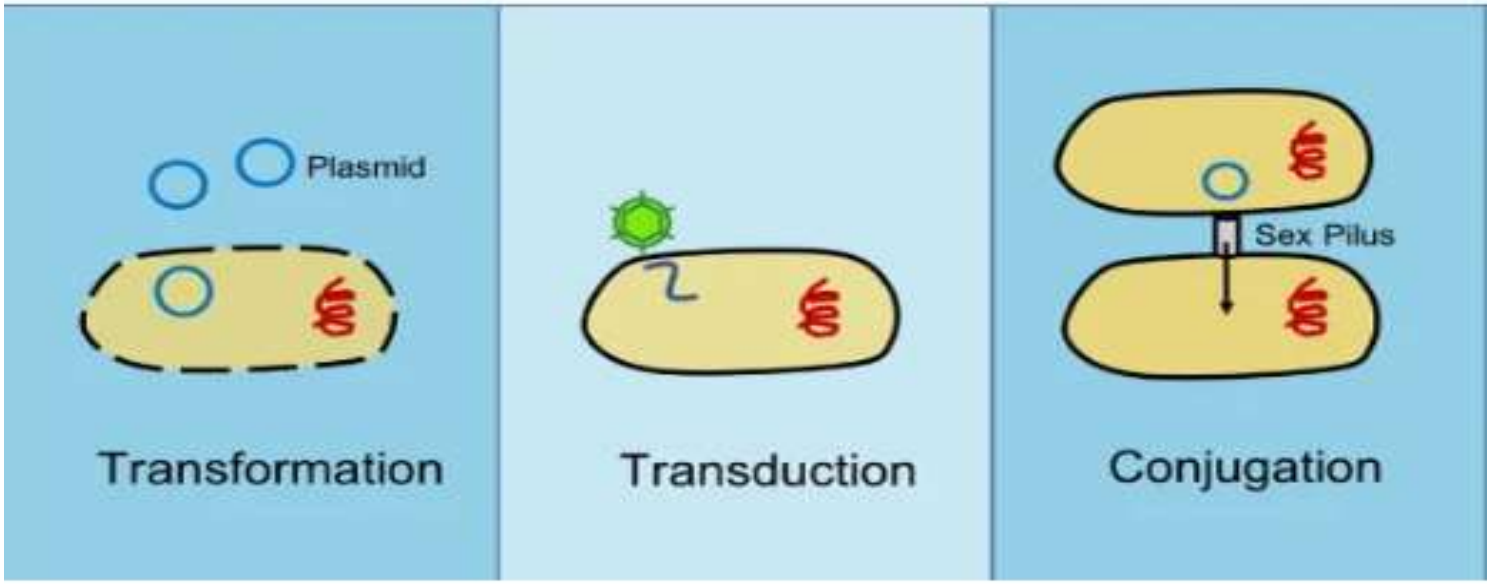
GENETIC RECOMBINATION IN BACTERIA (TRANSFORMATION AND TRANSDUCTION)



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For B.Sc. (Hons.) Part I Paper 1 Group C (Microbiology)

Lecture No. 28

Transformation

- Transformation takes place when a bacterium takes up DNA from the medium in which it is growing.
- After transformation recombination may occur between the introduced genes and those of the bacterial chromosome.
- The first demonstration of bacterial transformation was done with ***Streptococcus pneumoniae*** in 1928 by an english bacteriologist **F. Griffith** .
- It led to discovery that DNA is genetic material.
- Many bacteria can acquire new genes by taking up DNA molecules (e.g., a plasmid) from their surroundings.
- The cells of *S. pneumoniae* (also known as the pneumococcus) are usually surrounded by a gummy capsule made of a polysaccharide.
- When *S. pneumoniae* grown on the surface of a solid culture medium, the capsule causes the colonies to have a glistening, smooth appearance. These cells are called **"S"** cells.

➢ However, after prolonged cultivation on artificial medium, some cells lose the ability to form the capsule, and the surface of their colonies is wrinkled and rough ("**R**").

➢ With the loss of their capsule, the bacteria also **lose their virulence**.

➢ Injection of a single **S** *pneumococcus* into a mouse will kill the mouse in 24 hours or so.

➢ But an injection of millions (100×10^6) of **R** cells did not kill the bacteria.

Why?

➢The capsule prevents the pneumococci from being engulfed and destroyed by scavenging cells, neutrophils and macrophages, in the body.

➢The R forms are completely at the mercy of phagocytes.

➢ Pneumococci also occur in over 90 different **types**: **I**, **II**, **III** and so on.

➢The types differ in the chemistry of their polysaccharide capsule.

➢ In Griffith's experiment, the virulent *S. pneumoniae* that has a smooth (S) capsule in its appearance was capable of causing lethal infections upon injection into mice

➢ Because of their lack of a protective coat, the R-type bacteria are destroyed by the animal after the injection. Mice are still alive after the injection of R-type bacteria

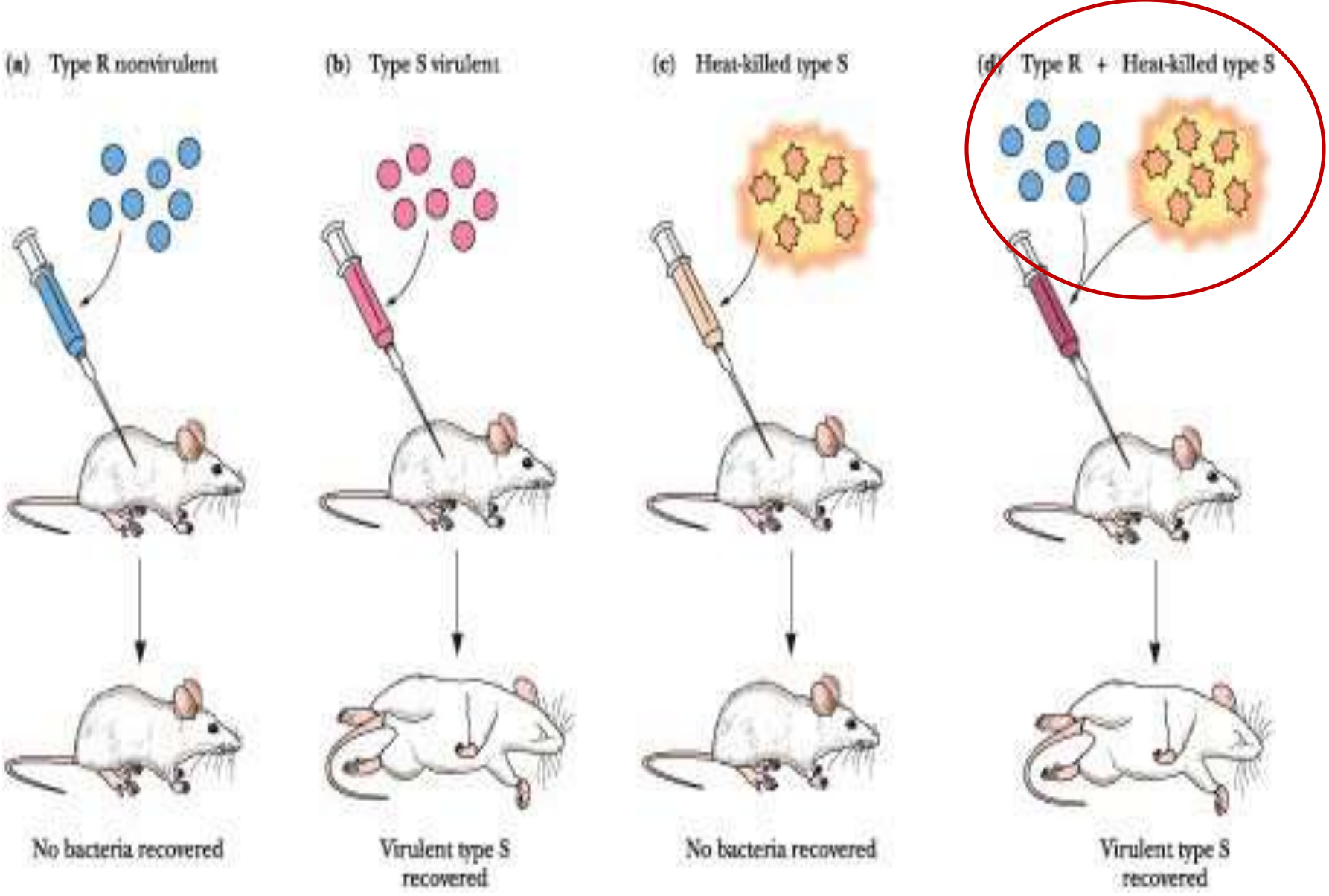


Figure: Griffith's Experiment

- When S-type bacteria were killed by the heat, they were no longer able to cause a lethal infection upon injection into mice alone.
- However, when the heat-killed S-type bacteria and live R-type bacteria were injected together, neither of which causes lethal infection alone, the mice died as a result of pneumonia infection.
- It was found that the virulent trait that was responsible for production of the polysaccharide capsule was passed from the heat-killed S-type cells into the live R-type cells, thus converting the R-type bacteria into S-type bacteria, allowing it to become virulent and lethal by evading the host's immune response.

- Griffith concluded that the heat-killed bacteria somehow converted live avirulent cells to virulent cells, and he called the component of the dead S-type bacteria the “transforming principle.”

- Transformation takes place to a limited extent in many species of bacteria , but laboratory techniques have been developed that increase the rate of DNA uptake.
- These techniques such as CaCl2 based approach or electroporation , are widey used in recombinant DNA technology.

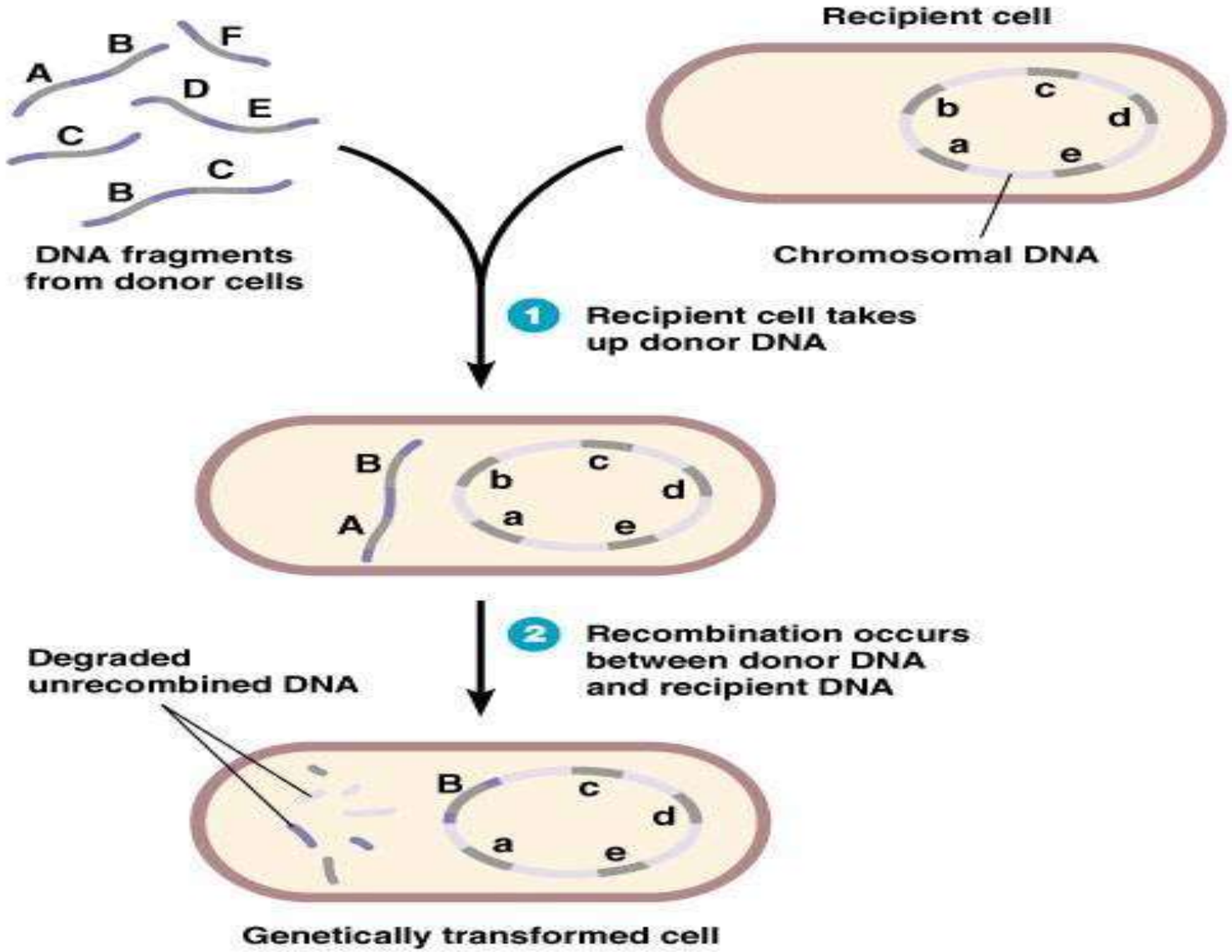
Factors affecting Transformation:

1. Size of DNA
2. Competence of the recipient: Some bacteria are able to take DNA naturally. However , these bacteria only take up DNA at particular time in their growth cycle, when they produce a specific protein called a competence factor. At this stage bacteria are said to be competent .

✓ Other bacteria are not able to take up DNA naturally. However in these bacteria competence can be induced in vitro by treatment with chemical (e.g. CaCl2)

Steps in Transformation

1. **Uptake of DNA:** Uptake of DNA differ in gram positive and gram negative bacteria. In gram positive bacteria the DNA is taken as a single stranded molecule and complementary strand is made in the recipient. In contrast gram negative bacteria take up double stranded DNA.
2. **Recombination:** After the donar DNA is taken up, a reciprocal recombination events occur between the chromosome and the donar DNA. Recombination require the bacterial recombination genes (Rec A,B and C) and homology between the DNA involved.



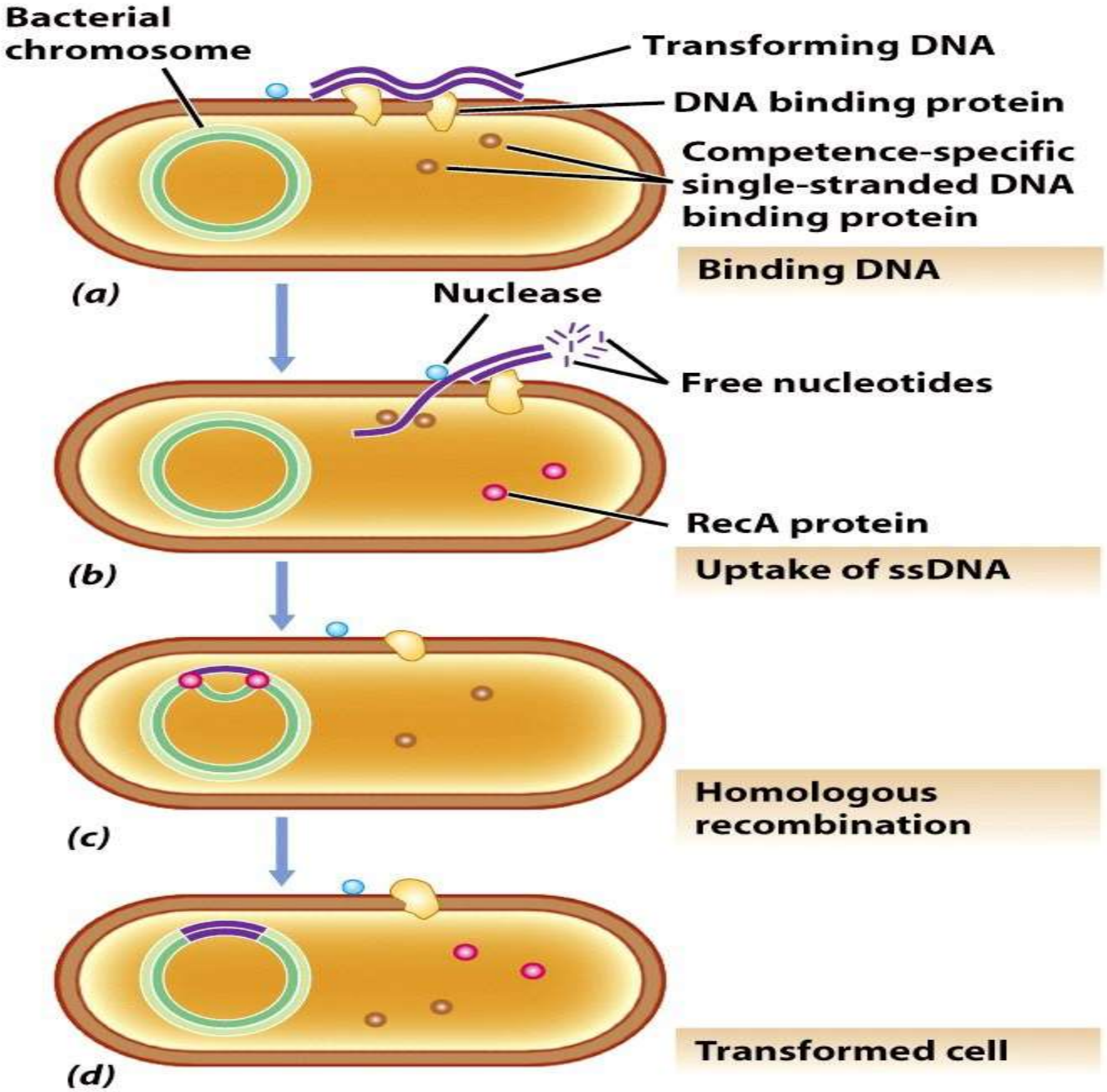


Figure 10-14 Brock Biology of Microorganisms 11/e

3. Transduction

- Transduction is the transfer of genetic information from a donar to the recipient by way of a bacteriophage.
- Transduction takes place when bacterial viruses (bacteriophages) carry DNA from one bacterium to another.
- Inside the bacterium, the newly introduced DNA may undergo recombination with the bacterial chromosome.
- Most bacteriophage has a limited host range, so transduction is normally between the bacteria of the same or closely related species only.
- The phage coat protect the DNA in the environment, So that transduction unlike transformation is not affected by nucleases in the environment.
- Not all phage can mediate transduction
- In most cases gene transfer is between members of the same bacterial species.
- If a particular phage has wide host range then transfer between species can occur.
- The ability of a phage to mediated transduction is related to the life cycle of the phage.

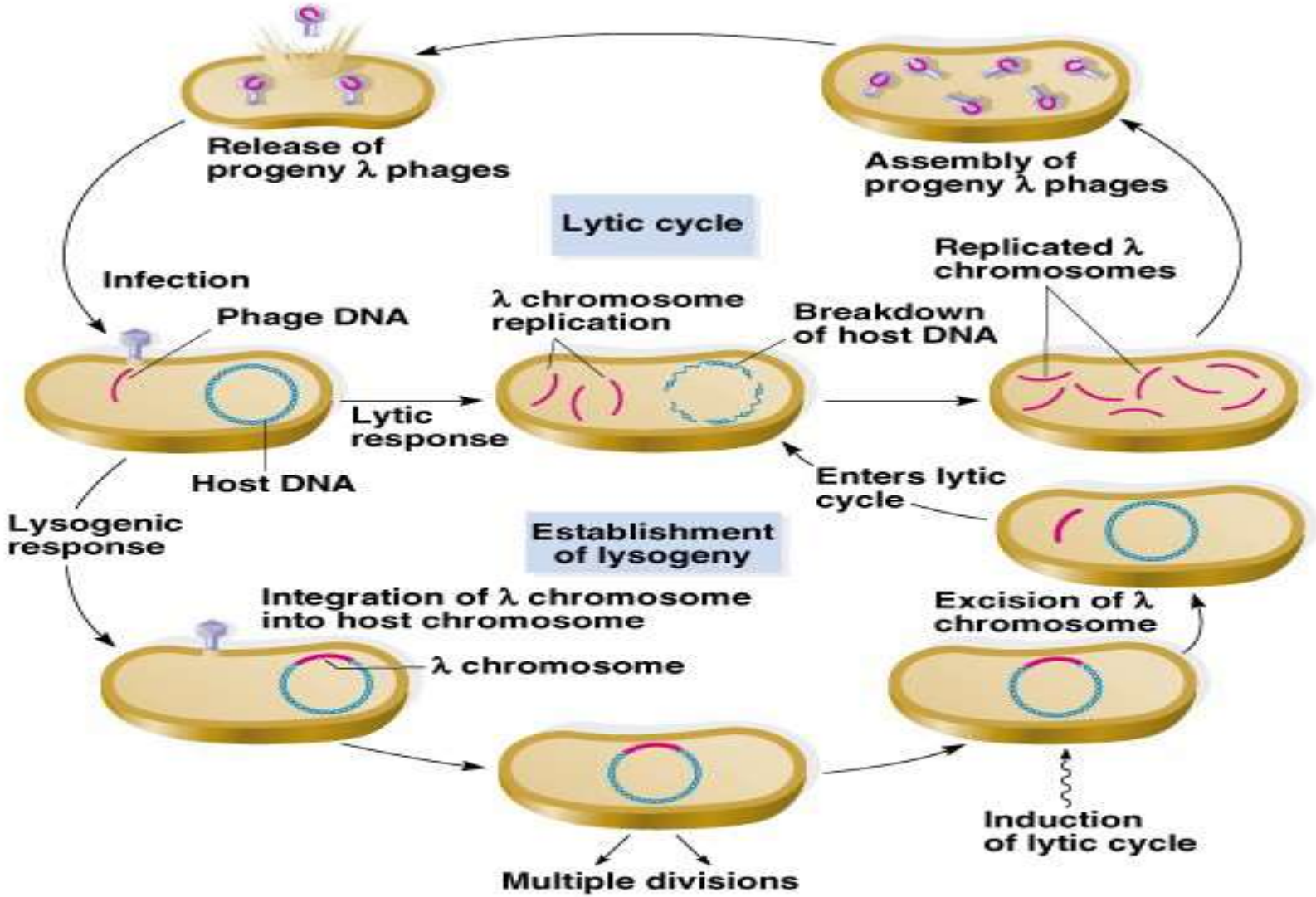


Figure: Lytic and Lysogenic cycle

Types of Transduction

Transduction are of two types

A. Generalized transduction.
B. Specialized transduction

A. Generalized transduction.

- Transduction in which potentially any bacterial gene from donar can be transferred to the recipient.
- Phages that mediate generalized transduction generally breakdown host DNA into smaller pieces and package their DNA into the phage particle by head full mechanism.
- Occasionally one of the pieces of host DNA is randomly packed into a phage coat.
- Thus, any donar gene can be potentially transferred but only enough DNA as can fit into the phase head can be transferred.
- If a recipient cell is infected by a phage that contains donar DNA, donar DNA enters the recipient.

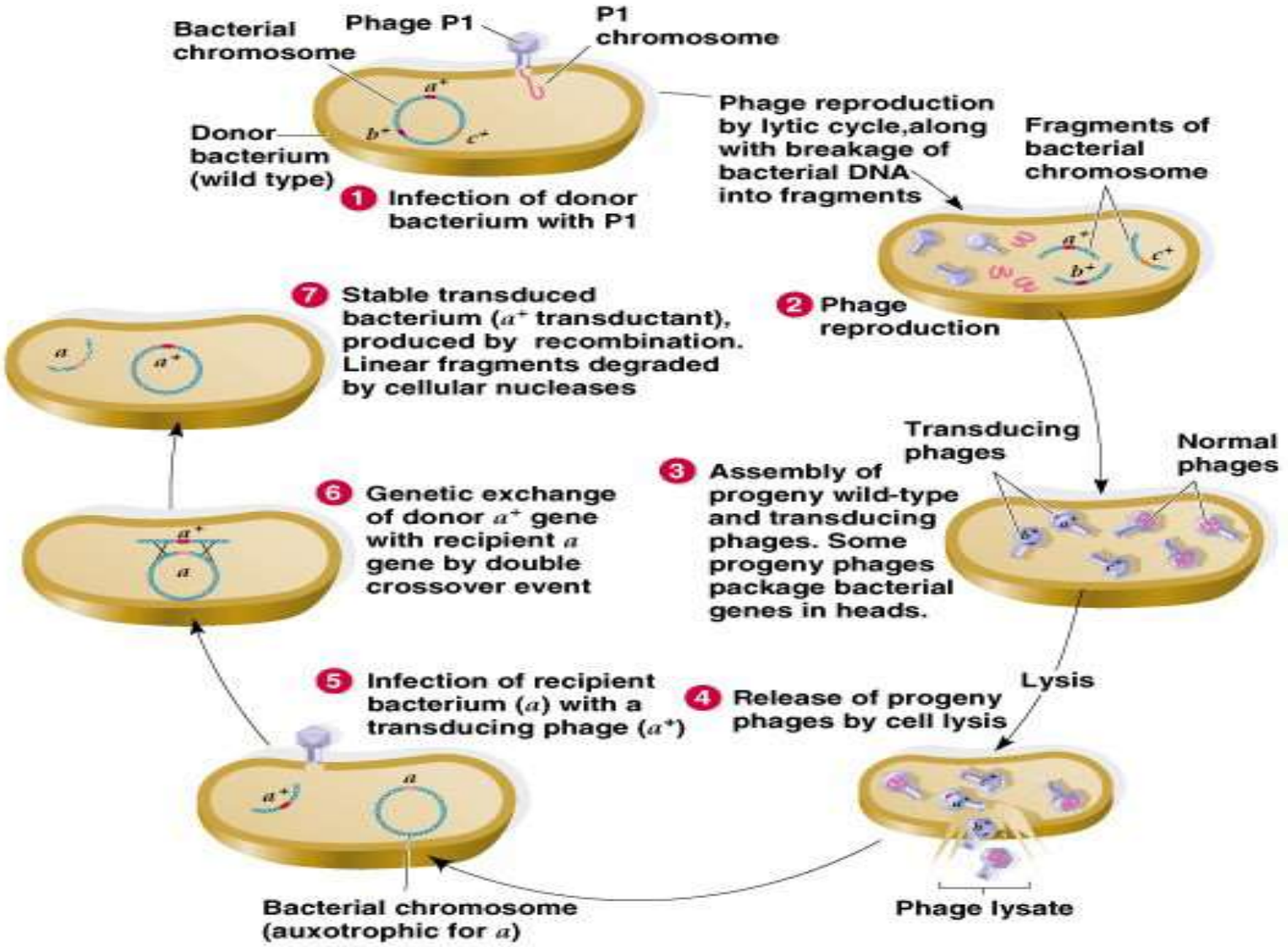


Figure: Generalized transduction of E. coli by phage P1

B. Specialized transduction:

- Transduction in which only certain donar genes is transferred to the recipient.
- Different phages may transfer different genes but an indivisual phage can only transfer certain genes.
- Specialized transduction is mediated by lysogenic or temperate phage and the genes that get transferred will depend on where the prophage has inserted in the chromosome.
- During excision of the prophage , occasionally an error occurs where some of the host DNA is excised with the phage DNA
- Only host DNA on either side of where the prophage has inserted can be transferred (i.e. specialized transduction).

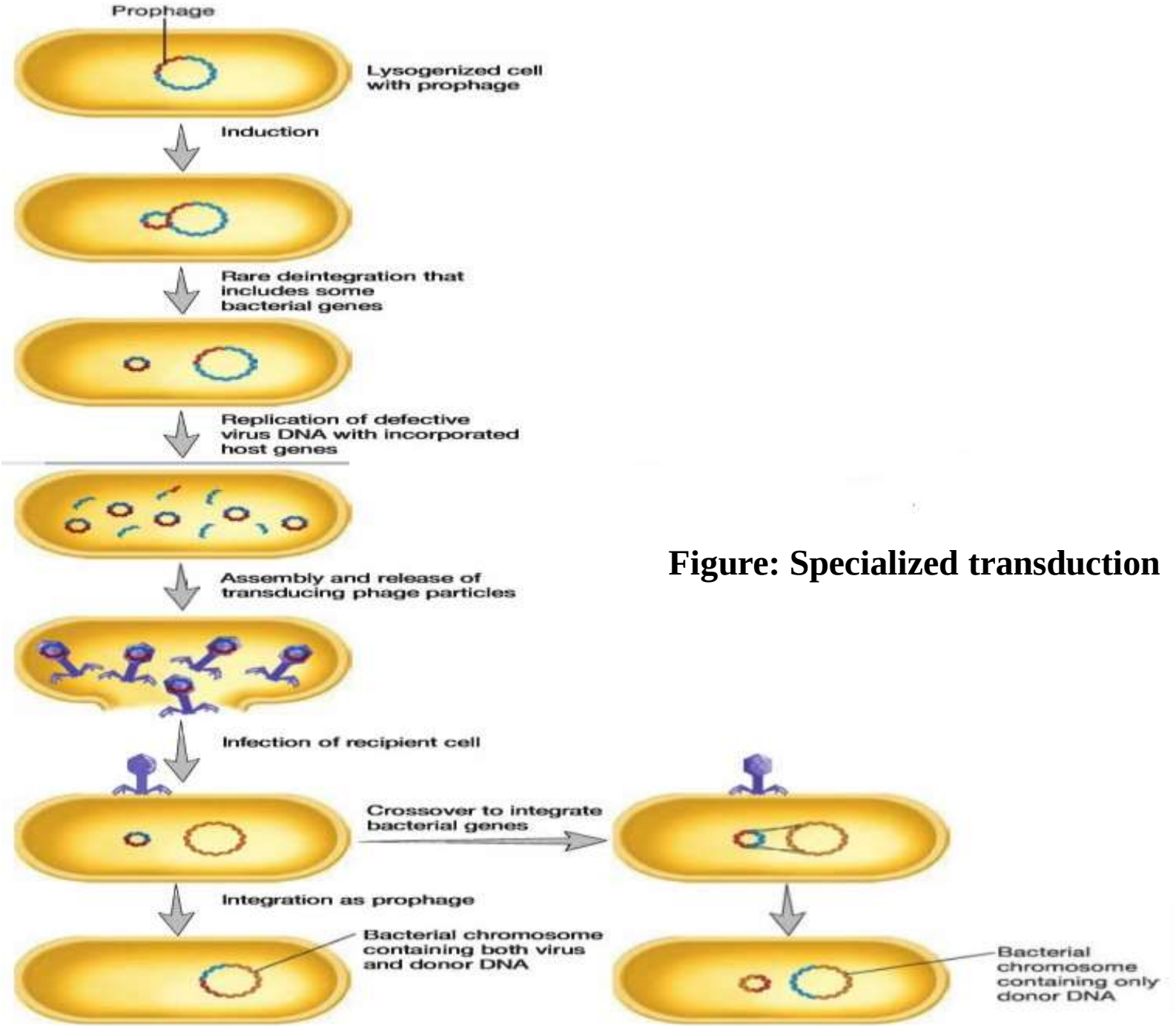


Figure: Specialized transduction

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Thank You!!!