ZO502 CELL AND MOLECULAR BIOLOGY

GENE TRANSFER METHODS IN PROKARYOTES

DR.S.S.KUNJWAL

GENE TRANSFER

- It is defined simply as a technique to efficiently and stably introduce foreign genes into the genome of target cells.
- The insertion of unrelated, therapeutic genetic information in the form of DNA into target cells.

INTRODUCTION

- Gene transfer in which bacteria genotype can be changed by transfer of genetic material from one bacterium to another.
- In such instances, the transferred DNA either:

a) Recombine with genome of the recipient bacterium or
b) Is on the plasmid capable of replication in the recipient bacterium without recombination.

- Genetic recombination it occurs when sequence of DNA from two separate sources are integrated.
- The directed desirable gene transfer from one organism to another and the subsequent stable integration & expression of foreign gene into the genome is referred as genetic transformation.
- The transferred gene is known as transgene and the organism that develop after a successful gene transfer is known as transgenic.

MECHANISM OF GENE TRANSFER

There are four mechanisms of gene transfer and recombination between bacteria cells.

1. Conjugation
2. Transformation
3. Transduction
4. Cell- cell fusion

DNA TRANSFER BY ARTIFICIAL METHODS

Physical methods

1. Microinjection
2. Biolistics transformation

Chemical methods

1. DNA transfer by calcium phosphate method
2. Liposome mediated transfer

Electrical methods

1. Electroporation

CONJUGATION

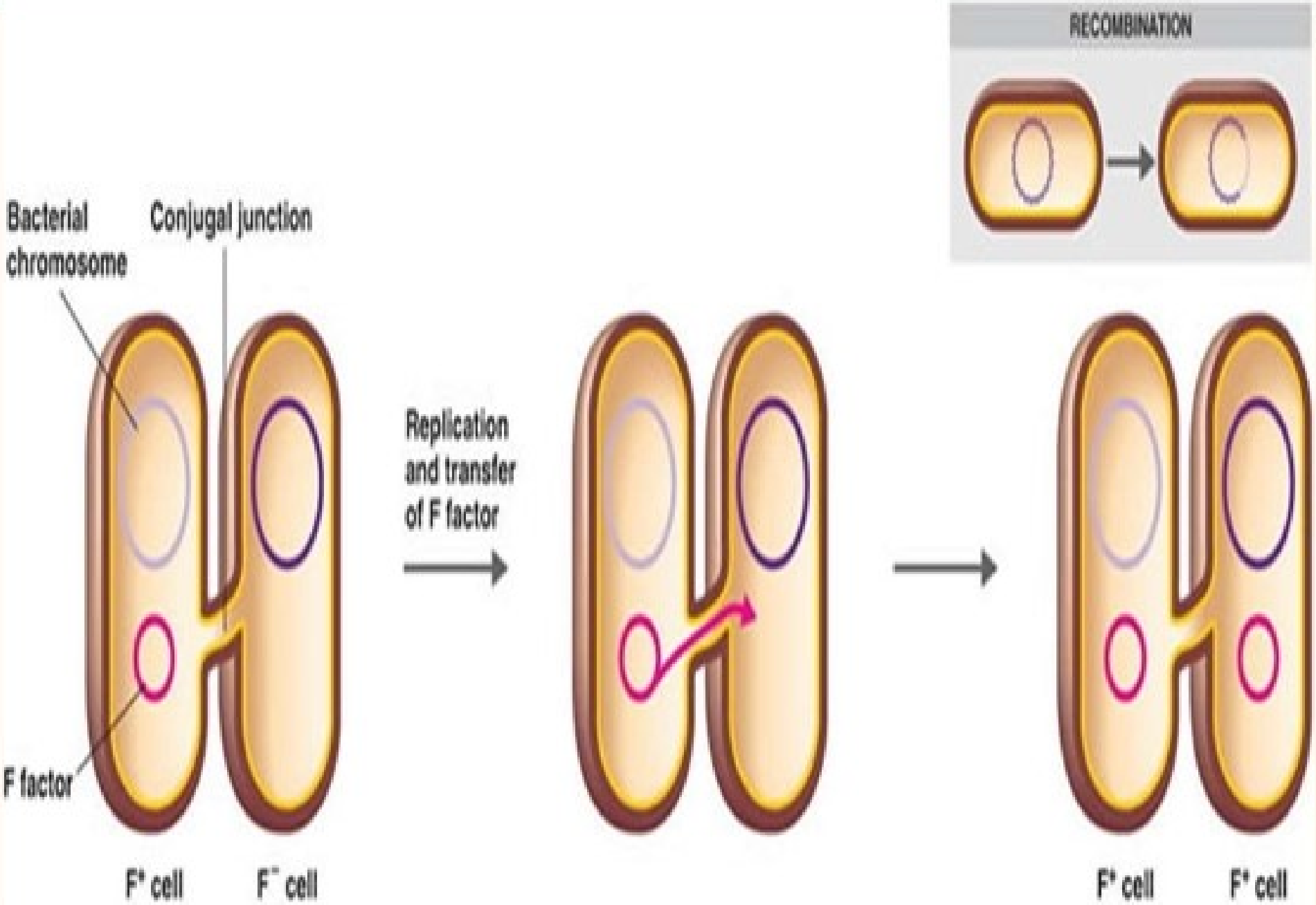
- Bacteria conjugation in E.coli was discovered by Lederberg and Tatum in 1946, when they observed sex like exchange between two mutants strains of E.coli called K12.
- Conjugation involves the transfer of plasmid from a donor(bacteria contain F plasmid) to the recipient(bacteria lacking F plasmid).

- Conjugation is more common in gram negative between strain of same species or closely related species.
- Some plasmid like F plasmid(fertility factor or sex factor) they carry tra gene that mediate their own transfer through sex pilus.
- Bacteria that have a F plasmid are referred to as as F+ or male. Those that do not have an F plasmid are F- of female.
- The F plasmid consists of 25 genes that mostly code for production of sex pilli.

Conjugation are of different types:

1) F+ conjugation- During conjugation the F+ bacteria synthesise a modified pilus (sex pilus) through which genetic material transfer, the result is two male cells.

- This attached pilus is a temporary cytoplasmic bridge through which a replicating F plasmid is transferred from the male to the female.
- Conjugation in gram positive does not depend on sex pili, the donor cells form a protein adhesin on the surface that causes donor and recipient cells to aggregate. Eg. *Bacillus subtillis, Streptocoocus lactis, Enterococcus fecaelis*



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted to an F^+ cell.

2) Resistant plasmid conjugation- Some gram negative bacteria(eg. *E.coli*, shigella) contain plasmids that contain antibiotic resistance gene called R factor. The R factor has two component-

i) RTF(resistance transfer factor) that codes for sex transfer like F factor.
ii) r determinant that codes for antibiotic resistance.

Sometimes RTF may dissociated from the r determinants and the two component may exist as separate entities. Under such condition, although the host cell remains drug resistant, the drug resistance is not transferable.

3) High frequency recombinant (Hfr) conjugation-

When the F+ plasmid is integrated within the bacterial chromosome, the cell is called an Hfr cell

Plasmid that are capable of integrating into the chromosome is called episome.

HFR cells are able to transfer chromosomal gene to recipient with high frequency.

The recipient F- cell usually remain F- after conjugation because only part of the F plasmid from the donor Hfr cell to the recipient have been transferred.

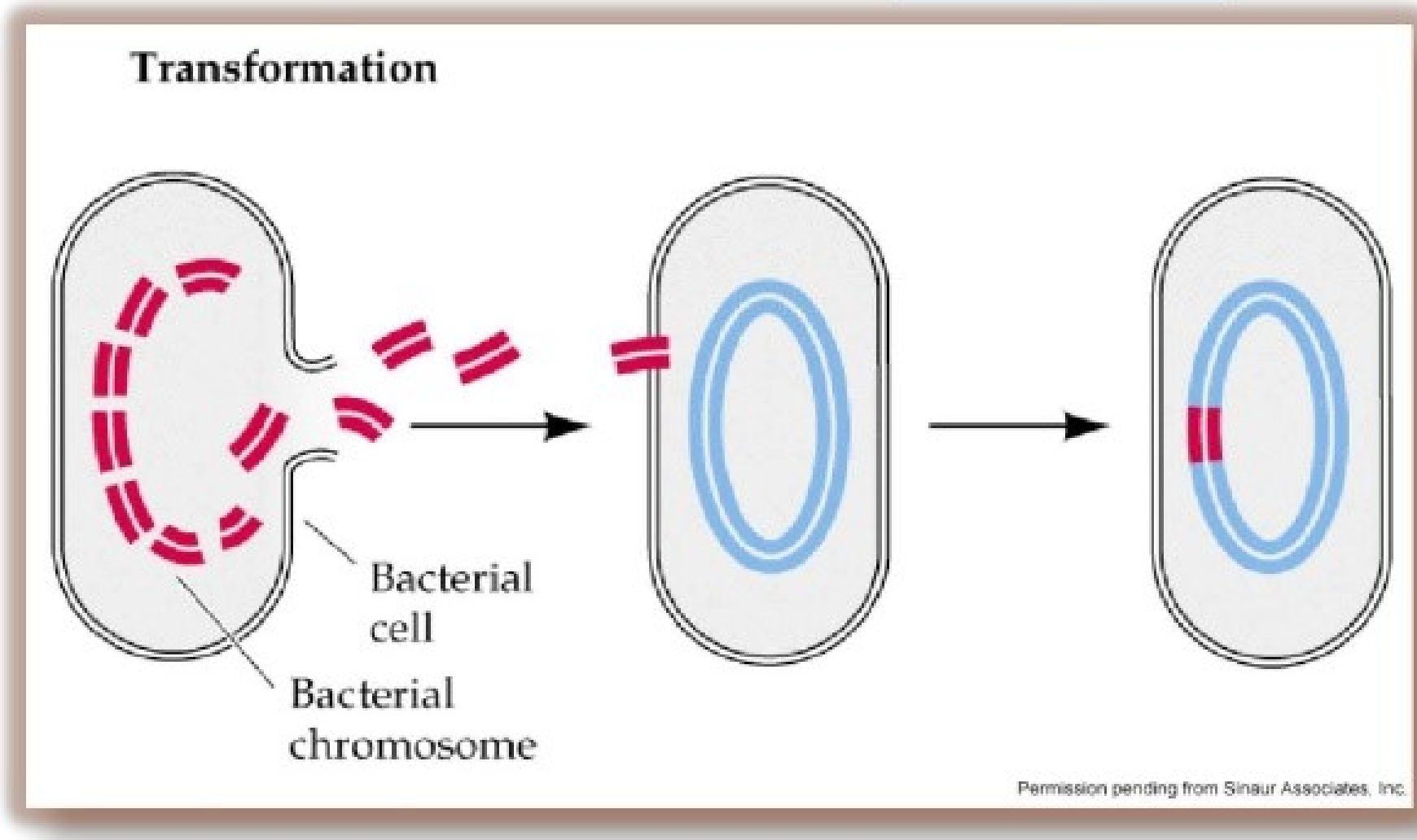
4) Sexduction- When F- factor reverts to free state from the integrated state, it may sometimes carry with it some genetic material from the host cell adjacent to the integration site of the F plasmid. Such type of hybrid molecule and F factor plasmid incorporating some host chromosomal gene is called F prime (F') factor. When F' cell mates with a recipient(F-) cell the recipient bacterium become F'. This process of gene transfer is termed as sexduction.

TRANSFORMATION

- Involves the uptake of free or naked DNA via a recipient released by the donor.
- Transformation, originally discovered in pneumococcus by Griffith(1928) known as Griffith's experiment, has also observed in Bacillus, Neisseria, Haemophilus bacteria.
- Griffith's experiment in Pneumococcus

Type I strain(smooth strain) injected to mice it dies

Type II strain (rough strain) injected to mice it survives


Transformation
Bacterial cell
Bacterial chromosome
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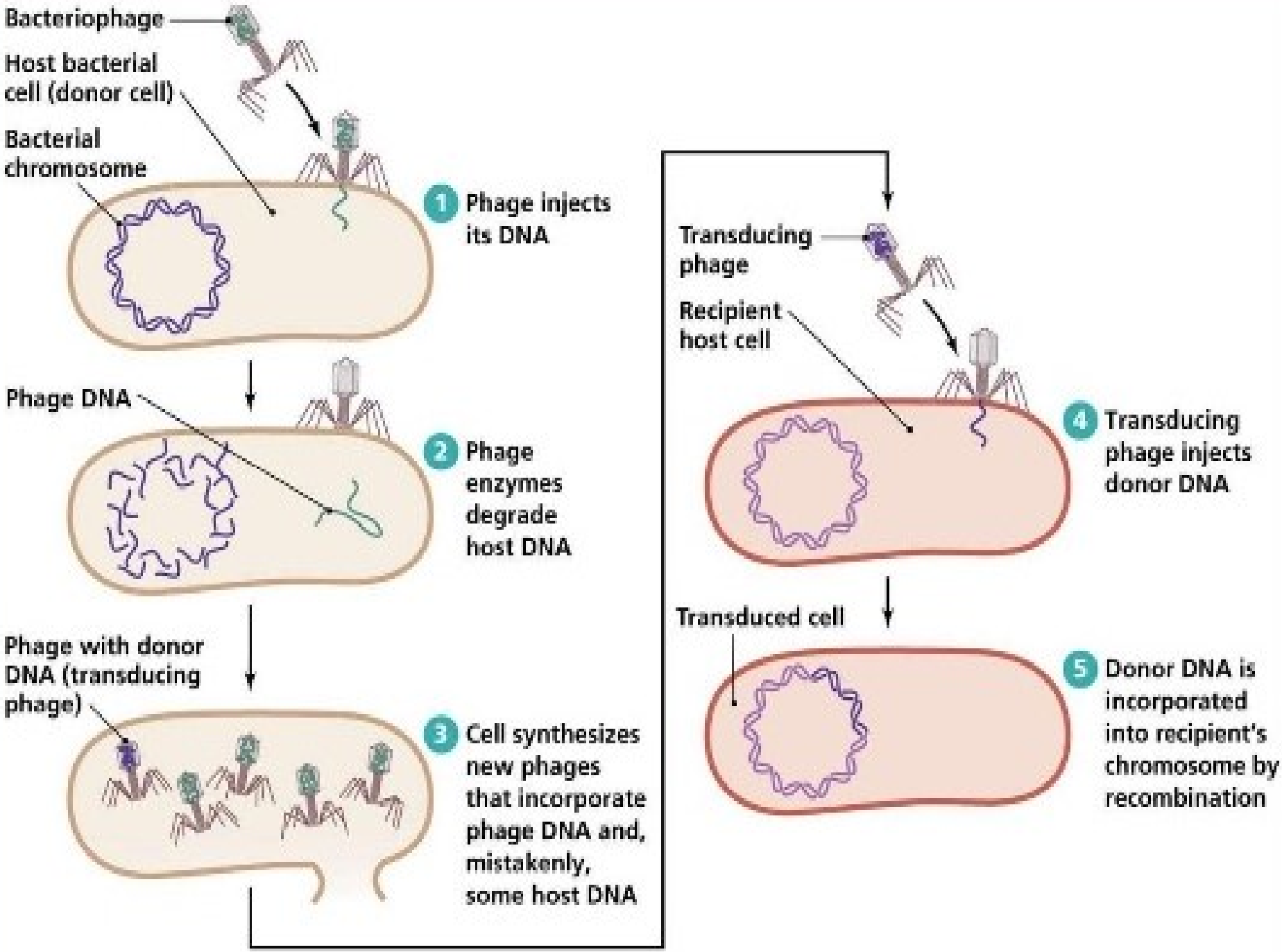
TRANSDUCTION

- Gene transfer from a donor to a recipient by way of a bacteriophage.
- If the lysogenic cycle is adopted, the phage chromosome is integrated (by covalent bonds) into the bacterial chromosome, this is called prophage.this process- lysogeny or lysogenic conversion,bacteria-lysogenic bacteria.
- The lytic cycle leads to the production of new phage particles which are released by lysis of the host.


Bacteriophage
Host bacterial cell (donor cell)
Bacterial chromosome
1 Phage injects its DNA
Phage DNA
2 Phage enzymes degrade host DNA
Phage with donor DNA (transducing phage)
3 Cell synthesizes new phages that incorporate phage DNA and, mistakenly, some host DNA
Transducing phage
Recipient host cell
4 Transducing phage injects donor DNA
Transduced cell
5 Donor DNA is incorporated into recipient's chromosome by recombination

- Transduction has been observed im a diverse group of bacteria- Escherichia, Salmonella, Shigella, klebsiella, proteus, psedomonas, bacillus and streptococcus.

Two ways in which transduction occurs:

1) Generalised transduction eg. Phages P22 of *Salmonella typhimurium*, phage P1 of *E.coli*
2) Restricted or specialised transduction eg. Lamda phage of *E.coli* transfers gal gene.

	Generalised transduction	Restricted transduction
Transfer	Any gene	Genes immediately adjacent to the site of prophage attachment
Induction	Transducing phage is produced upon induction of prophage and in infection with virulent phages.	Transducing phage is produced upon induction of only prophage
Content in transducing phage	Only bacterial DNA	Hybrid- contains both bacterial and phage DNA
Transducing paricles	Replace recipient gene when integrated in chromosome	Added to genome of recipient cell

Cell to cell fusion(protoplast fusion)

- In gram positive bacteria???
- Cell membrane of participating cells are joined together in protoplast fusion which inturn generates cytoplasmic bridge between the cells resulting in mixing of their cytoplasm and exchange of genetic material.
- Protoplast fusion is an artificial method of gene transfer.

SCREENING OF TRANSGENE

- The presence of transgene or gene of interest is detected by several methods:
- A selectable marker gene
- Southern blot techniques
- Northern bolt technique
- Western blot technique

APPLICATION

- Clinical gene transfer applications
- Vaccine Development
- Production of transgenic animals
- Treatment of Cancer, AIDS
- Gene Discovery
- Gene Therapy
- Enhancing the resistance of plants
- GMO


THANKU VERY MUCH