

Genetic Transfer &
Recombination In Bacteria

PROSANTA SAHA

DNA Recombination

- **Recombination** is a type of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA.
- It is used in horizontal gene transfer to exchange genetic material between different strains and species of bacteria and viruses.

Section 2 Bacterial Genetic Elements

- **Bacterial chromosome**
- **Plasmid**
- **Bacteriophage**
- **Transposable elements**

Genetic recombination produces new bacterial strains

- In addition to mutations, genetic recombination generates diversity within bacterial populations.
- Recombination occurs through three processes:
 1. Transformation
 2. Transduction
 3. Conjugation

- Genetic recombination - transfer of DNA from one organism (donor) to another recipient. The transferred donor DNA may then be integrated into the recipient's nucleoid by various mechanisms (homologous, non-homologous).
- Homologous recombination - homologous DNA sequences having nearly the same nucleotide sequences are exchanged by means of Rec A proteins. This involves breakage and reunion of paired DNA segments as seen in (Natural mechanisms of genetic recombination in bacteria include:
 - a. transformation
 - b. transduction
 - c. conjugation

1. TRANSFORMATION

- Transformation involves the uptake of free DNA molecules released from one bacterium (the donor cell) by another bacterium (the recipient cell).
- Discovered by Frederick Griffith in 1928 in *S.pneumoniae*.
- In this experiment Griffith found out that a virulent *Streptococcus pneumonia* became virulent when exposed to heat killed virulent cell.

Transformation

- We aren't going to speak much of this process, except to note that it is very important for recombinant DNA work. The essence of recombinant DNA technology is to remove DNA from cells, manipulate it in the test tube, then put it back into living cells. In most cases this is done by transformation.
- In the case of *E. coli*, cells are made "competent" to be transformed by treatment with calcium ions and heat shock. *E. coli* cells in this condition readily pick up DNA from their surroundings and incorporate it into their genomes.

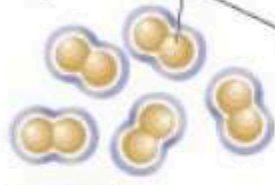
Griffith's Transformation Experiment

- Frederick Griffith in 1928 performed experiment with *Streptococcus pneumonia* bacteria in mice.
- This showed that something passed from dead bacteria into nearby living ones, allowing them to change their cell surface.
- He called this agent the transforming principle, but did not know what it was or how it worked.



Type IIR:
living,
nonvirulent

Bacteria with
polysaccharide
capsule



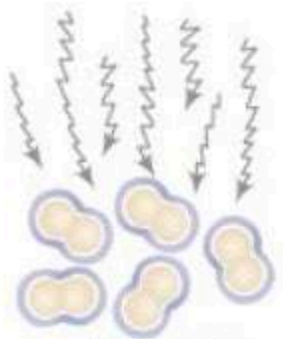
Type IIIS:
living, virulent



Type IIIS:
heat-killed,
nonvirulent



Type IIR:
living,
nonvirulent



Type IIIS:
heat-killed,
nonvirulent

+

Inject mice



Survives;
no bacteria
recovered



Dies; type IIIS
virulent
bacteria
recovered



Survives;
no bacteria
recovered



Dies; type IIIS
virulent
bacteria
recovered

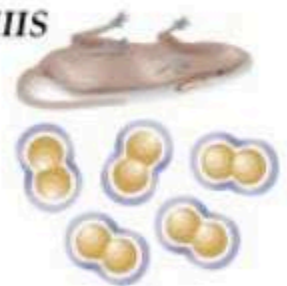
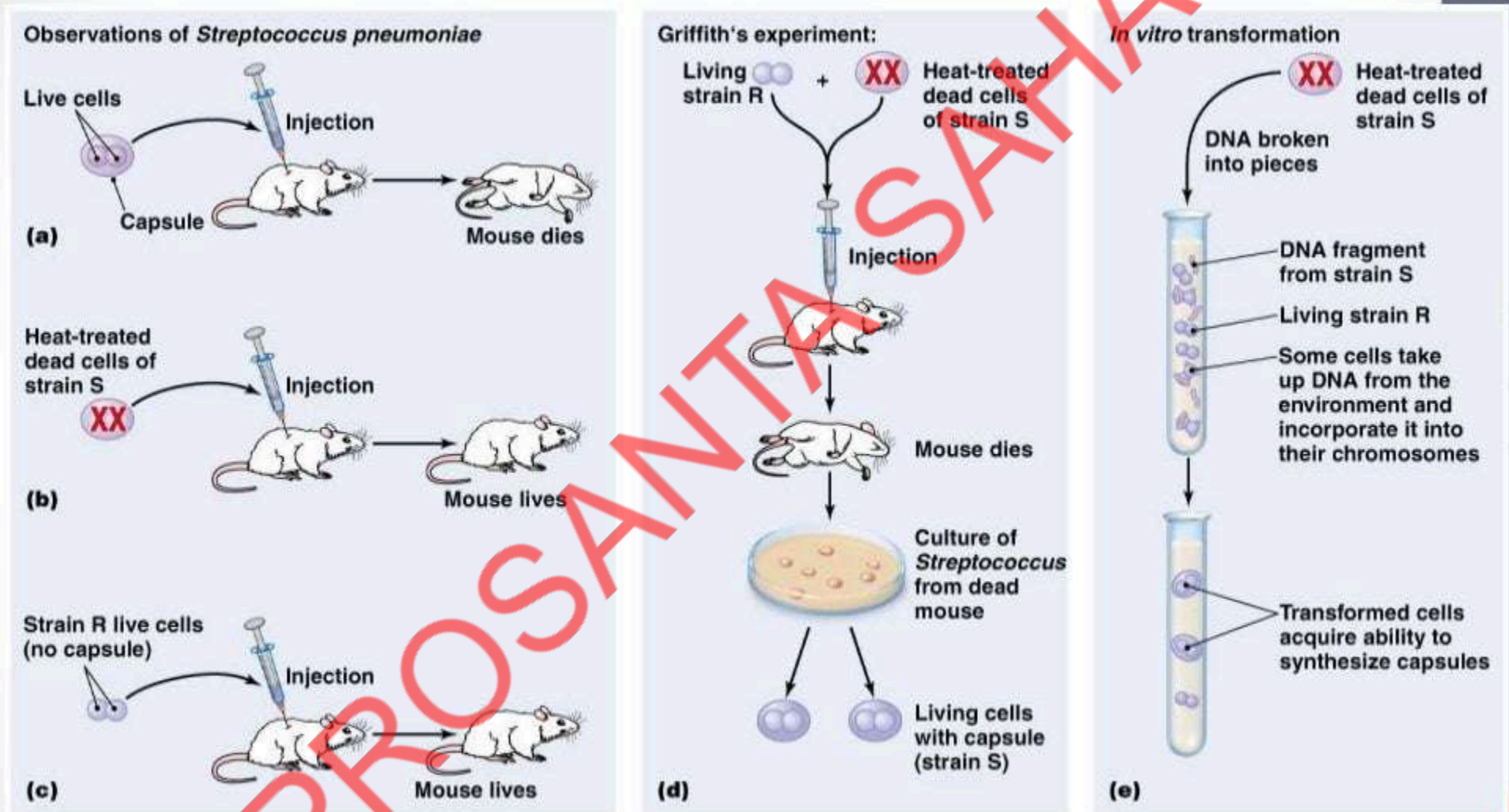


Figure 7.33 Transformation in *Streptococcus pneumoniae*-overview



COMPETENT CELLS & COMPETENCE

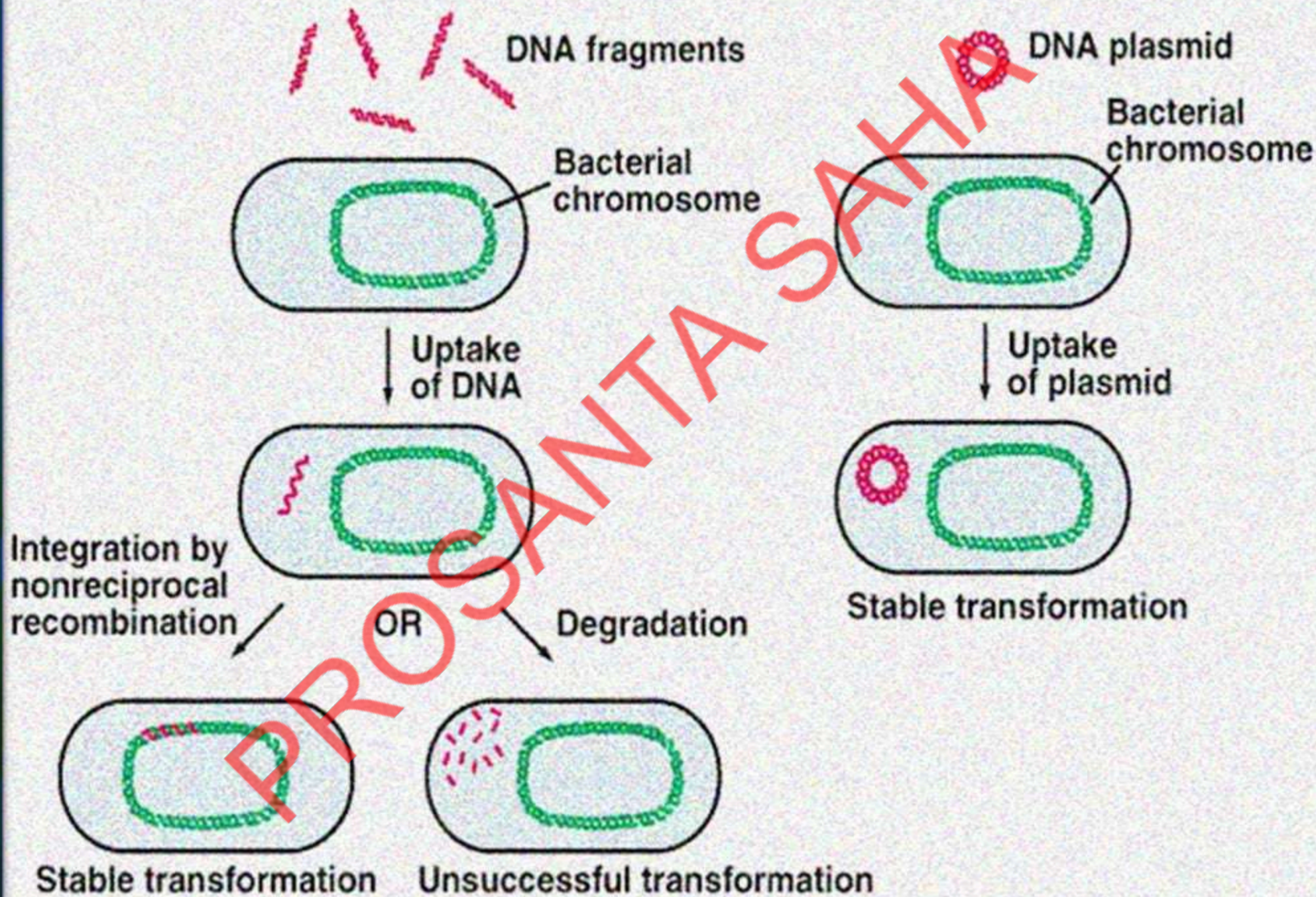
- The ability of a cell to be transformed, depends on its COMPETENCE.
- COMPETENCE is the ability of a recipient bacterium to take up DNA from the environment.
- COMPETENT CELL'S are those cells, which can be transformed.

Contd.

- The mechanism of transformation involves 2 steps which are as follows:
- Step1: The DNA binding receptor on a competent bacterium binds double stranded DNA. As the DNA enters the cell, one strand is degraded, & the other strand is coated with single-strand DNA-binding protein.
- Step2: The single strand of donor DNA is integrated into the chromosome of the recipient cell producing a DNA heteroduplex with different alleles in the two strands.

(a) Transformation with DNA fragments

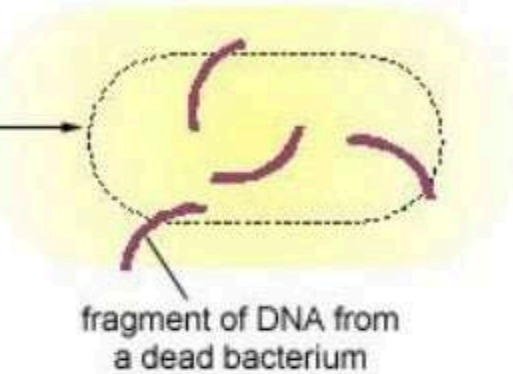
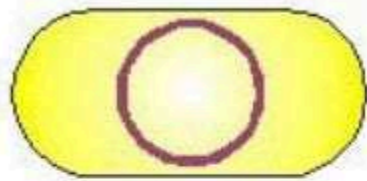
(b) Transformation with a plasmid



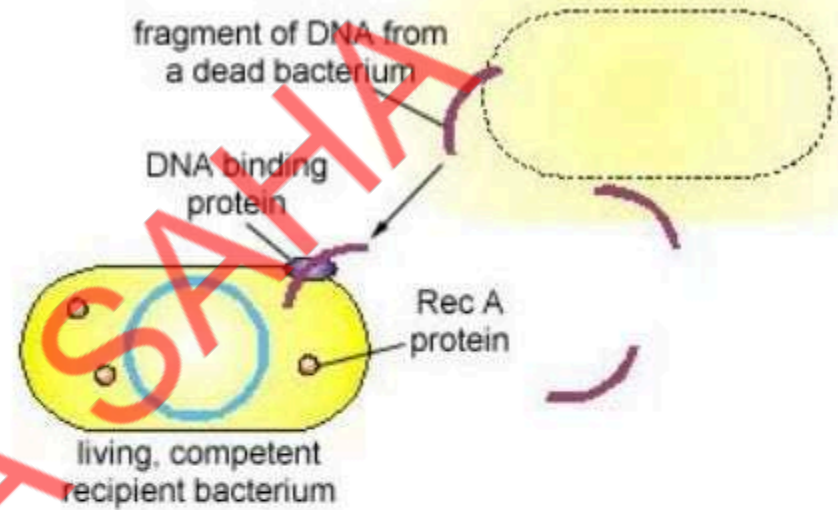
Transformation

- Genetic recombination in which a DNA fragment from a dead, degraded bacterium enters a competent recipient bacterium and it is exchanged for a piece of the recipient's DNA.
- Involves 4 steps

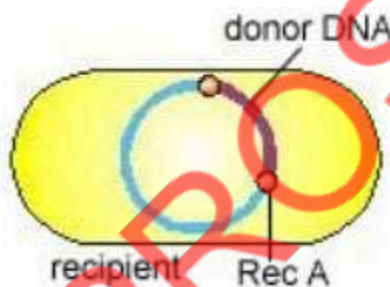
The 4 steps in Transformation



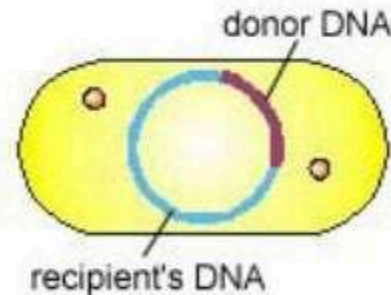
1. A donor bacterium dies and is degraded



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



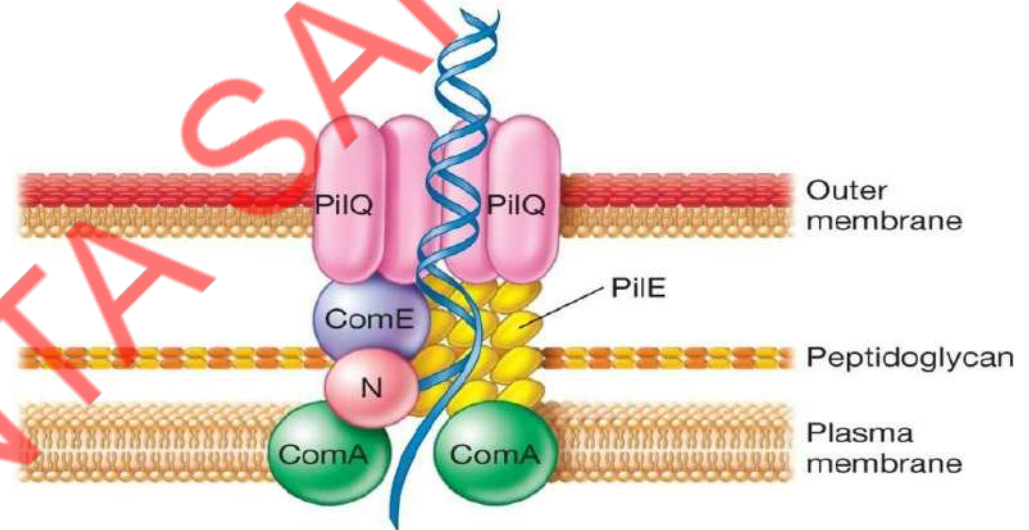
3. The Rec A protein promotes genetic exchange between a fragment of the donor's DNA and the recipient's DNA



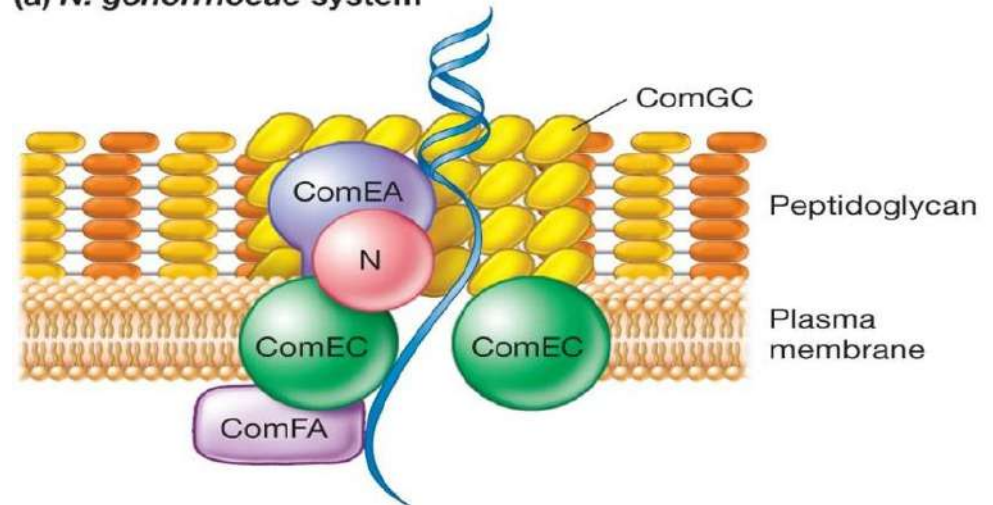
4. Exchange is complete

DNA Uptake in Bacterial Transformation (details of mechanism)

- Protein system allows DNA to move across cell walls
 - Gram-negatives
 - PilQ aids in movement across outer membrane
 - Pilin complex (PilE) moves DNA across periplasm and peptidoglycan
 - ComE is DNA binding protein
 - N is nuclease that degrades one strand
 - ComA forms transmembrane channel
 - Similar system in Gram-pos.



(a) *N. gonorrhoeae* system



(b) *B. subtilis* system

Transformation

- Transfer of naked DNA from donor to recipient cell.
- Transformation experiment by Griffith showed that DNA is the genetic material and can be transferred between host and recipient DNA.
- *E.coli* cannot undergo transformation naturally, hence it is made competent in the lab.
- The process is called '**Artificial Transformation**'.
- Physiological ability to take up DNA is called 'Competence' and such cells are Competent cells.
- E.coli cannot undergo transformation naturally, hence made competent in the lab.
- This procedure is comparatively easy and simple.
- Involves Calcium chloride or Electroporation.

Transformation by Calcium Chloride

- Cells are incubated in a solution containing divalent cations like calcium in cold condition and a rapid heat shock is given.
- Surface of *E.coli* is negatively charged (Phospholipids, Lipopolysaccharides) as well as DNA is negatively charged.
- The divalent cation shields the negative charges and hence DNA adheres to cell surface.
- Divalent cations might also weaken cell surface making it more permeable to DNA.
- Heat shock creates thermal imbalance within the cell.
- DNA enters the cell either by pores on the surface or damaged cell wall.

Transformation by Electroporation

- Electric shock is given to the cells which creates holes in the pores of the membrane.
- DNA enters through the pores.
- After the shock, pores are closed rapidly by repair mechanisms of cell membrane.

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Conjugation

- Conjugation is the closest analogue in bacteria to eukaryotic sex.
- The ability to conjugate is conferred by the F plasmid. A plasmid is a small circle of DNA that replicates independently of the chromosome. Bacterial cells that contain an F plasmid are called "F+". Bacteria that don't have an F plasmid are called "F-".
- F+ cells grow special tubes called "sex pilli" from their bodies. When an F+ cell bumps into an F- cell, the sex pilli hold them together, and a copy of the F plasmid is transferred from the F+ to the F-. Now both cells are F+.
- Why aren't all E. coli F+, if it spreads like that? Because the F plasmid can be spontaneously lost.

Mating of F+ and F- Bacterial Strains

**Animation by
Thomas M. Terry**

Bacterial Conjugation

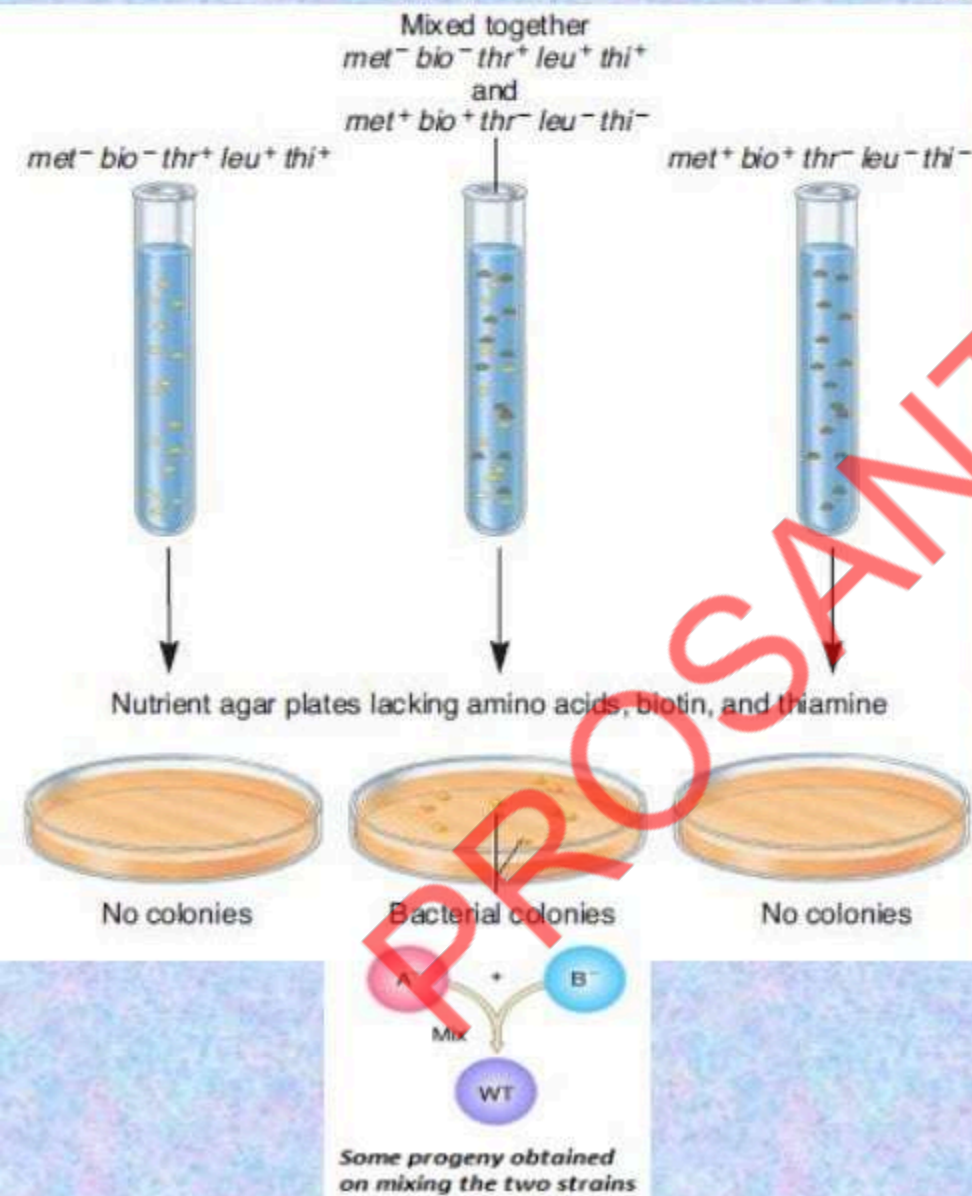
Bacterial Conjugation is genetic recombination in which there is a transfer of DNA from a living donor bacterium to a recipient bacterium. Often involves a sex pilus.

- The 3 conjugative processes
 - I. F⁺ conjugation
 - II. Hfr conjugation
 - III. Resistance plasmid conjugation

2. Conjugation

- ❖ “CONJUGATION is the transfer of genes between cell’s that are in physical contact with another”.
- ❖ The first demonstration of recombination in bacteria was achieved by LEDERBERG & TATUM IN 1946.
- ❖ JHOSUA LEDERBERG & EDWARD TATUM, combined two different strains of E. Coli and gave them opportunity to mate.
- ❖ They found that, genetic traits could be transferred among them, if physical contact occurred.

Experimental Work Of Joshua Lederberg and Edward Tatum

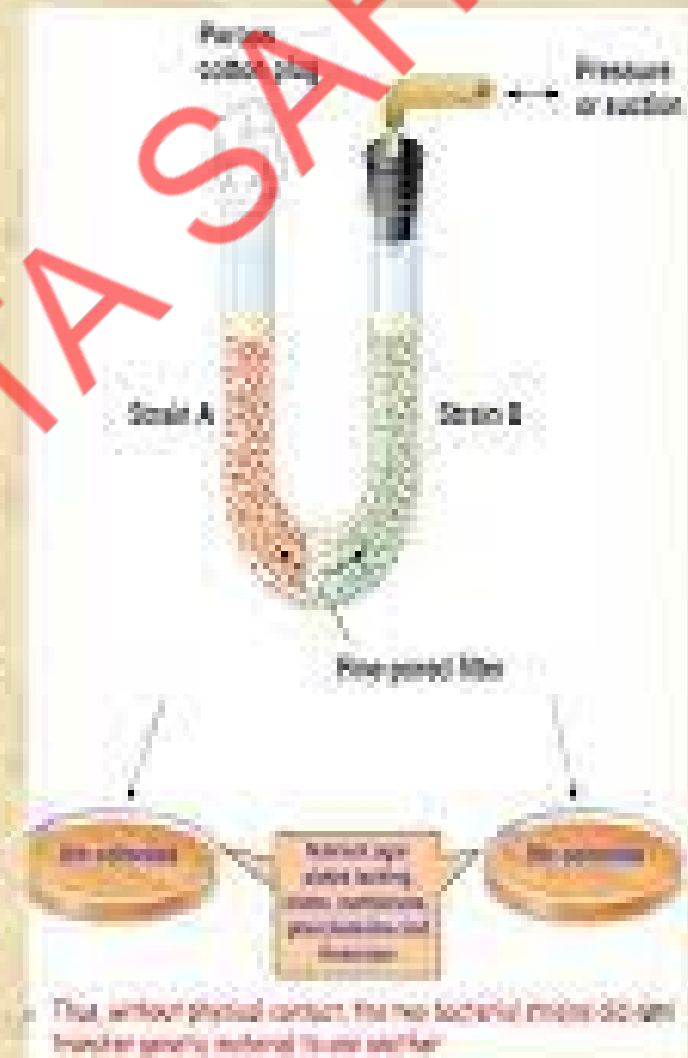


- No colonies arose on plates containing either strain A or strain B alone, showing that back mutations cannot restore prototrophy, the ability to grow on unsupplemented minimal medium.
- However, the plates that received the mixture of the two strains produced growing colonies at a frequency of 1 in every 10,000,000 cells plated (in scientific notation, 1×10^{-7}).
- This observation suggested that some form of recombination of genes had taken place between the genomes of the two strains to produce prototrophs.

2. Davis (U-tube) tested whether cell-to-cell contact was required

a. Strain A cells were placed on one side of a filter, and strain B on the other. Cells could not move through the filter but molecules moved freely; encouraged by alternating suction and pressure.

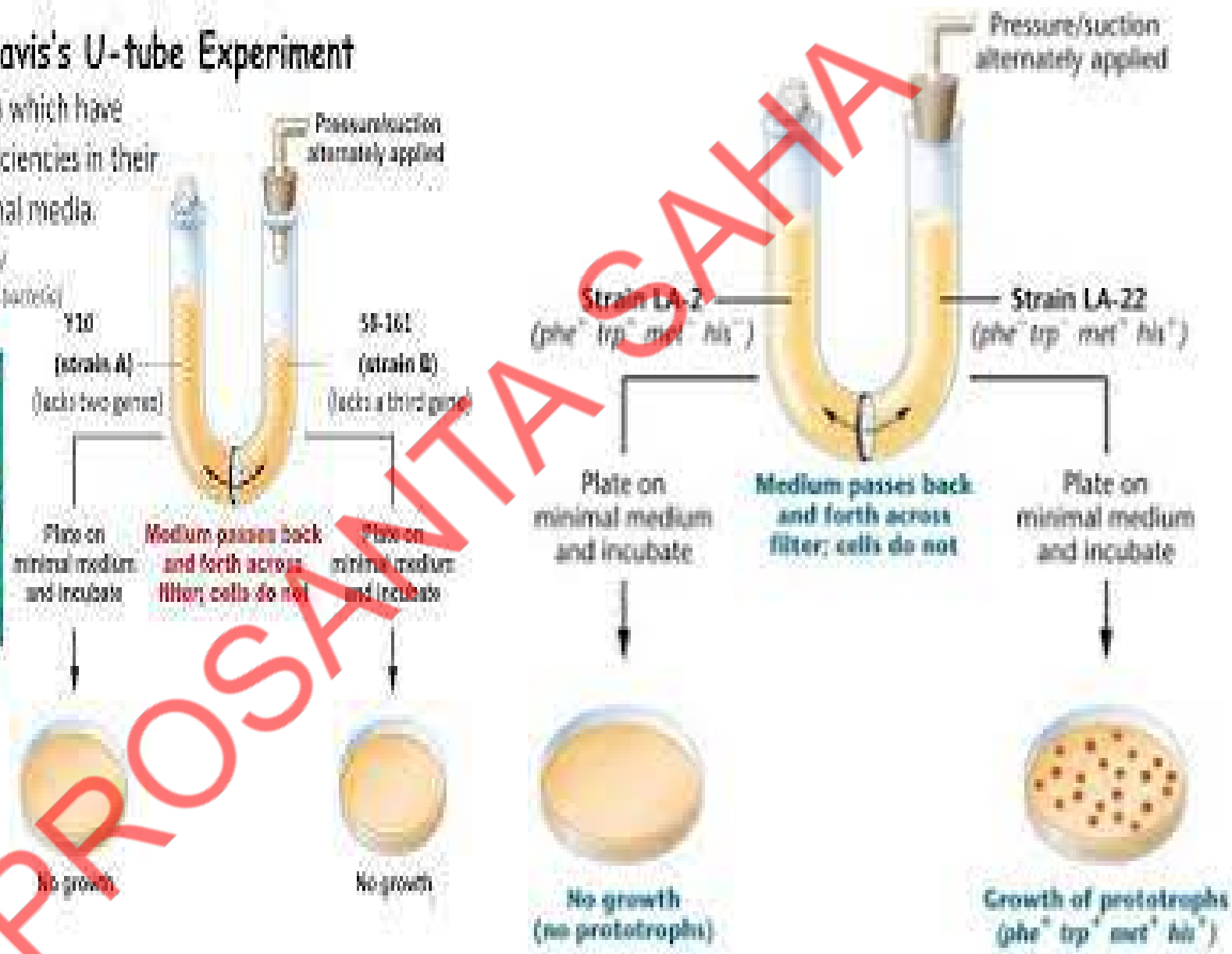
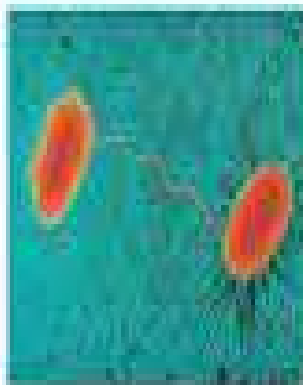
b. No prototrophic colonies appeared when the cells were plated on minimal medium. This indicates that cell-to-cell contact is required, and the genetic recombination results from conjugation.



Conjugation = Davis's U-tube Experiment

Two strains of bacteria which have "complementary" deficiencies in their ability to utilize minimal media.

(Davis drew Y10 and 58-161 mixed together and called "auxotrophic" bacteria)



The Fertility Factor (F)

- The Fertility Factor (F) is a plasmid.
- The F plasmid directs the synthesis of pili, projections.
- Pili,
 - initiate contact with a recipient
 - draw the donor and recipient cells closer, and
 - allows the F DNA, from the donor cell, to pass through a pore into the recipient cell.
- One strand of the double-stranded F DNA is transferred and then DNA replicates, restores the complementary strand in both the donor and the recipient.
- This replication results in a copy of F remaining in the donor and another appearing in the recipient.

F+ and F- FACTORS

- In 1950, WILLIAM HAYES, FRANCOIS JOCOB and Eli h. Wolman established that-
Conjugating bacteria are of two mating types:-
 - 1-male types which donates their DNA, these are called f+ cells.
 - 2-female types which are recipient of DNA donated by F+ cells and are called F- cells.
- These F+ and F- are called FERTILITY factor or F- factor or SEX factor.

PROCESS OF CONJUGATION

- The F Pili of the F⁺ donor cell make contact with the F⁻ recipient cell & pull the cell together.
- Rolling circle replication transfer one strand of the F factor into the recipient cell.
- Transfer of F factor is completed, yielding two F⁺ factor bacteria.

Terminology

The Donor

- ☞ F+
- Fertility factor
- ☞ Contains genetic material for gene exchange
- ☞ “male” cell

The Recipient

- ☞ F-
- No fertility factor
- ☞ Receives genetic material
- ☞ “Female” cell

The Conjugate

- ☞ HFR
- High frequency recombinant
- ☞ Resulting genetic recombinant
- ☞ Transfers entire genomic DNA when mixed with F-

1. Cell – Cell Contact

Attachment Via Pili

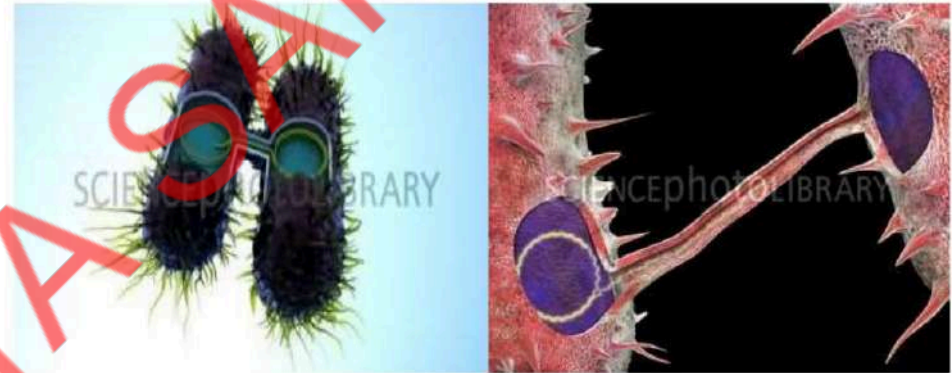
- Formation of “sex pilus” or F-bridge between



2. Mobilization

Genetic Transfer of ssDNA

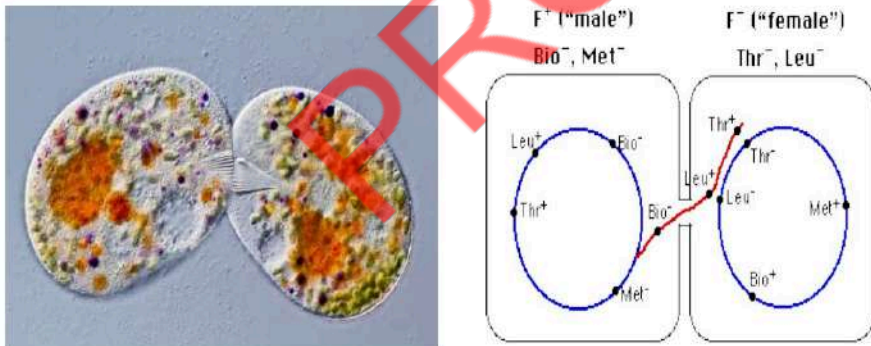
- Target DNA is replicated in F+
- Genetic transfer of ssDNA to F-



3. DNA Synthesis

Synthesis of Complementary DNA

- Complementary strands of DNA are replicated in both F- and F+
- Target DNA is incorporated into F- genome

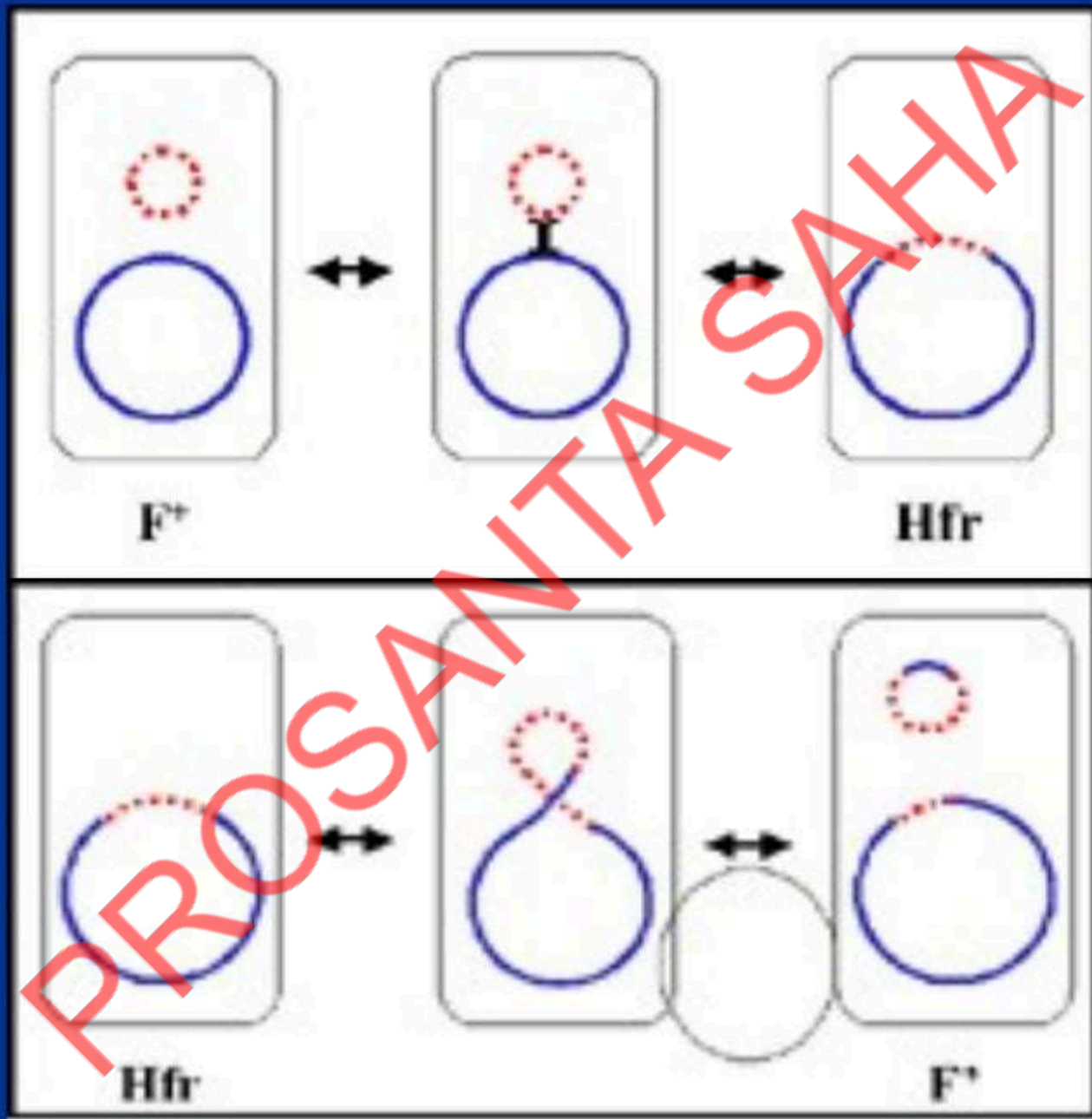


4. Separation

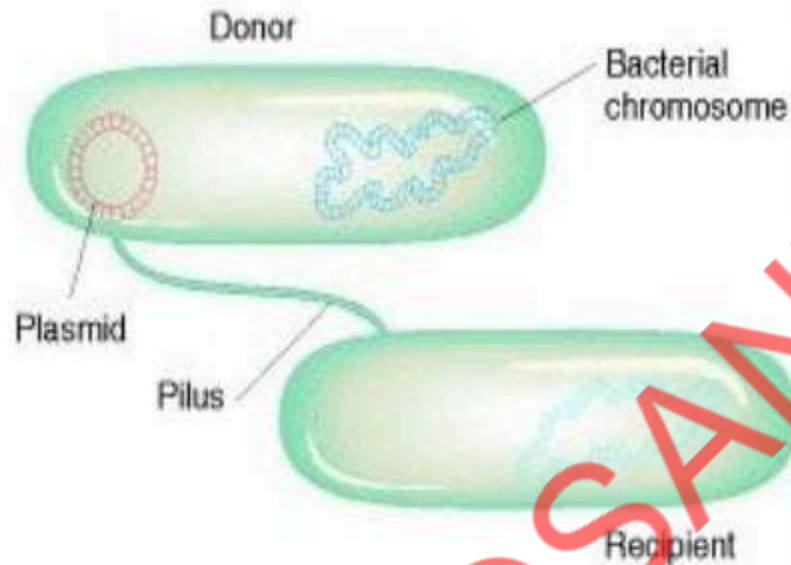
Separation and Formation of Two F+ cells

- Cells separate
- Form two F+ cells with completed recombinant plasmids
- Both serve as donor cells for further conjugation



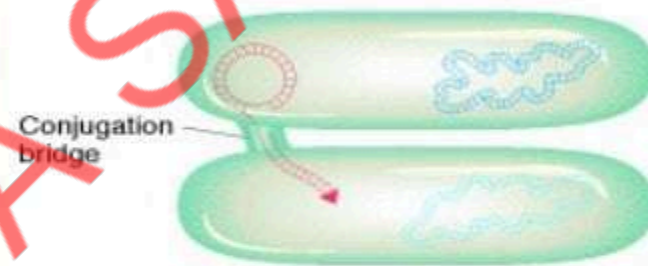


Conjugation in Bacteria: $F^+ \times F^-$ crosses



(a)

Two bacterial cells are pulled together, by a 'Pilus'



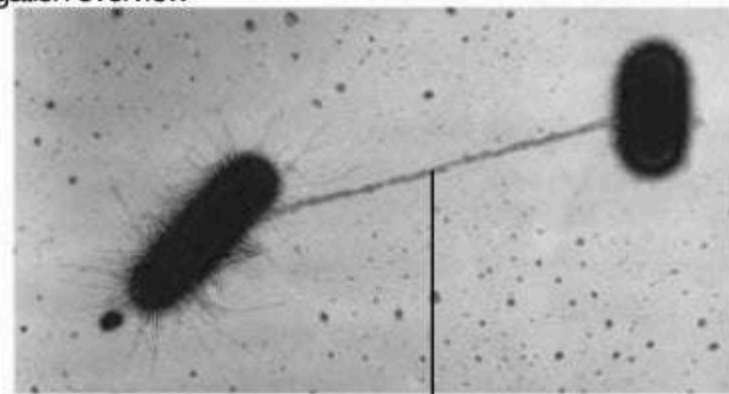
(b)

Conjugation bridge, having a pore, is formed between the two cells.

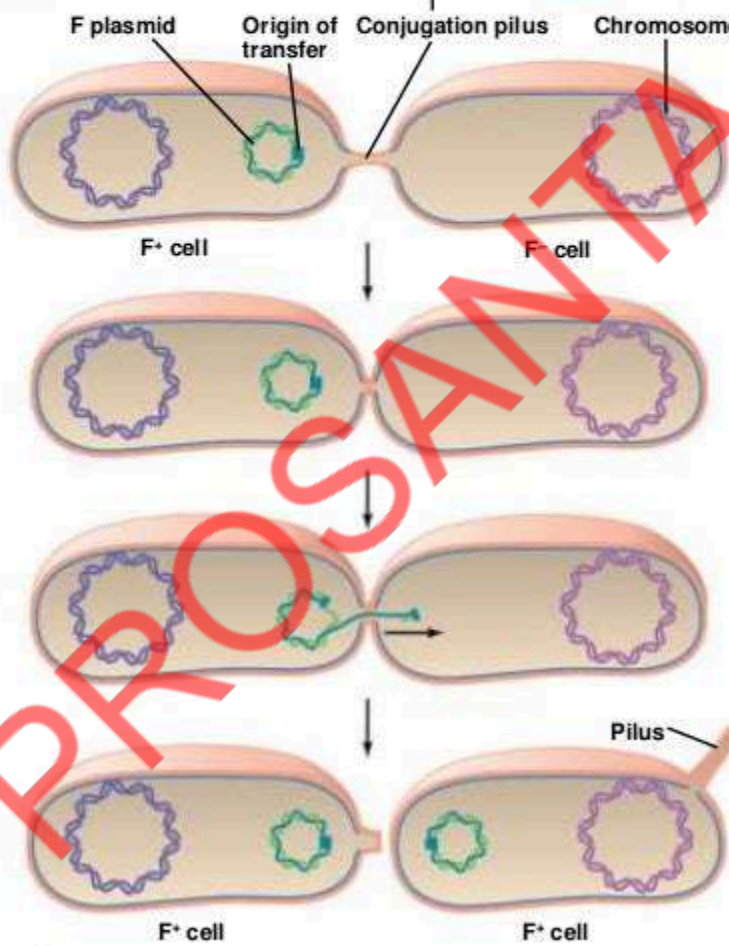
One strand of the plasmid (F) DNA, passes into the recipient bacterium

Then, the single strands replicate in both the bacterial cells.

Figure 7.35 Bacterial conjugation-overview



(a) TEM 1 μm



(b)

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1 Donor cell attaches to a recipient cell with its pilus.

2 Pilus may draw cells together.

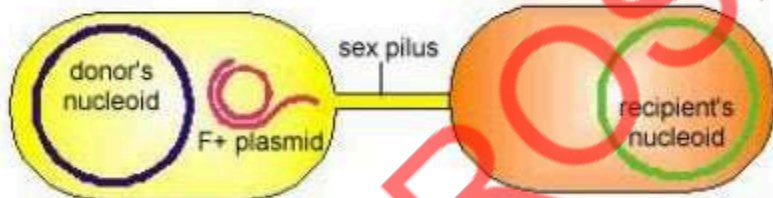
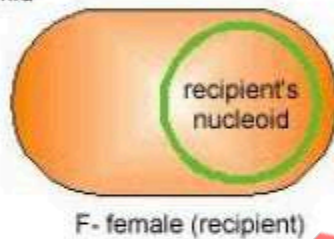
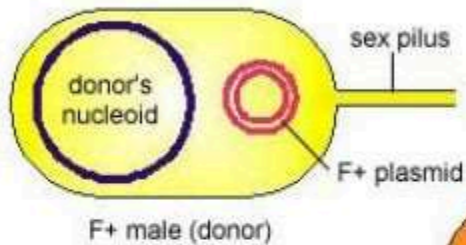
3 One strand of F plasmid DNA transfers to the recipient.

4 The recipient synthesizes a complementary strand to become an F+ cell with a pilus; the donor synthesizes a complementary strand, restoring its complete plasmid.

I. F+ Conjugation Process

F+ Conjugation- Genetic recombination in which there is a transfer of an F+ plasmid (coding only for a sex pilus) but not chromosomal DNA from a male donor bacterium to a female recipient bacterium. Involves a sex (conjugation) pilus. Other plasmids present in the cytoplasm of the bacterium, such as those coding for antibiotic resistance, may also be transferred during this process.

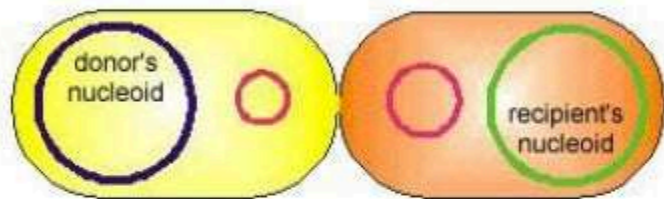
The 4 stepped F+ Conjugation



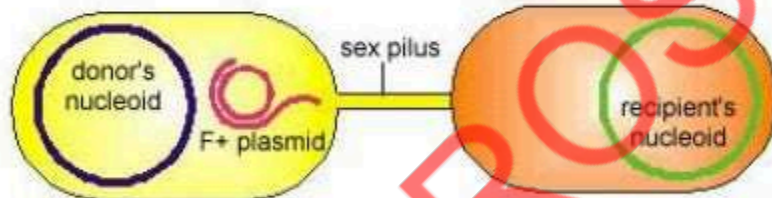
1. The F+ male has an F+ plasmid coding for a sex pilus and can serve as a genetic donor

2. The sex pilus adheres to an F- female (recipient). One strand of the F+ plasmid breaks

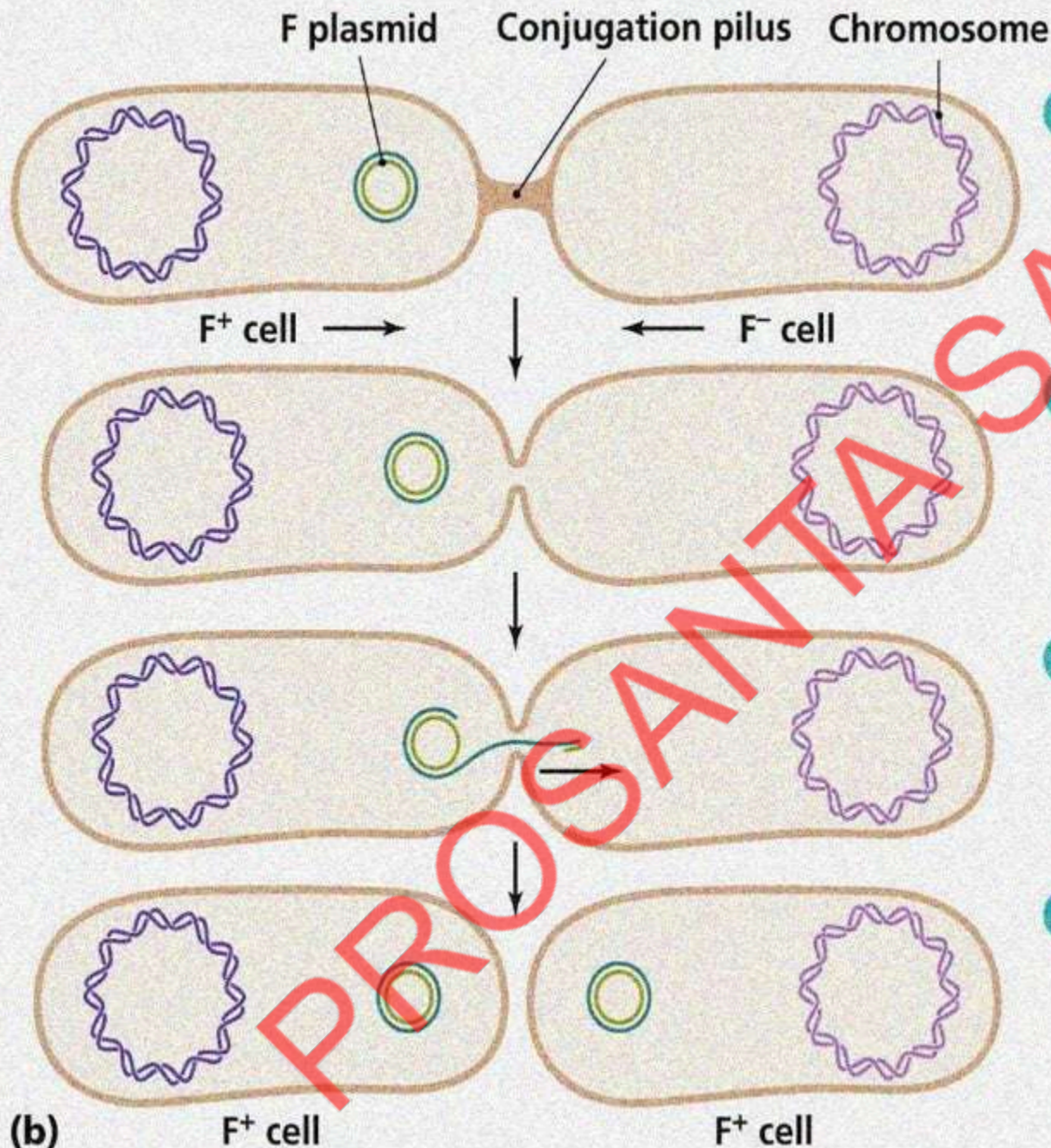
The 4 stepped F+ Conjugation (cont'd)



3. The sex pilus retracts and a bridge is created between the two bacteria. One strand of the F+ plasmid enters the recipient bacterium



4. Both bacteria make a complementary strand of the F+ plasmid and both are now F+ males capable of producing a sex pilus. There was no transfer of donor chromosomal DNA although other plasmids the donor bacterium carries may also be transferred during F+ conjugation.



1 Donor cell attaches to a recipient cell with its pilus. The pilus draws the cells together.

2 The cells contact one another.

3 One strand of plasmid DNA transfers to the recipient.

4 The recipient synthesizes a complementary strand to become an F⁺ cell; the donor synthesizes a complementary strand, restoring its complete plasmid.

(b) F⁺ cell

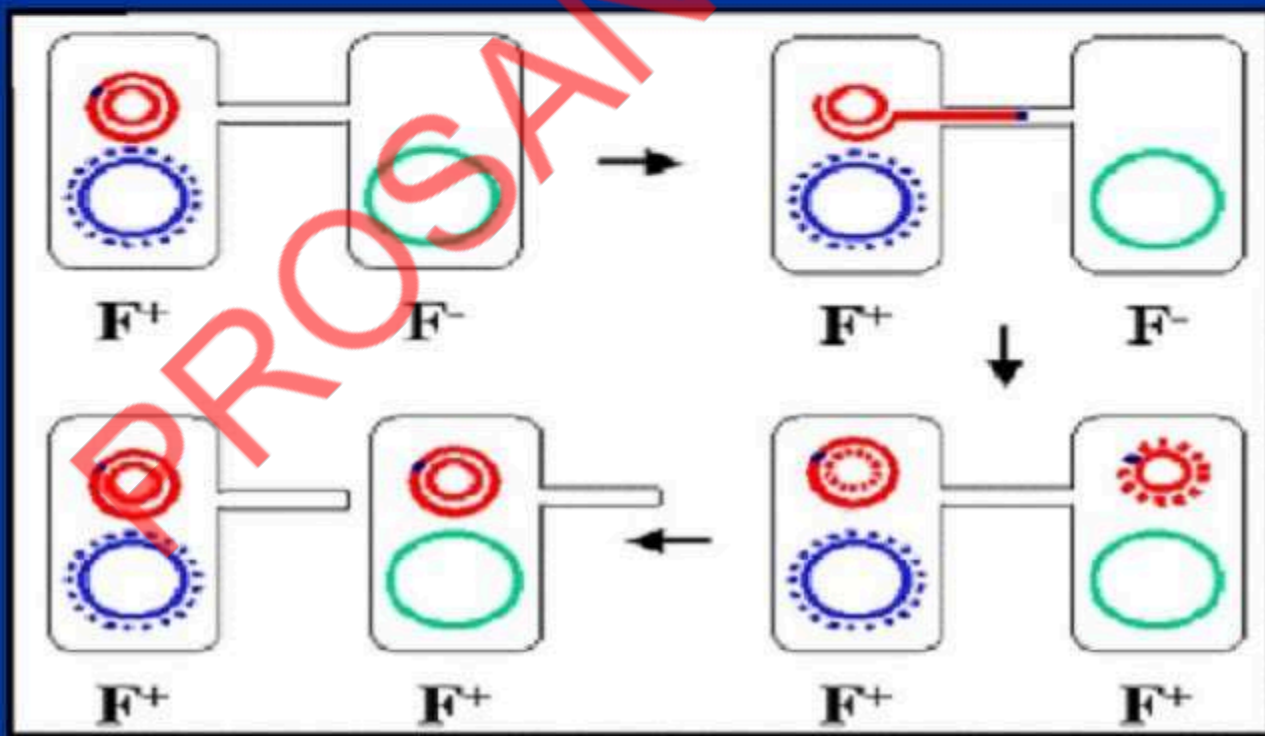
F⁺ cell

Physiological States of F Factor

Autonomous (F^+)

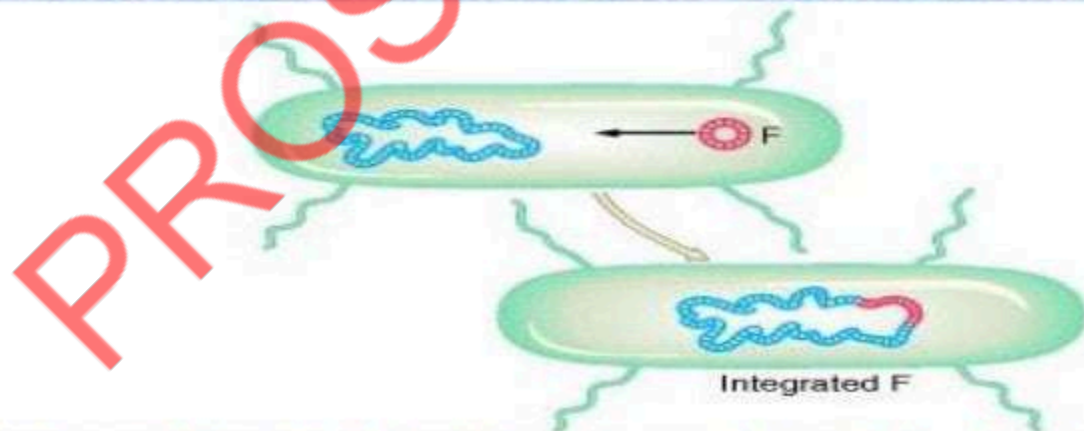
Characteristics of $F^+ \times F^-$ crosses:

- F^- becomes F^+ while F^+ remains F^+
- Low transfer of donor chromosomal genes



Hfr Strains

- Luca Cavalli-Sforza discovered a derivative of an F^+ strain, in 1950.
- On crossing with F^- strains this new strain produced 1000 times as many recombinants for 'genetic markers' as did a normal F^+ strain.
- Cavalli-Sforza designated this derivative an Hfr strain to indicate a high frequency of recombination.
- In $Hfr \times F^-$ crosses, virtually none of the F^- parents were converted into F^+ or into Hfr.
- This result is in contrast with $F^+ \times F^-$ crosses, where transfer of F results in a large proportion of the F^- parents being converted into F^+ .
- An Hfr strain results from the integration of the F factor into the chromosome.

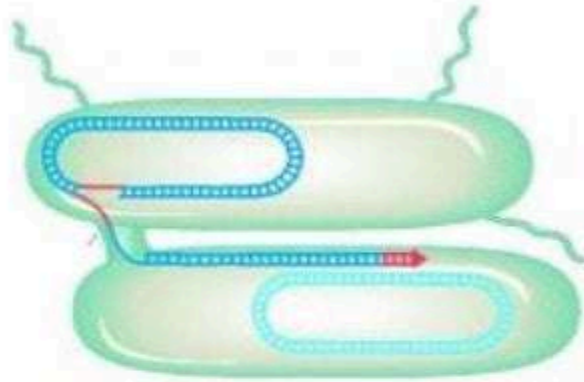


Fertility factor F integrates into the host chromosome

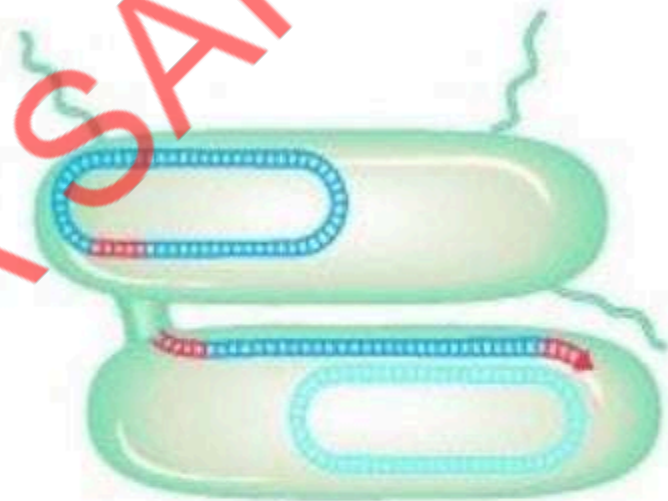
II. Hfr Conjugation

Genetic recombination in which fragments of chromosomal DNA from a male donor bacterium are transferred to a female recipient bacterium following insertion of an F⁺ plasmid into the nucleoid of the donor bacterium. Involves a sex (conjugation) pilus.

Conjugation in Bacteria: Hfr × F⁻



A single strand of F is transferred to a recipient cell, along with the copy of a part of the host chromosome. In the recipient cell, a second strand is synthesized, for the transferred DNA strand.

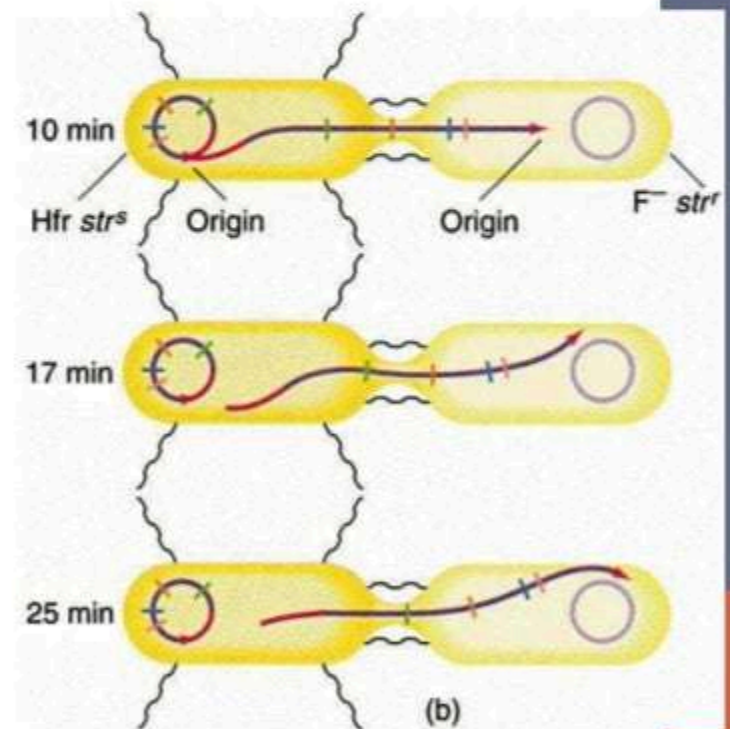
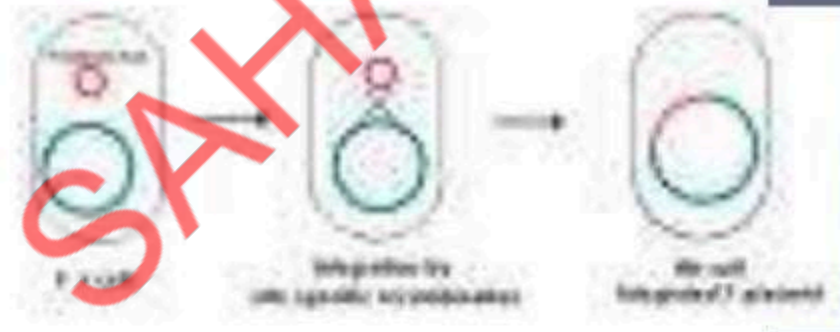


In the donor cell, host chromosome, a copy of F remains (due to replication of the remaining single strand)

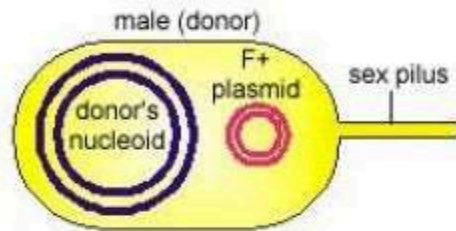
* The chromosomal fragment transferred, can then recombine with the recipient chromosome.

Hfr Conjugation

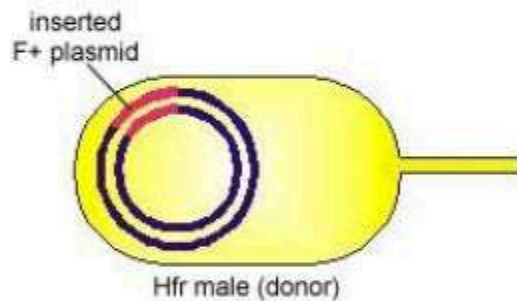
- When it exists as a free plasmid, the F plasmid can only transfer itself. This isn't all that useful for genetics.
- However, sometimes the F plasmid can become incorporated into the bacterial chromosome, by a crossover between the F plasmid and the chromosome. The resulting bacterial cell is called an "Hfr", which stands for "High frequency of recombination".
- Hfr bacteria conjugate just like F⁺ do, but they drag a copy of the entire chromosome into the F⁻ cell.



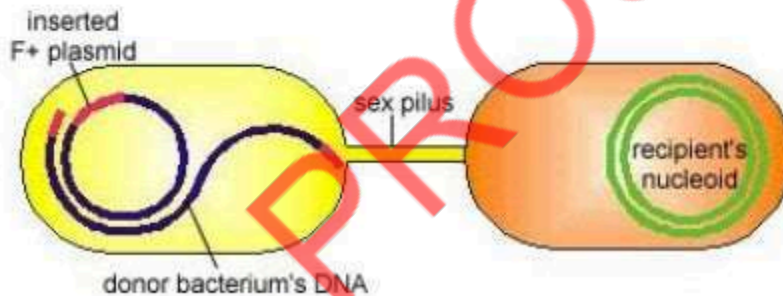
5 stepped Hfr Conjugation



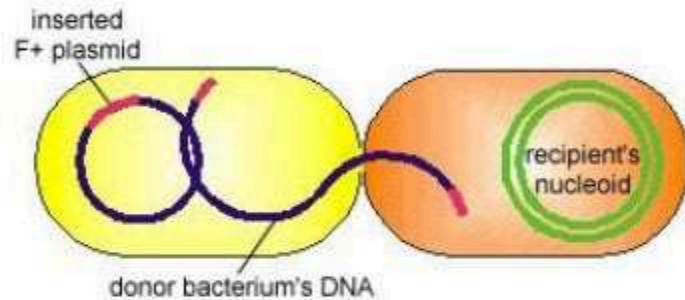
1. An F+ plasmid inserts into the donor bacterium's nucleoid to form an Hfr male.



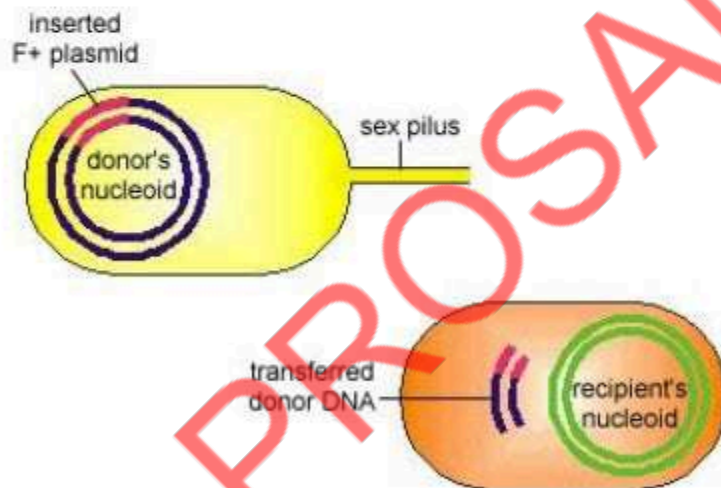
2. The sex pilus adheres to an F- female (recipient). One donor DNA strand breaks in the middle of the inserted F+ plasmid.



5 stepped Hfr Conjugation (cont'd)

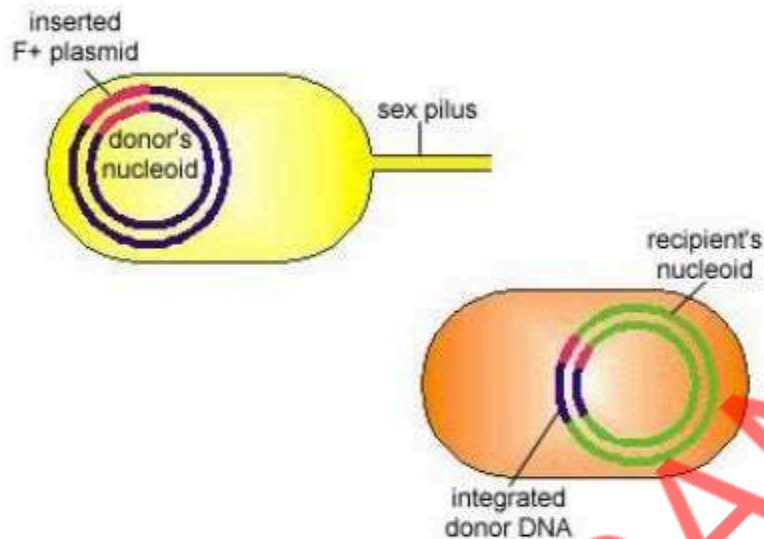


3. The sex pilus retracts and a bridge forms between the two bacteria. One donor DNA strand begins to enter the recipient bacterium. The two cells break apart easily so the only a portion of the donor's DNA strand is usually transferred to the recipient bacterium.



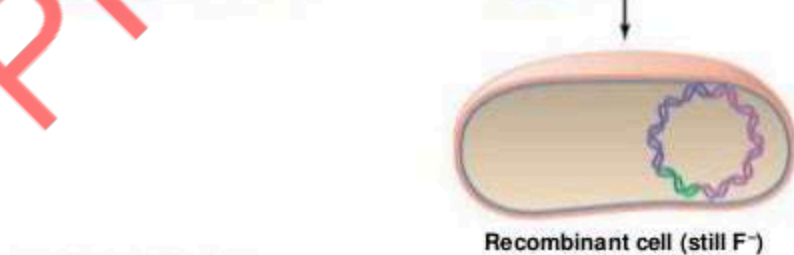
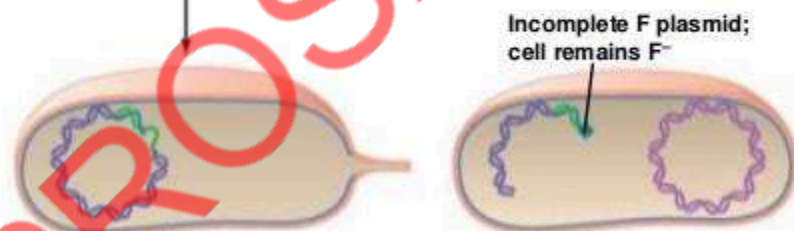
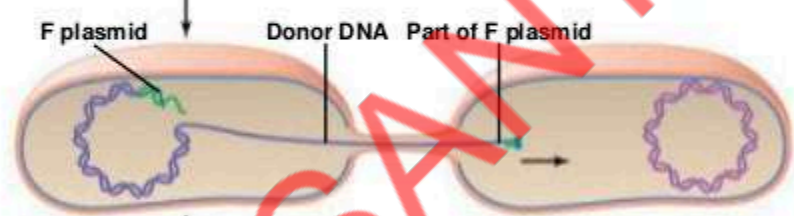
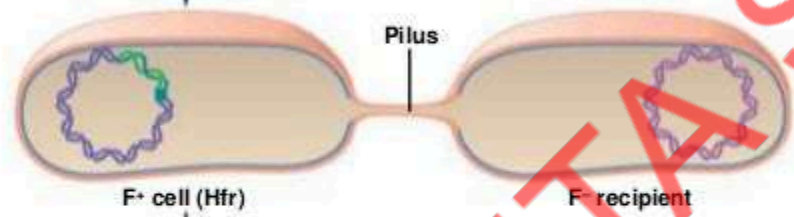
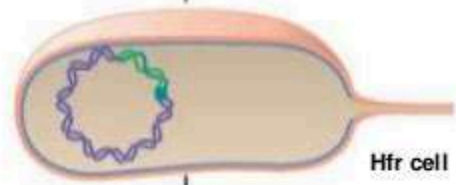
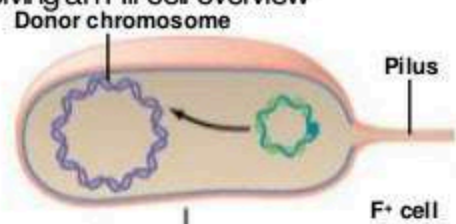
4. The donor bacterium makes a complementary copy of the remaining DNA strand and remains an Hfr male. The recipient bacterium makes a complementary strand of the transferred donor DNA.

5 stepped Hfr Conjugation (cont'd)



5. The donor DNA fragment undergoes genetic exchange with the recipient bacterium's DNA. Since there was transfer of some donor chromosomal DNA but usually not a complete F+ plasmid, the recipient bacterium usually remains F-

Figure 7.36 Conjugation involving an Hfr cell-overview



1 F plasmid integrates into chromosome by recombination.

2 Cells join via a conjugation pilus.

3 Portion of F plasmid partially moves into recipient cell trailing a strand of donor's DNA.

4 Conjugation ends with pieces of F plasmid and donor DNA in recipient cell; cells synthesize complementary DNA strands.

5 Donor DNA and recipient DNA recombine, making a recombinant F⁻ cell.

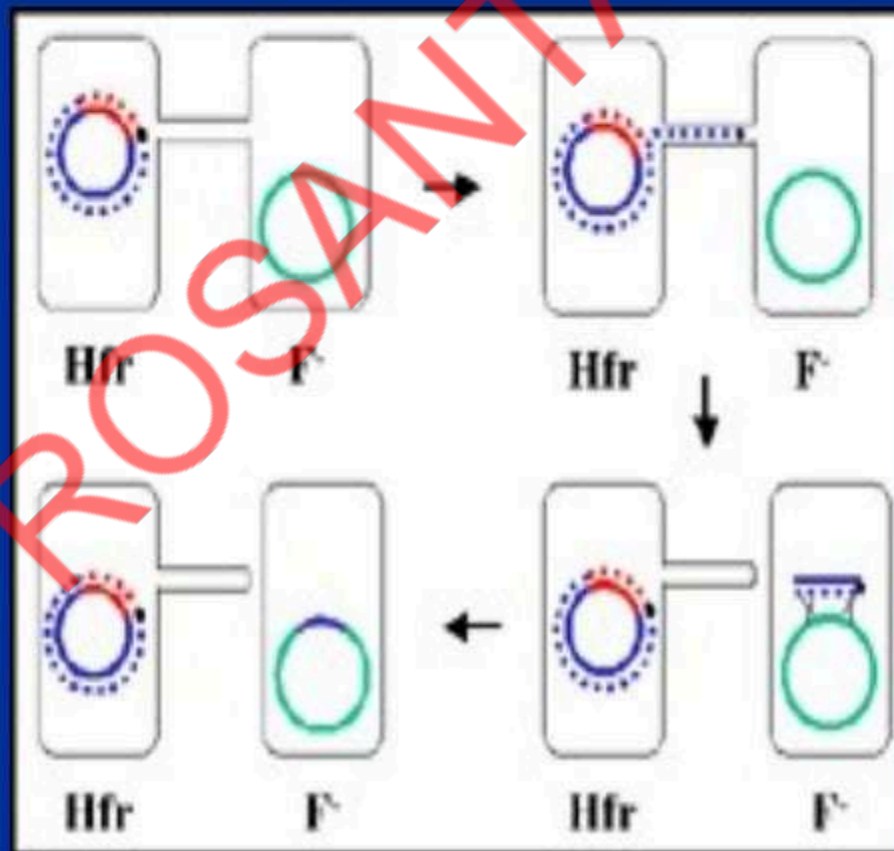
PROSANTA SARKHA

Physiological States of F Factor

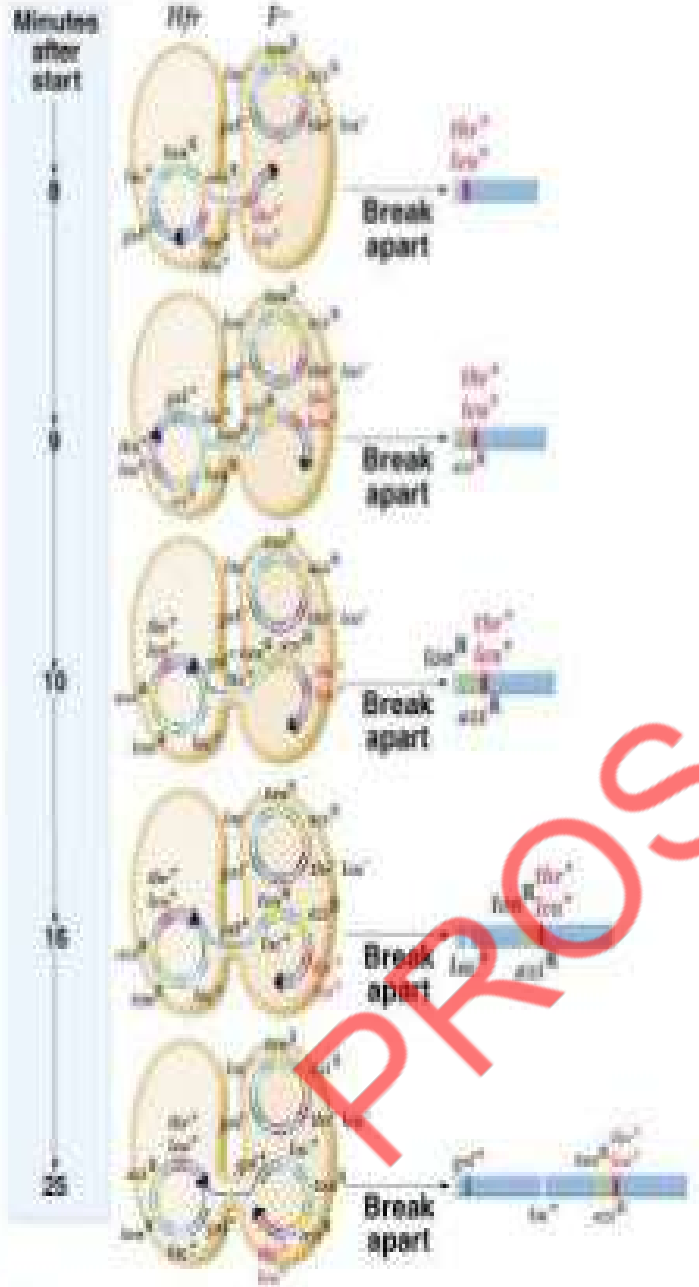
Integrated (Hfr)

Characteristics of Hfr \times F⁻ crosses:

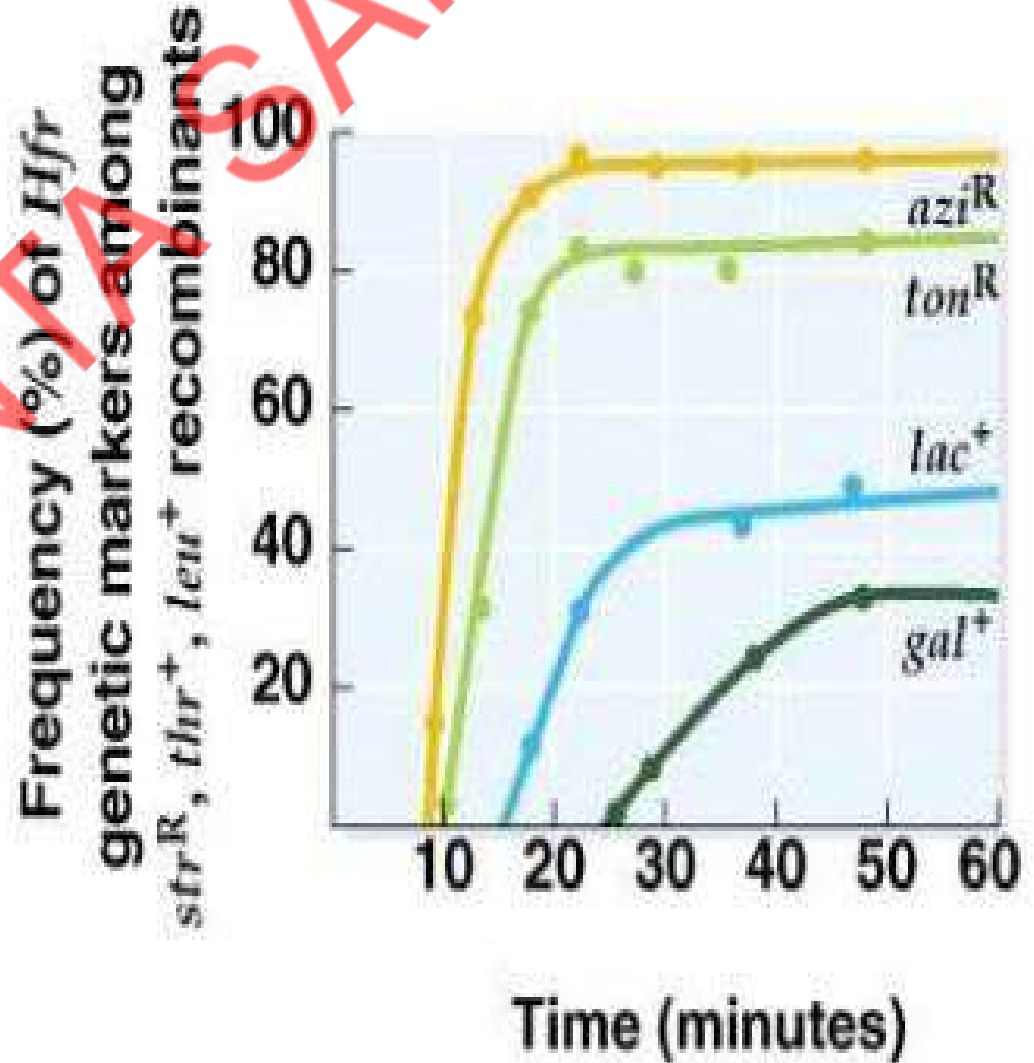
- F⁻ rarely becomes Hfr while Hfr remains Hfr
- High transfer of certain donor chromosomal genes

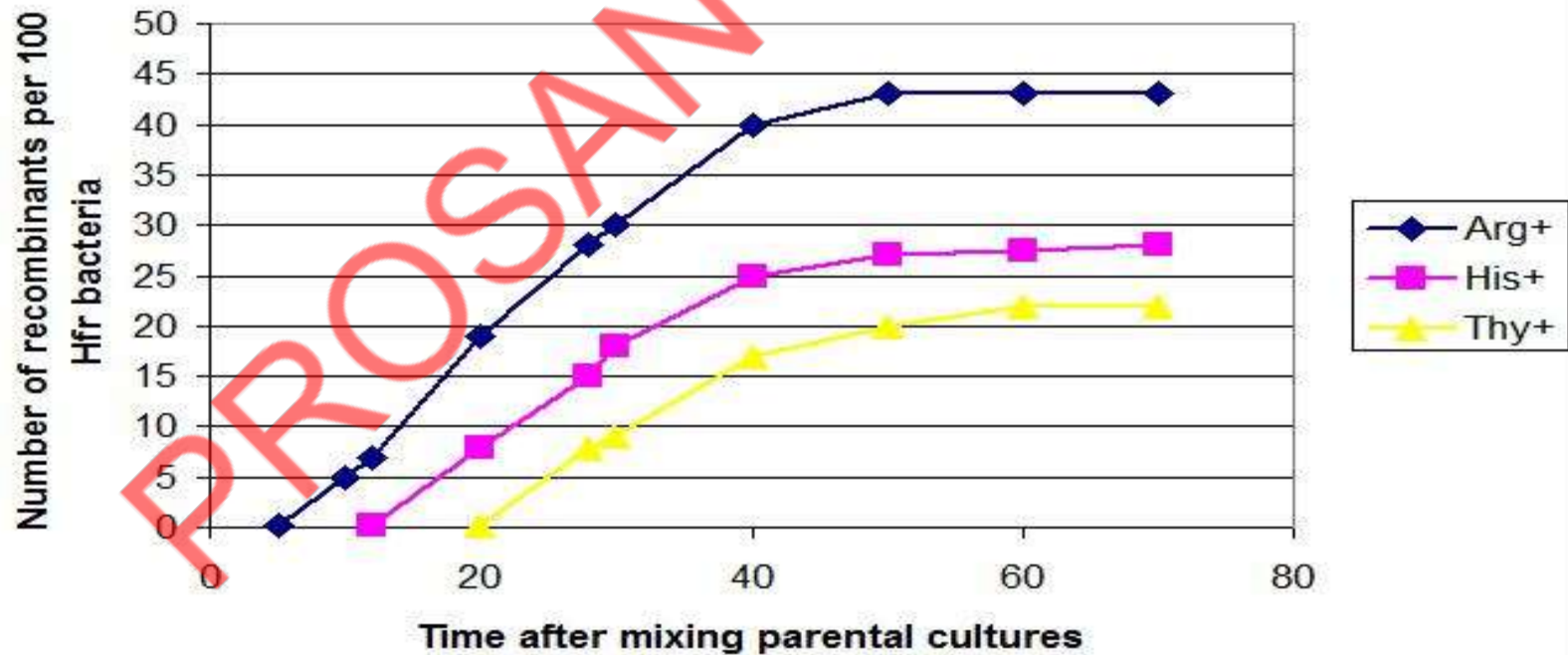
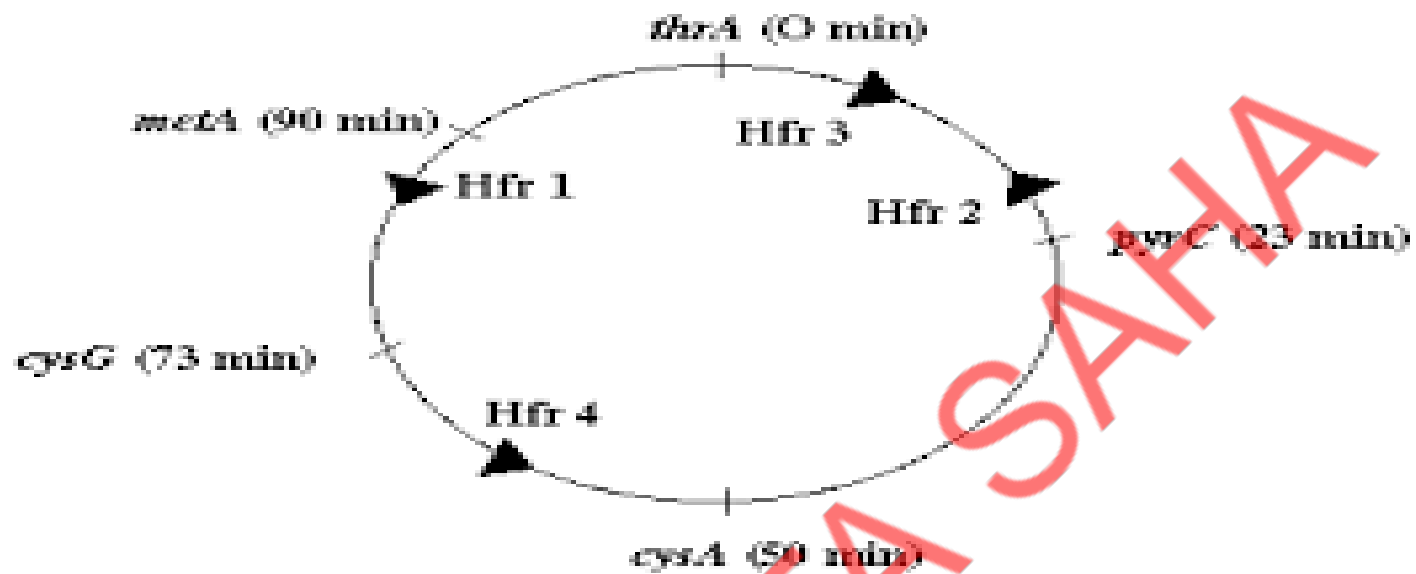


a) Progressive transfer of donor genes to recipient during *Hfr* × *F*⁻ conjugation

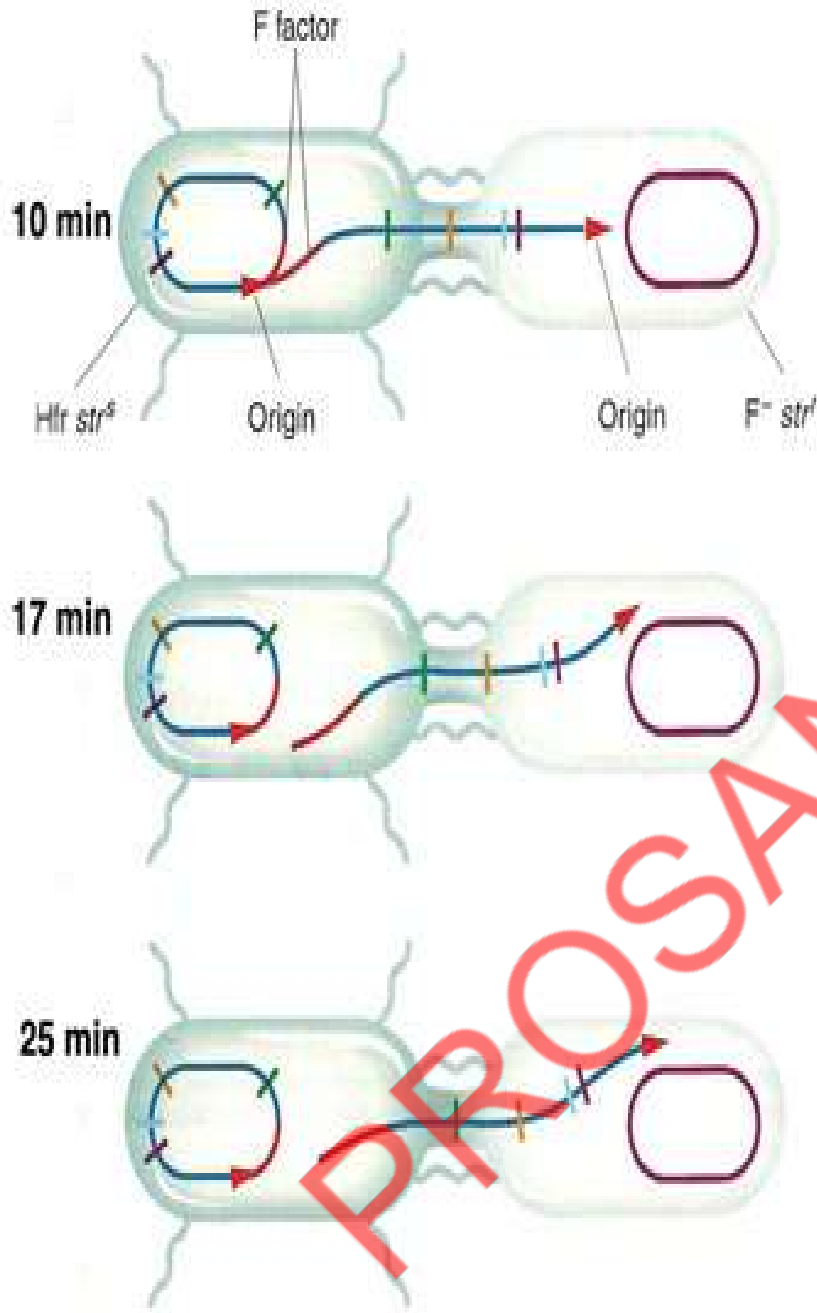


b) Appearance of donor genetic markers in recipient as a function of time

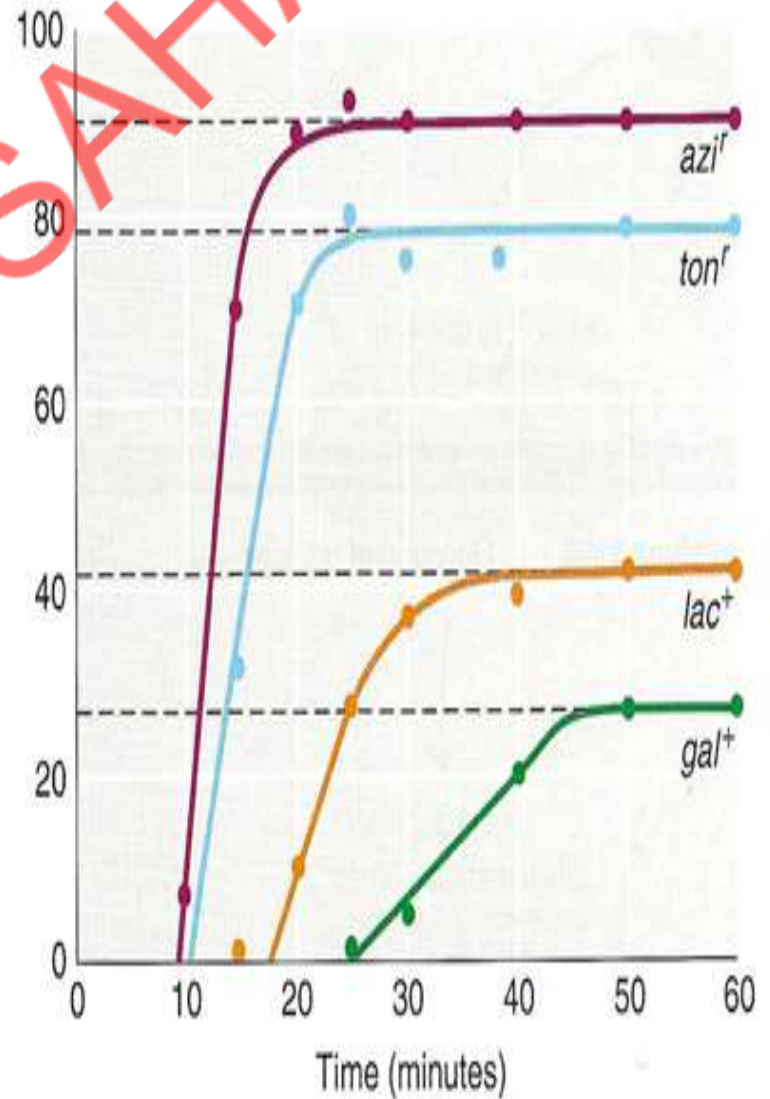




Interrupted mating shows markers transfer at different times

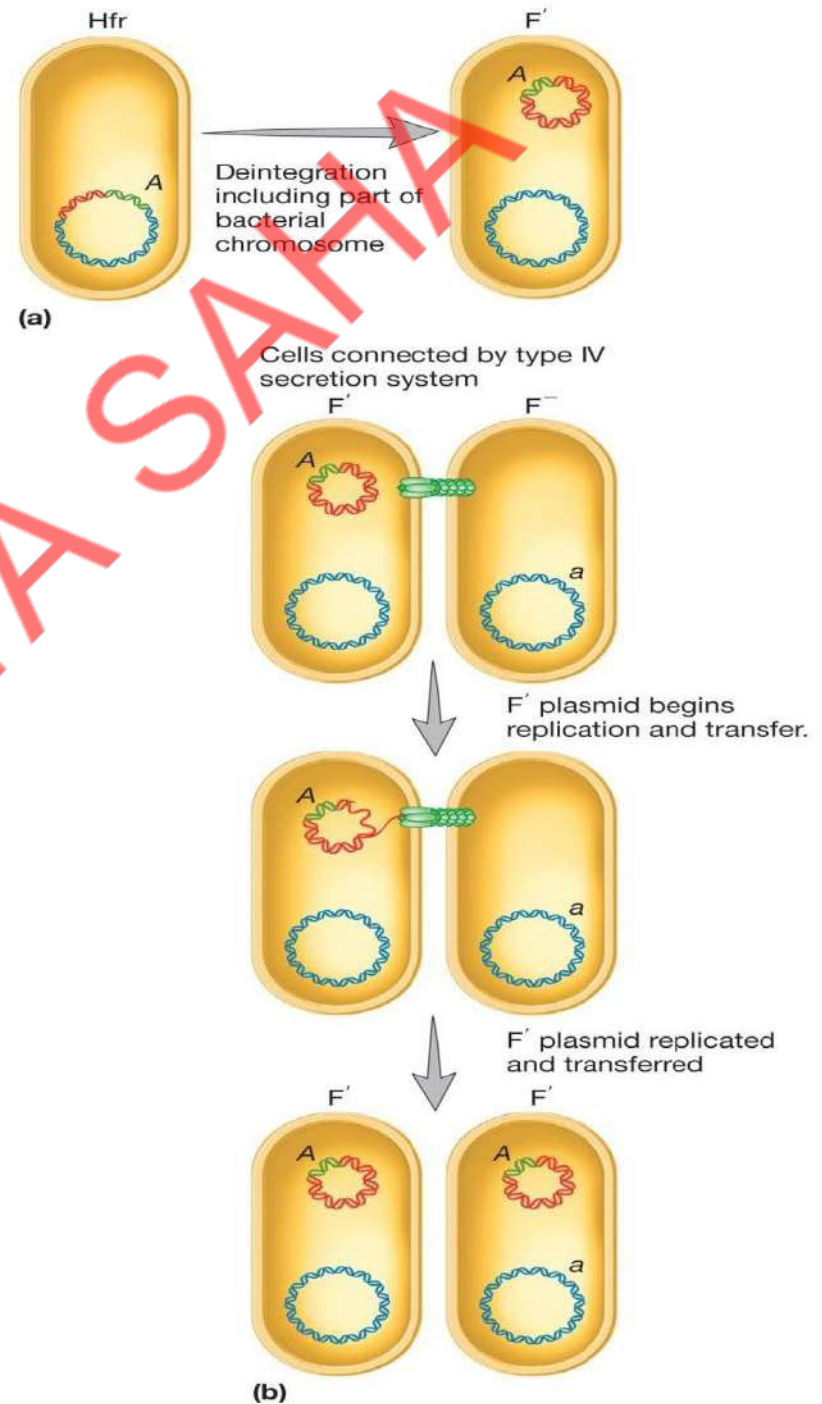


frequency (%)
of Hfr traits
among
exconjugants



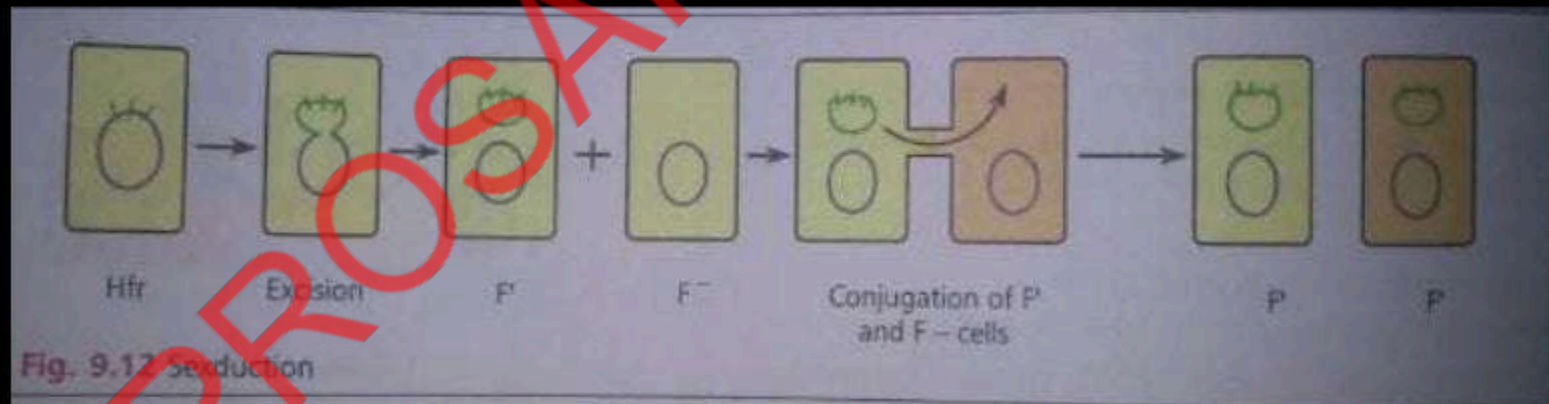
F' Conjugation

- Result when the F factor incorrectly leaves the host chromosome
- Some of the F factor is left behind in the host chromosome
- Some host genes have been removed along with some of the F factor
- these genes can be transferred to a second host cell by conjugation





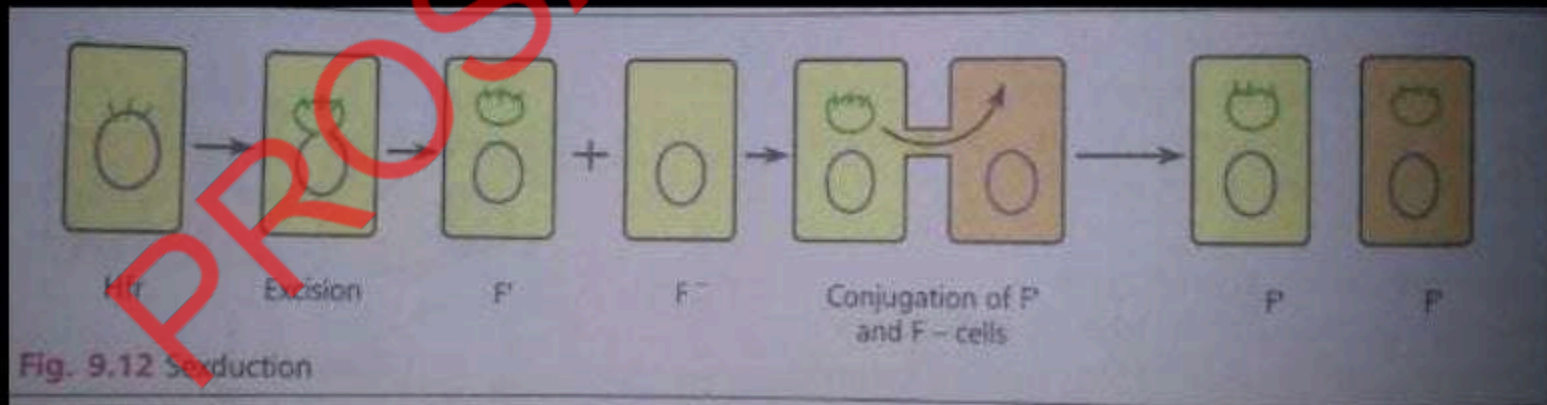
WHEN THE F FACTOR REVERTS FROM INTEGRATED \longrightarrow FREE STATE IT MAY SOMETIMES CARRY WITH IT SOME CHROMOSOMAL DNA FROM ADJACENT SITE OF ITS ATTACHMENT



SUCH AN F FACTOR IS KNOWN AS **F' FACTOR**

$(F') + (F^-) \longrightarrow$ SEXDUCTION

WHEN (F') CONJUGATES WITH A RECIPIENT (F^-) , IT TRANSFERS, ALONG WITH THE F FACTOR, THE HOST DNA INCORPORATED WITH IT.

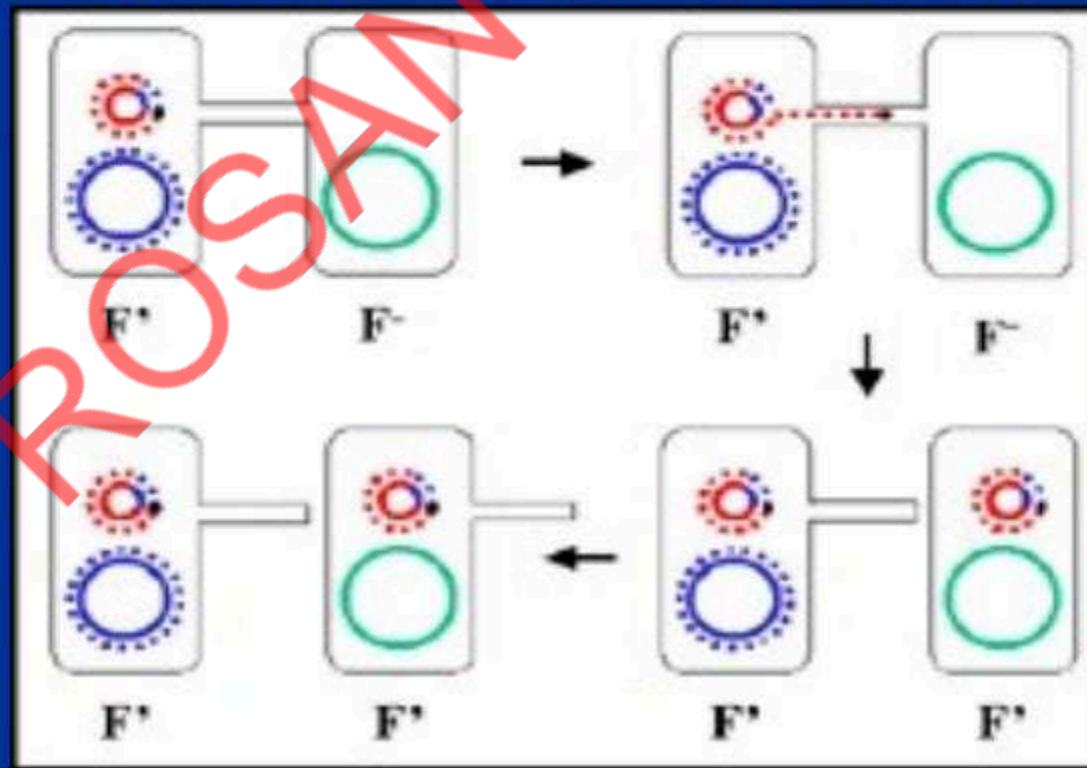


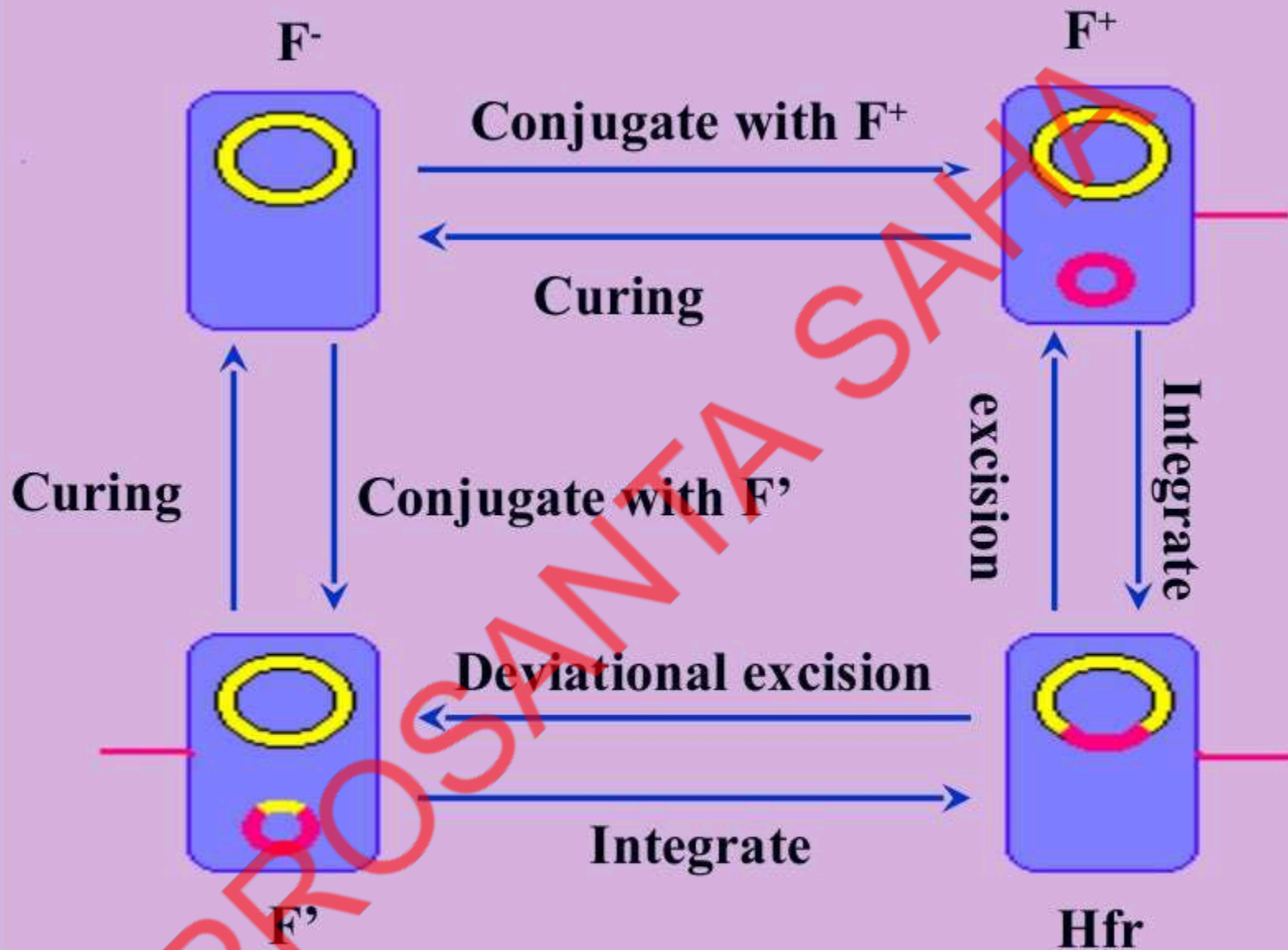
Physiological States of F Factor

Autonomous with donor genes (F')

Characteristics of F' x F- crosses:

- F- becomes F' while F' remains F'
- High transfer of donor genes on F' and low transfer of other donor chromosomal genes





Transform of F plasmid

3. TRANSDUCTION

- **Transduction** occurs when a phage (virus) carries bacterial genes from one host cell to another.
- TRANSDUCTION IS A PHENOMENON, BY WHICH, BACTERIAL DNA IS TRANSFERRED FROM ONE CELL TO ANOTHER WITH THE HELP OF BACTERIOPHAGE.
- Transduction was discovered by Norton Zinder and Joshua Lederberg at the University of Wisconsin–Madison in 1952.

Transduction

- Transduction is the process of moving bacterial DNA from one cell to another using a bacteriophage.
- Bacteriophage or just “phage” are bacterial viruses. They consist of a small piece of DNA inside a protein coat. The protein coat binds to the bacterial surface, then injects the phage DNA. The phage DNA then takes over the cell’s machinery and replicates many virus particles.
- Two forms of transduction:
 - 1. generalized: any piece of the bacterial genome can be transferred
 - 2. specialized: only specific pieces of the chromosome can be transferred.

What are Bacteriophages?

Bacteriophage (phage) are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery (i.e., viruses that infect bacteria)

- Two types:

1. Bacteriophage T4

2. Bacteriophage Lambda

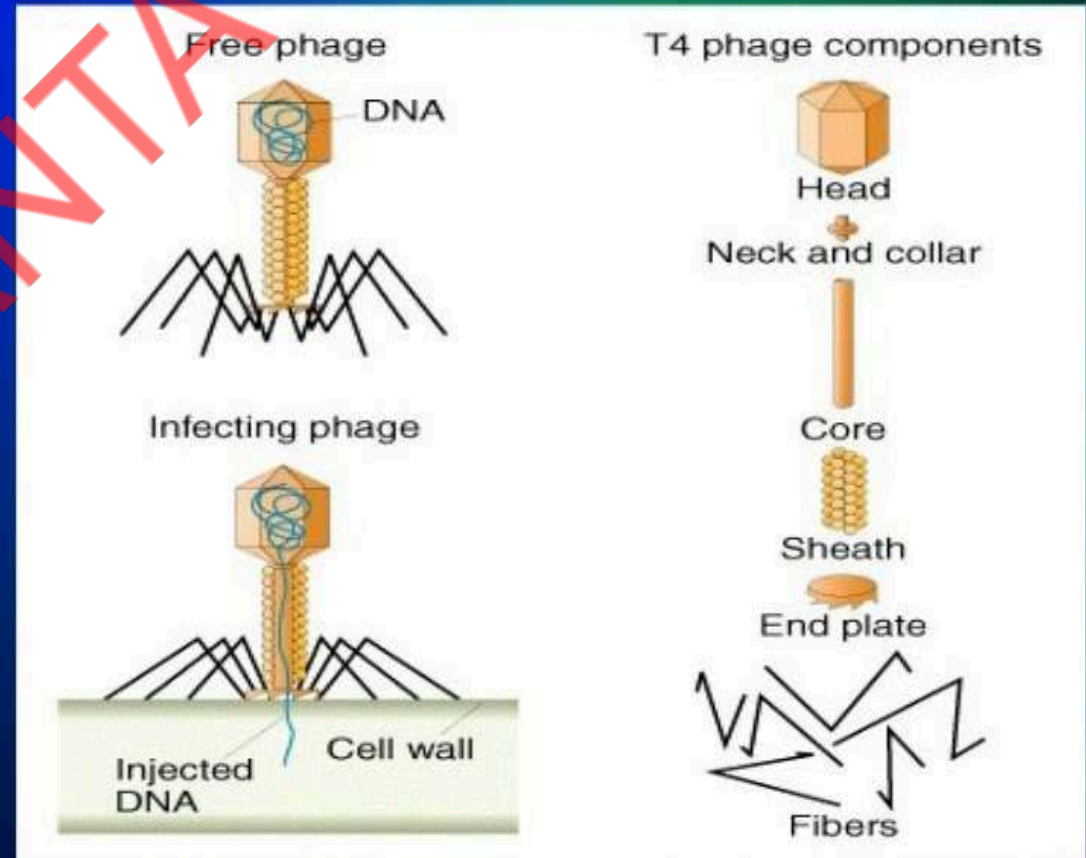
- The life cycle of these two involve:

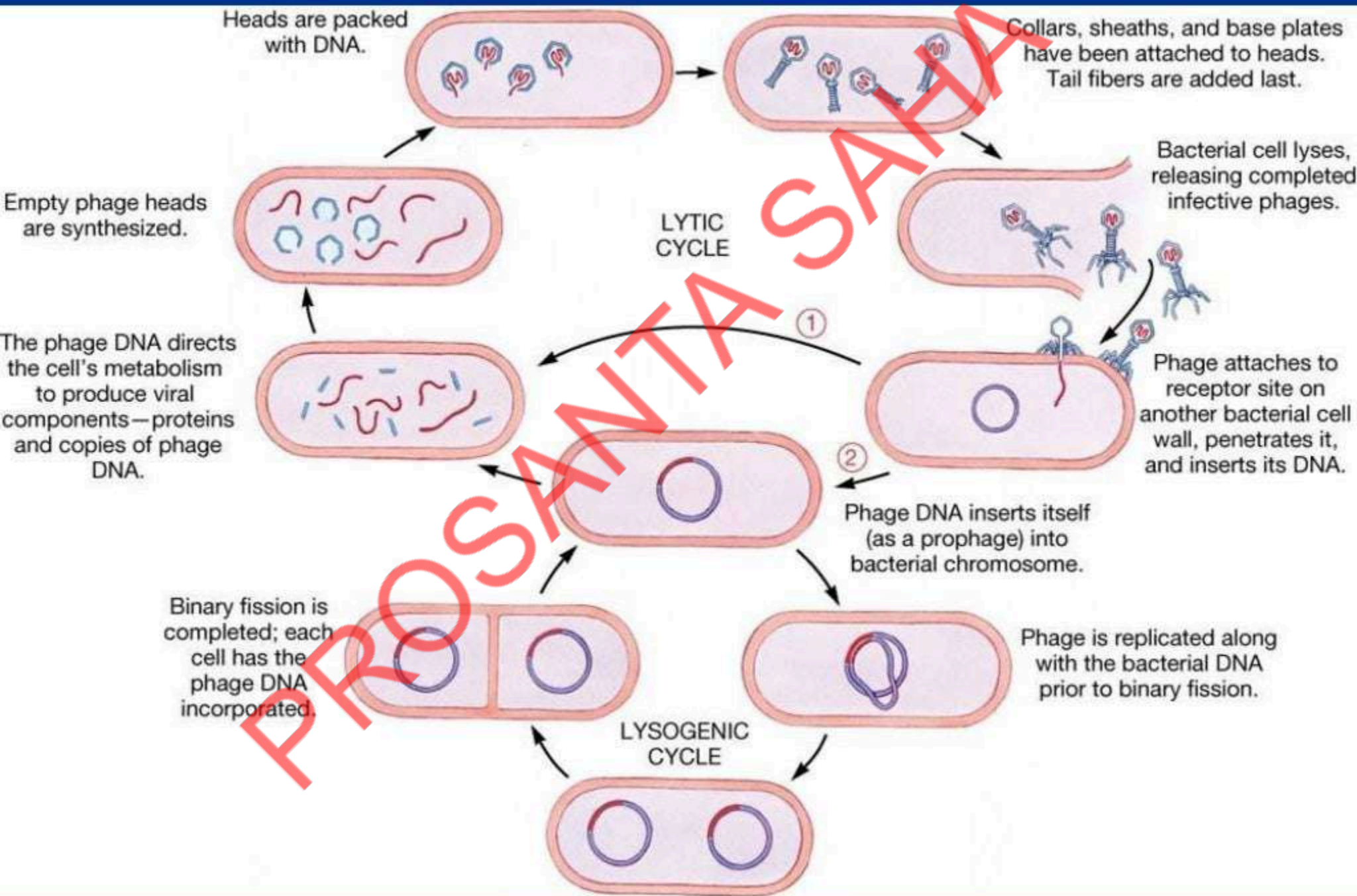
1. Lytic cycle

2. Lysogenic cycle

BACTERIOPHAGE

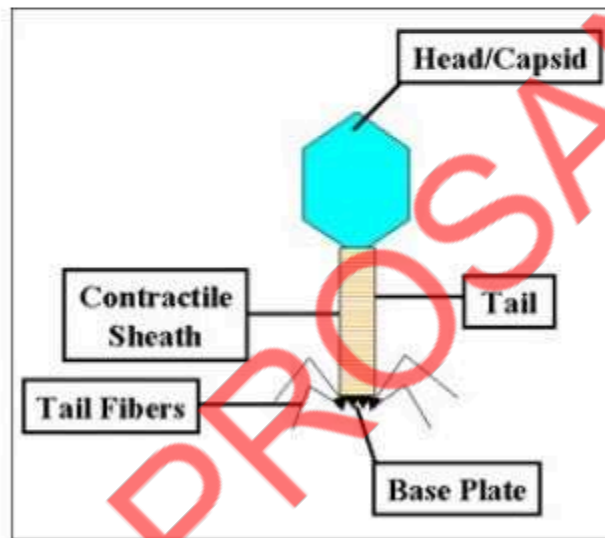
- Bacteriophages are viruses (PHAGE) which infects the bacteria.....



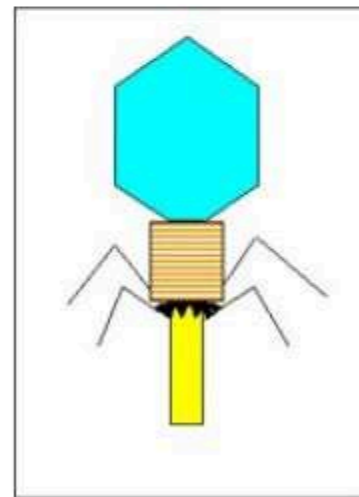


Transduction

- Genetic recombination in which a DNA fragment is transferred from one bacterium to another by a bacteriophage



Structure of T4 bacteriophage



Contraction of the tail sheath of T4

PROCESS OF TRANSDUCTION

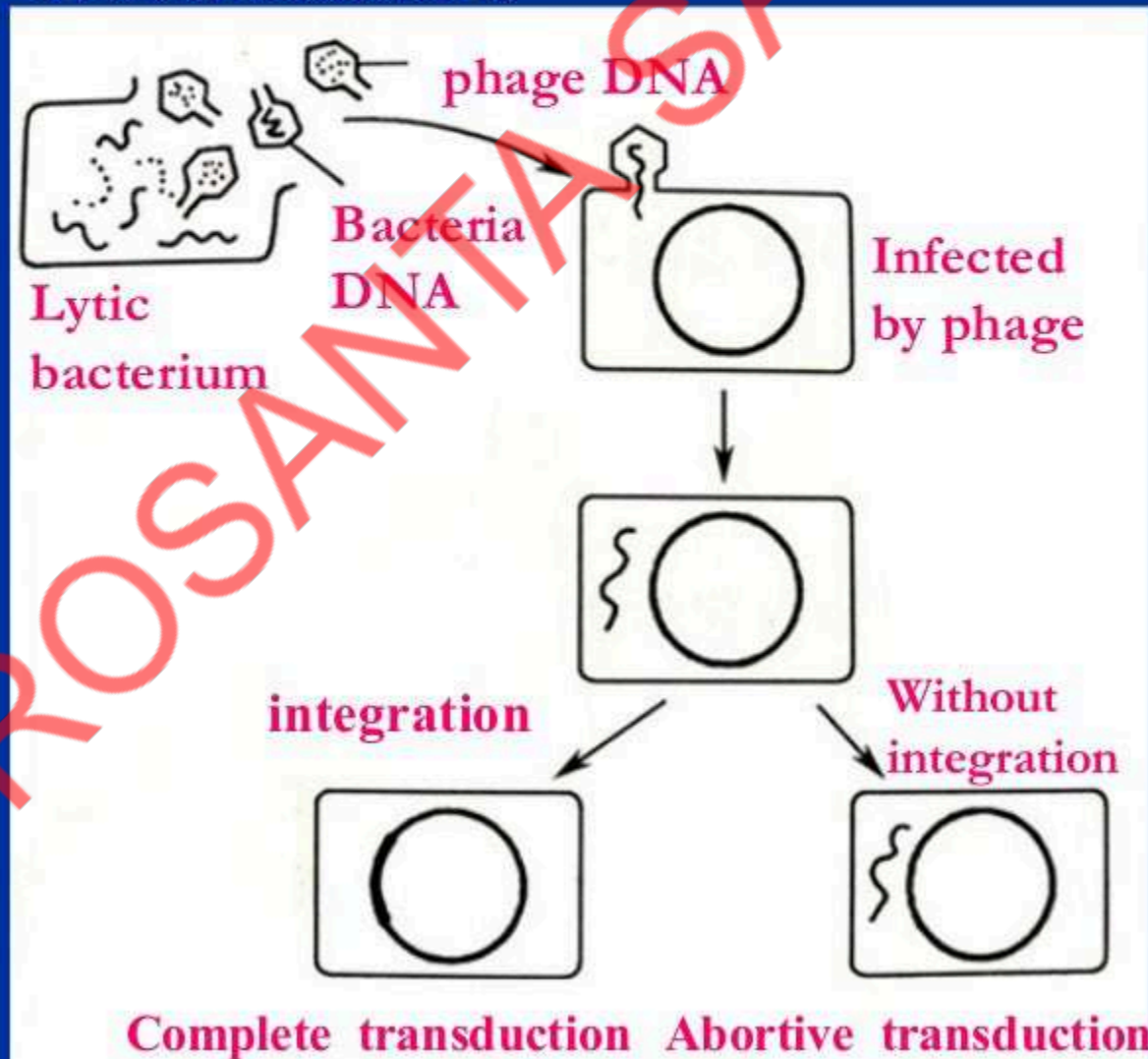
- First of all, bacteriophage attaches to donor bacteria.
- They inject their nucleic acid (DNA) into bacterium.
- This DNA replicates rapidly, and also directs the synthesis of new phage protein.
- Then, the new DNA combines with new proteins, to make whole phage particles.
- These are then released by destruction of cell wall and lysis of the cell.

Contd.

- These phases are composed of its DNA together with the donors DNA.
- Now Then this phage attacks the another host and infect it.
- the recipient DNA integrates with this DNA.
- And it results in the transfer of DNA.
- Recipient cell is now called TRANSDUCED CELL.

The fate of exogenous DNA in Generalized Transduction:

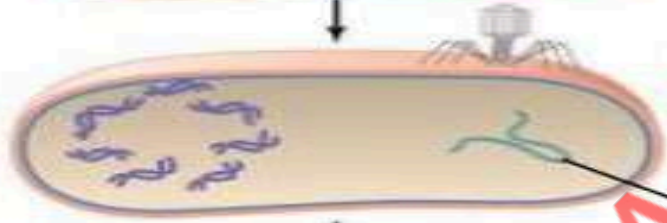
- 1) Complete transduction
- 2) Abortive transduction



Bacteriophage
Host bacterial cell
(donor cell)
Bacterial chromosome



1 Phage injects its 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.

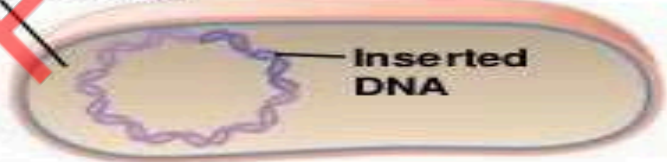
Transducing phage



Recipient host cell

4 Transducing phage injects donor DNA.

Transduced cell



5 Donor DNA is incorporated into recipient's chromosome by recombination.

TYPES OF TRANSDUCTION

- Mainly there are two types of transduction:-

i.e.

- ❖ GENERALISED or NON-SPECIALISED TRANSDUCTION
- ❖ RESTRICTED or SPECIALIZED TRANSDUCTION

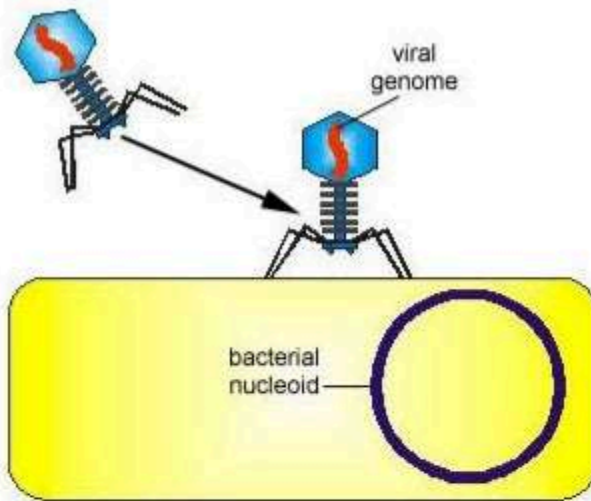
Transduction (cont'd)

- There are two types of transduction:
 - **generalized transduction:** A DNA fragment is transferred from one bacterium to another by a lytic bacteriophage that is now carrying donor bacterial DNA due to an error in maturation during the lytic life cycle.
 - **specialized transduction:** A DNA fragment is transferred from one bacterium to another by a temperate bacteriophage that is now carrying donor bacterial DNA due to an error in spontaneous induction during the lysogenic life cycle

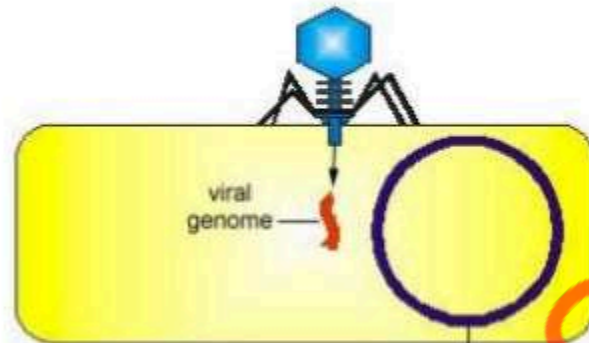
GENERALISED TRANSDUCTION

- If all fragments of bacterial DNA have a chance to enter a transducing phage, the process is called **GENERALISED TRANSDUCTION**.
- It mediates the exchange of any bacterial gene.

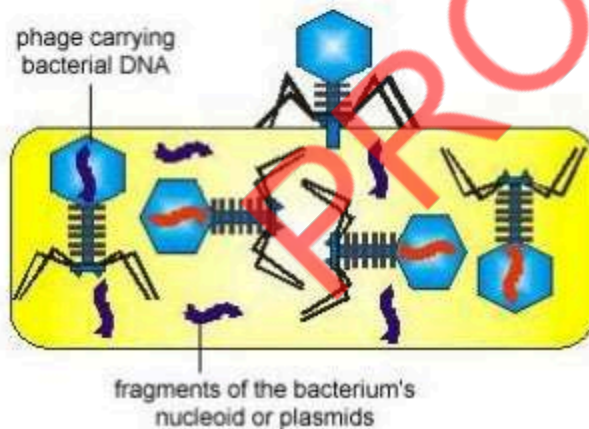
Seven steps in Generalised Transduction



1. A lytic bacteriophage adsorbs to a susceptible bacterium.

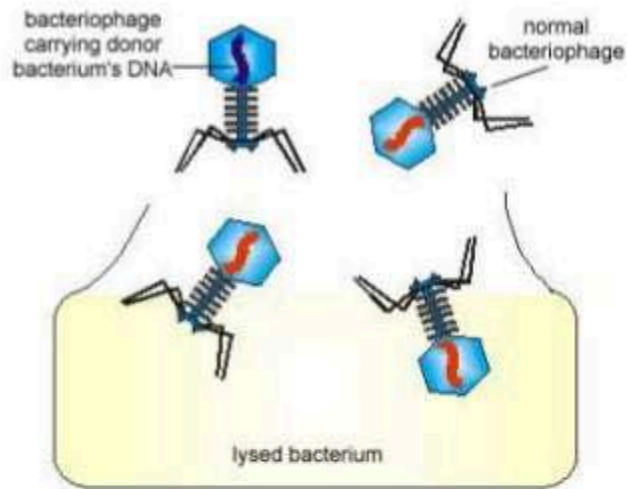


2. The bacteriophage genome enters the bacterium. The genome directs the bacterium's metabolic machinery to manufacture bacteriophage components and enzymes

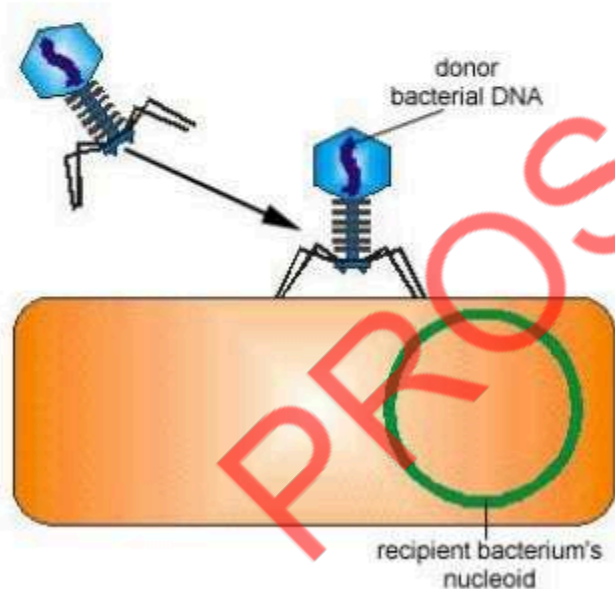


3. Occasionally, a bacteriophage head or capsid assembles around a fragment of donor bacterium's nucleoid or around a plasmid instead of a phage genome by mistake.

Seven steps in Generalised Transduction (cont'd)

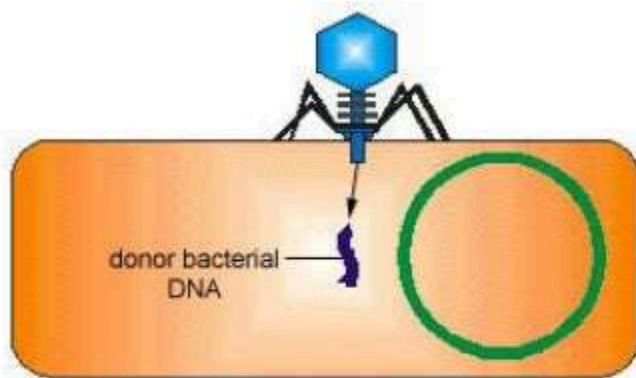


4. The bacteriophages are released.

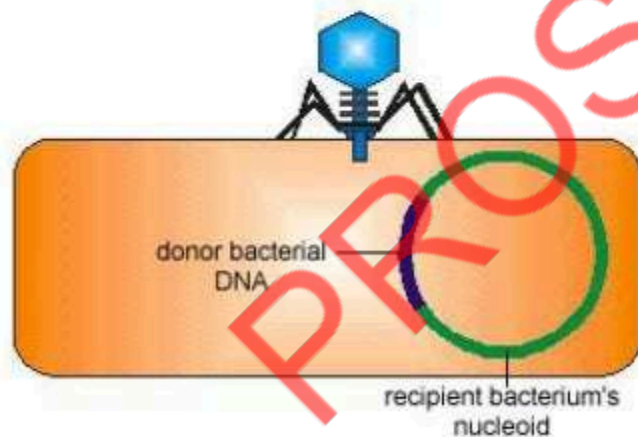


5. The bacteriophage carrying the donor bacterium's DNA adsorbs to a recipient bacterium

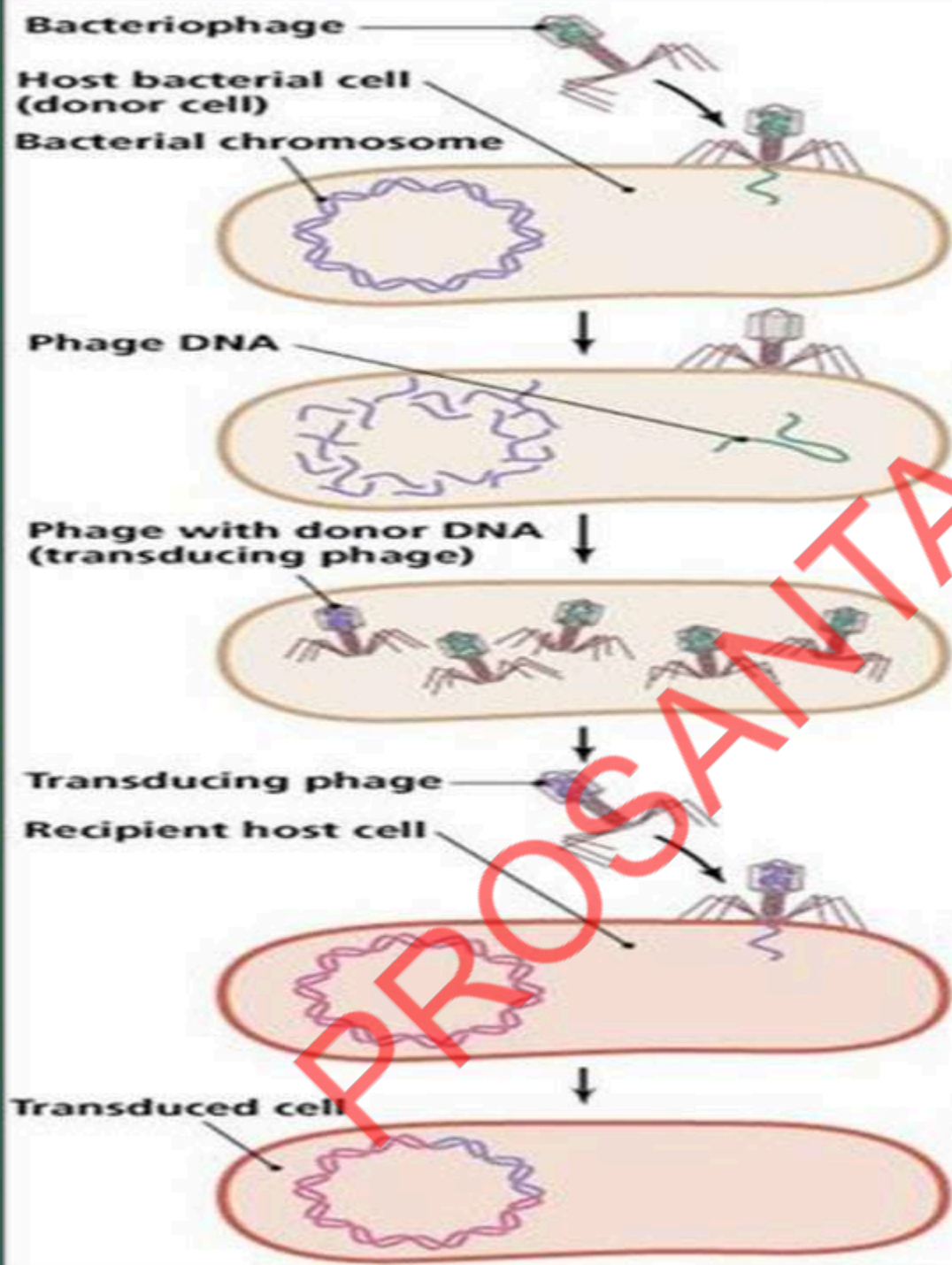
Seven steps in Generalised Transduction (contd)



6. The bacteriophage inserts the donor bacterium's DNA it is carrying into the recipient bacterium .



7. The donor bacterium's DNA is exchanged for some of the recipient's DNA.



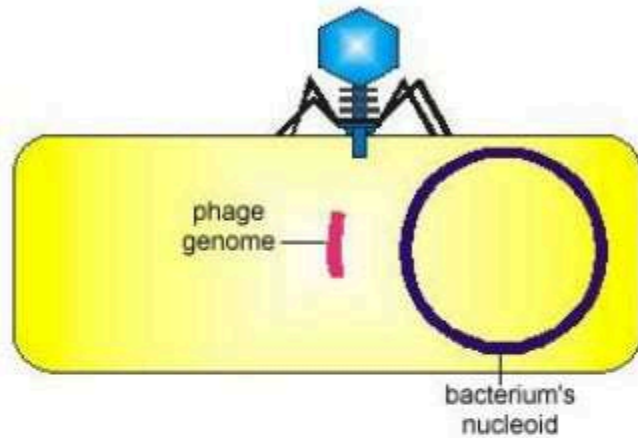
- 1 Phage injects its DNA
- 2 Phage enzymes degrade host DNA
- 3 Cell synthesizes new phages that incorporate phage DNA and, mistakenly, some host DNA
- 4 Transducing phage injects donor DNA
- 5 Donor DNA is incorporated into recipient's chromosome by recombination

PROSANTA SAHA

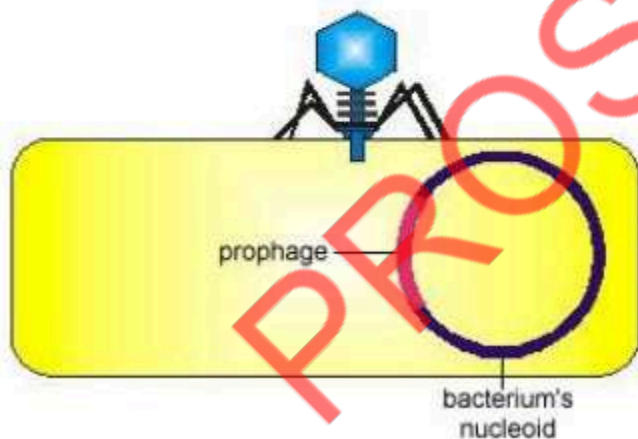
SPECIALISED TRANSDUCTION

- “In this phenomenon, certain phages can transfer only a few restricted genes of the bacterial chromosomes.”
- Here, the phages transduce only those bacterial genes adjacent to the phage in the bacterial chromosomes.
- Thus the process is called restricted transduction.
- It mediates the exchange of only limited numbers of specific genes.
- This is mediated by LAMBDA PHAGE VIRUS.

Six steps in Specialised Transduction

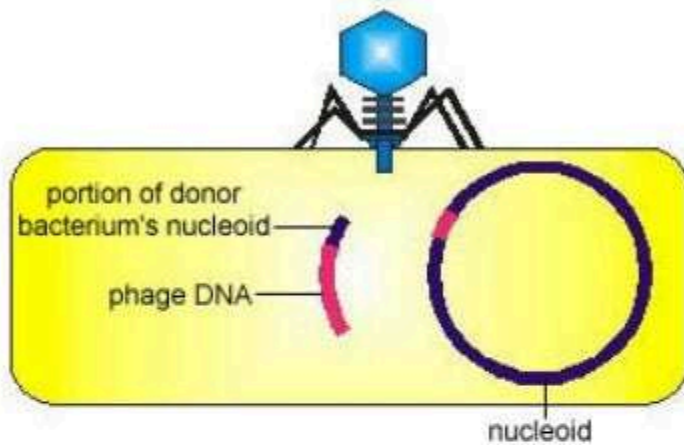


1. A temperate bacteriophage adsorbs to a susceptible bacterium and injects its genome.

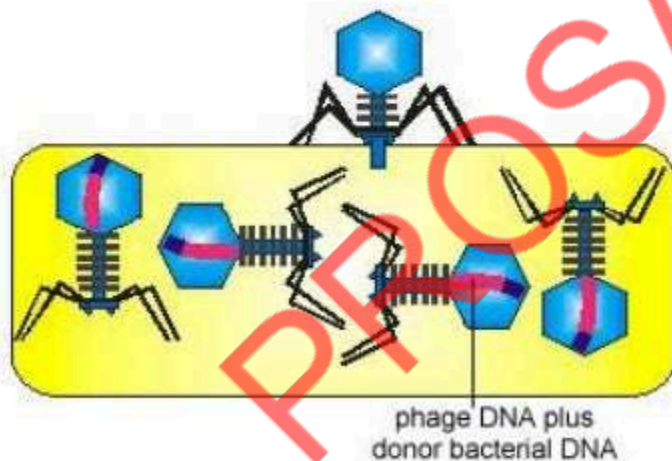


2. The bacteriophage inserts its genome into the bacterium's nucleoid to become a prophage.

Six steps in Specialised Transduction (cont'd)

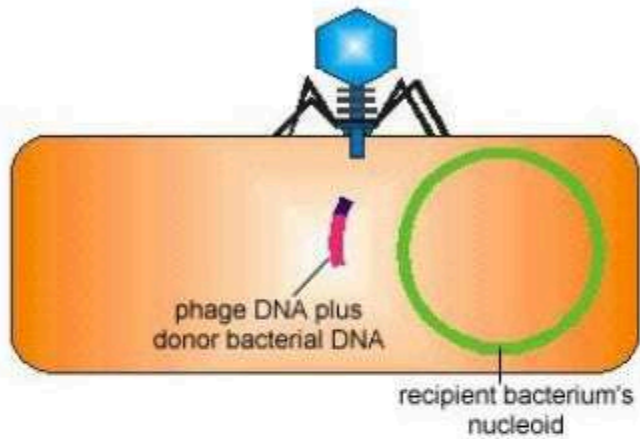


3. Occasionally during spontaneous induction, a small piece of the donor bacterium's DNA is picked up as part of the phage's genome in place of some of the phage DNA which remains in the bacterium's nucleoid.

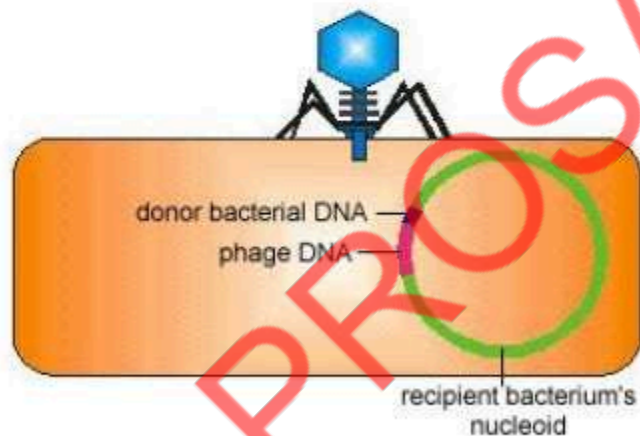


4. As the bacteriophage replicates, the segment of bacterial DNA replicates as part of the phage's genome. Every phage now carries that segment of bacterial DNA.

Six steps in Specialised Transduction (cont'd)



5. The bacteriophage adsorbs to a recipient bacterium and injects its genome.



6. The bacteriophage genome carrying the donor bacterial DNA inserts into the recipient bacterium's nucleoid.

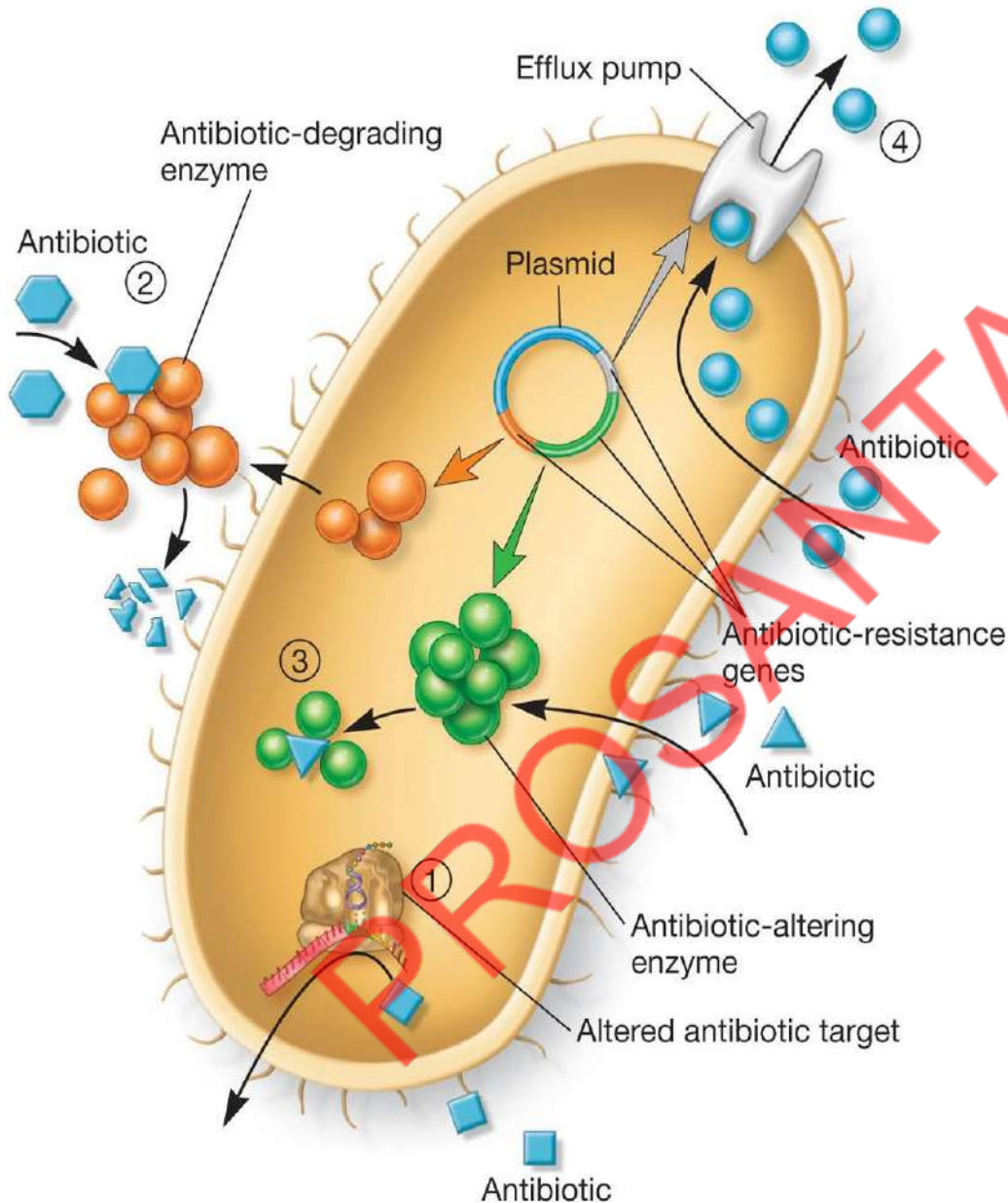
Drug Resistance

- An increasing problem
 - once resistance originates in a population it can be transmitted to other bacteria
 - a particular type of resistance mechanism is not confined to a single class of drugs
- Microbes in abscesses or biofilms may be growing slowly and not be susceptible
- Resistance mutants arise spontaneously and are then selected

Drug Resistant “Superbug”

- A methicillin-resistant *Staphylococcus aureus* (MRSA) that developed resistance to vancomycin
 - this new vancomycin-resistant *S. aureus* (VRSA) was also resistant to most other antibiotics
 - isolated from foot ulcers on a diabetic patient
 - Acquired from conjugation with vancomycin-resistant enterococci (VRE) were isolated from same patient
- These drug resistant organisms are a serious threat to human health

Mechanisms of Drug Resistance



- Prevent entrance of drug
- Drug efflux (pump drug out of cell)
- Inactivation of drug
 - chemical modification of drug by pathogen
- Modification of target enzyme or organelle
- Use of alternative pathways or increased production of target metabolite

The Origin and Transmission of Drug Resistance

- Immunity genes
 - resistance genes that exist in nature to protect antibiotic producing microbes from their own antibiotics
- Horizontal gene transfer
 - transferred immunity genes from antibiotic producers to non-producing microbes

Origin and Transmission...

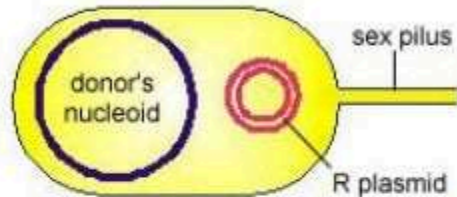
- Chromosomal genes
 - resistance from (rare) spontaneous mutations (usually result in a change in the drug target)
- R (resistance) plasmids
 - can be transferred to other cells by conjugation, transduction, and transformation
 - can carry multiple resistance genes



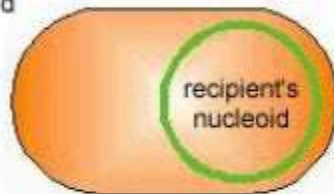
III. Resistant Plasmid Conjugation

Genetic recombination in which there is a transfer of an R plasmid (a plasmid coding for multiple antibiotic resistance and often a sex pilus) from a male donor bacterium to a female recipient bacterium. Involves a sex (conjugation) pilus

4 stepped Resistant Plasmid Conjugation

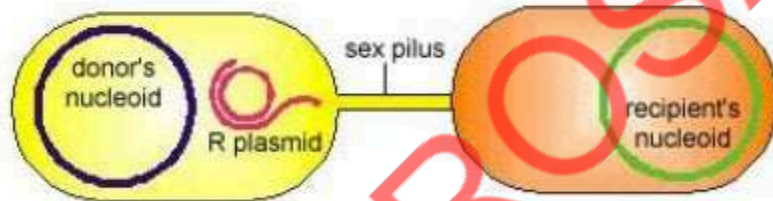


Bacterium with R plasmid: multiple antibiotic resistant and male (donor).



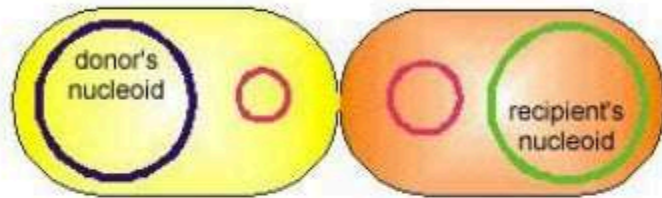
Bacterium without R plasmid: antibiotic sensitive and female (recipient).

1. The bacterium with an R-plasmid is multiple antibiotic resistant and can produce a sex pilus (serve as a genetic donor).

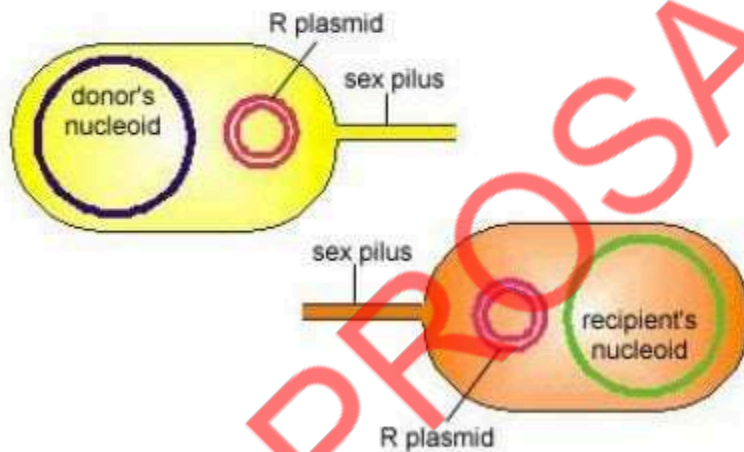


2. The sex pilus adheres to an F- female (recipient). One strand of the R-plasmid breaks.

4 stepped Resistant Plasmid Conjugation (cont'd)



3. The sex pilus retracts and a bridge is created between the two bacteria. One strand of the R-plasmid enters the recipient bacterium.



4. Both bacteria make a complementary strand of the R-plasmid and both are now multiple antibiotic resistant and capable of producing a sex pilus.

THANK YOU

PROSANTA SAHA