BACTERIAL GENETICS
(Mutation & Recombination)

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BACTERIAL MUTATION

Mutation

- A heritable change in the nucleotide sequence of a gene is called a **mutation.**

- Mutations are usually detrimental, but they can also lead to beneficial changes.

- A mutant is called an **auxotroph** if the mutation leads to a new nutrient requirement, while the wild type strain is known as **prototroph**.

Mutation

- Two types – spontaneous and induced
  - **Spontaneous mutation** occurs naturally, about one in every million to one in every billion divisions, and is probably due to low level natural mutagens present in the environment.
  - **Induced mutation** is caused by **mutagens** that cause a much higher rate of mutation; induced by **chemicals** or **radiations**
- Other mutations are caused by transposable genetic element

Chemical mutagens

- **chemical modifications of purine and pyrimidine bases** that alter their hydrogen-bonding properties, e.g. nitrous acid converts cytosine to uracil which then forms hydrogen bonds with adenine rather than guanine.
- **incorporation of base analogs** (compounds that chemically resemble a nucleotide base closely, but do not have the hydrogen-bonding properties of the natural base) during DNA replication in place of the natural base, e.g. 2-amino purine, a compound that resembles adenine, and 5-bromouracil, a compound that resembles thymine.
- **intercalating agents** (planar three-ringed molecules that are about the same size as a nucleotide base pair) can insert or intercalate between adjacent base pairs during DNA replication thus pushing the nucleotides far enough apart so that an extra nucleotide is often added to the growing chain during DNA replication, e.g. ethidium bromide.
- **activation of SOS repair** that can lead to further mistakes in DNA base pairing

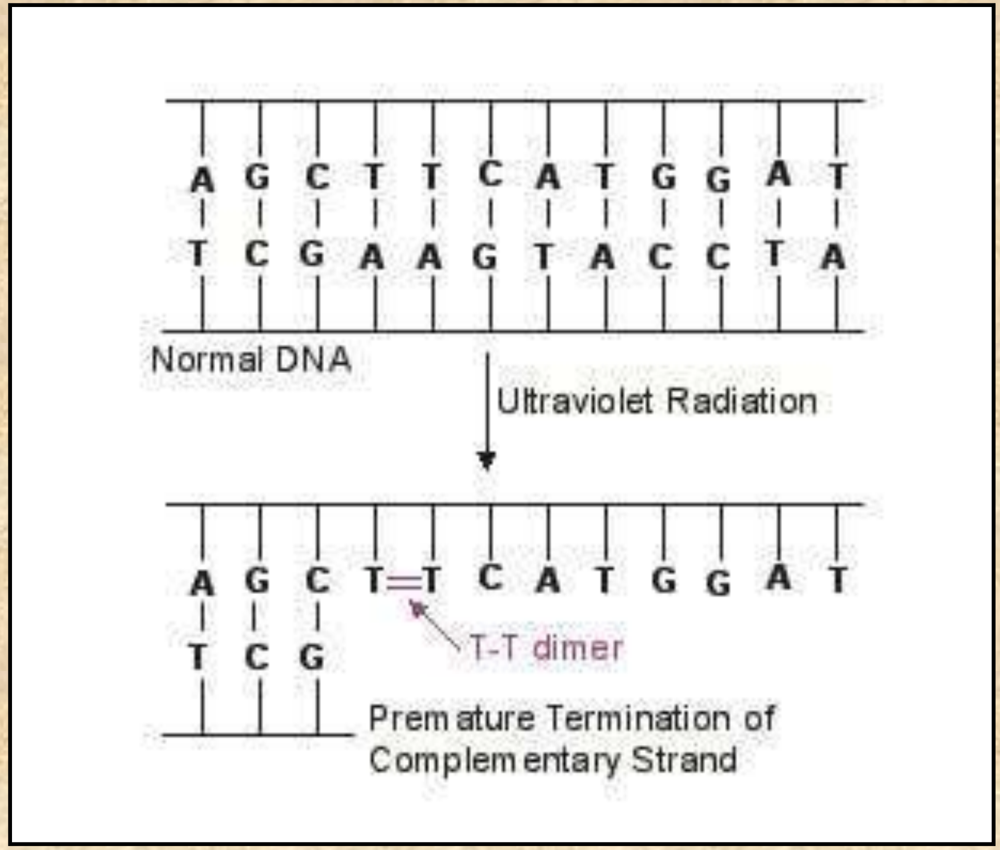
Radiations as mutagens

Non-ionizing radiations (e.g. UV rays)

- **Induce formation of thymine-thymine dimers,** which do not complementary base pair with the nucleotides and this terminates the replication of DNA strand.
- **Activation of SOS repair system**, which binds to DNA polymerase. However, this altered DNA polymerase loses its proofreading ability resulting in the synthesis of DNA that itself now contains many mis-incorporated bases.

Ionizing Radiation (e.g. X-rays & γ-rays)

- has much more energy and penetrating power than ultraviolet radiation;
- ionizes water and other molecules to form free radicals that can **break DNA strands and alter purine and pyrimidine bases**.


A G C T T C A T G G A T
T C G A A G T A C C T A
Normal DNA
Ultraviolet Radiation
A G C T=T C A T G G A T
T C G
T-T dimer
Premature Termination of
Complementary Strand

Types of Mutations

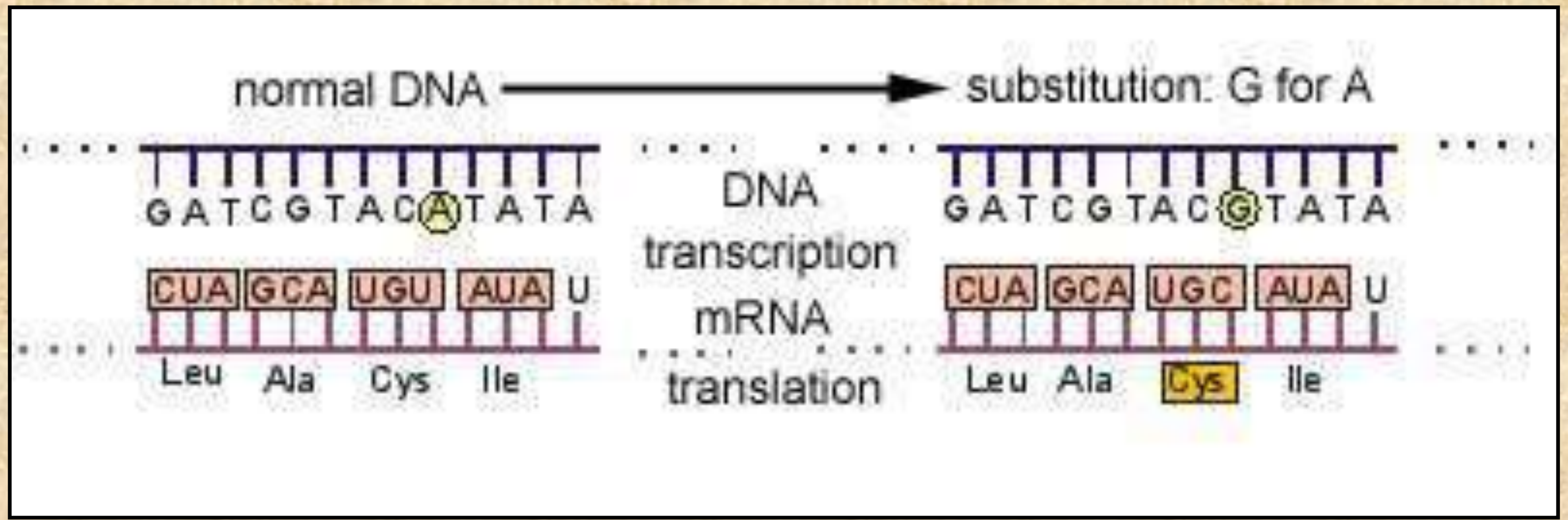
Point mutation

- substitution of one base for another during DNA replication;
- most common mechanism of mutation;
- substitution of one nucleotide for another may be a result of **tautomeric shift**, **a process by which the hydrogen atoms of a base shift in a way that changes the properties of its hydrogen bonding**
- for example, a shift in the hydrogen atom of adenine enables it to form hydrogen bonds with cytosine rather than thymine

Results of point mutation

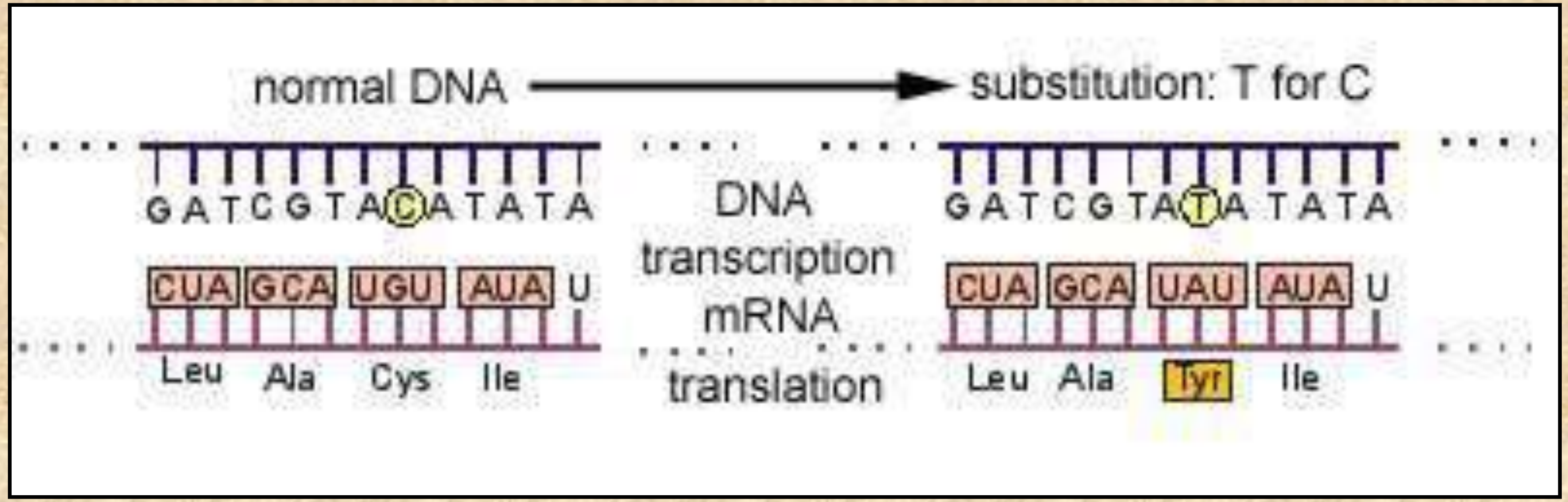
A sense mutation

A single substitution mutation which results in a new codon still coding for the same amino acid.



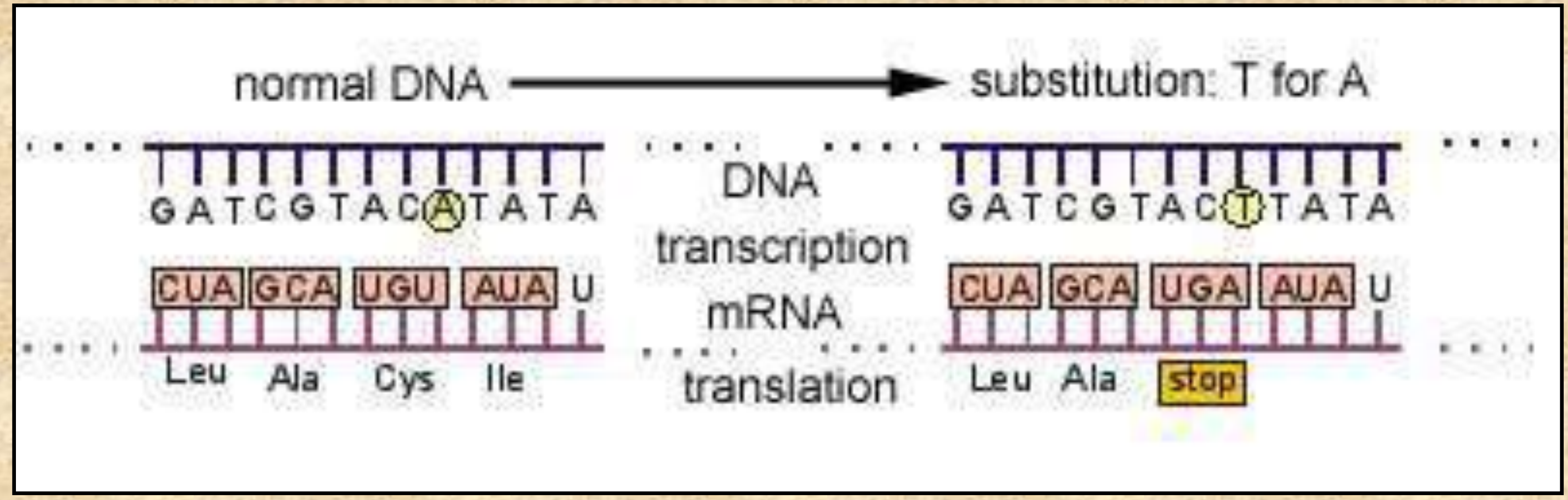
A mis-sense mutation

A single substitution mutation which results in one wrong codon and, therefore, one wrong amino acid.



A non-sense mutation

A single substitution mutation which results in transcription of a stop or nonsense codon resulting in the termination of polypeptide chain

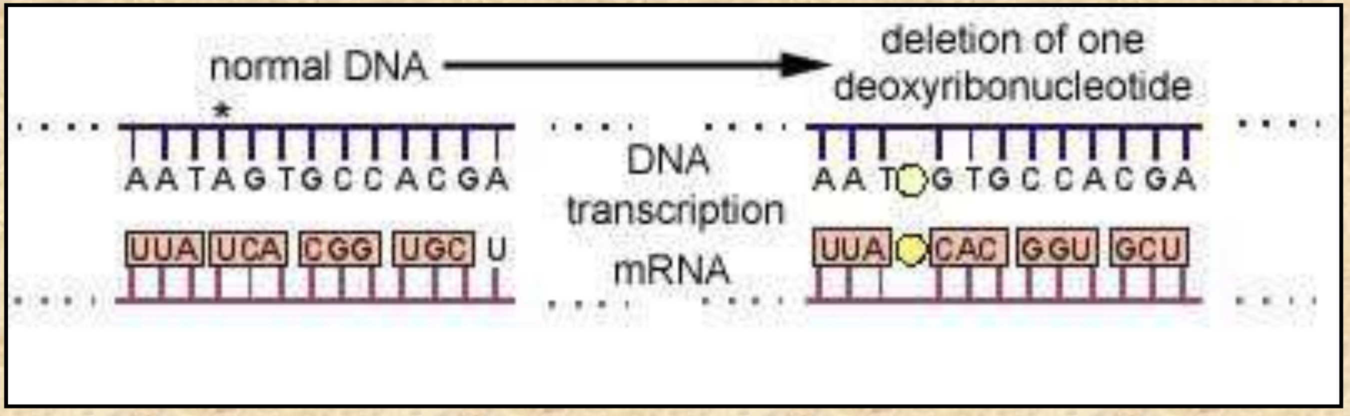


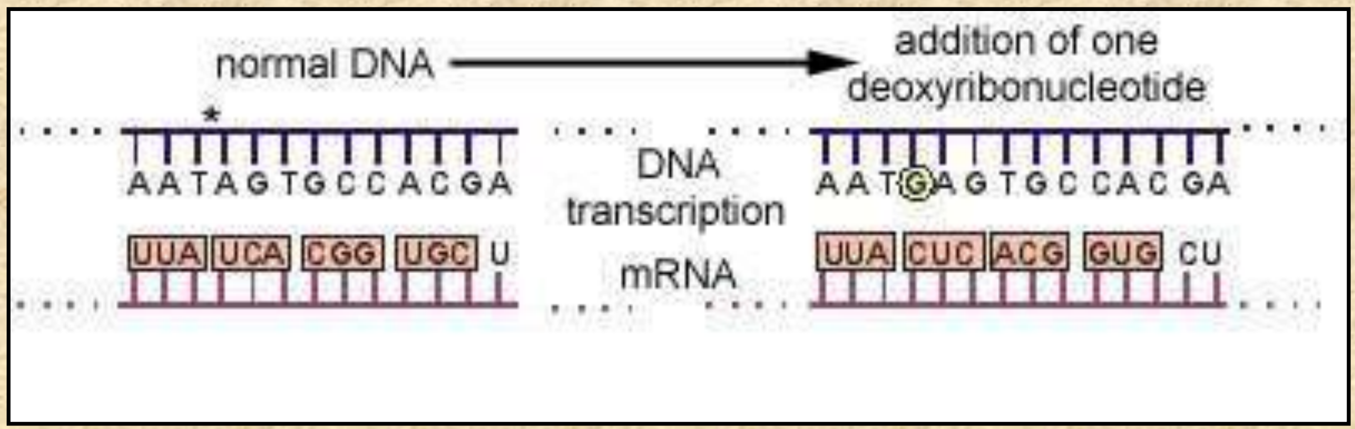
Types of Mutations

Deletion or addition of a nucleotide (Frame shift mutation)

- **DNA nucleotides not divisible by three are added or deleted.**
- **causes a shift in open reading frame and all of the codons and all of the amino acids after that mutation are usually wrong**
- **frequently one of the wrong codons turns out to be a stop or nonsense codon and the protein is terminated at that point**

Frame shift mutation





BACTERIAL RECOMBINATION

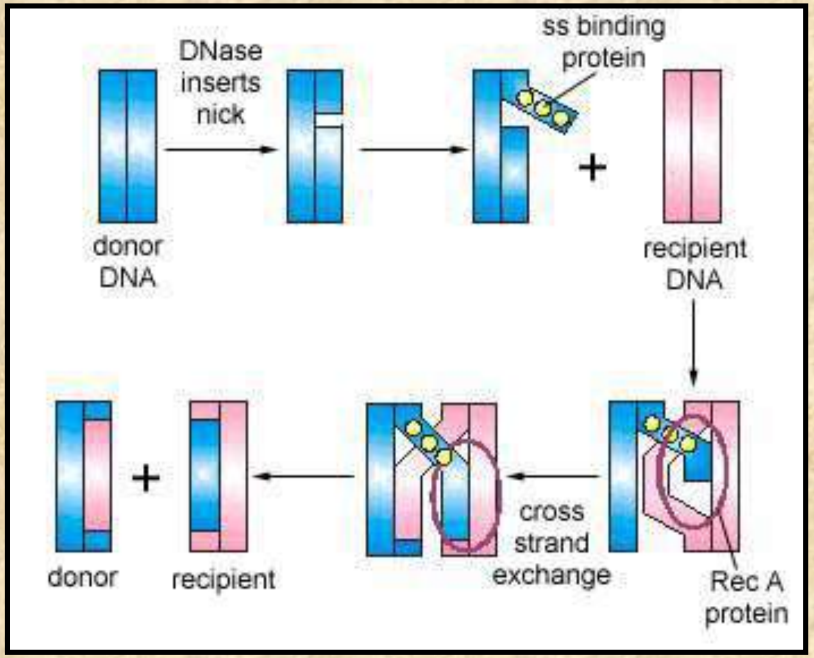
Genetic Recombination in Bacteria

- **Genetic recombination** is the transfer of DNA from one organism to another.
- The donor's DNA may then be integrated into the recipient's DNA by various mechanisms - homologous or heterologous

General Features of Recombination

- Unidirectional
  - Donor to recipient
- Donor does not give an entire chromosome
  - Merozygotes
- Gene transfer can occur between same or different species

Homologous recombination of bacterial DNA



- DNase inserts a nick in one strand of the donor DNA.
- Then single-stranded binding protein (yellow) bind to nicked strand to stabilise
- Rec A protein then binds to the single-strand fragment
- Strand exchange between donor and recipient DNA followed by annealing.

Other Mechanisms of Genetic Recombination n Bacteria

- **Transformation**
- **Transduction**
- **Conjugation**

NOTE THAT THESE ARE NOT METHODS OF REPRODUCTION

TRANSFORMATION

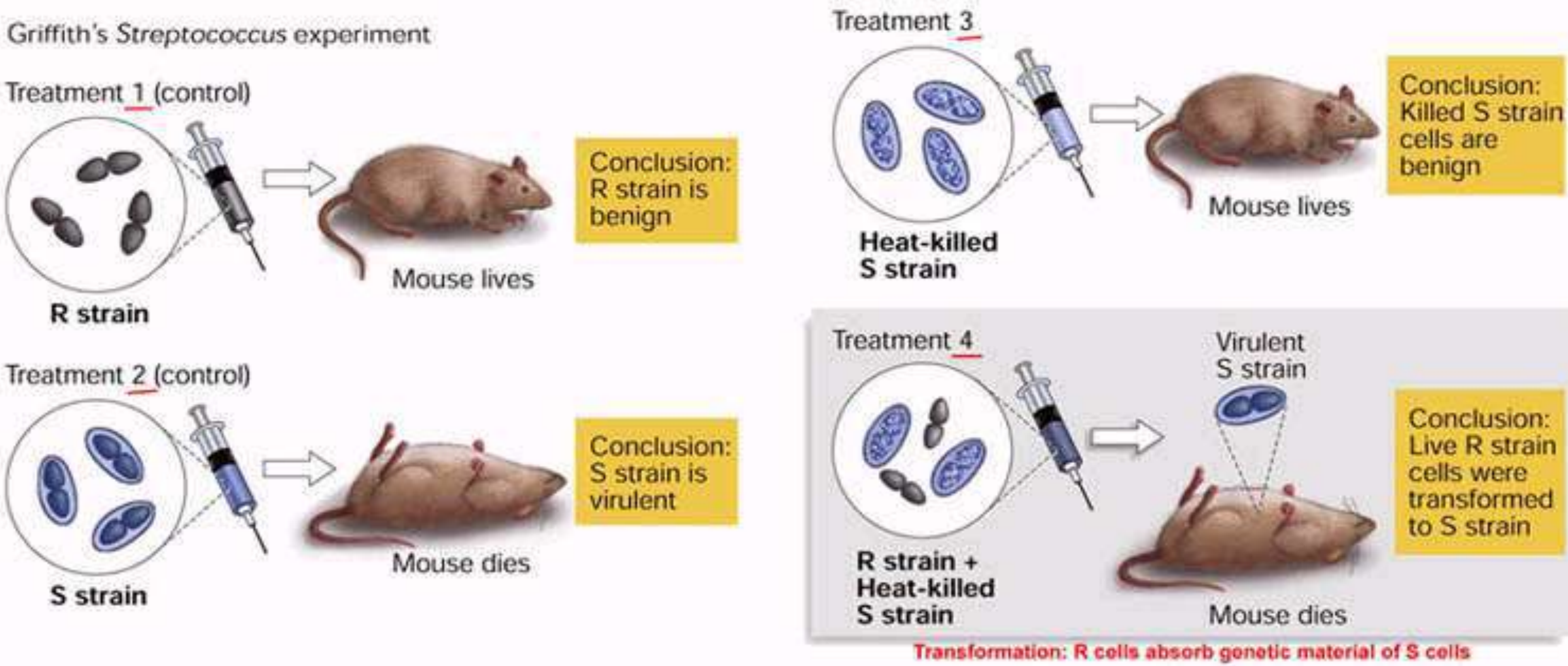
- **Transformation is a method of genetic recombination in which a naked DNA from a donor bacteria is transferred to a competent recipient bacteria and incorporated into chromosome of the latter, *e.g.* in *Bacillus, Haemophilus, Neisseria, Pneumococcus*.**

- **Transformation occurs in nature.**

- **It is widely used in recombinant DNA technology.**

- **In Gram+ve bacteria the DNA is taken up as a single stranded molecule and the complementary strand is synthesized in the recipient.**

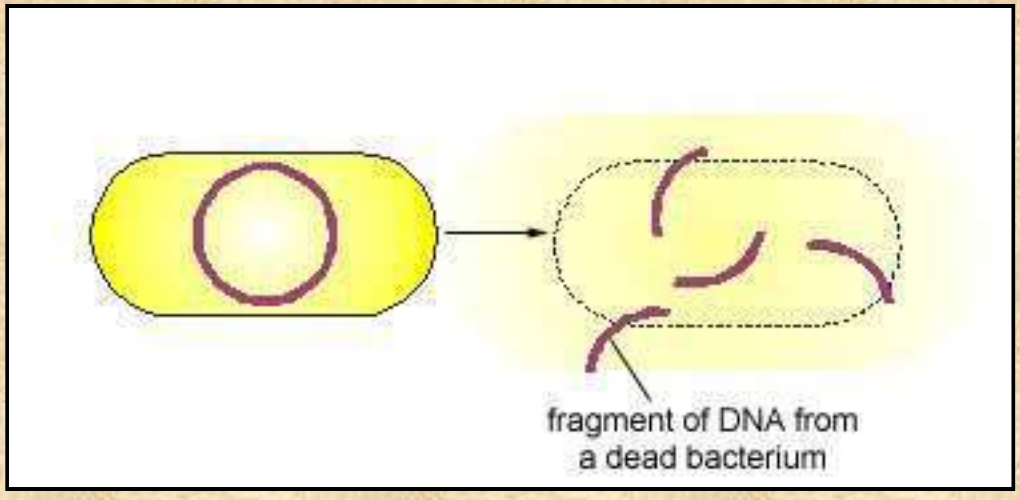
- **In Gram-ve bacteria double stranded DNA is transformed.**


Griffith's Streptococcus experiment
Treatment 1 (control)
R strain
Mouse lives
Conclusion: R strain is benign
Treatment 2 (control)
S strain
Mouse dies
Conclusion: S strain is virulent
Treatment 3
Heat-killed S strain
Mouse lives
Conclusion: Killed S strain cells are benign
Treatment 4
R strain + Heat-killed S strain
Virulent S strain
Mouse dies
Conclusion: Live R strain cells were transformed to S strain
Transformation: R cells absorb genetic material of S cells

Mechanism of Transformation

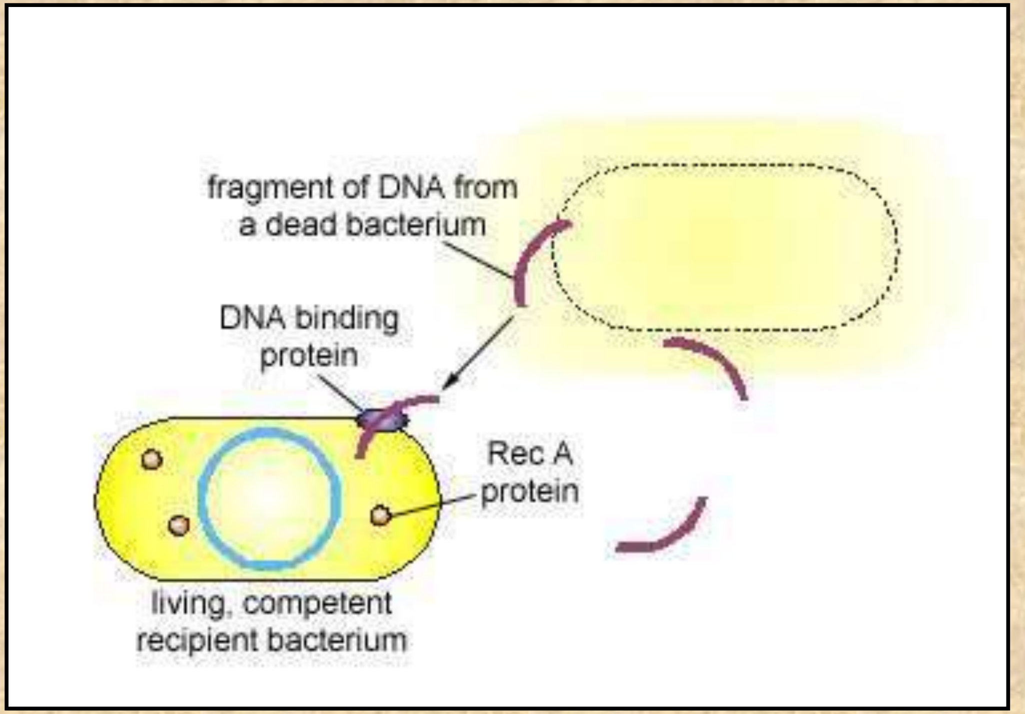
- **A bacterial cell dies or is degraded releasing its dsDNA molecule in environment.**
- **Nuclease enzymes cut the released DNA into fragments of usually about 20 genes long.**
- **The fragments bind to DNA binding proteins present on the surface of a competent recipient bacterium and subsequently 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.**

Transformation – step I



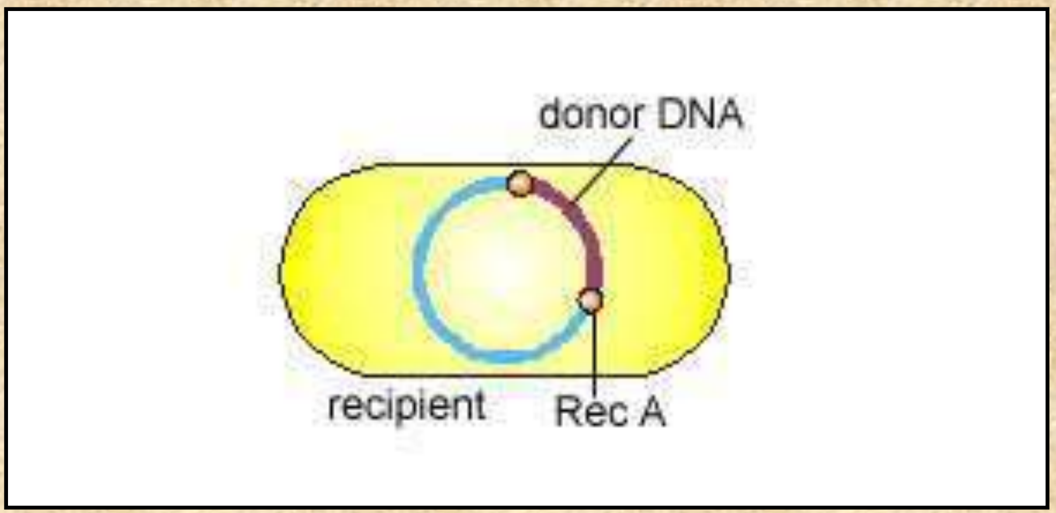
A donor bacterium dies and is degraded.

Transformation – step II



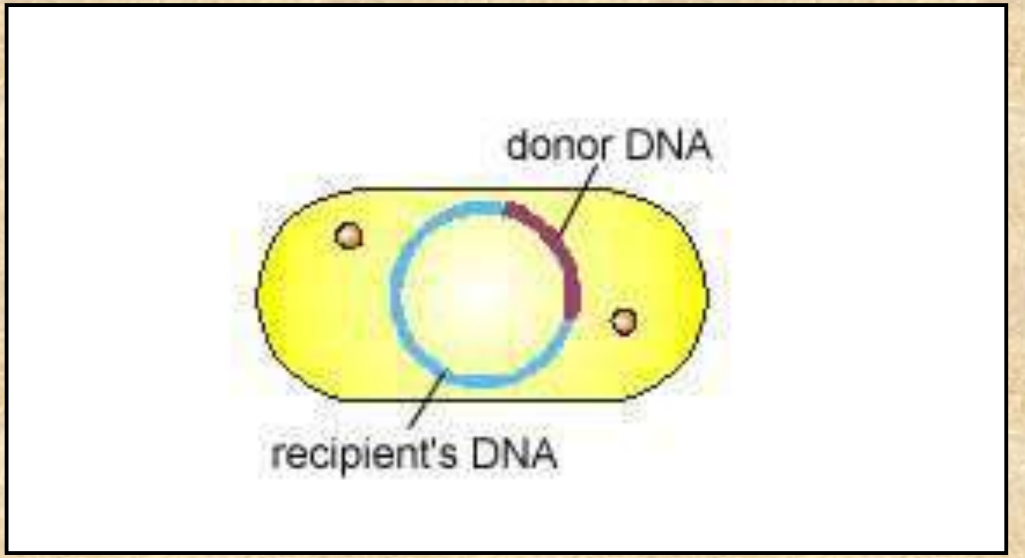
A fragment of DNA from the dead donor bacterium binds to DNA binding proteins on cell wall of a competent live recipient bacterium

Transformation – step III



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

Transformation – step IV



Exchange is complete.

Factors affecting transformation

- **DNA size and state**
  - Sensitive to nucleases (at least 5 X 10^5 daltons)
- **Competence of the recipient** (*Bacillus*, *Haemophilus*, *Neisseria*, *Streptococcus*)
  - The ability to take up DNA from the environment is known as **competence**
  - **only DNA from closely related bacteria (competent cells) would be successfully transformed**
  - **Competence factor** (a specific protein produced at a particular time in the growth cycle of competent bacteria and enable it to take up DNA naturally)
  - **Induced competence,** *e.g.* by $CaCl_2$

TRANSDUCTION

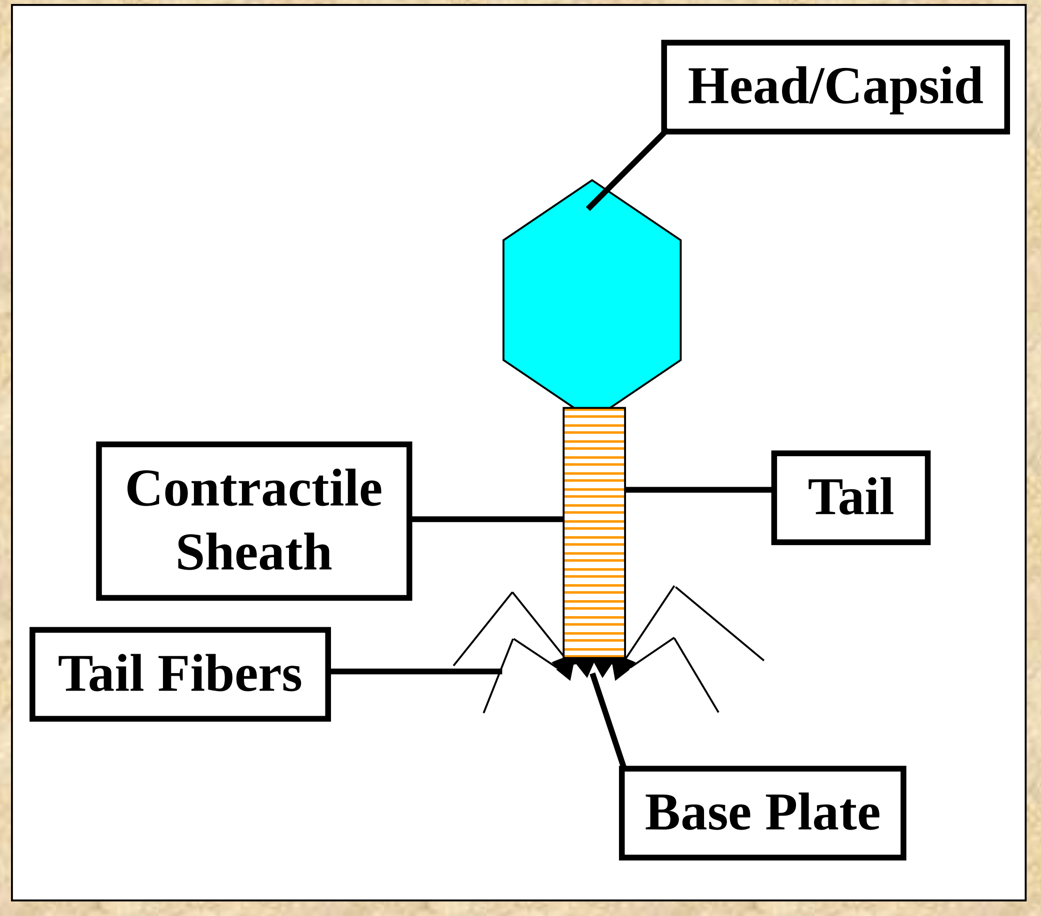
- **Definition**: Gene transfer from a donor to a recipient bacteria through a bacteriophage

- **Bacteriophage** (phage): A virus that infects bacteria

- **Types of transduction**
  - **Generalized**
  - **Specialized**

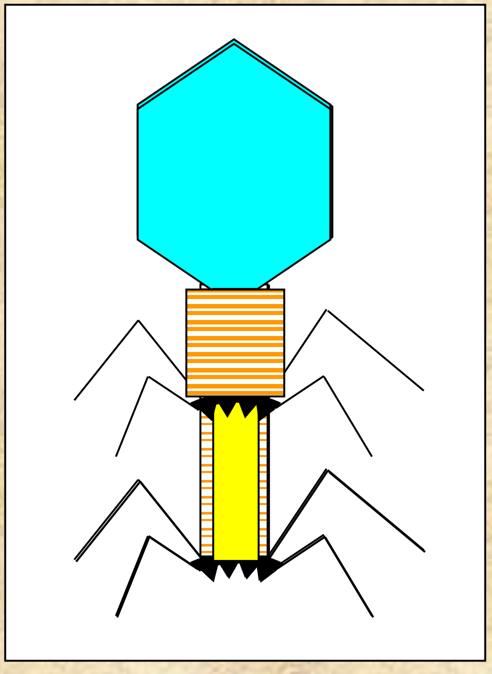
Phage Composition and Structure

- Composition
  - Nucleic acid (DNA/RNA)
    - Genome size
    - Modified bases (protect from host nucleases)
  - Protein
    - Protection
    - Infection
- Structure (T_4)
  - Size (80 X 100 nm)
  - Head or capsid
  - Tail (contractile sheath, base plate, tail fibres)



Infection of Host Cells by Phages

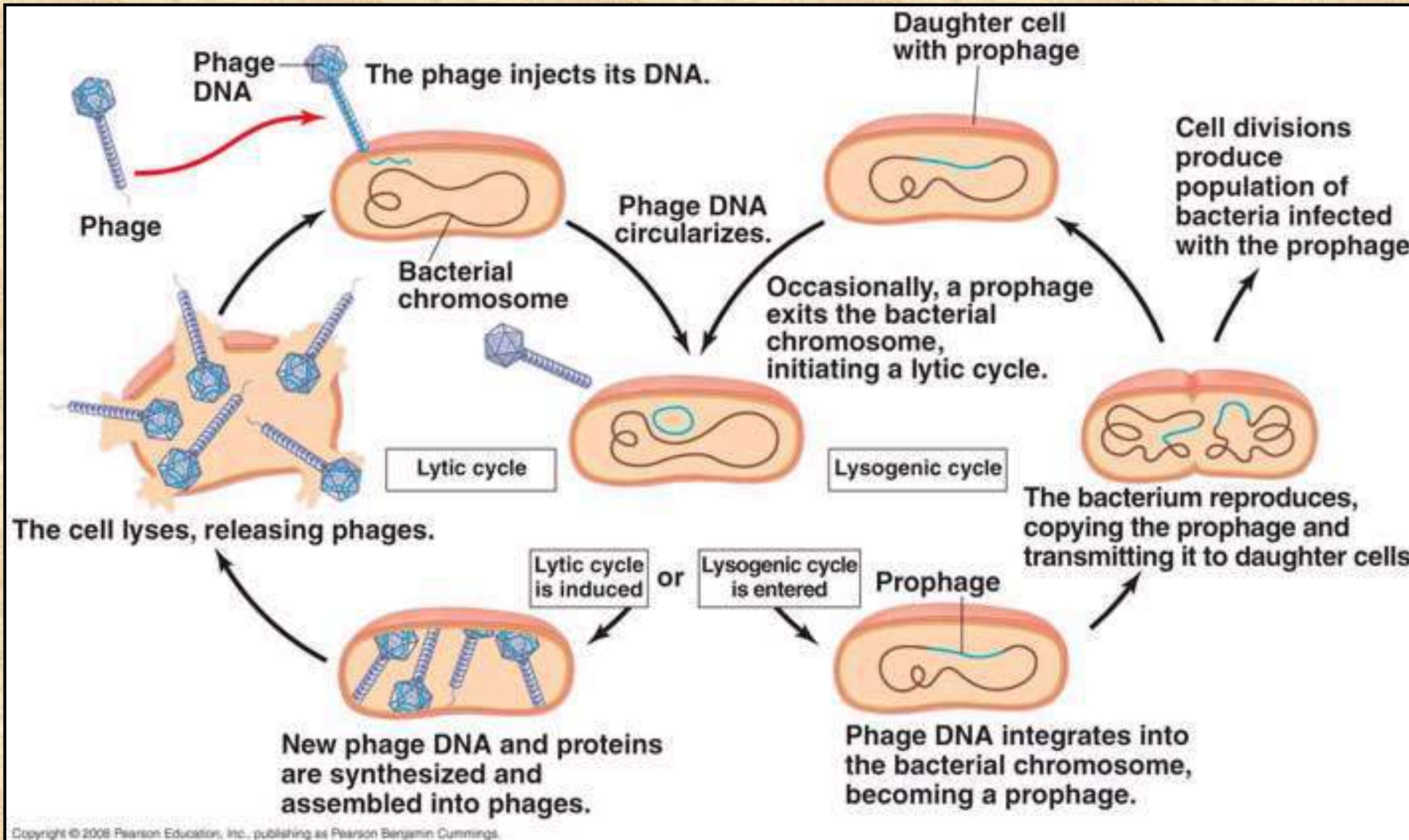
- Adsorption
  - Tail fibers
  - Receptor is LPS for T4
- Irreversible attachment
  - Base plate
- Sheath Contraction
- Nucleic acid injection
- DNA uptake



Types of Bacteriophage

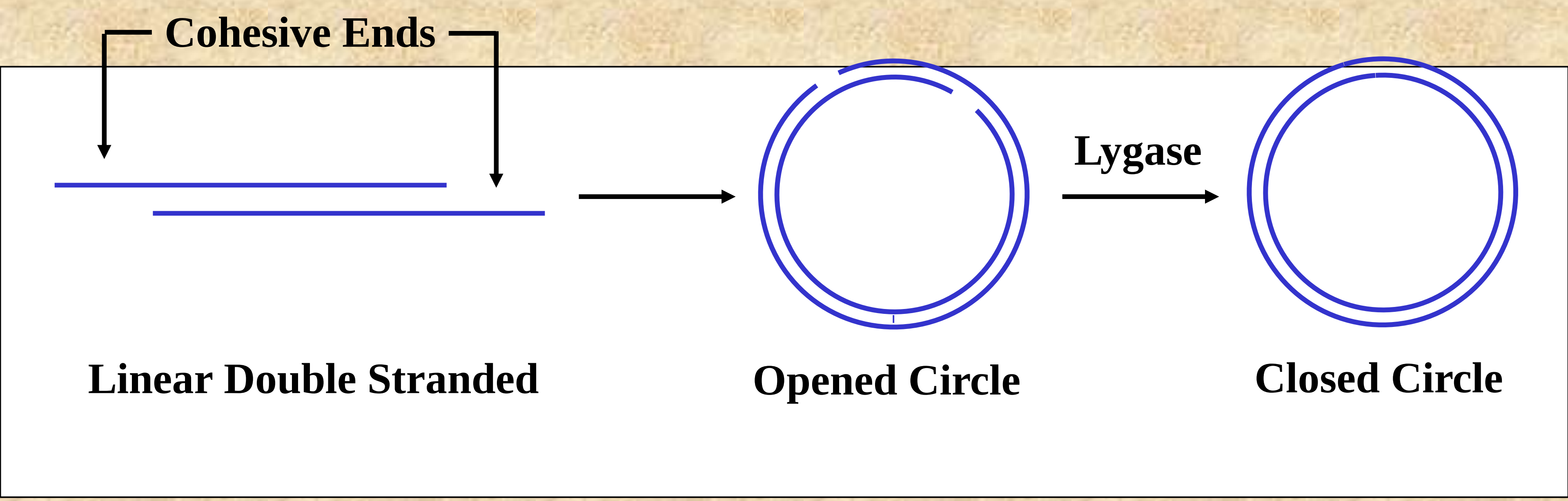
- **Virulent phage:** a phage that multiply within the host cell, lyse the cell and release progeny phage (*e.g.* T4) – **lytic cycle**
- **Temperate phage:** a phage that can either multiply via the lytic cycle or enter a quiescent integrated state in the bacterial cell. (*e.g.*, 🖰) – **lysogenic cycle**
  - **Expression of most phage genes repressed**
  - **Prophage: Phage DNA in the quiescent integrated state**
  - **Lysogen – Bacteria harboring a prophage**

The phage cycle

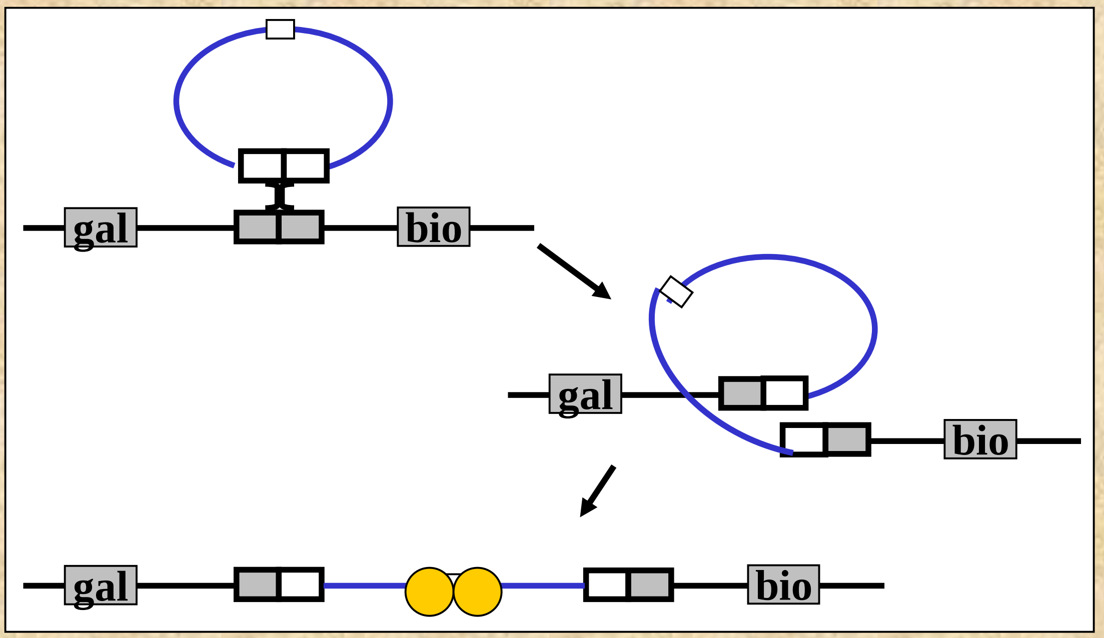


Events leading to Lysogeny

- **1. Circularization of the phage DNA**
  - Cohesive ends: double stranded linear molecule with small single stranded regions at the 5' ends.
  - Cohesive ends promotes circularization of phage DNA
  - e.g. lambda phage



- **2. Site-specific recombination**
  - recombination occurs between a particular site on the circularized phage DNA and a particular site on the host chromosome DNA
  - phage coded enzyme helps



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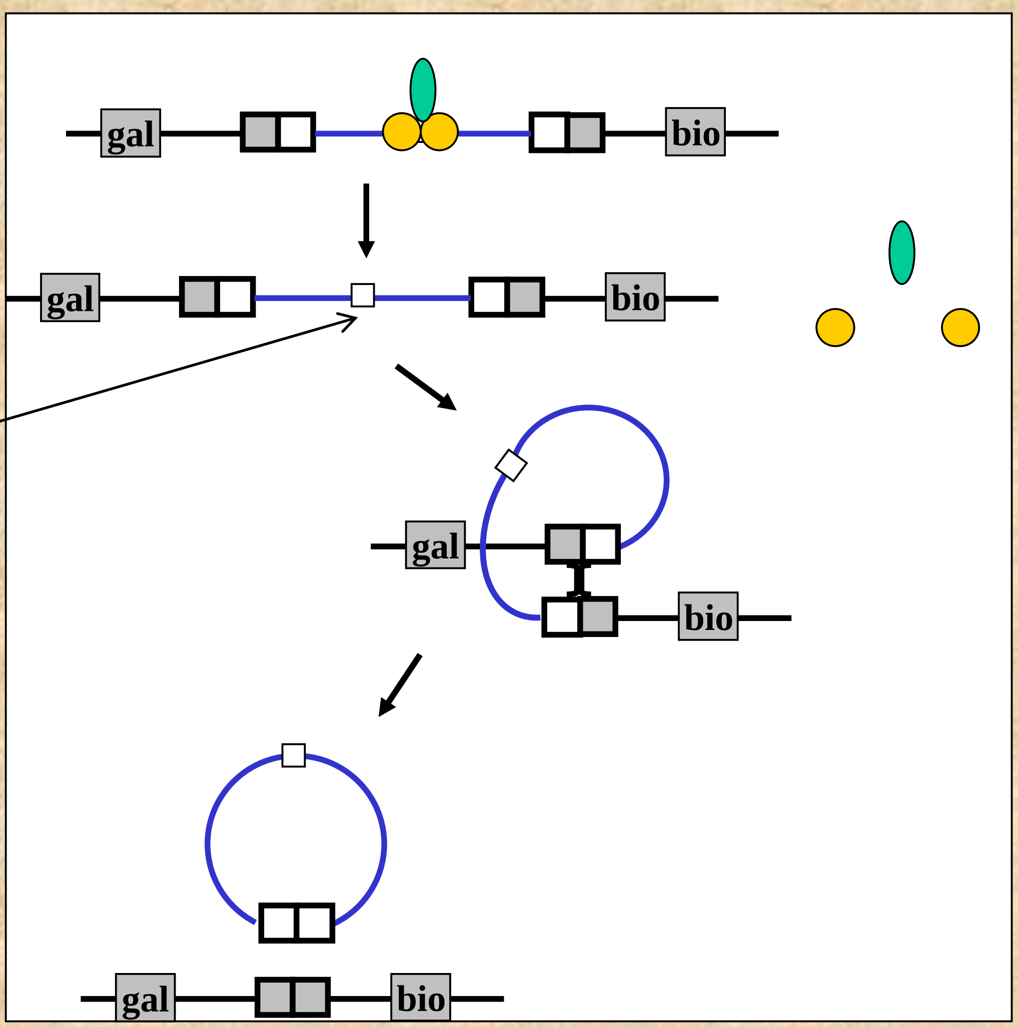
- 3. **Repression of the phage genome**
  - phage coded **repressor protein**
  - binds to a particular site on the phage DNA, called the **operator**, and shuts off transcription of most phage genes EXCEPT the repressor gene resulting in a stable repressed phage genome which is integrated
  - Specific
  - Immunity to superinfection

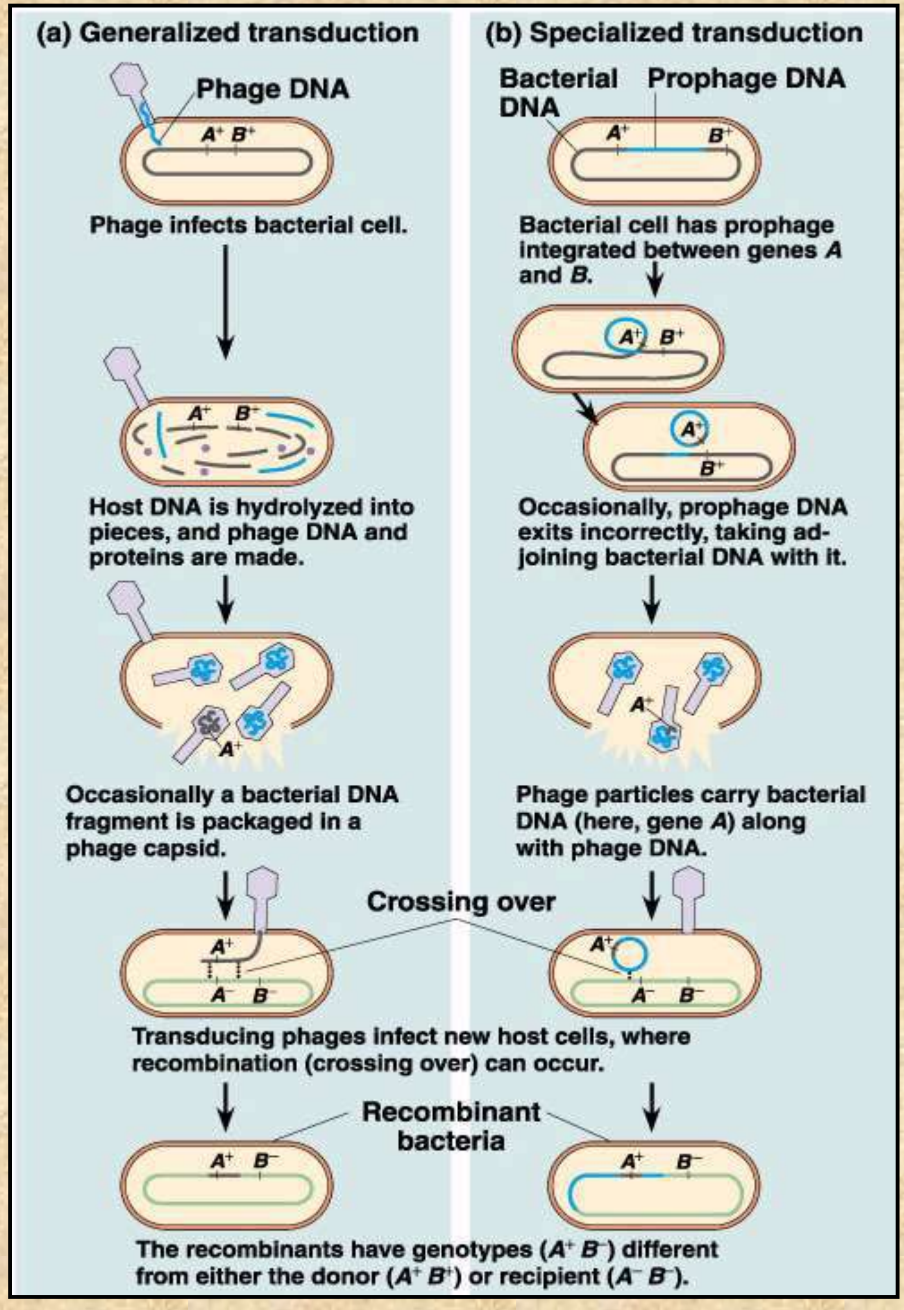
Termination of Lysogeny

- **Induction**
  - Adverse conditions
- **Role of proteases**
  - recA protein
  - destruction of phage repressor
- **Phage Gene expression**
- **Excision of phage**
- **Lytic growth**



TYPES OF TRANSDUCTION

- **Generalised Transduction**
- **Specialised Transduction**


(a) Generalized transduction
Phage DNA
A^+ B^+
Phage infects bacterial cell.
A^+ B^+
Host DNA is hydrolyzed into pieces, and phage DNA and proteins are made.
A^+
Occasionally a bacterial DNA fragment is packaged in a phage capsid.
A^+
A^- B^-
(b) Specialized transduction
Bacterial DNA
Prophage DNA
A^+ B^+
Bacterial cell has prophage integrated between genes *A* and *B*.
A^+ B^+
A^+
B^+
Occasionally, prophage DNA exits incorrectly, taking adjoining bacterial DNA with it.
A^+
Phage particles carry bacterial DNA (here, gene *A*) along with phage DNA.
A^+
A^- B^-
Crossing over
Transducing phages infect new host cells, where recombination (crossing over) can occur.
Recombinant bacteria
A^+ B^-
A^+ B^-
The recombinants have genotypes (A^+ B^-) different from either the donor (A^+ B^+) or recipient (A^- B^-).

Generalized Transduction

- **Generalized transduction can transfer any gene of donor bacteria to recipient bacteria**

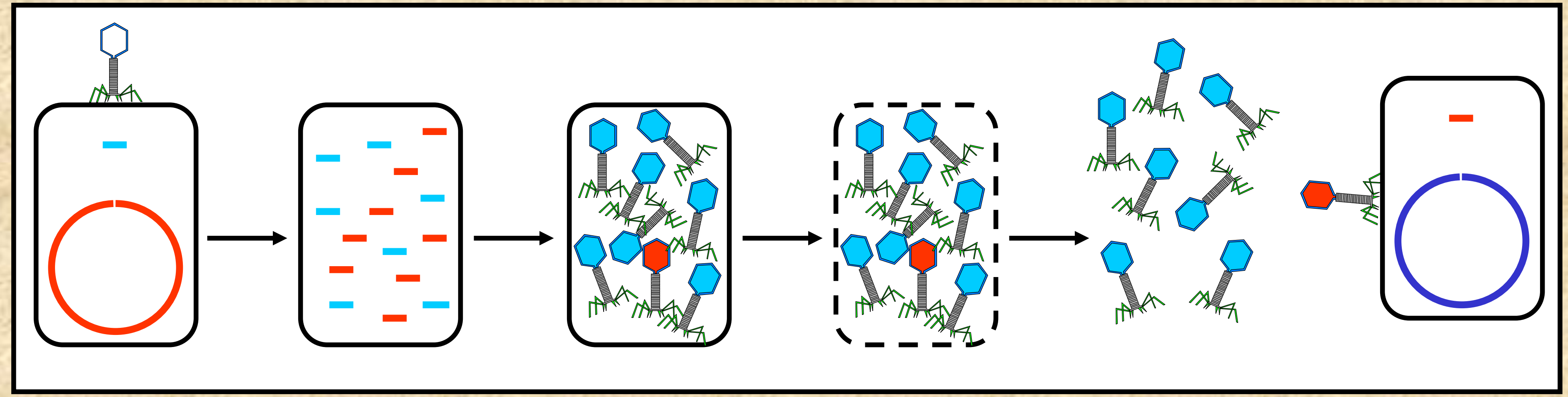
- During the replication of a **lytic phage** the capsid sometimes enclose a small fragment of lysed bacterial DNA, instead of phage DNA, by a "head-full" mechanism. **This is a defective phage**

- 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



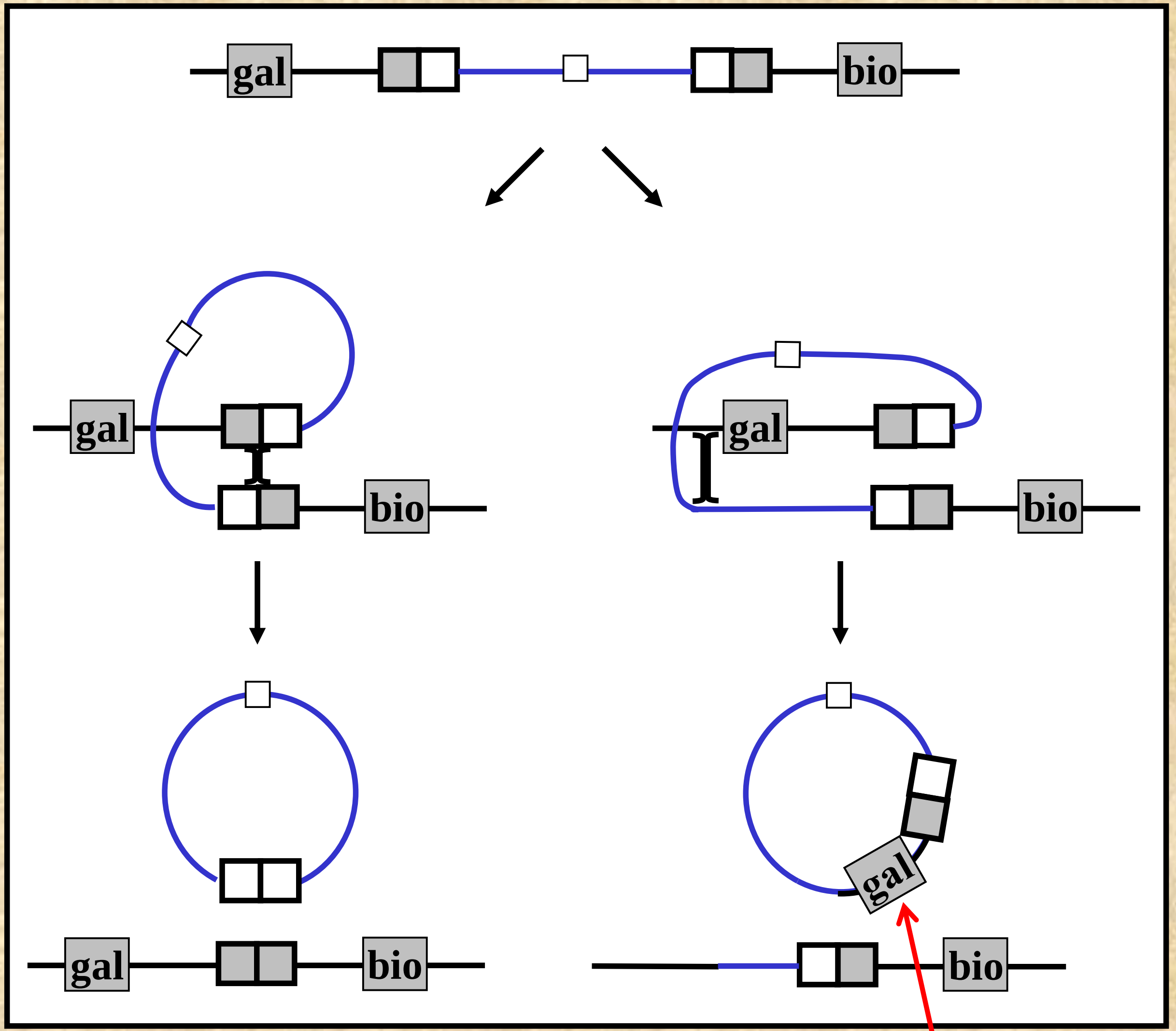
Potentially any donor gene can be transferred

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 bacerium 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

Steps in Specialized Transduction

- Excision of the prophage
- Replication and release of phage
- Infection of the recipient
- Lysogenization of the recipient
  - Homologous recombination also possible

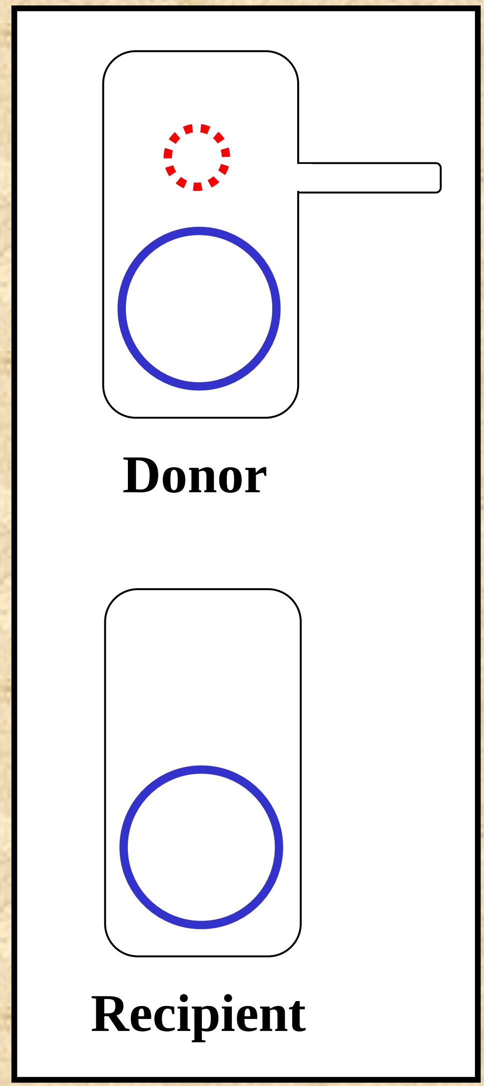


(Left normal excision of the prophage, right abnormal excision resulting in a specialized transducing)

CONJUGATION

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

- Significance
  - Gram - bacteria
    - Antibiotic resistance
    - Rapid spread
  - Gram + bacteria
    - Production of adhesive material by donor cells

Physiological states of F factor

- **Autonomous (F+)** - In this state the F factor carries only its own genes. There are no chromosomal genes associated with the F factor in F+. In crosses of the type F+ X F-, the F- becomes F+ while F+ remains F+. Thus, the F factor is infectious. In addition, there may be only low-level transfer of chromosomal genes.

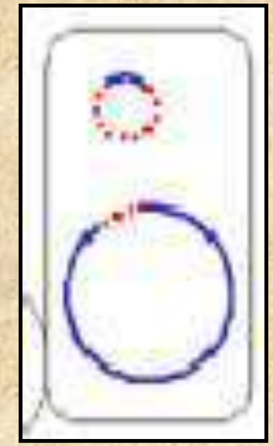
- **Integrated (Hfr)** - In this state the F factor has integrated (episomal) into the bacterial chromosome via a recombination. In crosses of the type Hfr X F-, the F- rarely becomes Hfr and Hfr remains Hfr. In addition, there is a high frequency of transfer of donor chromosomal genes.

- **Autonomous with chromosomal genes (F')** - In this state the F factor is autonomous, but it carries some chromosomal genes and is designated as F' (F prime) factor. In crosses of the type F' X F-, the F- becomes F' while F' remains F'. In addition there is high frequency of transfer of chromosomal genes present on F’, but low frequency transfer of other chromosomal genes.

Physiological states of F factor

F+

HFr



F’

Mechanisms of conjugation

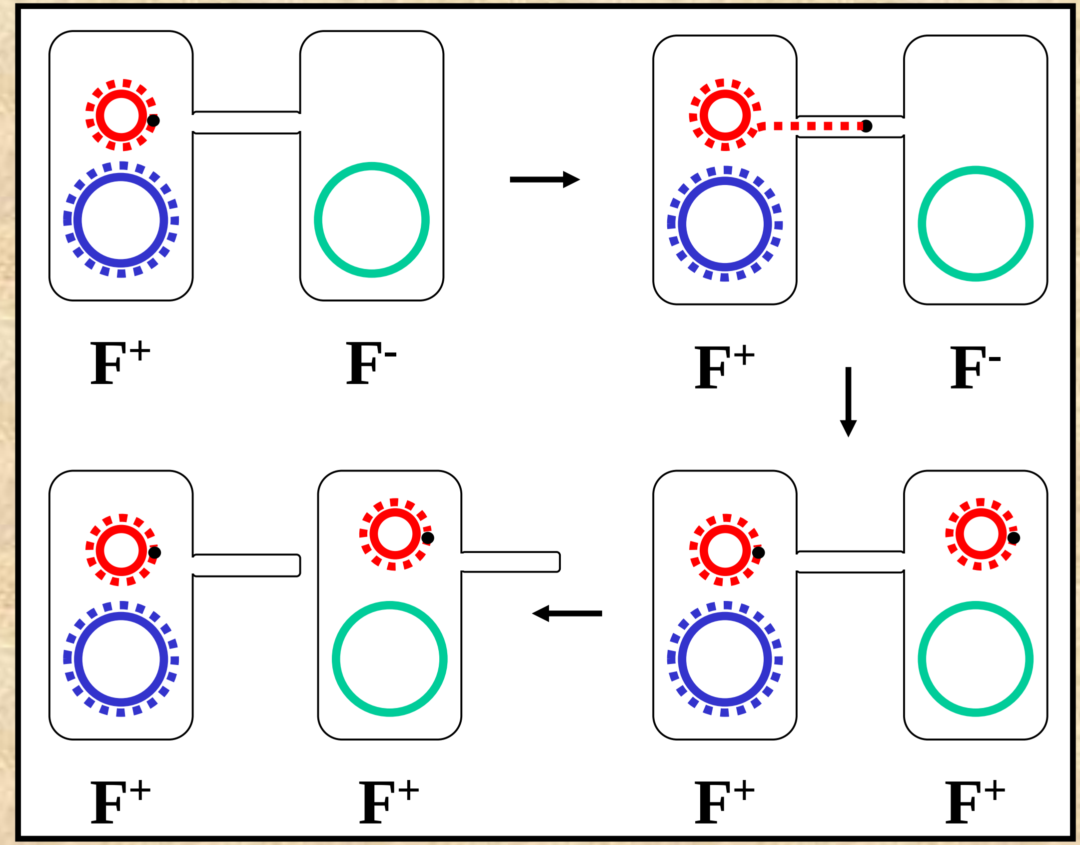
- **F+ X F- crosses**
- **Hfr X F- crosses**
- **F' X F- crosses**

F^+ x F^- conjugation

- The plasmid DNA is nicked at a specific site called the origin of transfer.
- Pair formation - The tip of the sex pilus comes in contact with the recipient and a conjugation bridge is formed between the two cells, through which the plasmid DNA will pass from the donor to the recipient
- A single strand of plasmid DNA passes through the conjugation bridge and enters the recipient where the complimentary strand is synthesized by a rolling circle mechanism
- This results in the transfer of a F+ plasmid (coding only for a sex pilus), **but not chromosomal DNA**, from a male (F+) donor bacterium to a female recipient (F-) bacterium.
- The recipient F- then becomes a F+ (male) and can make a sex pilus.
- Other genes present on the plasmid, such as those coding for antibiotic resistance, may also be transferred during this process.

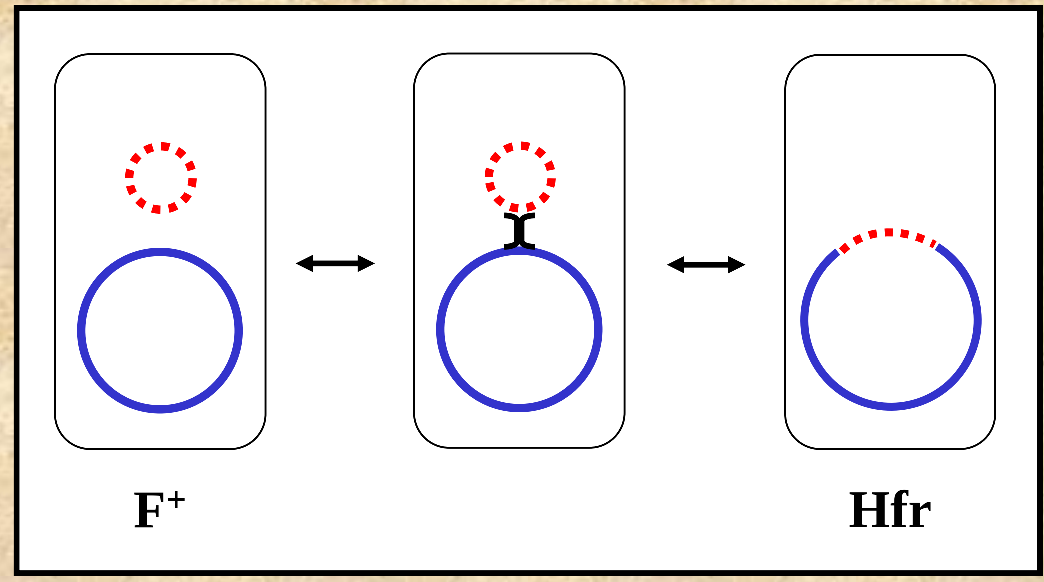
Mechanism of F^+ x F^- Crosses

- Pair formation
  - Conjugation bridge
- DNA transfer
  - Origin of transfer
  - Rolling circle replication

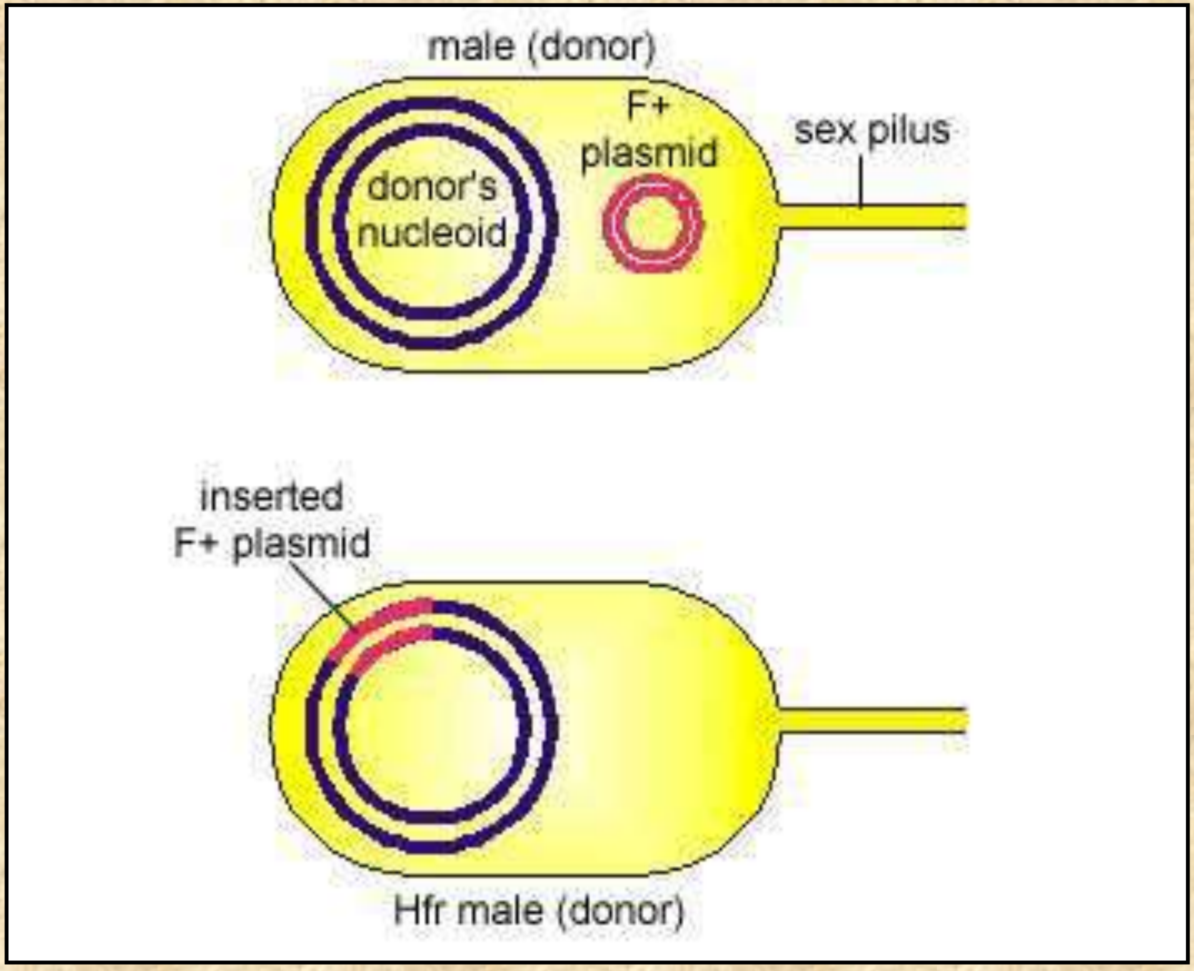


Hfr formation

- An F+ plasmid inserts or integrates into the bacterial chromosome via a recombination event to form an Hfr male.



Mechanism of Hfr formation



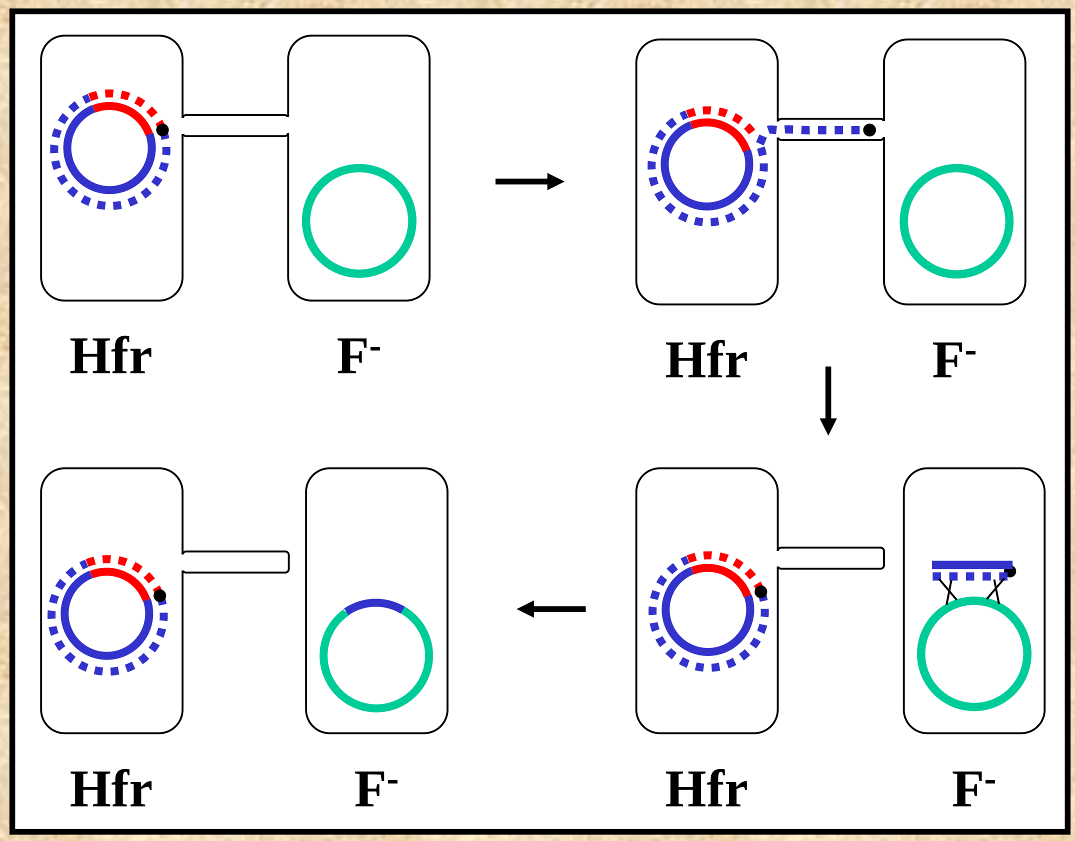
An F+ plasmid inserts into the donor bacterium's nucleoid to form an Hfr male

Hfr x F^- crosses

- The DNA of Hfr male (donor) breaks in the middle of the inserted F+ plasmid and one DNA strand begins to enter the F-(recipient) bacterium.
- The connection usually breaks before the transfer of the entire chromosome is completed so the F+ plasmid seldom enters the recipient.
- As a result, there is a transfer of some chromosomal DNA, which may be exchanged for a piece of the recipient's DNA, but not maleness.
- Thus, F- rarely becomes Hfr while Hfr remains Hfr
- High transfer of certain donor chromosomal genes

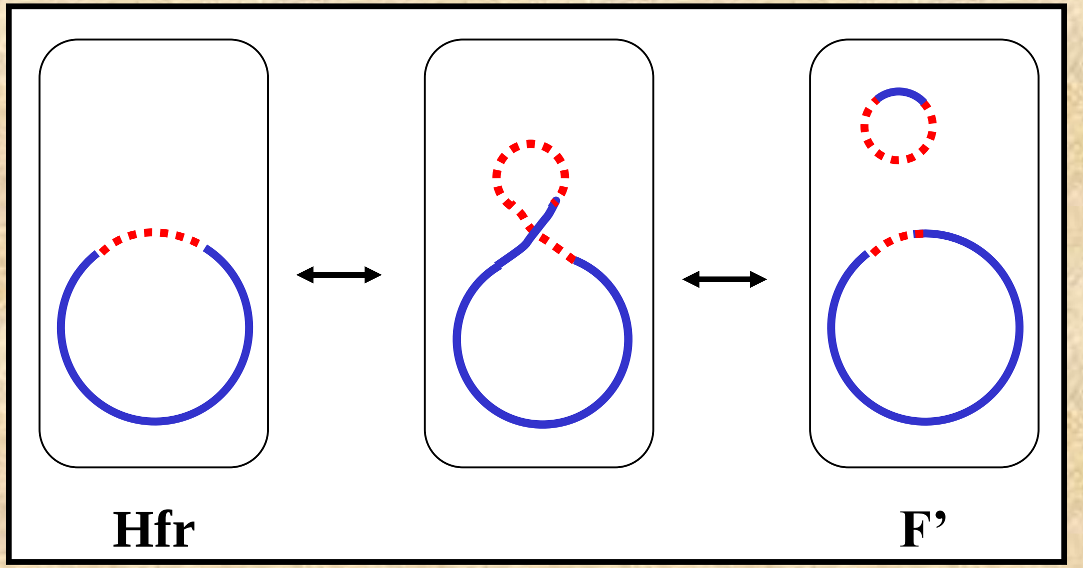
Mechanism of Hfr x F$^-$ Crosses

- Pair formation
  - Conjugation bridge
- DNA transfer
  - Origin of transfer
  - Rolling circle replication
- Homologous recombination



F’ formation

- **Sometimes the F factor breaks free from the chromosome of an Hfr cell, and takes a segment of the chromosomal DNA.**
- **The factor is now called F' factor (F prime).**
- **When the F' factor is transferred during conjugation, the process is called as Sexduction.**



Mechanism of F' x F$^-$ Crosses

- Pair formation
  - Conjugation bridge
- DNA transfer
  - Origin of transfer
  - Rolling circle replication
- Homologous recombination
  - Not necessary
  - May occur