BACTERIAL GENETICS

I MSc Botany Based on Prescott

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Key Words

- Genetics
- Bacterial genetics
- Mutation & its types
  - Point mutation
  - Frameshift mutation
  - Lethal mutation
  - Suppressor mutation
- Missense & nonsense mutation
- Bacteriophage
- Lysogenic cycle
- Mechanisms of gene transfer
  - Transformation
  - Transduction
  - Lysogenic conversion
  - Conjugation
  - Transposition (Jumping Genes)

Bacterial Genetics

- **Genetics** is the study of heredity and variation.

  The unit of heredity is ***gene***, which is a segment of DNA specifying for a particular polypeptide.

  Introns - non coding sequences on a gene.

  Exons - coding sequences on a gene translated into gene products.

- Bacterial genetics is used as a model to understand DNA replication, genetic characters, their changes & transfer to next generations.

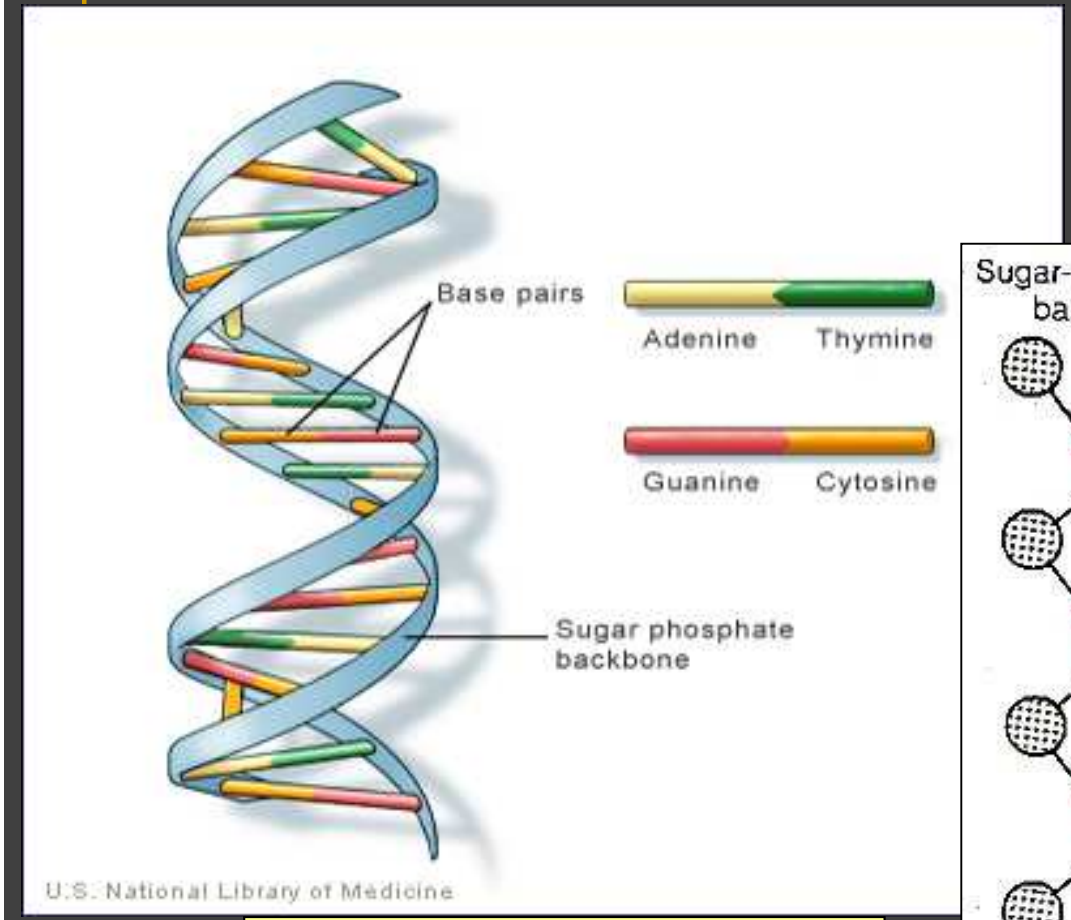
Nucleic Acids

- DNA (deoxy ribonucleic acid) : stores information for protein synthesis.
- RNA (ribonucleic acid) : transcription & translation of information for protein synthesis.
- Central Dogma : DNA → RNA → Protein

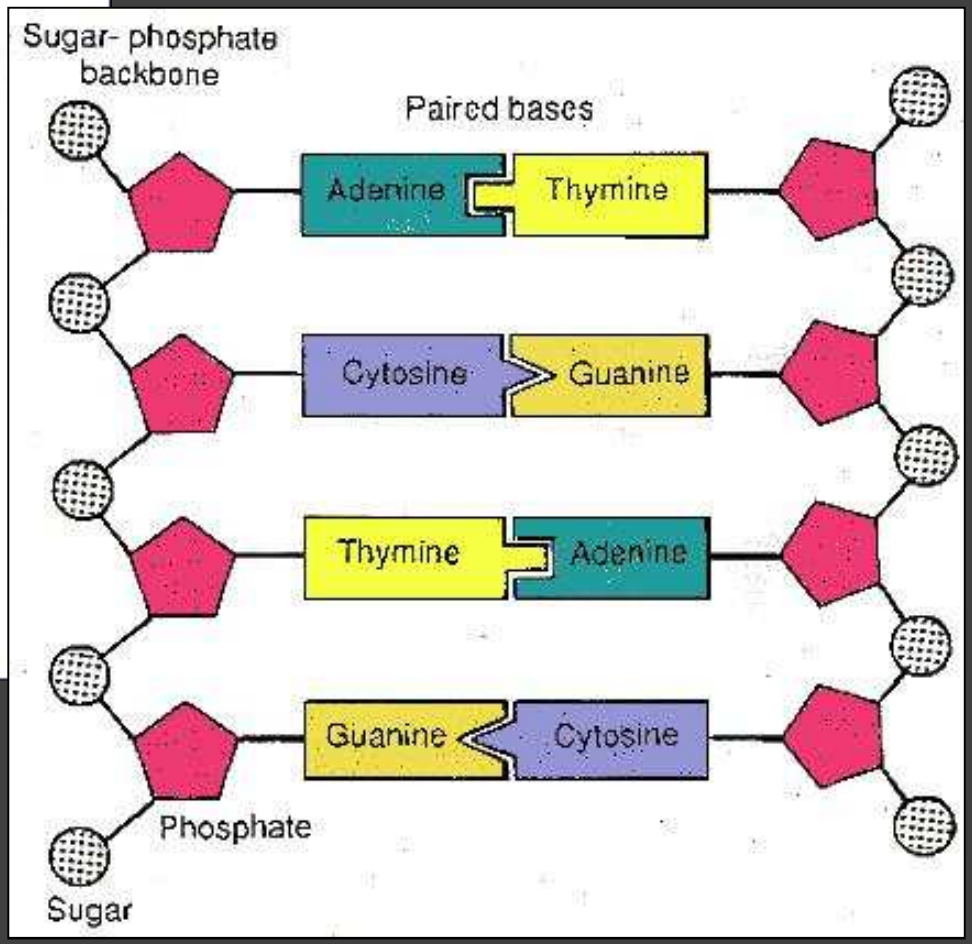
Structure Of DNA

- Proposed by Watson & Crick.
- Double helix model.
- Composed of 2 chains of polypeptides, each chain has a backbone of deoxyribose sugar and phosphate residues arranged alternately.
- 4 nitrogenous bases:

Adenine (A)	Purine
Guanine (G)	
Thymine(T)	Pyrimidine
Cytosine (C)	



Double helical structure of DNA by Watson & Crick



Structure Of RNA

- Structurally similar to DNA, except for 2 major differences:
  - ribose sugar
  - uracil in place of thymine.

- 3 types of RNA
  - m RNA (messenger RNA)
  - t RNA (transfer RNA)
  - r RNA (ribosomal RNA)

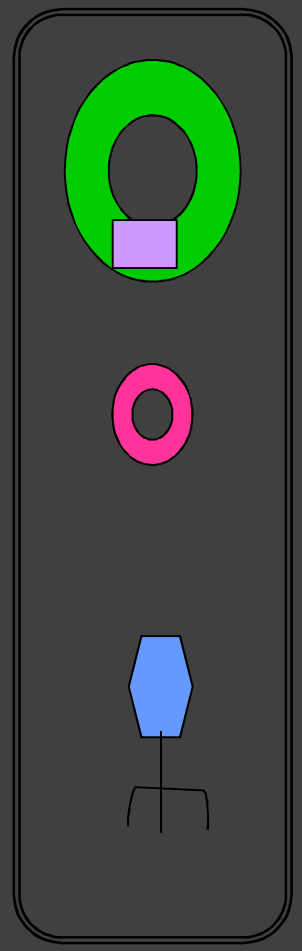
Genetic Information In Bacteria

Chromosome — Carries properties like virulence, pathogenicity & resistance

Plasmid — Extrachromosomal genetic material in the cytoplasm

Replicate independently

Bacteriophage — Virus infecting bacteria



PLASMIDS

- Circular DNA molecules
- Important vectors in genetic engineering
- **EPISOME**
  - Plasmid DNA integrated with chromosomal DNA.
- **Types of plasmids**
  - R plasmid (drug resistance): RTF* + r determinant
  - F plasmid (maleness)

* Resistance Transfer Factor

Genotypic & Phenotypic Variations

- Genotype – genetic constitution of a cell that is transmitted to its progeny

- Phenotype – physical expression of the genotype in a given environment

- Variations

1. Phenotypic variations –
   - influenced by the environment
   - temporary & not heritable

2. Genotypic variations –
   - Not influenced by the environment
   - Stable & heritable

Mechanisms Of Genetic Variations

- Mutation
- Transfer or exchange of genetic material
  1. Transformation
  2. Transduction
  3. Conjugation
  4. Lysogenic conversion
  5. Transposition

Mutation

- Random, undirected heritable variation
- Caused by a change in the nucleotide base sequence of the DNA
- Types of mutation:
  1. Point mutation
  2. Frame shift mutation
  3. Lethal mutation
  4. Suppressor mutation
- Mutagens - Agents which can induce mutation e.g. UV rays, 5 bromouracil, alkylating agents, etc.

1. Point Mutation

- Cause - due to addition, deletion or substitution of one or more bases.

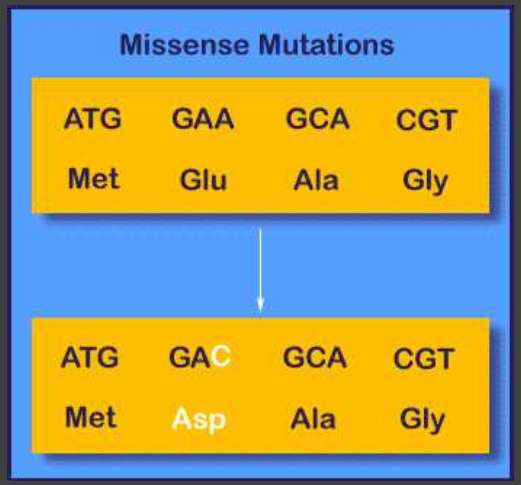
- Types -
  - **Transition** : a purine base is replaced by a purine base or a pyrimidine base is replaced by another pyrimidine base.

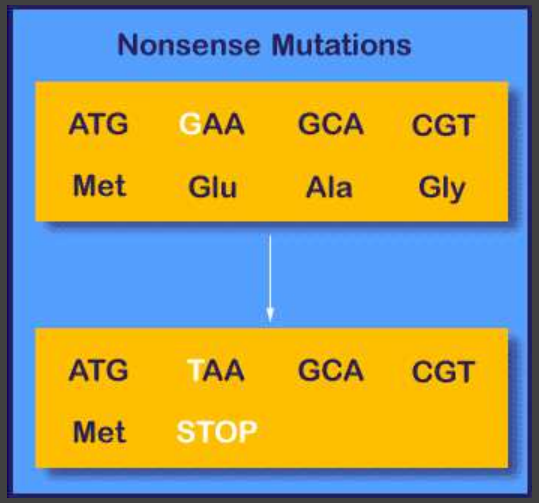
    Most common type.

  - **Transversion** : substitution of a purine base by a pyrimidine base & vice versa

1. Point Mutation

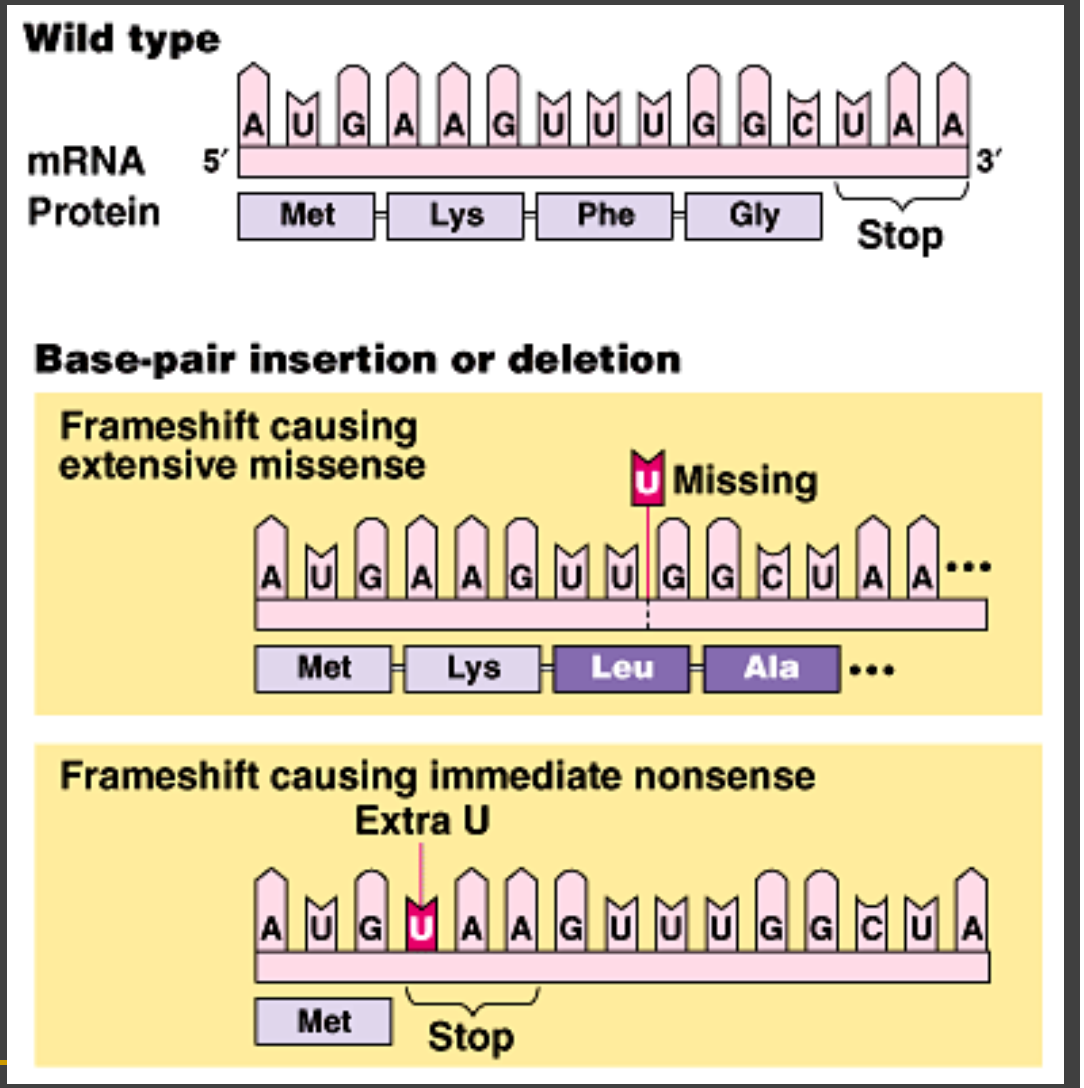
- Results of mutation -
  - Missense mutation – triplet code is altered so that a different amino acid is present at a particular position in the protein.
  - Nonsense mutation – converts a codon that specifies an amino acid into a termination codon.





2. Frame Shift Mutation

- Cause - Deletion or insertion of a base - changes all of the codons downstream from the change



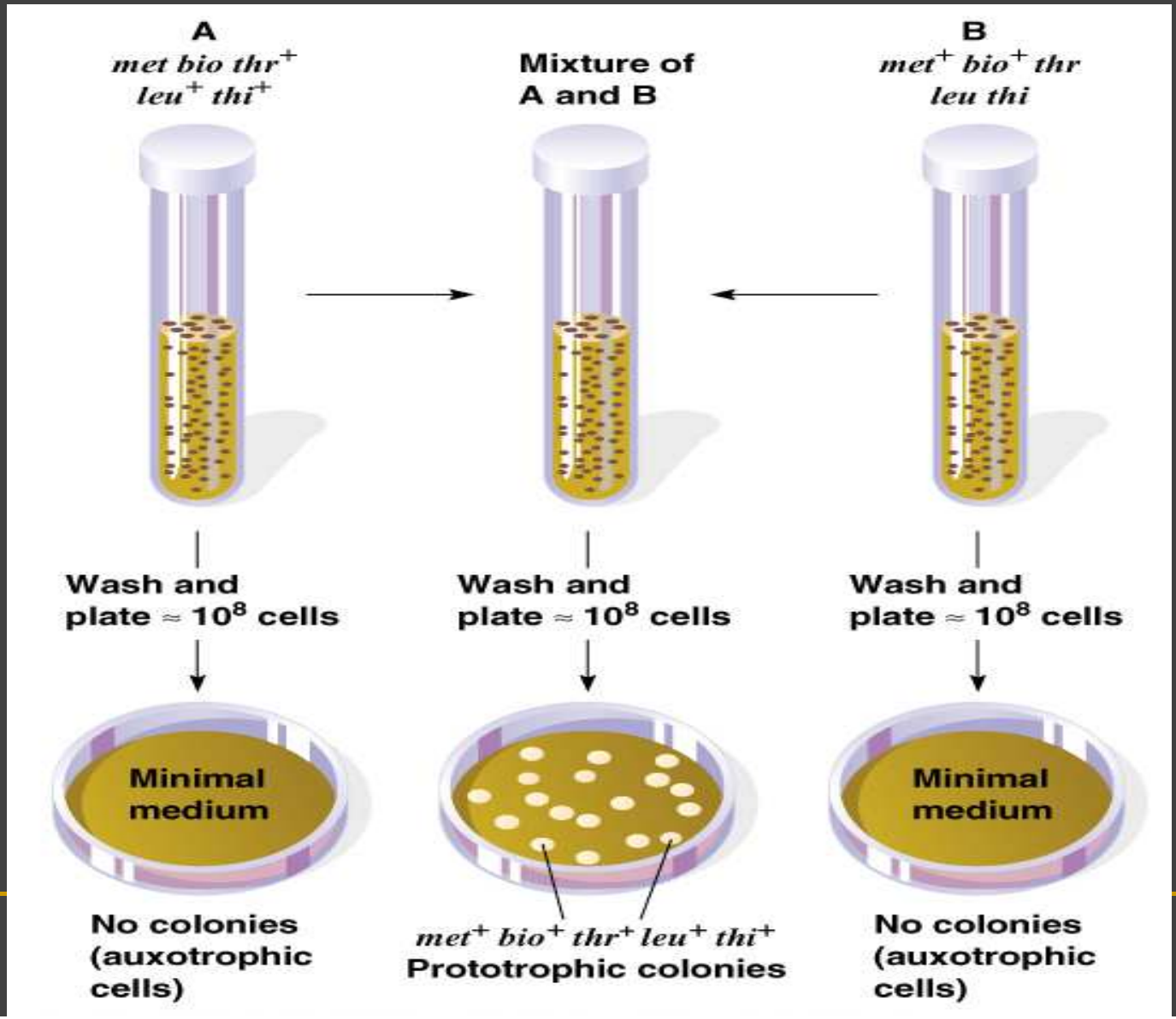
3. Lethal Mutation

- Mutation which resulting **involve vital functions in the death of the organism** – nonviable mutation.
  - A **conditional lethal mutant** may be able to live under certain conditions – permissive conditions.
  - Commonest type of conditional mutant is the **temperature sensitive (t_s) mutant** which is able to live at the permissive temperature of 35°C but not at the restrictive temp (39°C).

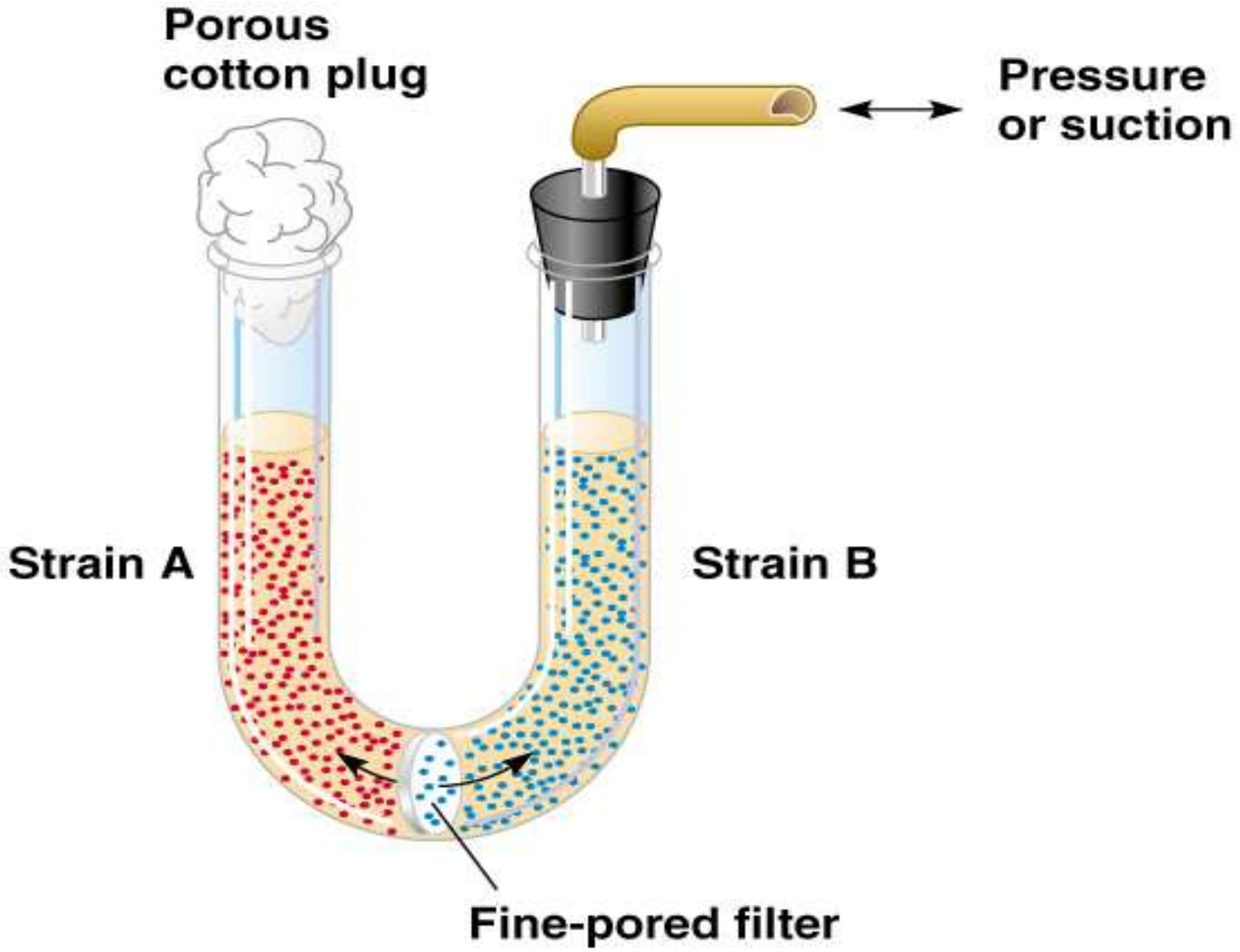
Suppressor Mutation

- Reversal of a mutant phenotype by another mutation at a position on the DNA, distinct from that of the original mutation.

Lederberg & Tatum (1946) Experiment demonstrating recombination in *E. coli*. Recombination of 2 complimentary auxotrophs gives rise to a strain that can synthesize all nutrients.



Bernard Davis experiment demonstrated that physical contact is required for bacterial recombination.



Conjugation-transfer of the sex factor *F*:

1. William Hayes (1953) demonstrated that genetic exchange in *E. coli* occurs only in one direction.

2. Genetic transfer is mediated by sex factor *F*.

3. Donor is F^+ and recipient is F^-.

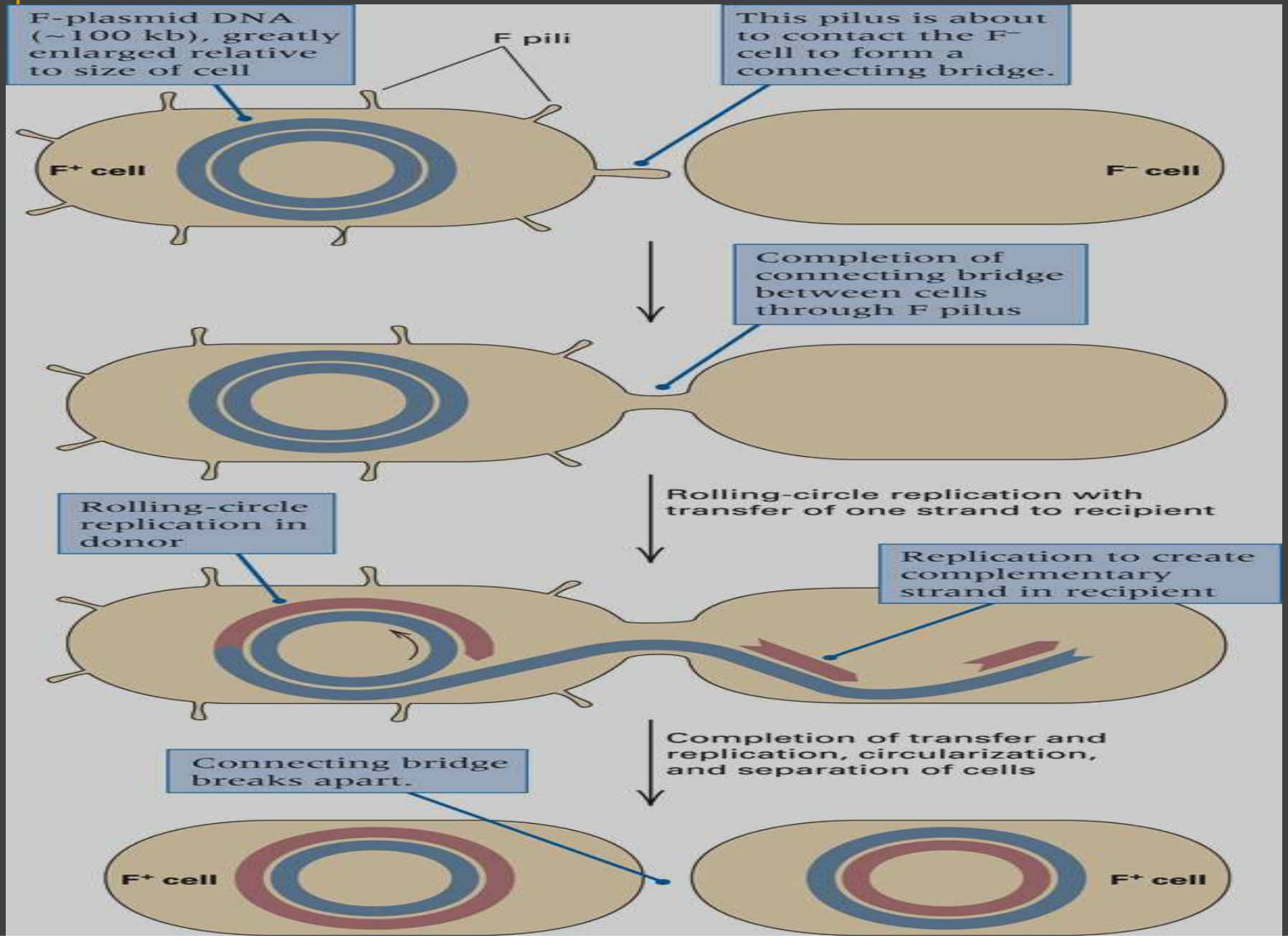
4. *F* is a self-replicating, circular DNA plasmid (1/40 the size of the main chromosome).

5. *F* plasmid contains an origin sequence (*O*), which initiates DNA transfer. It also contains genes for hair-like cell surface (*F*-pili or sex-pili), which aid in contact between cells..

F factor and Conjugation

- F (fertility) factor is a conjugative plasmid transferred from cell to cell by conjugation
- F factor is an episome = genetic element that can insert into chromosome or replicate as circular plasmid
- The F plasmid is a low-copy-number plasmid ~100 kb in length, and is present in 1–2 copies per cell
- It replicates once per cell cycle and segregates to both daughter cells in cell division

F factor and Conjugation

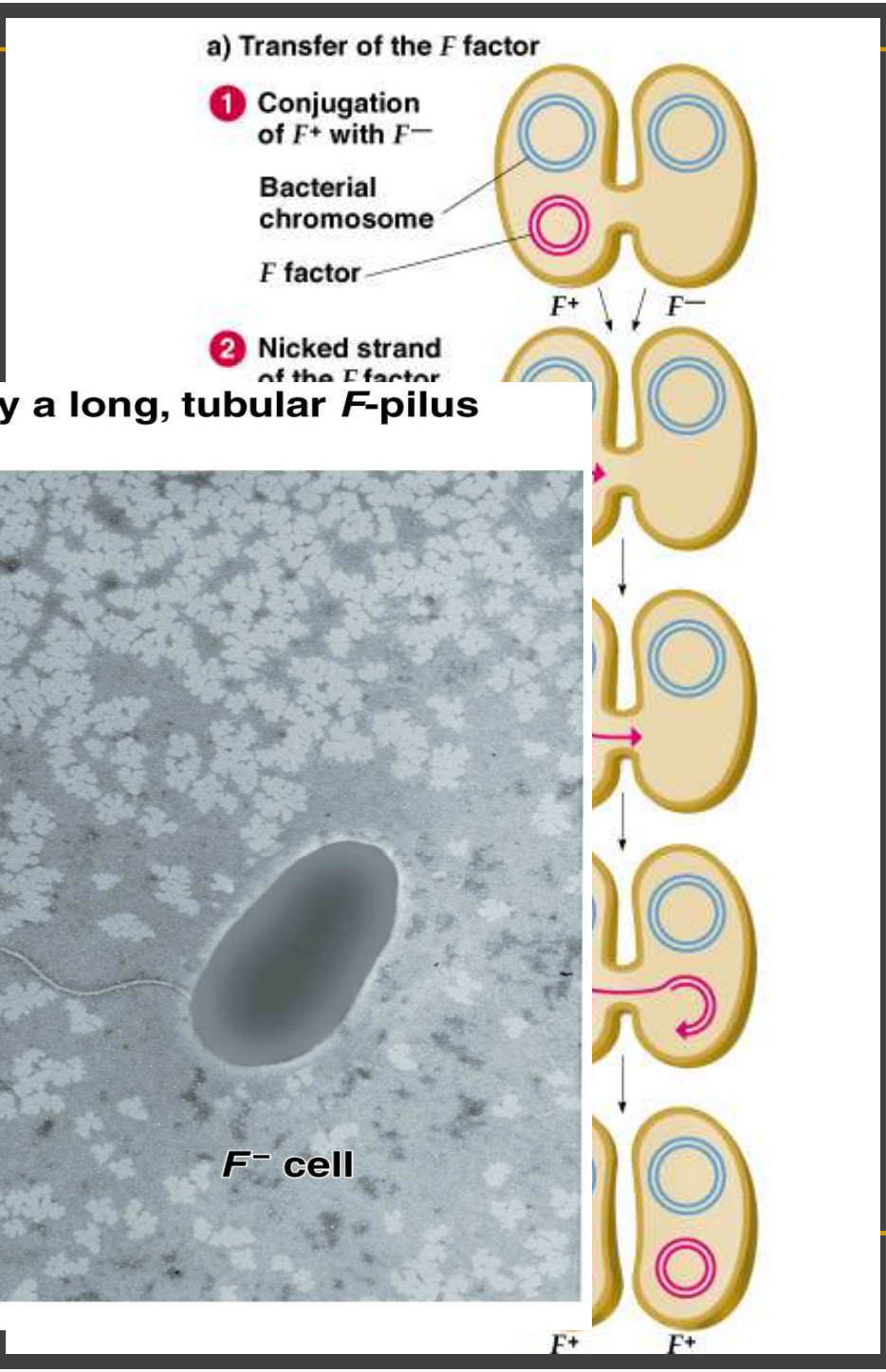


1. **No conjugation can occur between cells of the same mating type.**

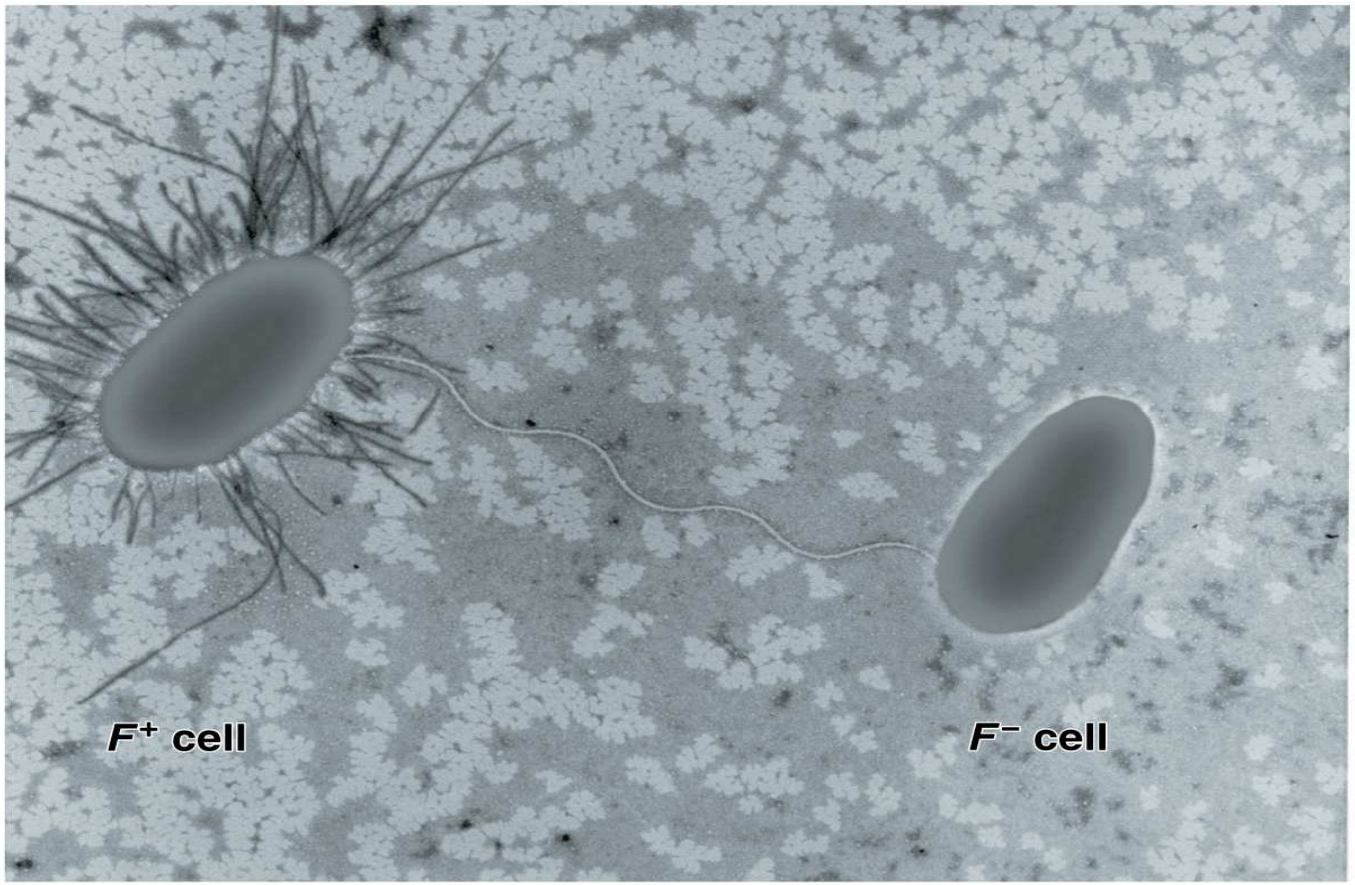
2. **Conjugation begins when the *F* plasmid is nicked at the origin, and a single strand is transferred using the rolling circle mechanism.**

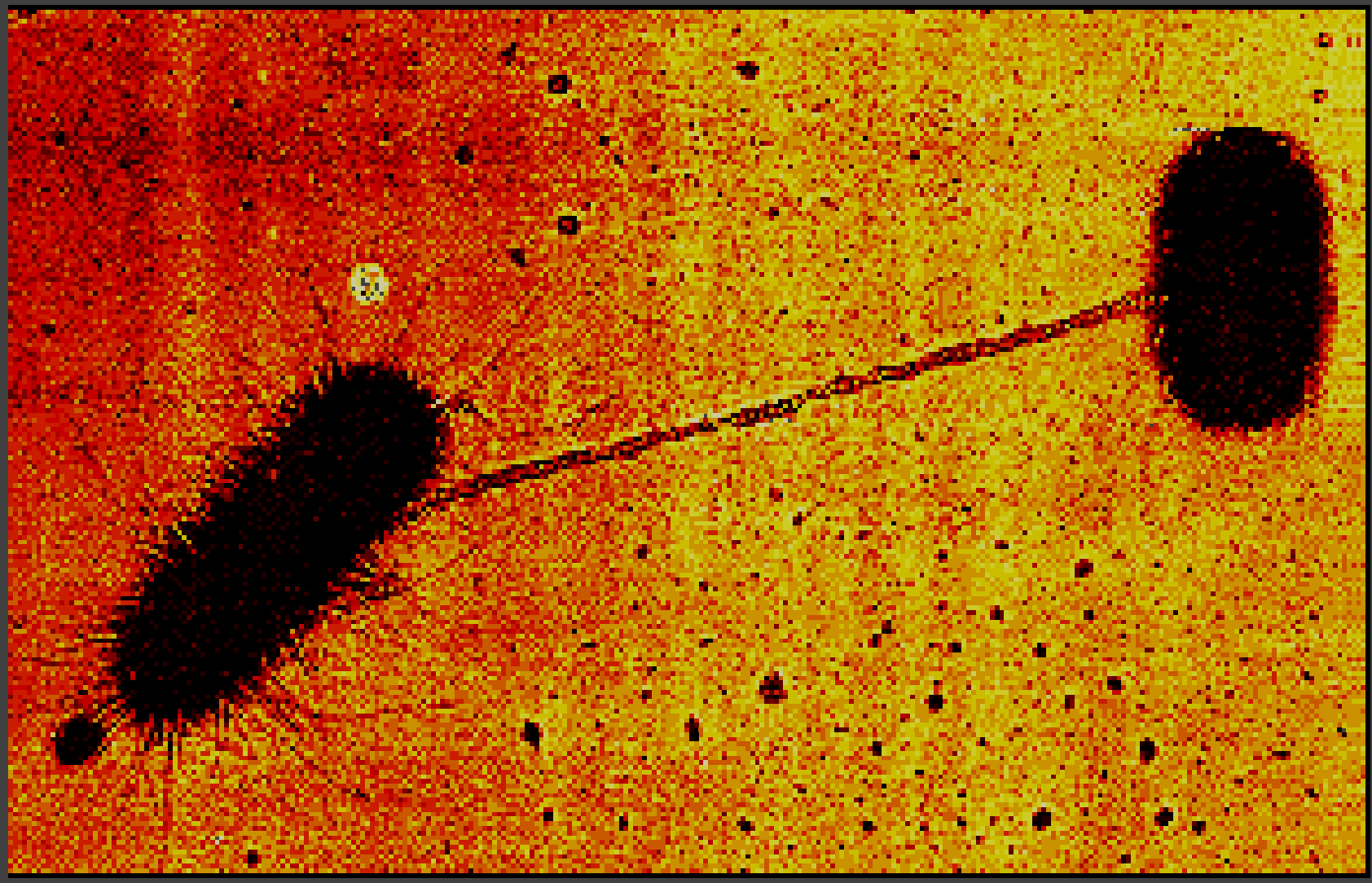
3. **When transfer is complete, both cells are F^+ double-stranded**

Transfer of the *F* factor



a) Two bacterial cells connected by a long, tubular *F*-pilus





Conjugation of high-frequency recombinant strains:

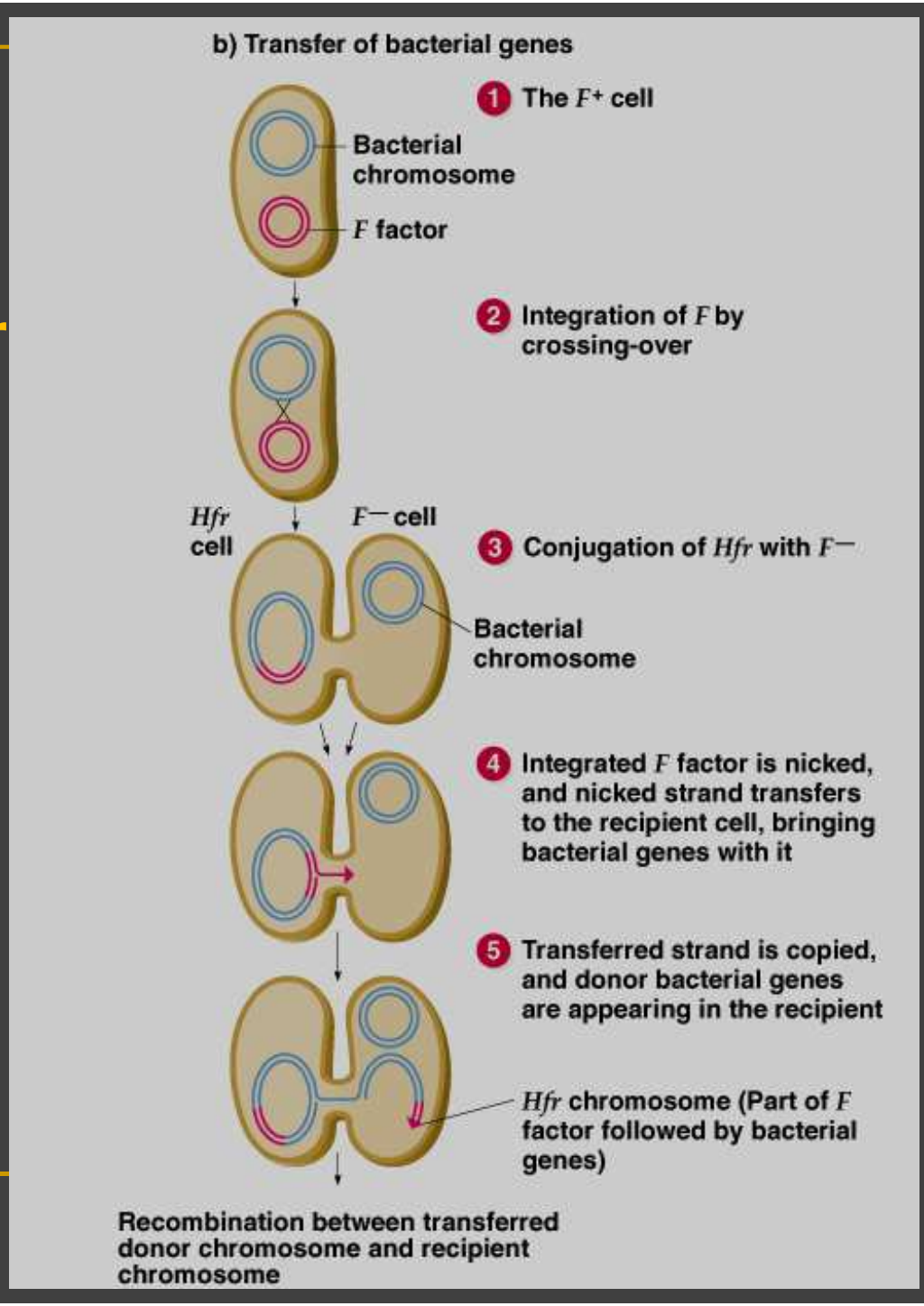
1. No chromosomal DNA is transferred by standard sex factor *F*.
2. Transfer of chromosome DNA is facilitated by special strains of F^+ integrated into the bacteria chromosome by crossing over.
3. *Hfr* strains = high frequency recombination strains.
4. Discovered by William Hayes and Luca Cavalli-Sforza.
5. *Hfr* strains replicate *F* factor as part of their main chromosome.

1. Conjugation in *Hfr* strains begins when F^+ is nicked at the origin, and F^+ and bacteria chromosomal DNA are transferred using the rolling circle mechanism.

2. Complete F^+ sequence (or complete chromosomal DNA) is rarely transferred (1/10,000) because bacteria separate randomly before DNA synthesis completes.

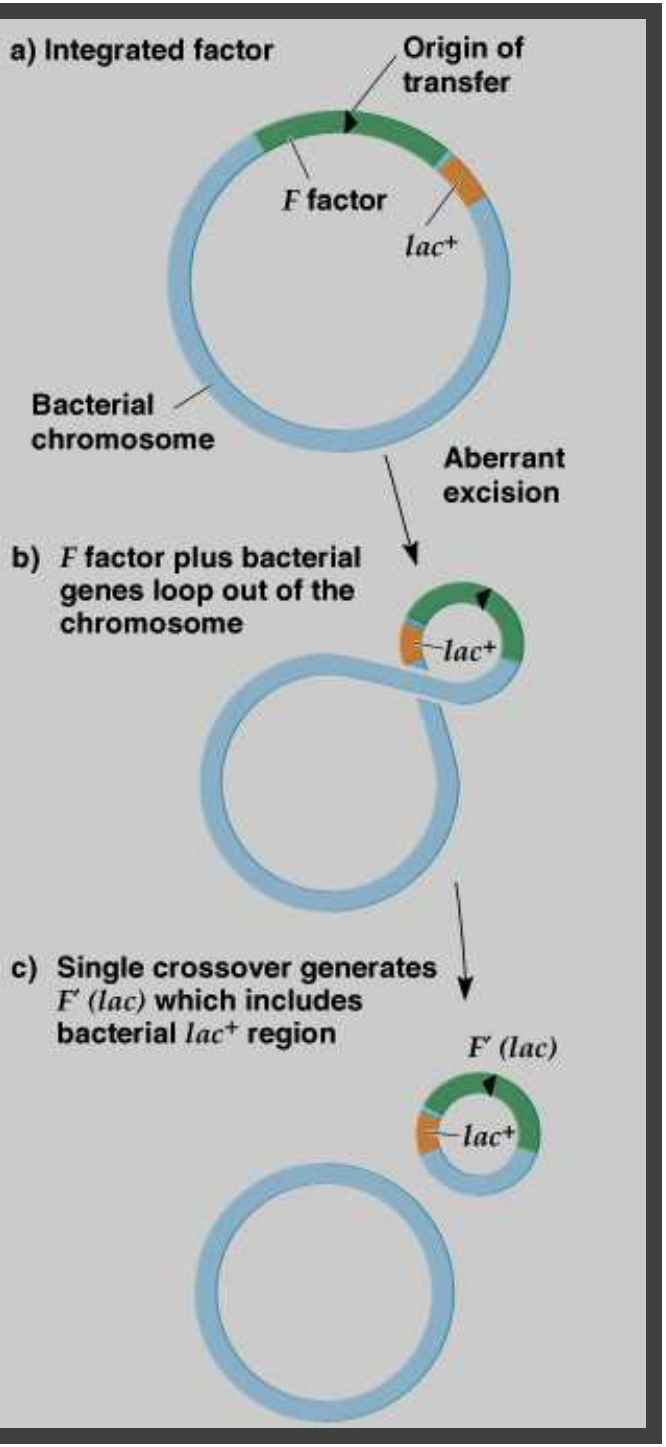
3. Recombinants are produced by crossover of the recipient chromosome and donor DNA containing F^+.

Transfer of the *Hfr* F^+ factor



Excision of the *F*+ factor also occurs spontaneously at low frequency.

1. **Begin with *Hfr* cell containing *F*+.**
2. **Small section of host chromosome also may be excised, creating an *F′* plasmid.**
3. ***F′* plasmid is named for the gene it carries, e.g., *F′(lac)***



Transformation (Griffith, 1928)

Transfer of genetic information by free DNA. i.e. by direct uptake of donor DNA by the recipient DNA.

Live noncapsulated (R) pneumococci + heat killed capsulated (S) pneumococci

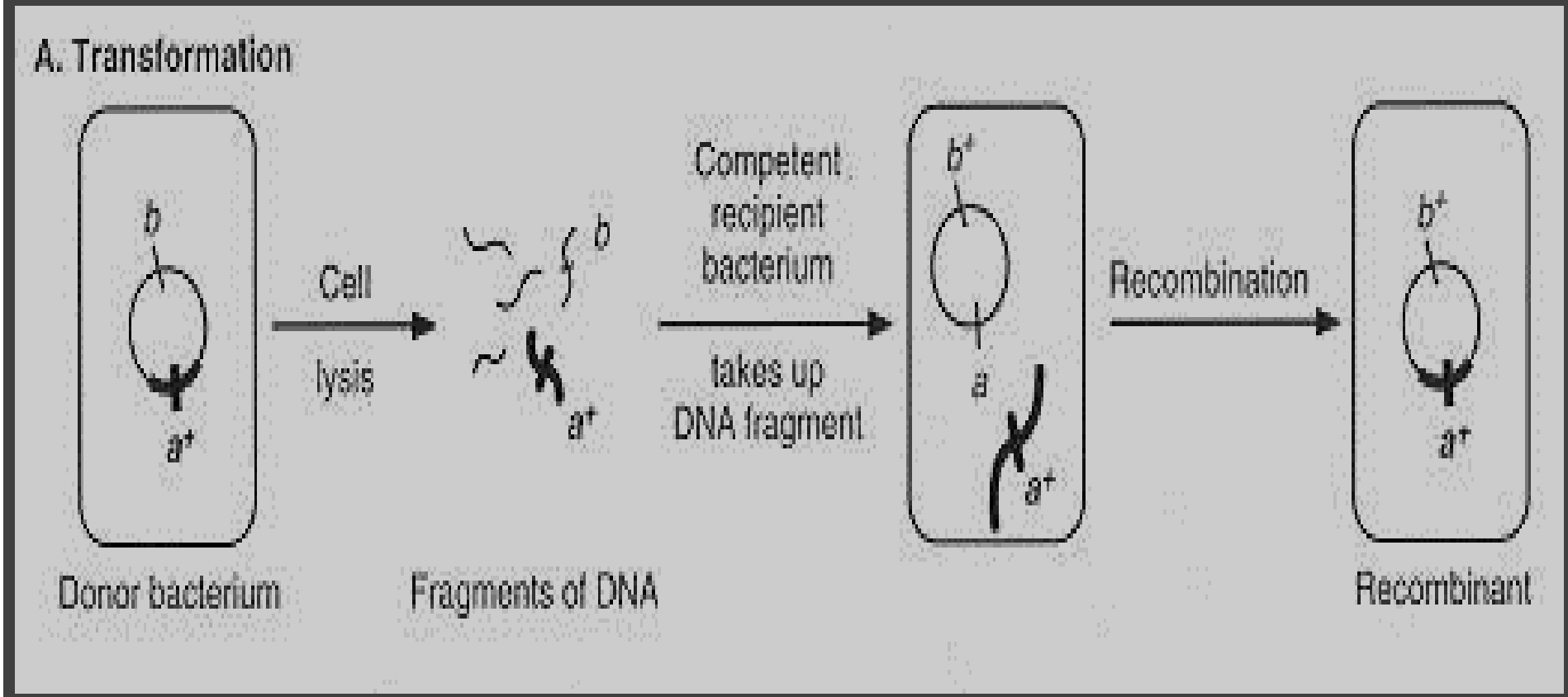
↓

Injected into mice

↓

Death of mice

- Live capsulated pneumococcus isolated from the blood of mice.


A. Transformation
b
a+
Donor bacterium
Cell
lysis
b
a+
Fragments of DNA
Competent
recipient
bacterium
takes up
DNA fragment
b+
a
a+
Recombination
b+
a+
Recombinant

Transduction

- Transfer of a portion of the DNA from one bacterium to another by a bacteriophage.

Packaging error within the infected bacteria during the assembly of progeny phages – presence of a segment of host DNA along with the phage nucleic acid in the core of phage

↓

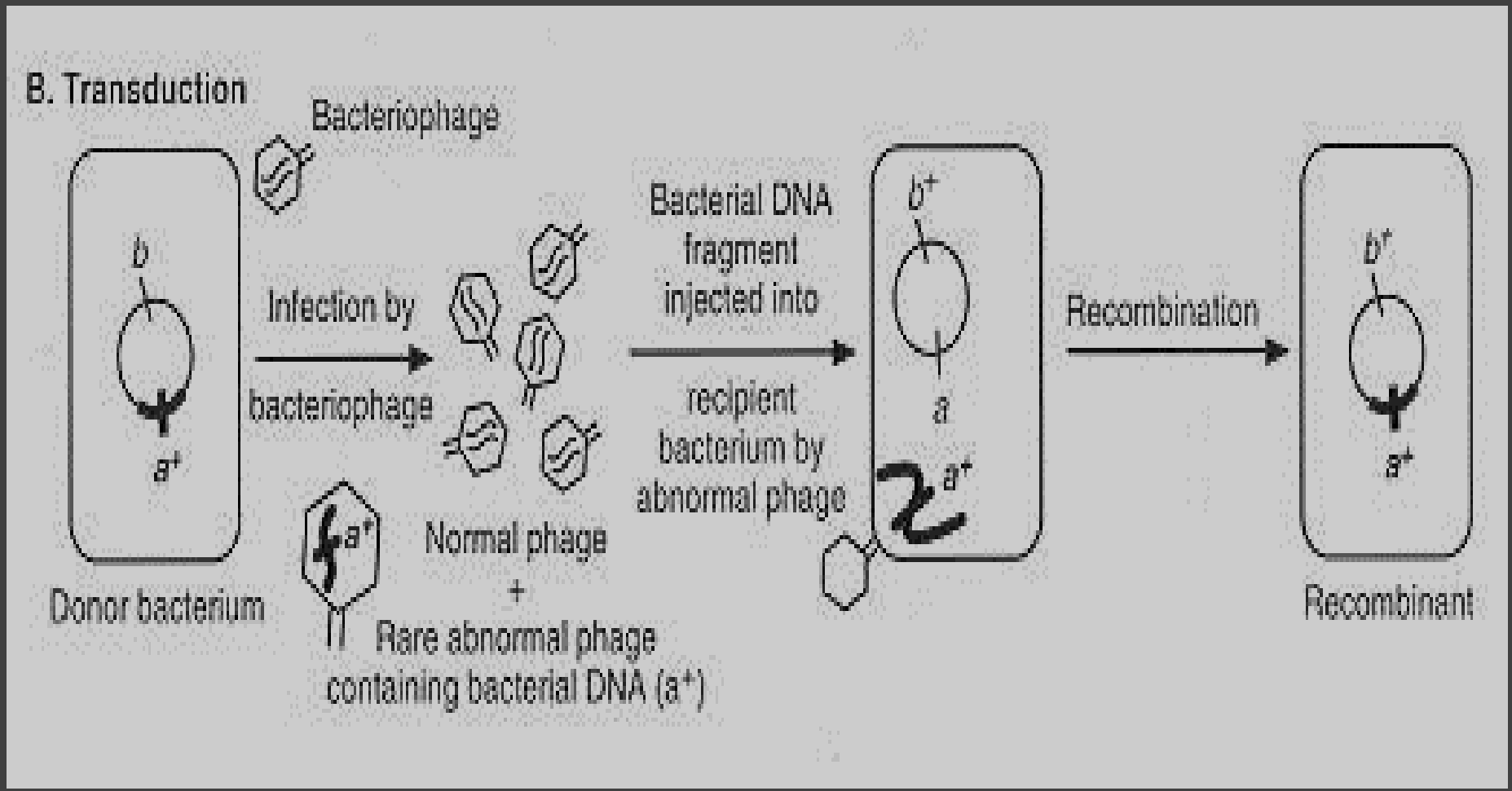
Infection of another bacterium

↓

Transfer of host bacterial DNA to the new bacterium

↓

Acquisition of new characteristics coded by the donor DNA.


B. Transduction
Bacteriophage
b
a+
Donor bacterium
Infection by
bacteriophage
a+
Normal phage
+
Rare abnormal phage
containing bacterial DNA (a+)
Bacterial DNA
fragment
injected into
recipient
bacterium by
abnormal phage
b+
a
a+
Recombination
b+
a+
Recombinant

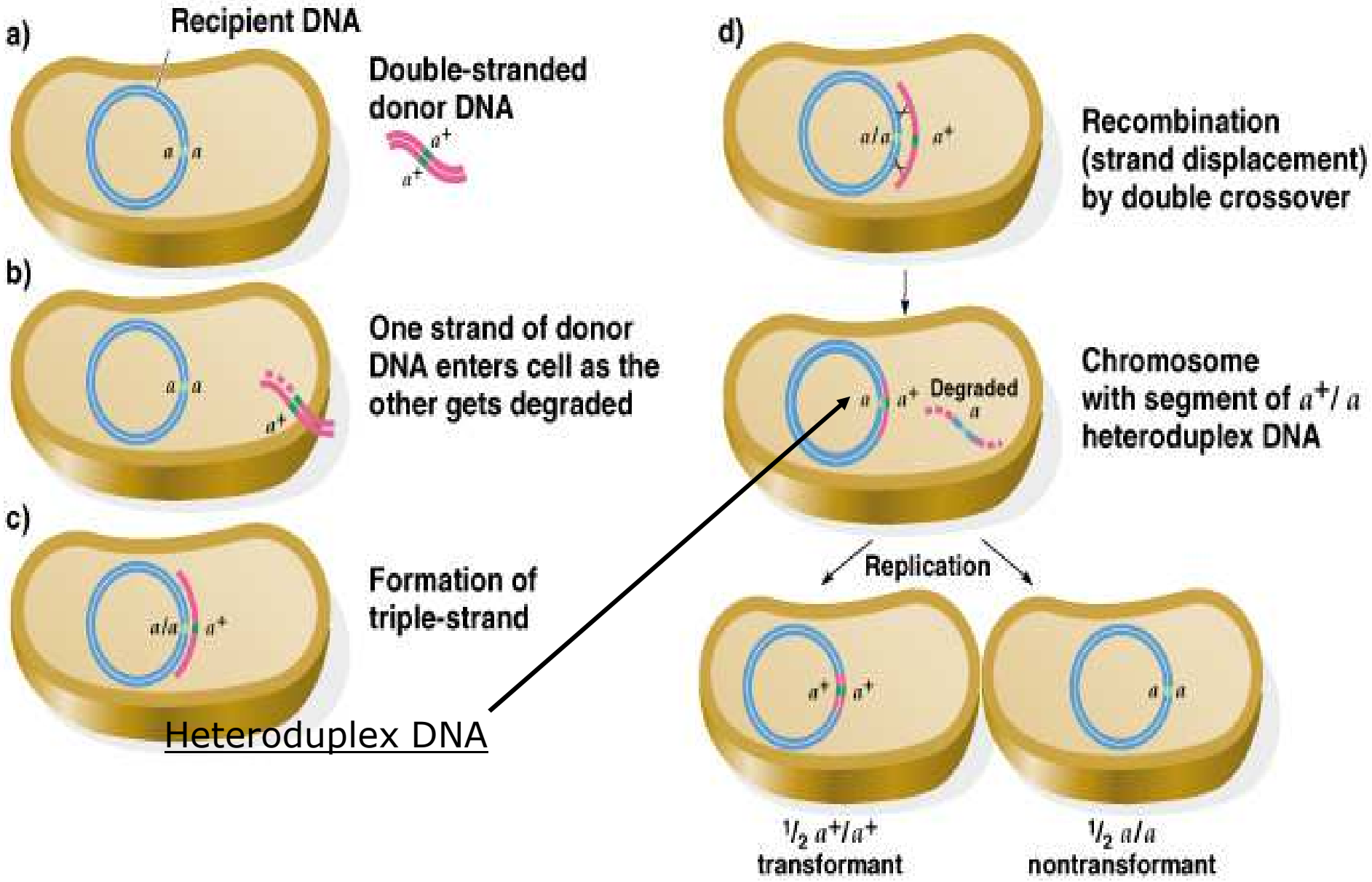
Transformation

1. Unidirectional transfer of extracellular DNA into cells, resulting in a phenotypic change in the recipient.
2. First discovered by Frederick Griffith (1928).
3. DNA from a donor bacteria is extracted and purified, broken into fragments, and added to a recipient strain.
4. Donor and recipient have different phenotypes and genotypes.
5. If recombination occurs, new recombinant phenotypes appear.

Transformation:

1. **Bacteria vary in their ability to take up DNA.**
2. **Bacteria such as *Bacillus subtilis* take up DNA naturally.**
3. **Other strains are engineered (i.e., competent cells).**
4. **Competent cells are electroporated or treated chemically to induce *E. coli* to take up extracellular DNA.**

Transformation of *Bacillus subtilus*



Transduction

1. Bacteriophages (bacterial viruses) transfer genes to bacteria (e.g., T2, T4, T5, T6, T7, and λ).
    1. Generalized transduction transfers any gene.
    1. Specialized transduction transfers specific genes.
2. Phages typically carry small amounts of DNA, ~1% of the host chromosome.
3. Viral DNA undergoes recombination with homologous host chromosome DNA.
4. Most widely used mechanism of gene transfer among prokaryotes

Fig. 15.12

Life cycle of phage λ

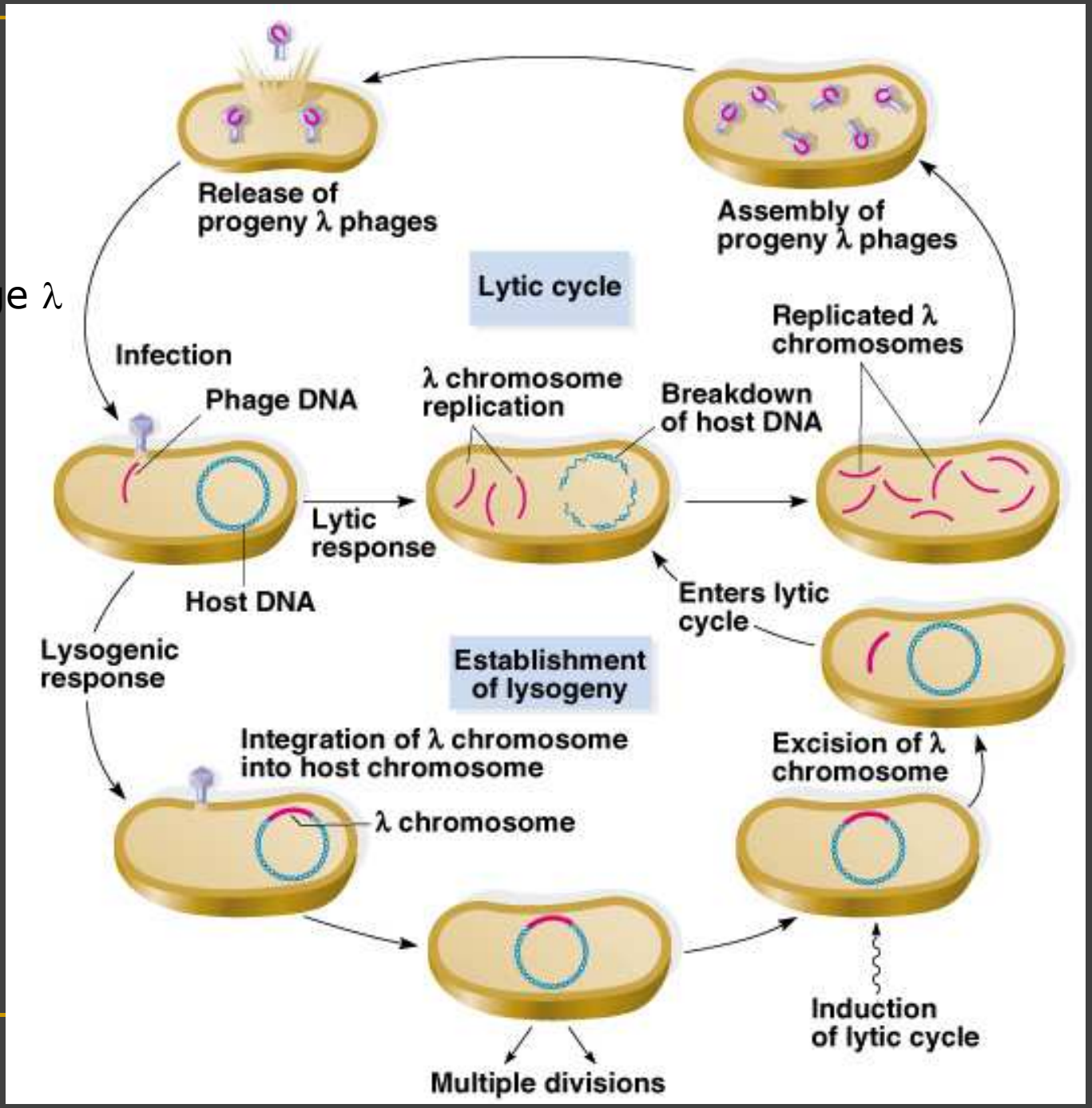
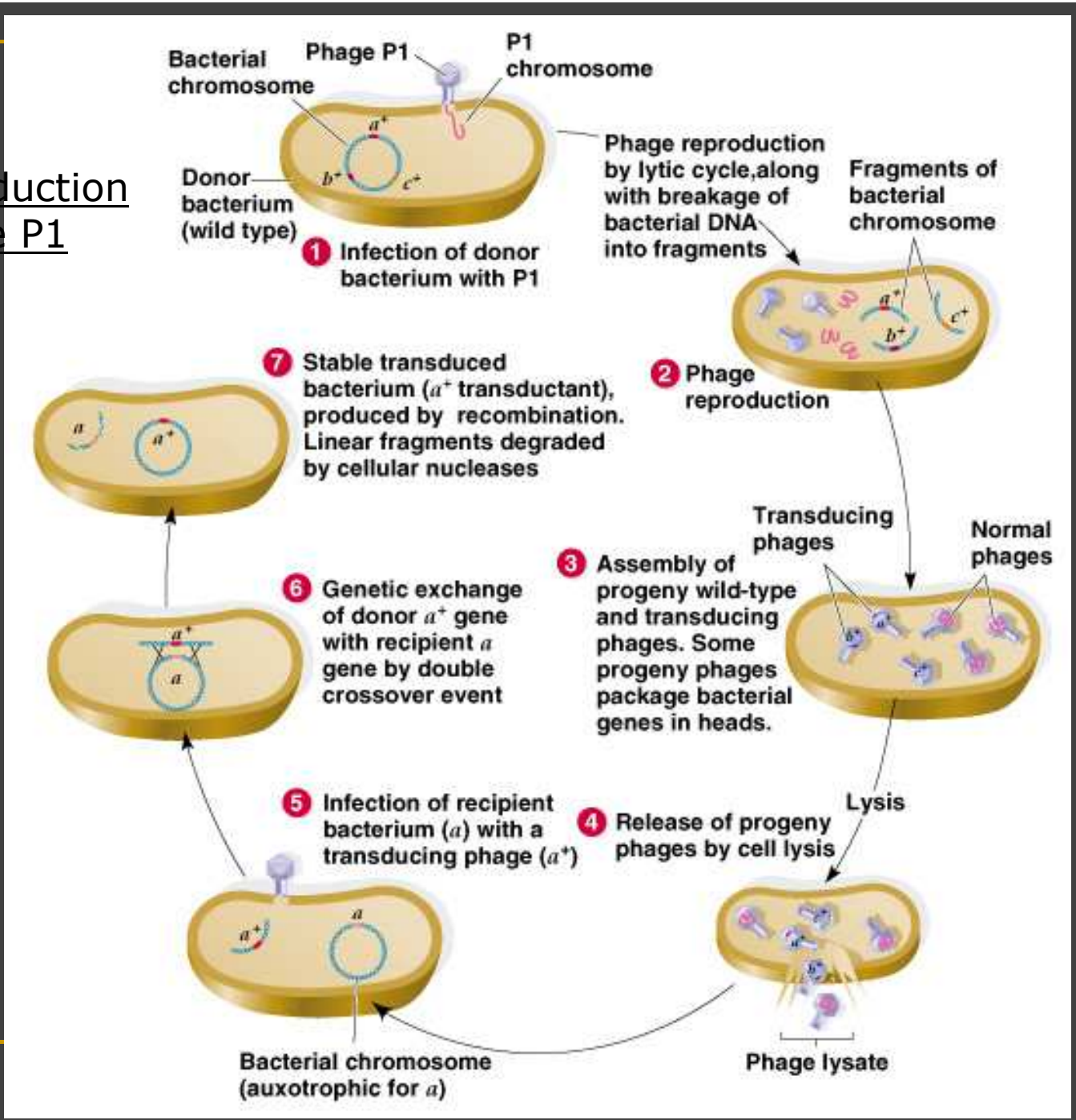


Fig. 15.13

Generalized transduction of *E. coli* by phage P1

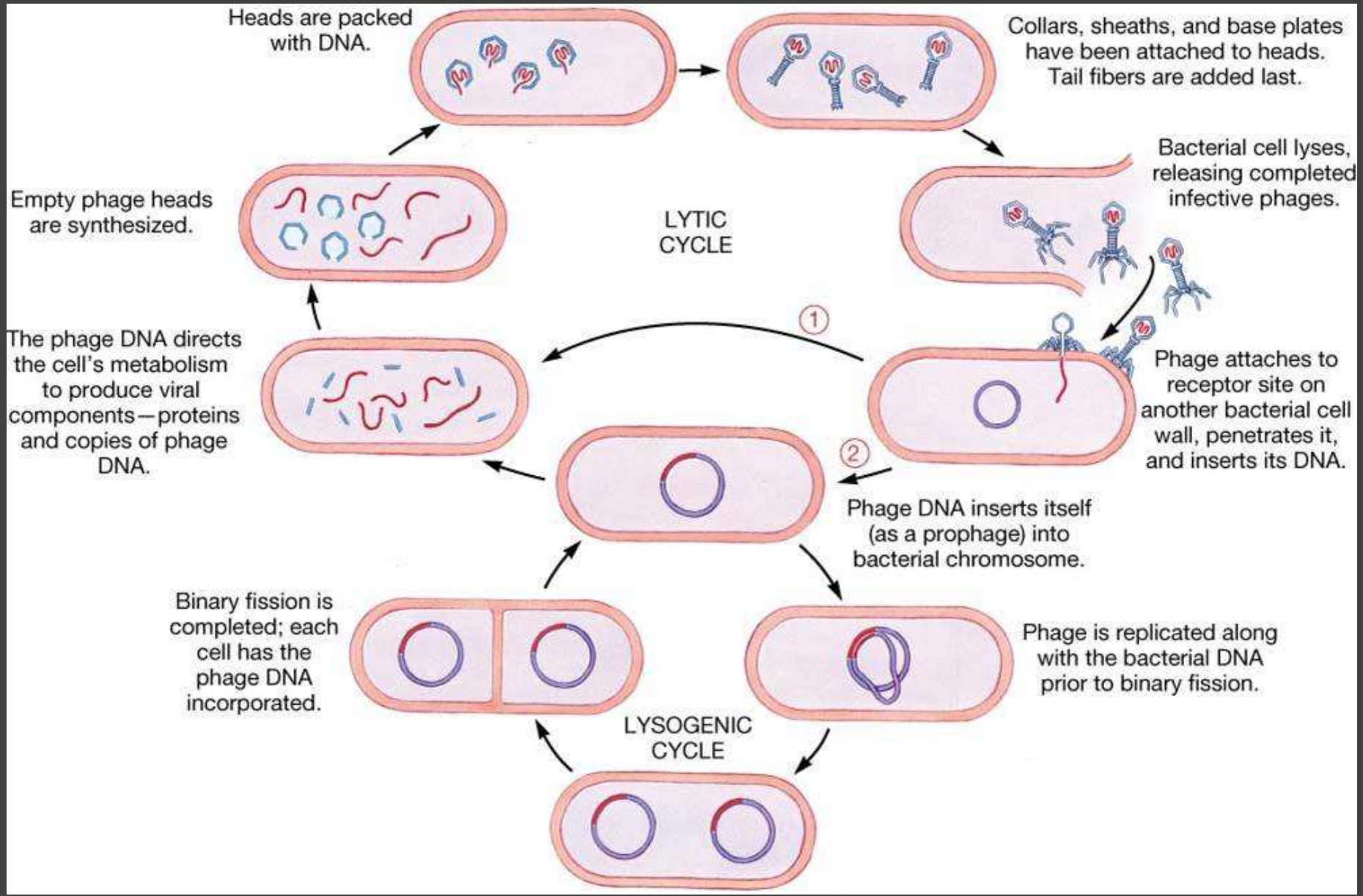


Lysogenic Conversion

Phage DNA itself is the new genetic element.

Bacteriophages – 2 Types of life cycle

- **Lytic or virulent cycle** – progeny viruses build up inside host bacterium, which rupture to release them.
- **Temperate or nonlytic or lysogenic cycle** – host bacterium is unharmed.

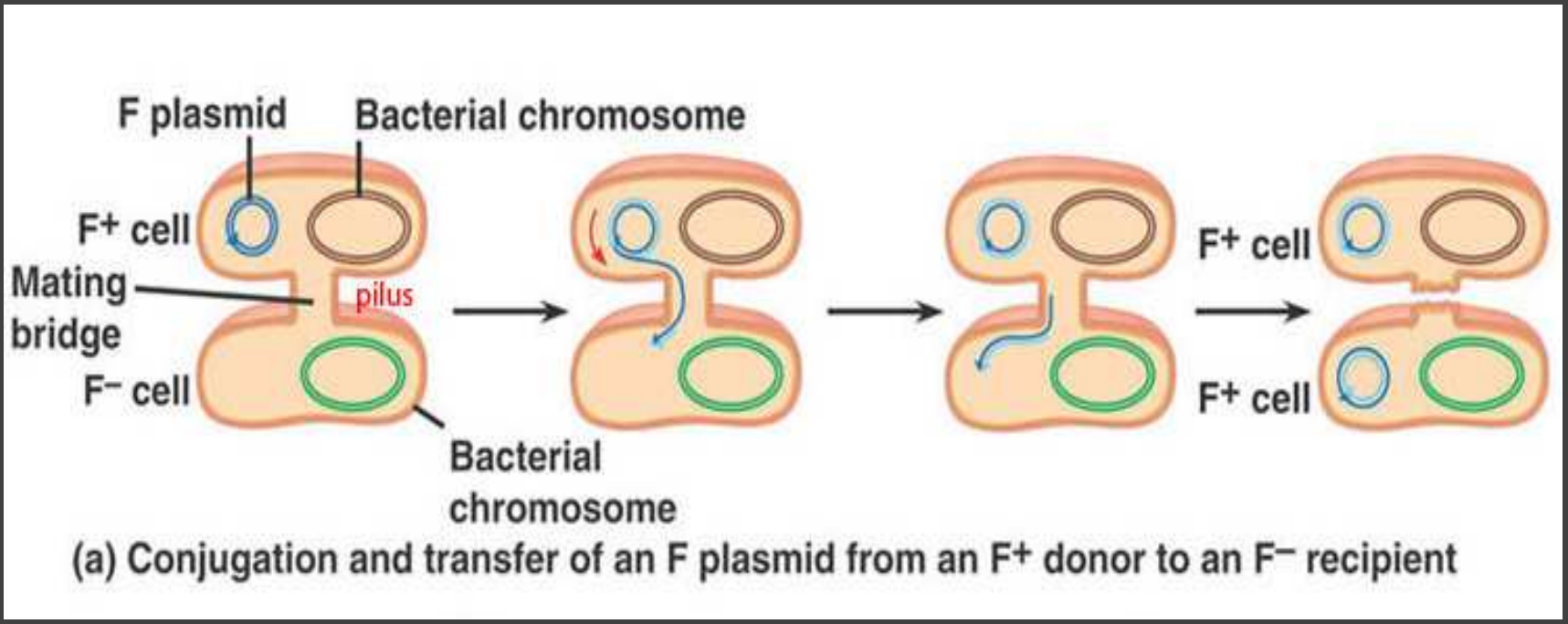

Heads are packed with DNA.
Collars, sheaths, and base plates have been attached to heads. Tail fibers are added last.
Bacterial cell lyses, releasing completed infective phages.
Empty phage heads are synthesized.
LYTIC CYCLE
The phage DNA directs the cell's metabolism to produce viral components—proteins and copies of phage DNA.
1
Phage attaches to receptor site on another bacterial cell wall, penetrates it, and inserts its DNA.
2
Phage DNA inserts itself (as a prophage) into bacterial chromosome.
Binary fission is completed; each cell has the phage DNA incorporated.
Phage is replicated along with the bacterial DNA prior to binary fission.
LYSOGENIC CYCLE

- Lysogeny
- Lysogenic bacteria
- Prophage behaves as an additional segment of bacterial chromosome, coding for new characteristics. This process by which prophage confers genetic information to a bacterium is called Lysogenic conversion .

Conjugation

- First described by Lederburg & Tatum in 1946 in a strain of E.coli called K12.
- A donor or male bacterium passes DNA directly to a recipient or female bacterium by a conjugation tube (sex pili). The female bacterium attains donor status & in turn can conjugate with other female cells.
- Maleness is determined by the presence of a plasmid which codes for sex pili.
- The plasmid is called the sex factor or fertility factor (F factor)
- R (resistance) factor can also be transferred by conjugation

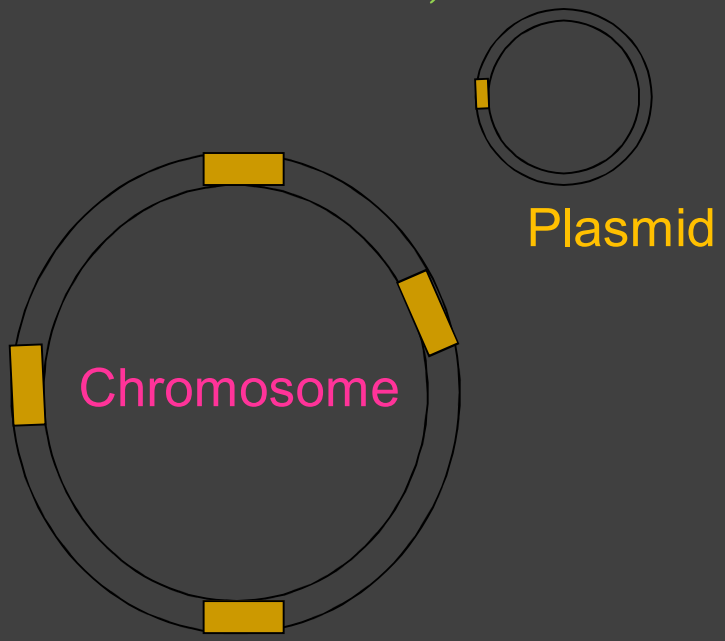
Process of Conjugation



(a) Conjugation and transfer of an F plasmid from an F+ donor to an F− recipient

Transposon (Jumping Genes, Barbara McClintock)

DNA segment that can move between chromosome & plasmids



Insertion of transposon into a functional gene would destroy the function of the gene (internal mutagenic agents)

Transposons are not self replicative, they depend on chromosomal or plasmid DNA for replication