A study material for M.Sc. Biochemistry (Semester: IV) Students
on the topic (EC-1; Unit III)

Genetic Exchanges in Prokaryotes

Causes of variation in the invisibles

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Genetic Recombination

- Recombination is the process in which one or more nucleic acids molecules are rearranged or combined to produce a new nucleotide sequence.
- Movement of DNA from a donor bacterium to the recipient can take place in three ways:
  - direct transfer between two bacteria temporarily in physical contact (conjugation),
  - transfer of a naked DNA fragment (transformation),
  - transport of bacterial DNA by bacteriophages (transduction).

Type IV secretion system

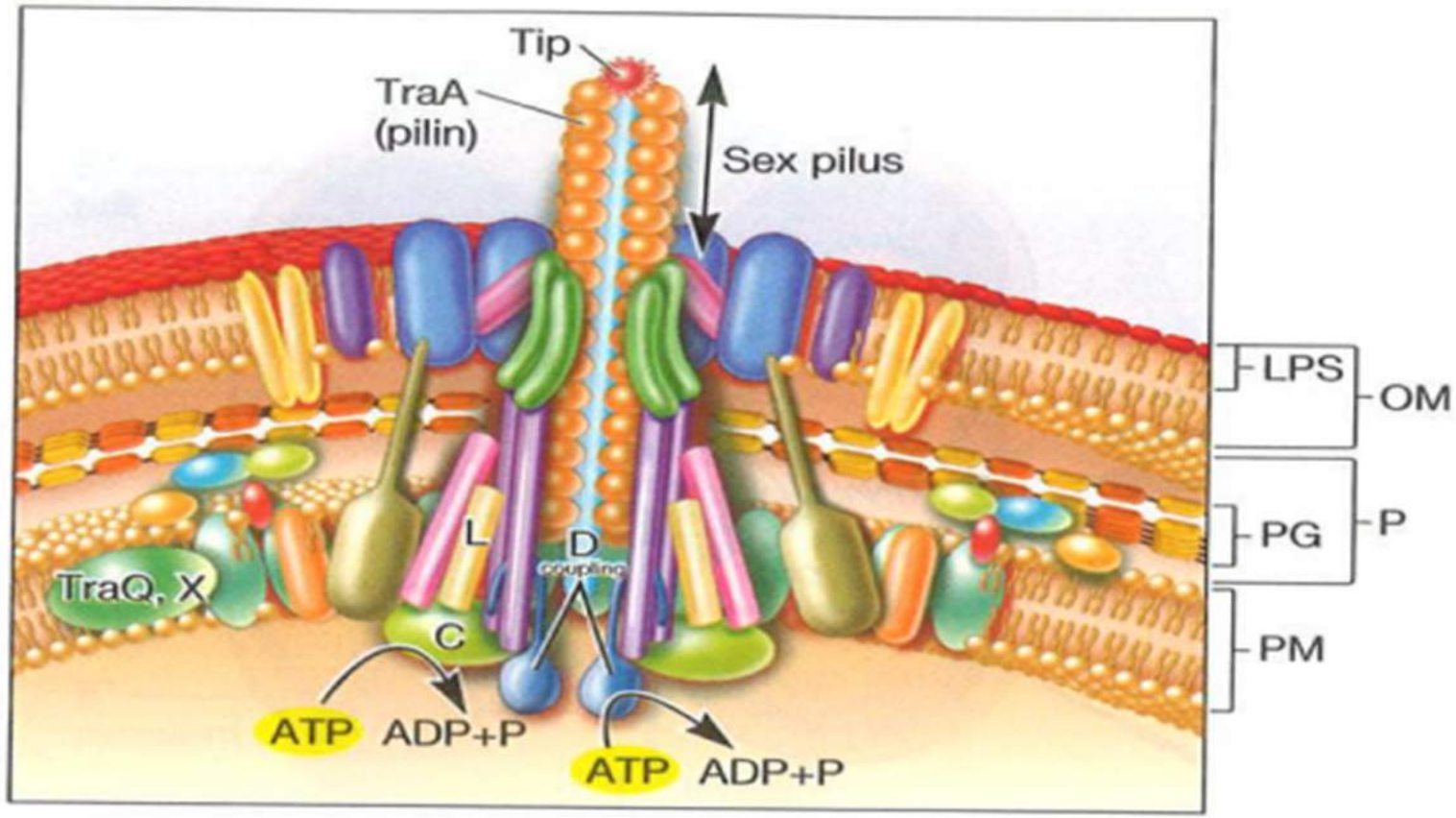


Figure 13.29 The Type IV Secretion System Encoded by F Factor. The F factor-encoded type IV secretion system is composed of numerous Tra proteins, including TraA proteins, which form the sex pilus, and TraD, which is the coupling factor. Some Tra proteins are located in the plasma membrane (PM), others extend into the periplasm (P) and pass through the peptidoglycan layer (PG) into the outer membrane (OM) and its lipopolysaccharide (LPS) layer.

Conjugation

- Direct transfer between two bacteria temporarily in physical contact.
- The initial evidence for bacterial conjugation, the transfer of genetic information by direct cell to cell contact, came from an elegant experiment performed by Joshua Lederberg and Edward L. Tatum in 1946.

Figure 13.26 Evidence for Bacterial Conjugation.
Lederberg and Tatum's demonstration of genetic recombination using triple auxotrophs. See text for details.

(They mixed two auxotrophic strains, incubated the culture for several hours in nutrient medium, and then plated it on minimal medium. To reduce the chance that their Results were due to simple reversion, they used double and triple auxotrophs on the assumption that two or three reversions would not often occur simultaneously. For example, one strain required biotin (Bio-), phenylalanine (Phe-), and cysteine (Cys-) forgrowth, and another needed threonine (Thr-), leucine (Leu-),and thiamine (Thi-). Recombinant prototrophic colonies appeared on the minimal medium after incubation)

- Lederberg and Tatum did not directly prove that physical contact of the cells was necessary for gene transfer.
- This evidence was provided by Bernard Davis (1950), who constructed a U tube consisting of two pieces of curved glass tubing fused at the base to form a U shape with a fritted glass filter between the halves. The filter allows the passage of media but not bacteria. The U tube was filled with nutrient medium and each side inoculated with a different auxotrophic strain of *E. coli*

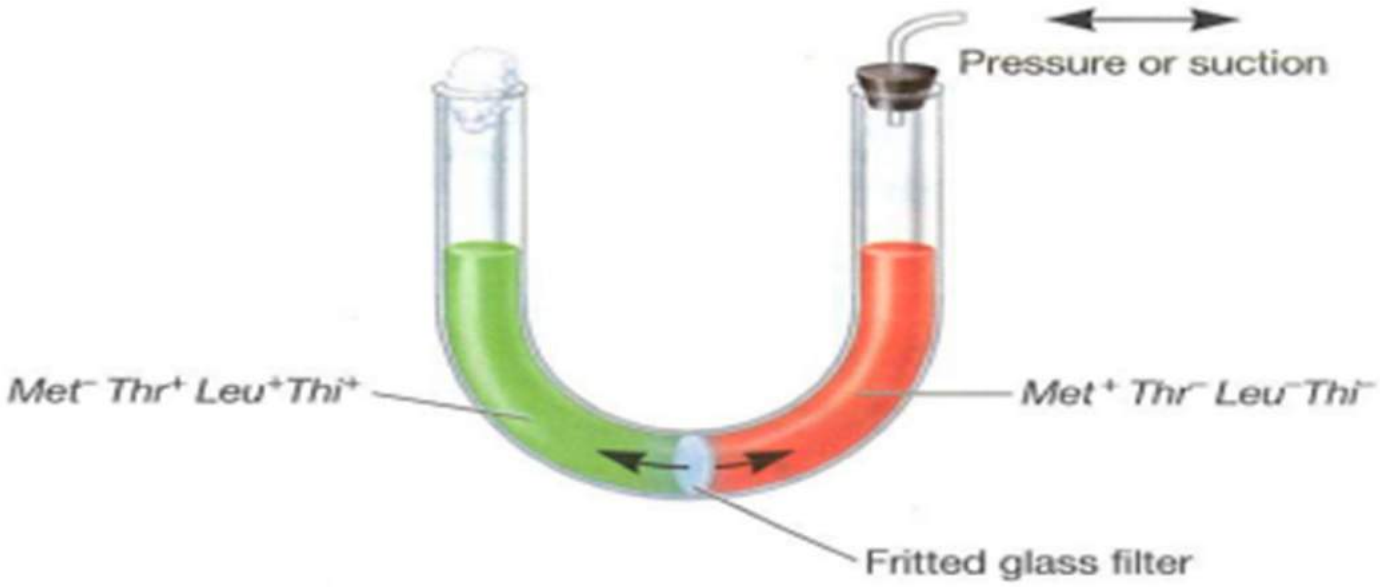
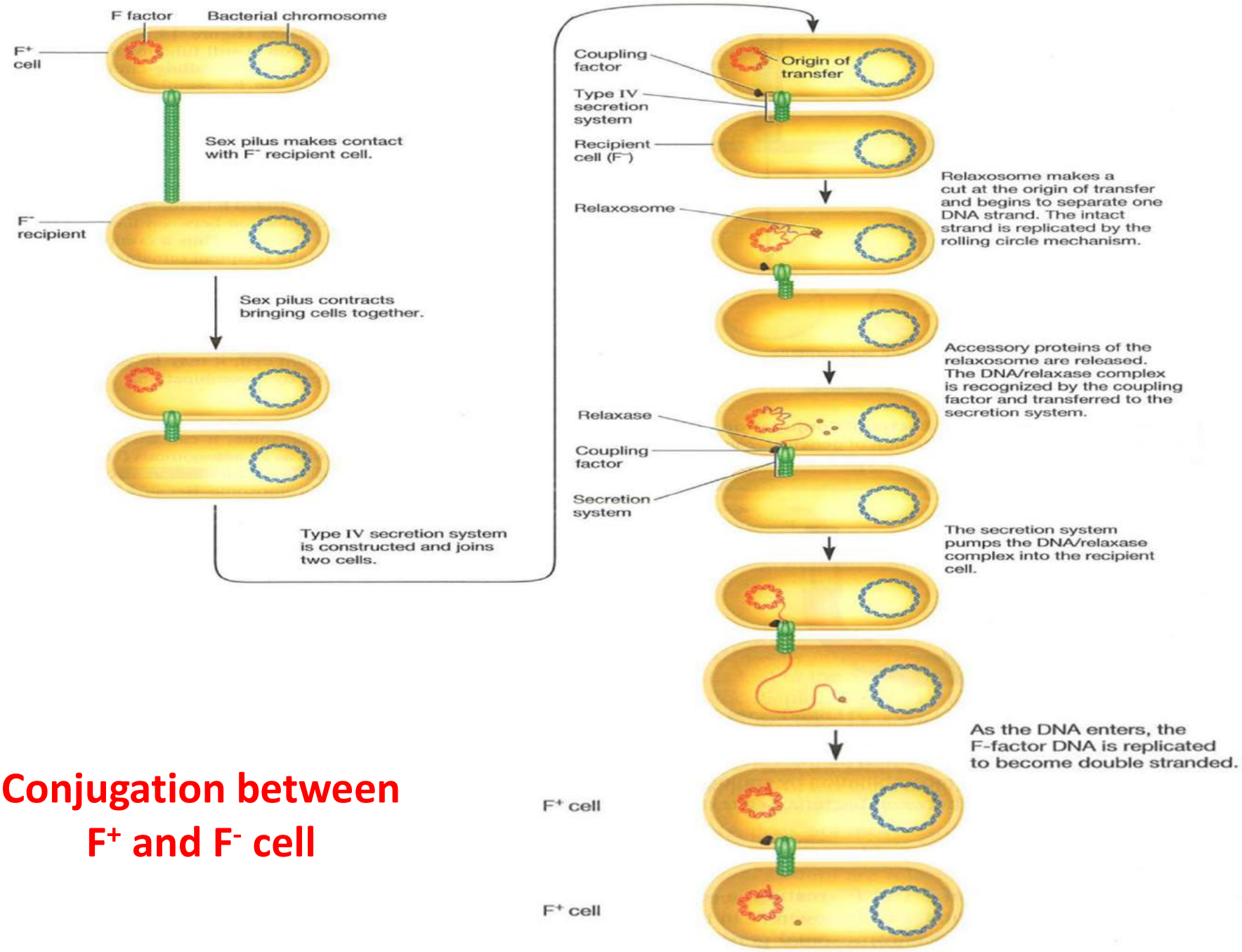


Figure. The U-Tube Experiment. The U-tube experiment used to show that genetic recombination by conjugation requires direct physical contact between bacteria.

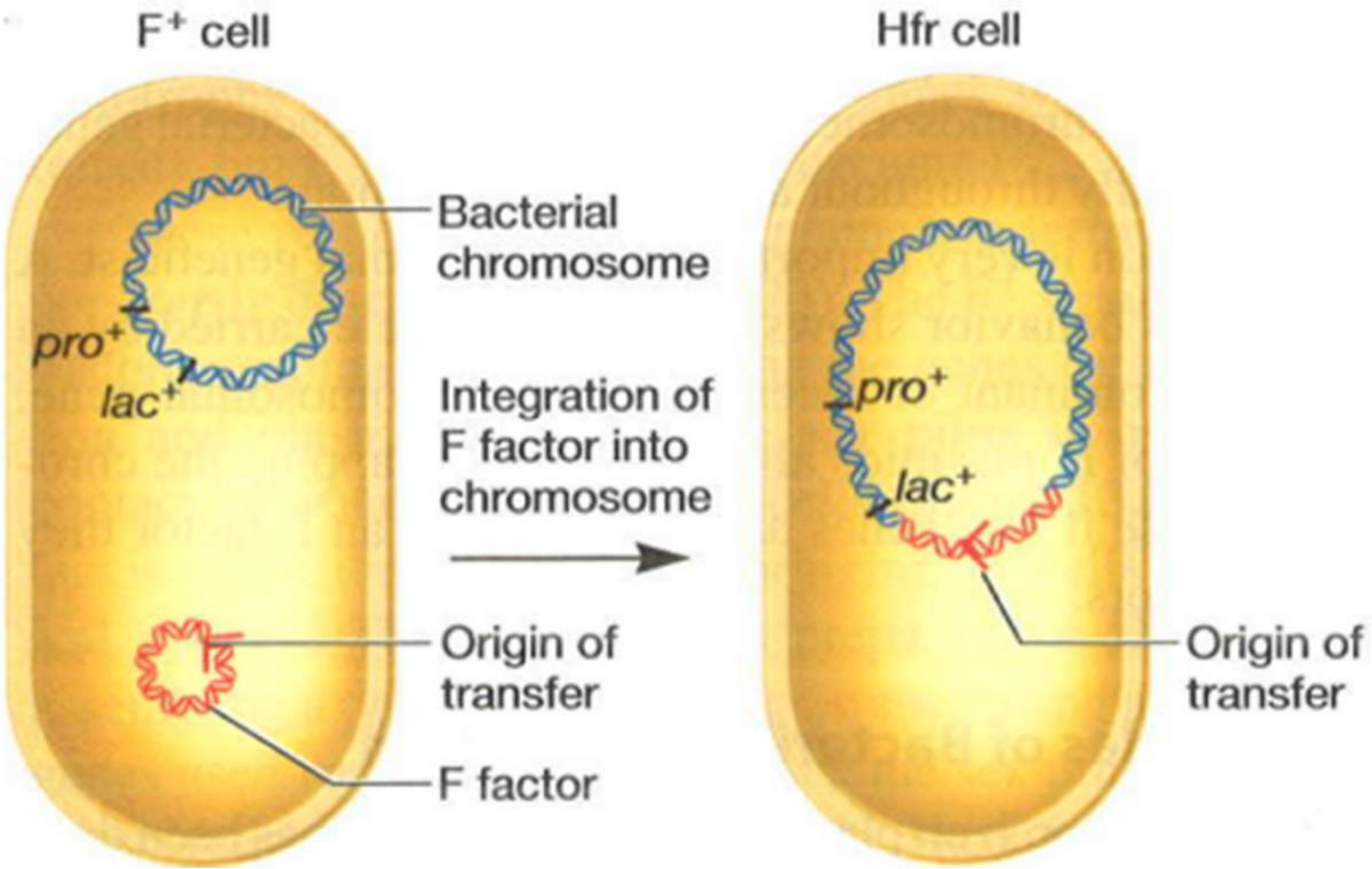
Mechanism

- The conjugative plasmid is the **F-plasmid**, or F-factor.
- The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome by homologous recombination) with a length of about 100 kb.
- It carries its own origin of replication, the *oriV*, and an origin of transfer, or *oriT*. There can only be one copy of the F-plasmid in a given bacterium, either free or integrated, and bacteria that possess a copy are called *F-positive* or*F-plus* (denoted F^+). Cells that lack F plasmids are called *F-negative* or *F-minus* (F^-) and as such can function as recipient cells.
- Among other genetic information the F-plasmid carries a *tra* and *trb* locus, which together are about 33 kb long and consist of about 40 genes.
- The *tra* locus includes the *pilin* gene and regulatory genes, which together form pili on the cell surface. The locus also includes the genes for the proteins that attach themselves to the surface of F^- bacteria and initiate conjugation.
- When conjugation is initiated by a signal the **relaxase** enzyme creates a nick in one of the strands of the conjugative plasmid at the *oriT*. Relaxase may work alone or in a complex of over a dozen proteins known collectively as a **relaxosome**.
- In the F-plasmid system the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM and the integrated host factor IHF.
- This T-DNA/relaxase complex is recognized by a coupling factor, then nicked strand, or *T-strand*, is then unwound from the unbroken strand and transferred to the recipient cell in a 5'-terminus to 3'-terminus direction.
- The remaining strand is replicated by *rolling circle replication.*

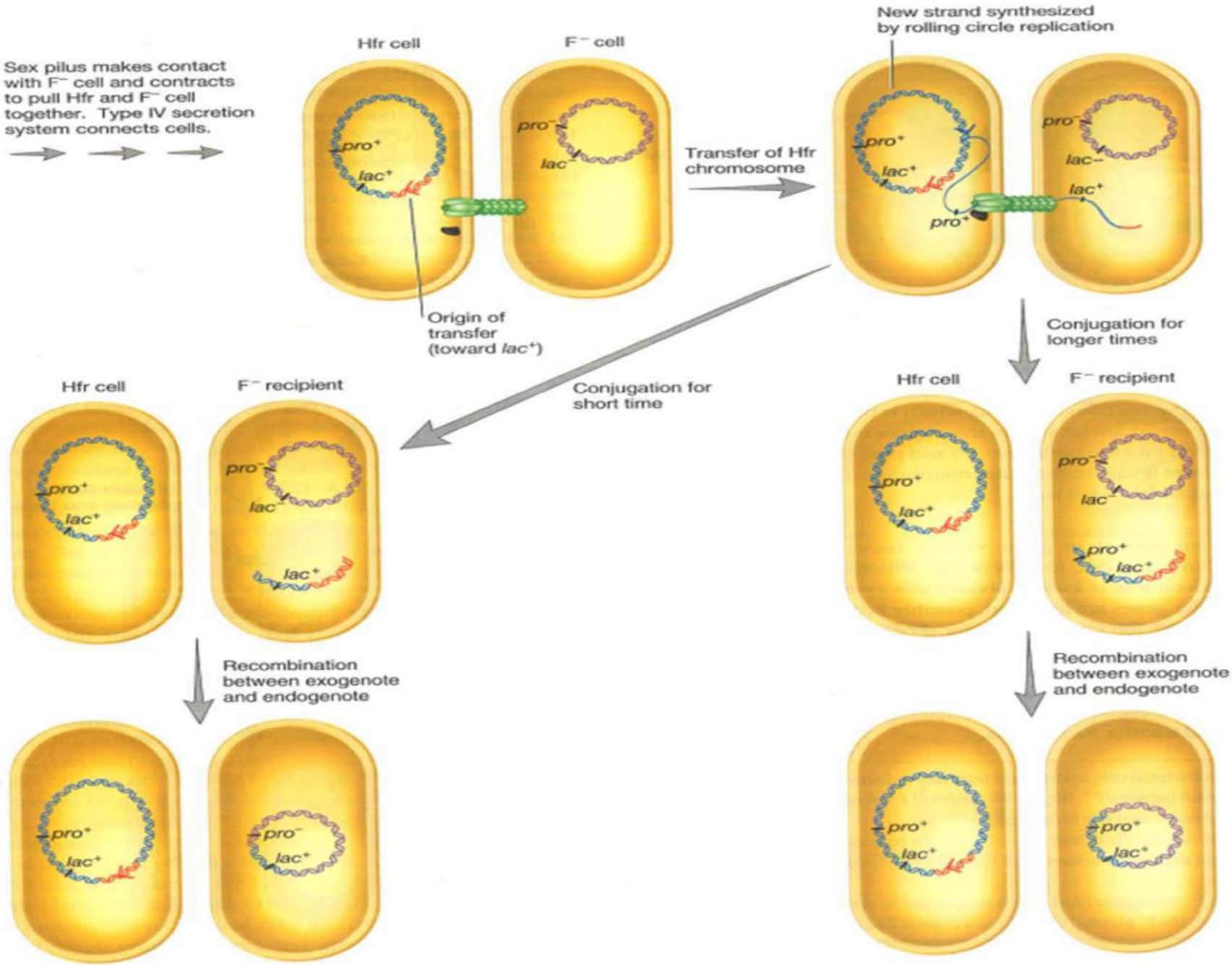


Conjugation between F^+ and F^- cell

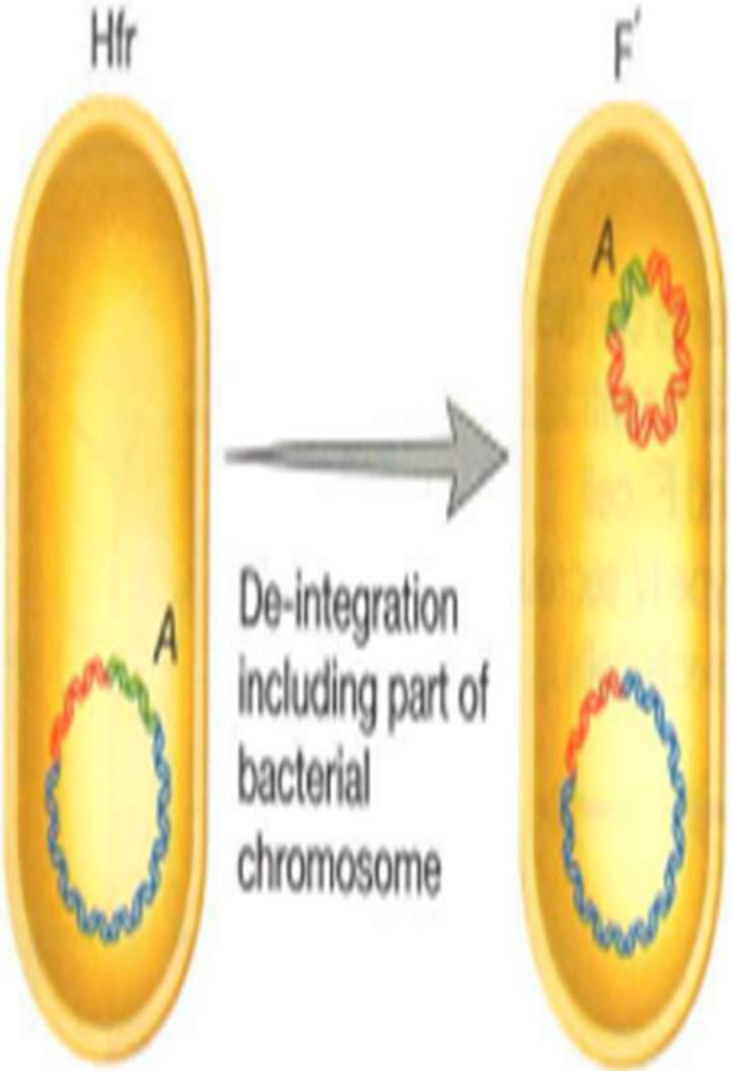
Hfr cell (High-frequency recombination cell)

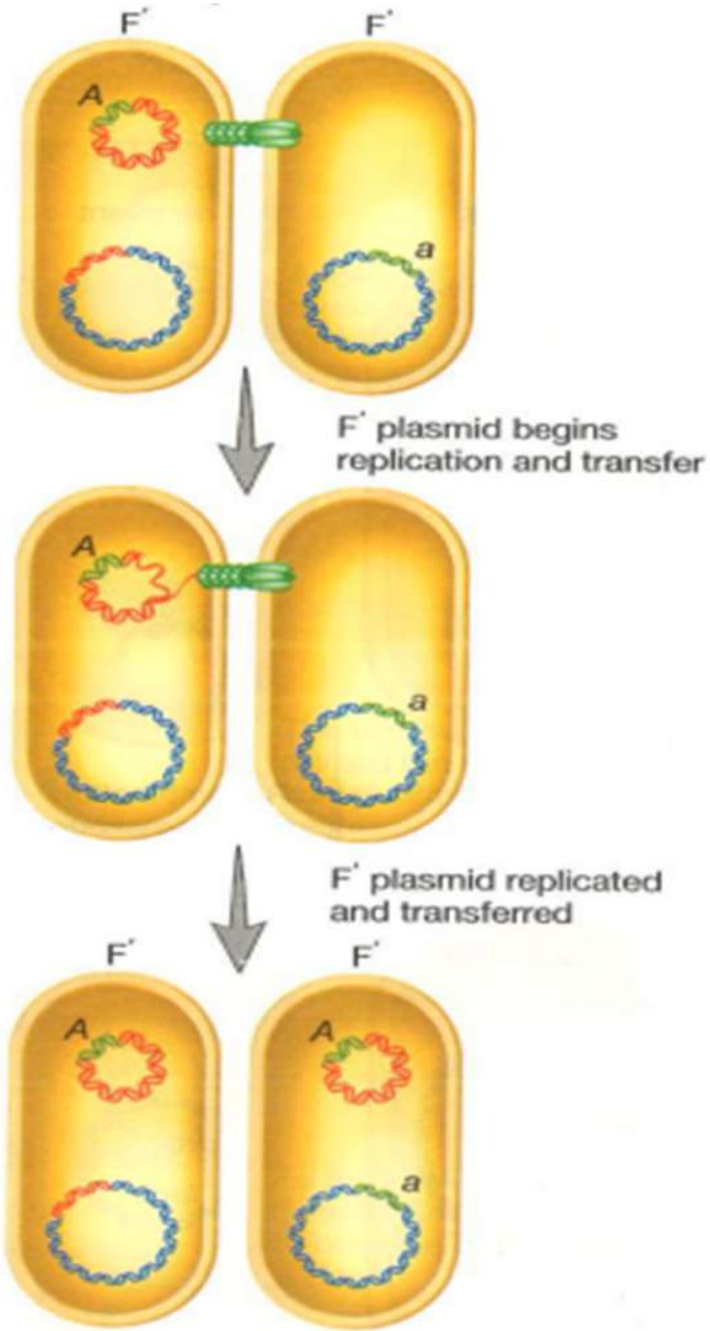


Conjugation between Hfr and F⁻ cell



F’ Cell





F’ conjugation

Transformation

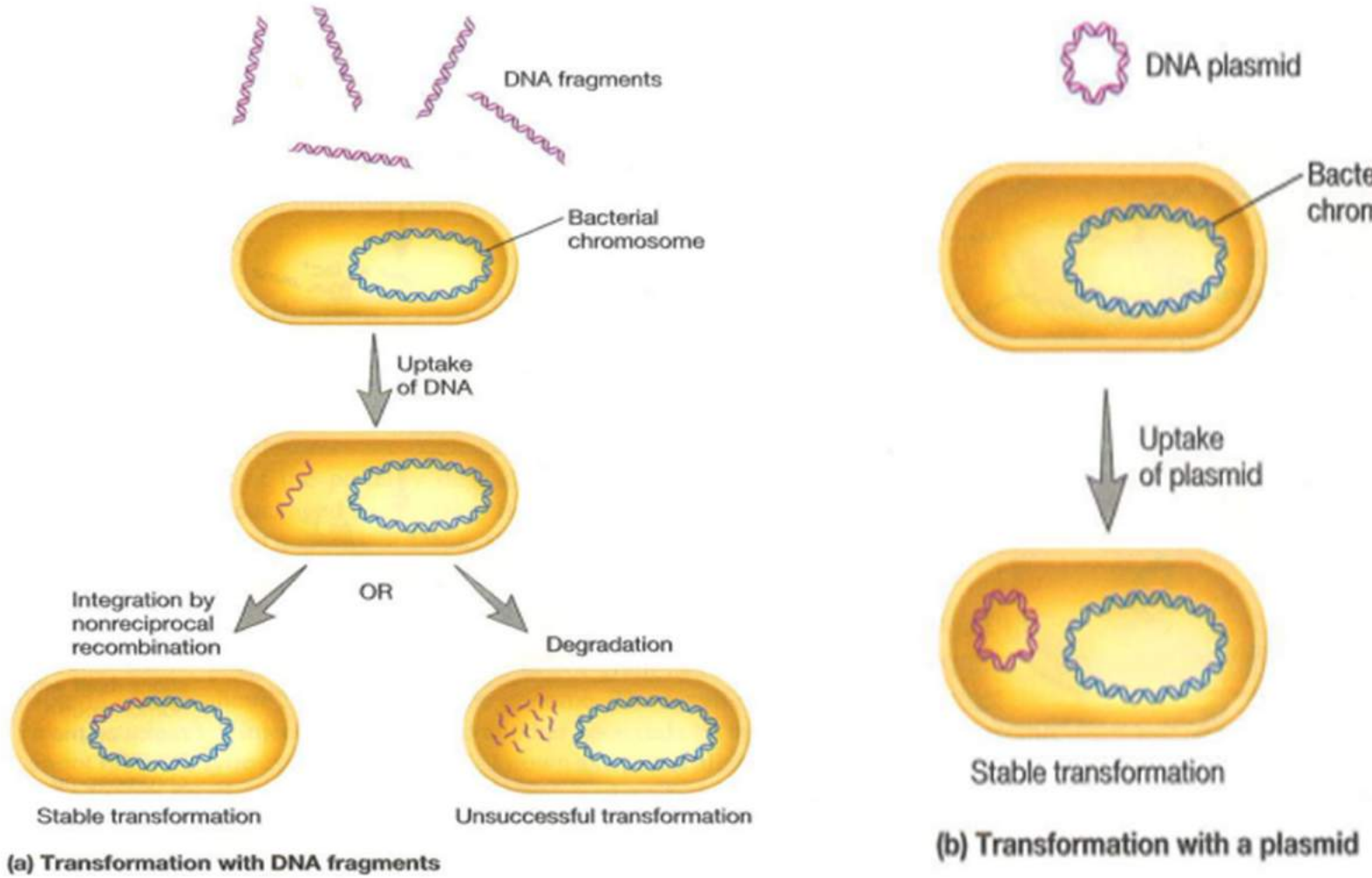
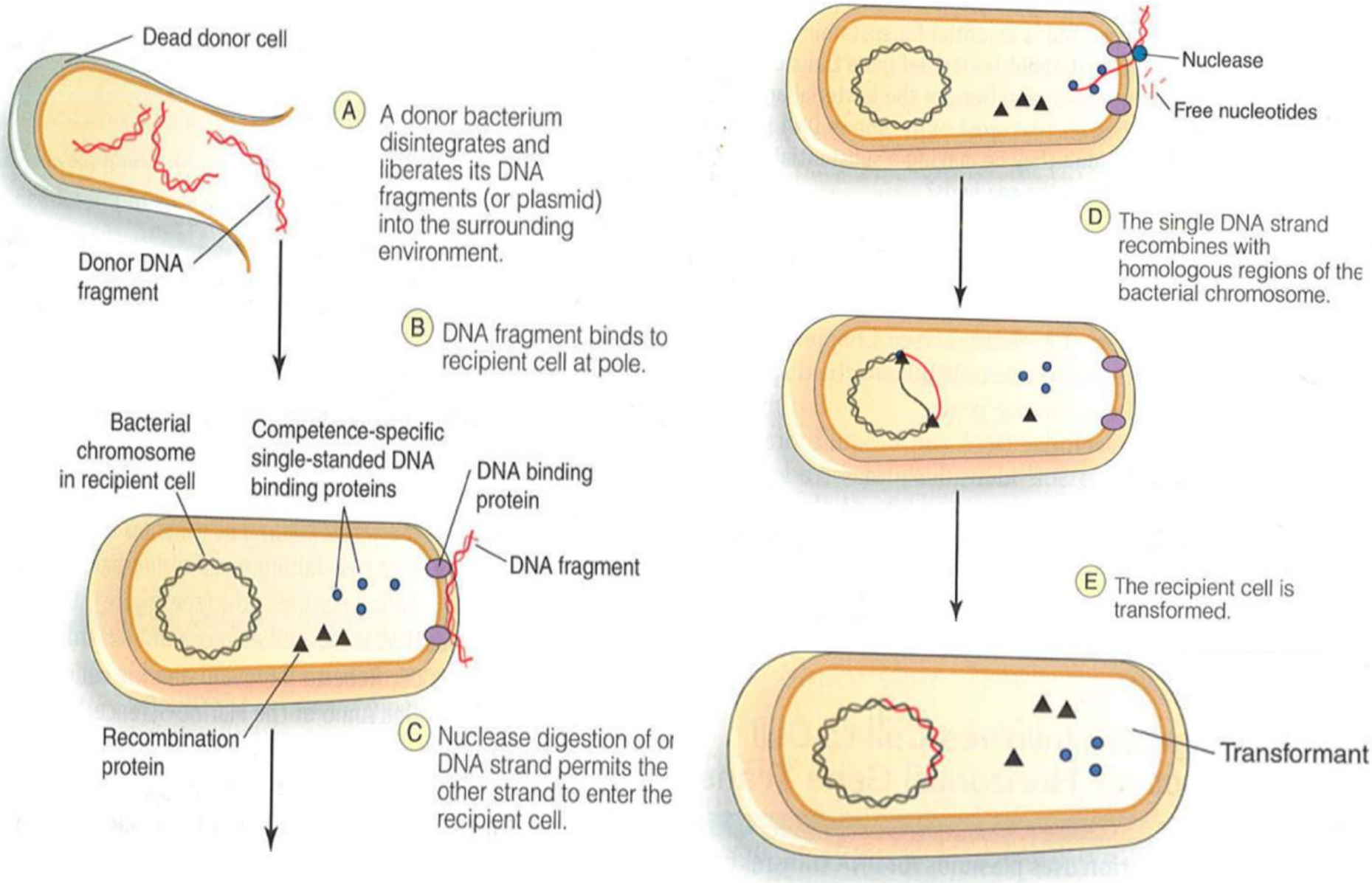
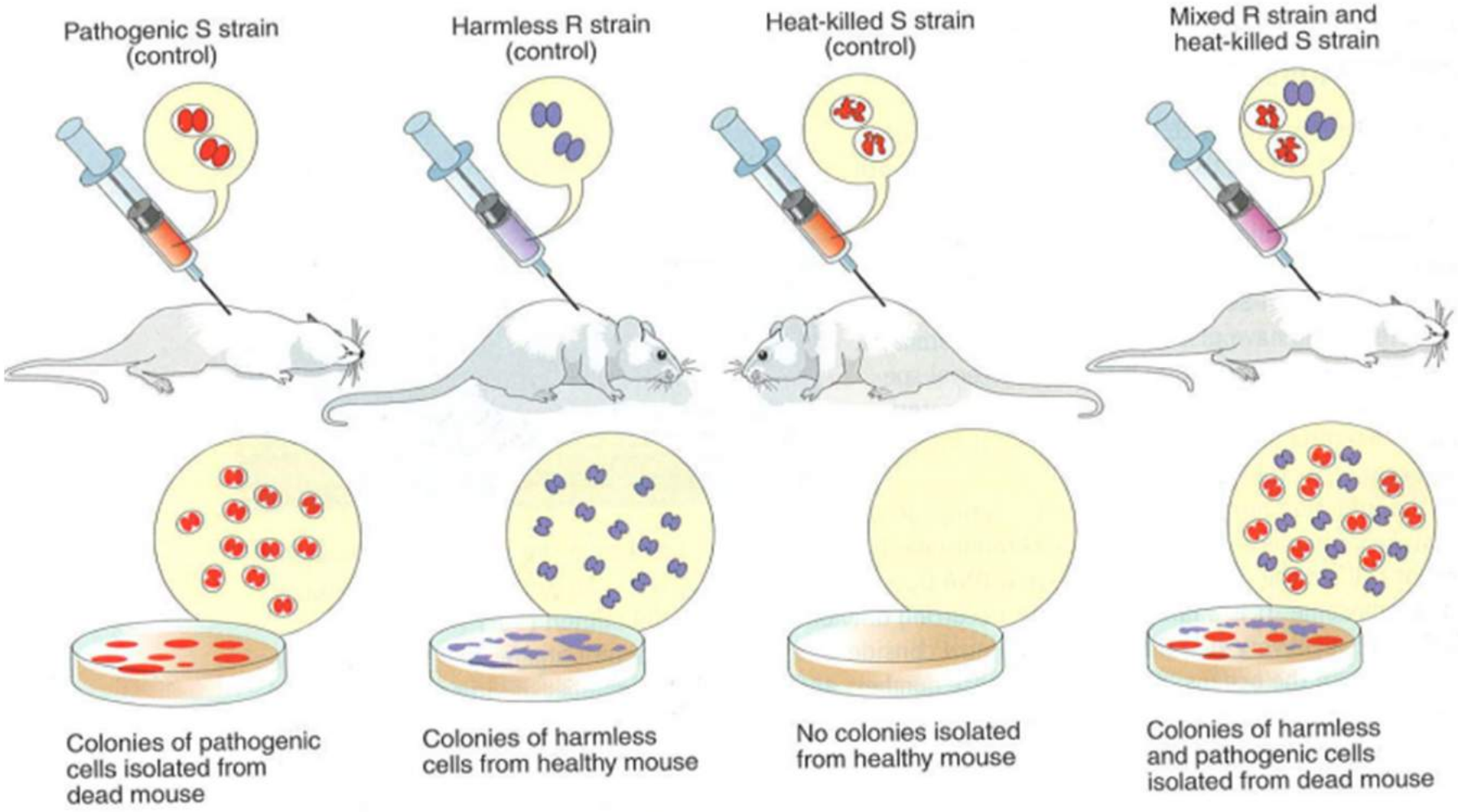


Figure 13.31 Bacterial Transformation. Transformation with **(a)** DNA fragments and **(b)** plasmids. Transformation with a plasmid often is induced artificially in the laboratory. The transforming DNA is in purple and integration is at a homologous region of the genome.

Bacterial Transformation



The Transformation Experiments of Griffith.



(A) When Griffith injected S strain (encapsulated, pathogenic) cells into the mouse, it developed pneumonia and died.

(B) An injection of R strain (unencapsulated,harmless) cells did no harm to the mouse.

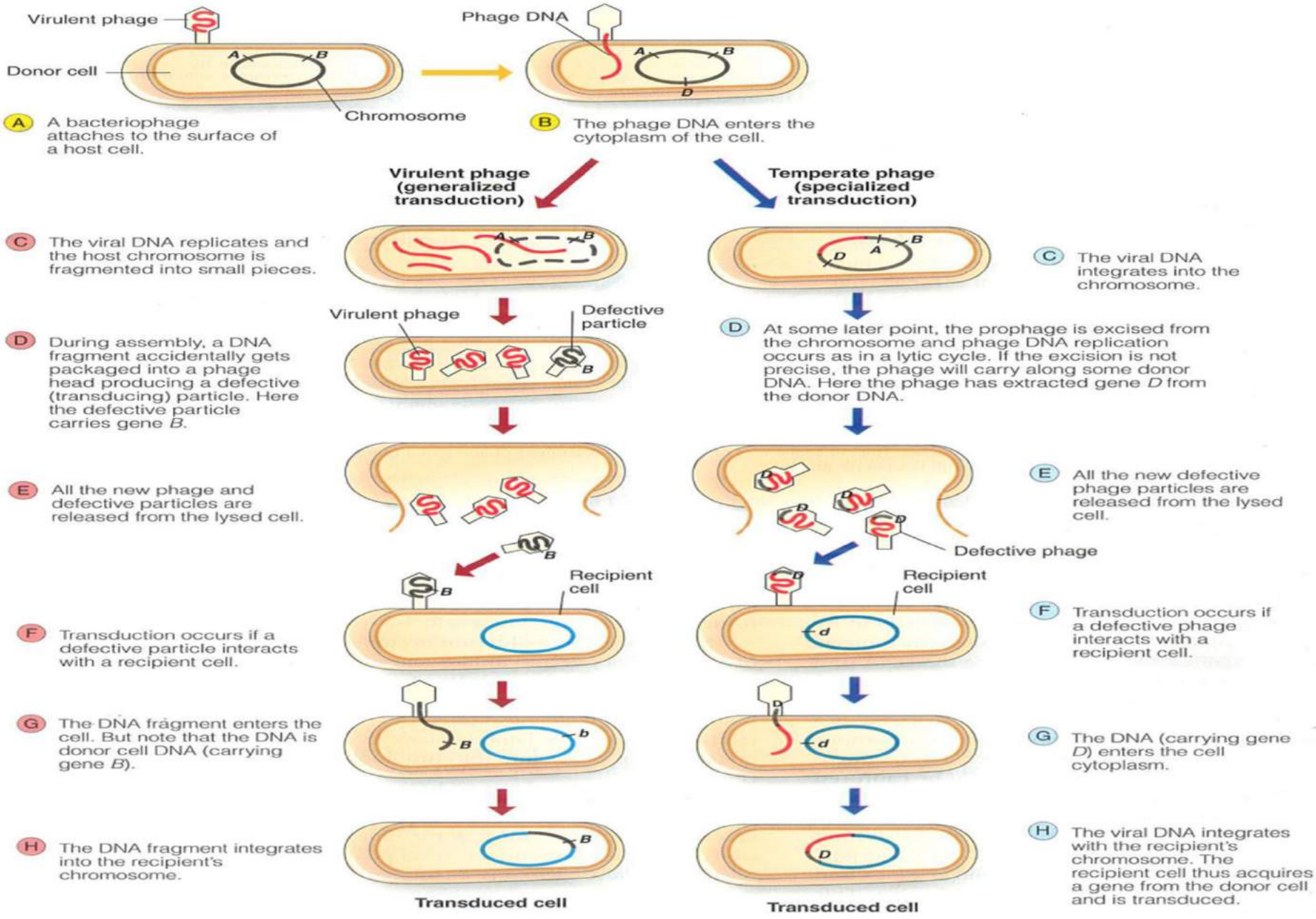
(C) Furthermore, an injection of heat-killed S strain cells did no harm because the cells were dead.

(D) But when Griffith injected a mixture of live R strain and heat-killed S strain cells into the mouse, it died. When Griffith cultivated the organism from the blood, he found live S strain cells.

Transduction

- It requires a virus to carry chromosomal DNA fragments from donor to recipient cell.
- In the replication cycle of a bacteriophage, different phages can interact with bacterial cell in two ways:
  - **Lytic cycle**: The phage DNA penetrates the cell, destroy the host chromosome, replicates itself within the cell, and then destroy (lyse) the cell as new phages are released. Because the phages kill the cell, they are call virulent phages.
  - **Lysogenic cycle**: The phages invade the host but donot always directly cause cell lysis. Instead the phages DNA integrates into the host chromosome as a prophage and phages participating in this cycle are known as temprate phages.

Generalized and Specialized Transduction



Acknowledgement and Suggested Readings:

1. Microbiology, An Introduction; Tortora, Funke and Case; Pearson Publication
2. Microbiology; Prescott, Harley and Klein; The MacGraw-Hill Companies
3. Microbiology: Principles and Explorations; Jacquelyn G Black; John Wiley and Sons Inc.
4. Brock Biology of Microorganisms; Madigan, Martinko, Stahl and Clark; Benjamin Cummings (Pearson Publication)

Thanks