Microbial genetics
Application microbial genetics
Evolution and molecular structure of cell and its organelles

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Basic Concepts

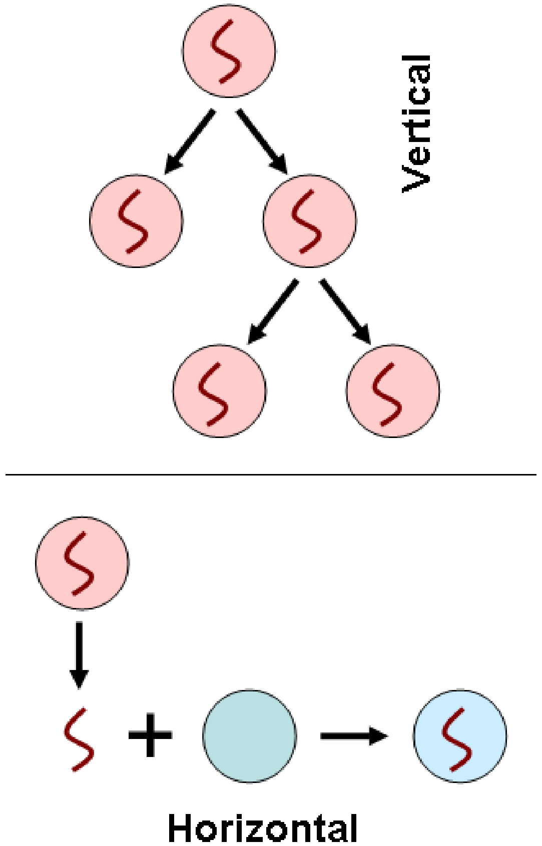
- Microbial Genotypes & Phenotypes
  - Microbial phenotypes are usually designated by a non italicized 3-letter abbreviation that in some way refers to the appearance or effect of the phenotype. Variation in the phenotype may be designated by superscripts such as "+" or "–" for auxotrophic mutations, "R" or "S" for antibiotic resistance mutations, etc.
  - Microbial genes are usually designated by an italicized, 3-letter abbreviation (that often refers to the phenotypic effect by which the gene was discovered) plus a letter that distinguishes one gene in a family from other genes that produce the same phenotype.

Basic Concepts

- Microbial Genotypes & Phenotypes (cont.)
  - For example:
    - A lysine prototroph of *E. coli*, capable of making its own lysine, is designated lys^{+}. An auxotrophic mutant, incapable of making its own lysine, is designated lys^{-}.
    - There are several different genes responsible for lysine production in *E. coli*. These genes encode the different enzymes in the metabolic pathway for lysine synthesis. The genes are designated *lysA*, *lysB*, *lysC*, and so on.
    - Mutation in <u>any</u> of the genes responsible for lysine production may block the lysine pathway and produce the Lys^{-} phenotype.

Genetic Transfer & Recombination

- Vertical
  - Parent to offspring
- Horizontal
  - Lateral transfer to same generation
  - Donor to recipient
  - DNA transfer
  - Plasmid transfer
  - Types
    - Transformation
    - Transduction
    - Conjugation



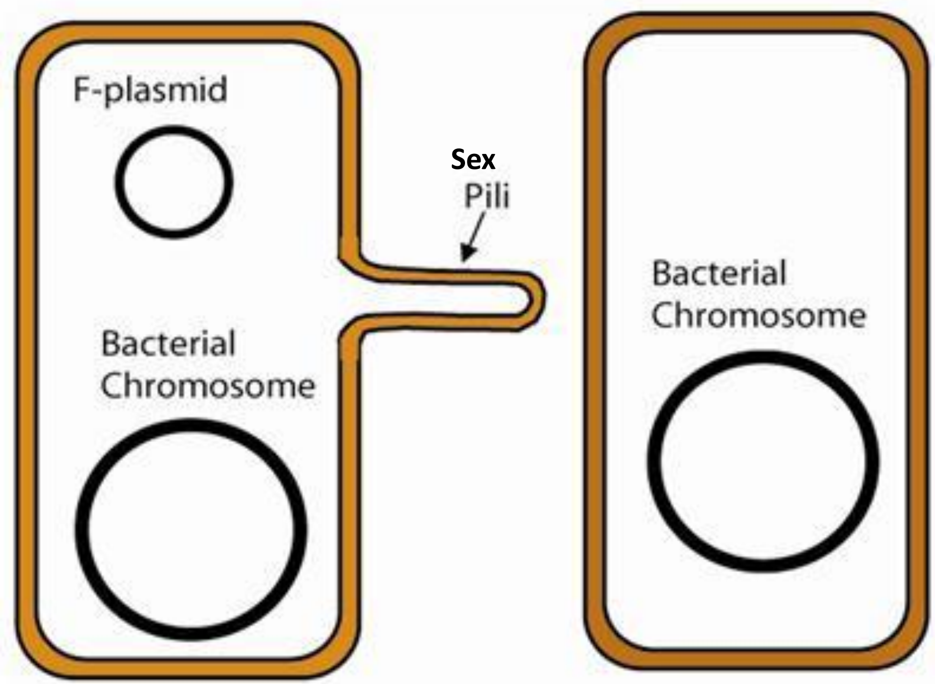
Genetic Transfer in Bacteria

- Genetic transfer-results in genetic variation
- Genetic variation-needed for evolution
- Three ways:
  - Transformation: genes transferred from one bacterium to another as "naked" DNA
  - Conjugation: plasmids transferred 1 bacteria to another via a pilus
  - Transduction: DNA transferred from 1 bacteria to another by a virus

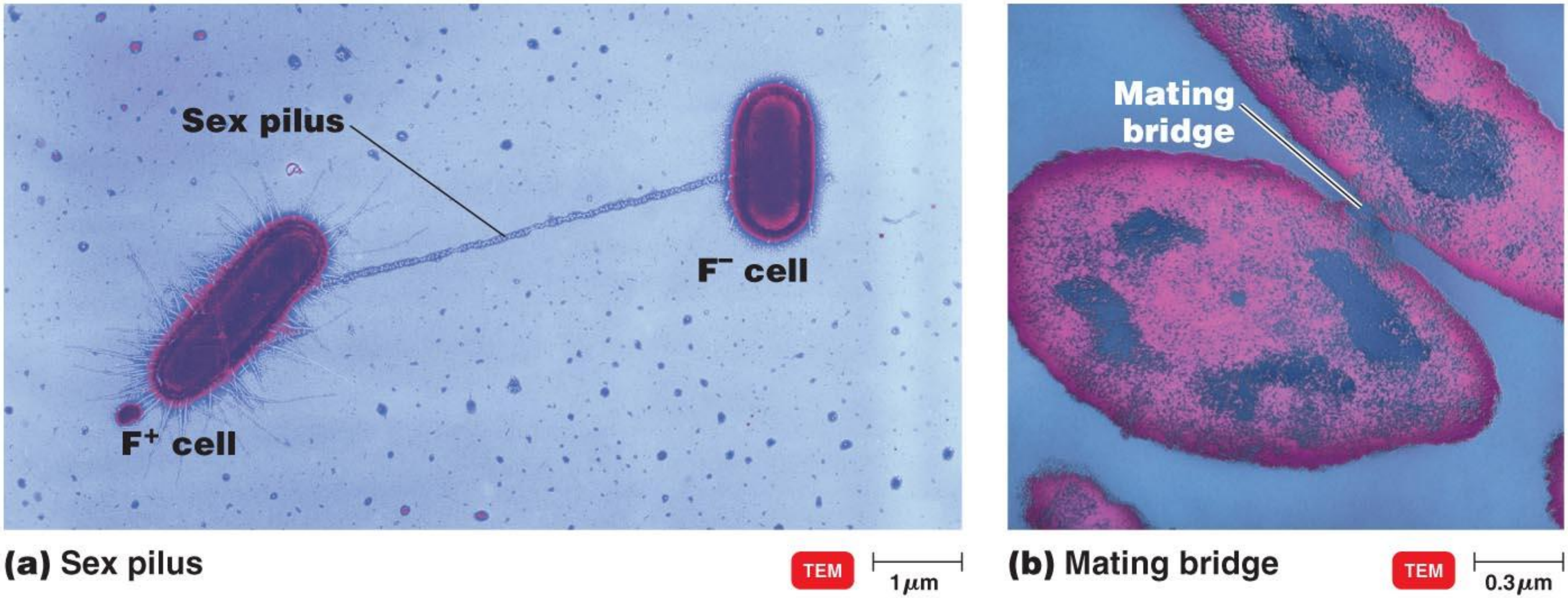
Gene Transfer in Bacteria

- Conjugation
  - A process of gene transfer from a living donor cell to a living recipient cell
  - Typically, the donor cell will possess conjugative structures on its surface that attach the donor cell to the recipient cell. The conjugative structures will also mediate the transfer of DNA from the donor to the recipient.
  - The ability to conjugate is often encoded on a plasmid.
  - For example: In *Escherichia coli*, conjugation is mediated by the F pili that are encoded for by genes on the F plasmid.

Conjugation



Conjugation



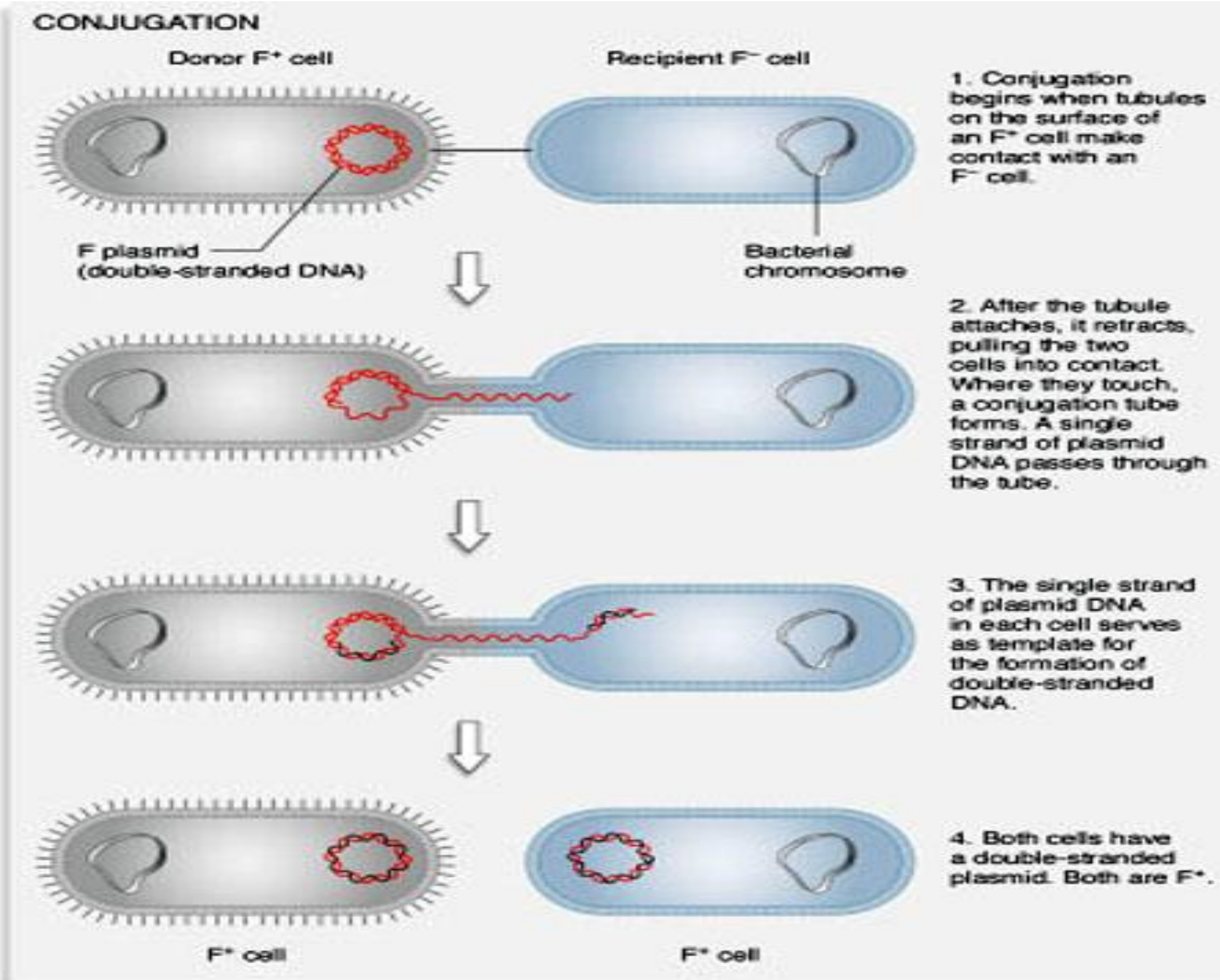
(a) Sex pilus

(b) Mating bridge

Gene Transfer in Bacteria

- Conjugation (cont.)
  - A strain of *E. coli* having F plasmids and pili is called an F^+ strain; a strain lacking F plasmids or pili is F^-.
  - When an F^+ cell (the donor) is mated with an F^- cell (the recipient), a copy the F plasmid is transferred to the F^- cell, so that after the process is complete, both cells will be F^+.
  - In a cross of F^+ x F^-, only the plasmid is transferred. None of the chromosomal genes are transferred. Therefore, an F^+ x F^- does not give us any information about the position of genes on the bacterial chromosome.

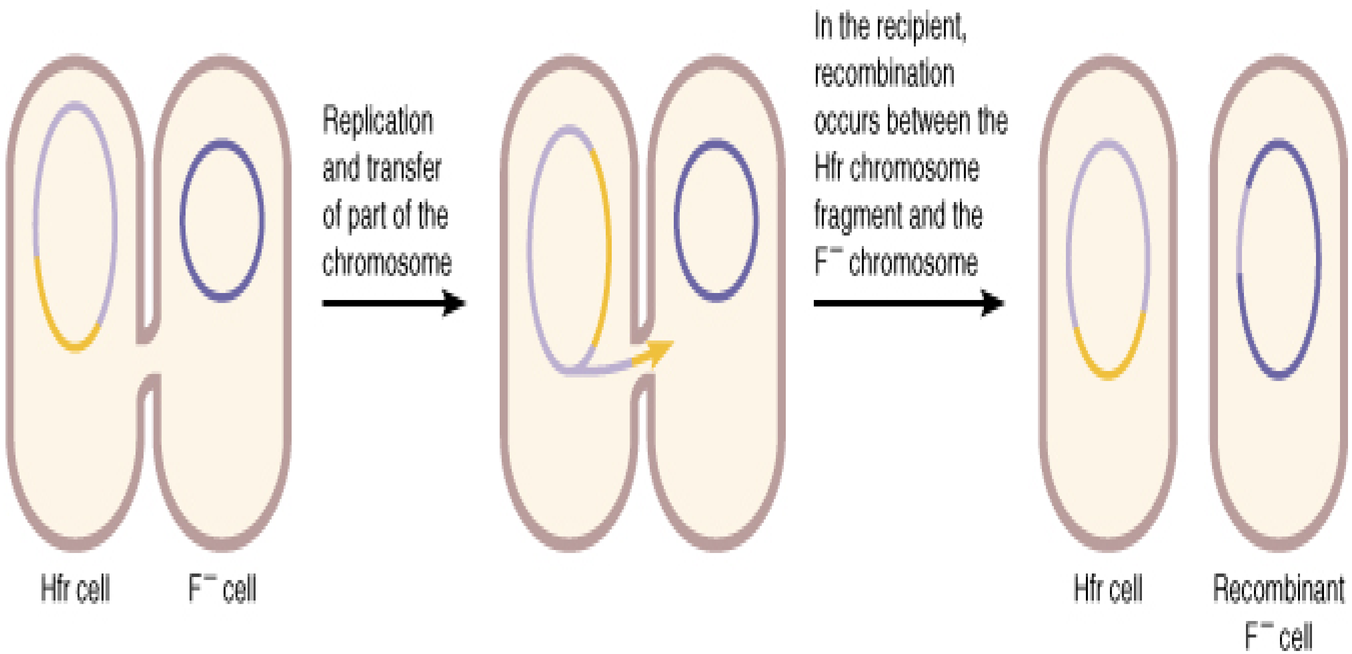
Conjugation



Gene Transfer in Bacteria

- Conjugation (cont.)
  - In some strains of *E. coli*, an F plasmid DNA sequence has become inserted into the chromosome through genetic recombination. These are called Hfr strains. Different Hfr strains have the F sequence inserted at different locations on the chromosome.
  - The cells of Hfr strains have F pili, and are capable of conjugating with F^- cells. In an Hfr x F^- mating, the F sequence is transferred first, followed by the chromosomal DNA.
  - Genes from the Hfr (donor) chromosome can replace genes in the F^- chromosome by genetic recombination.

Conjugation continued…



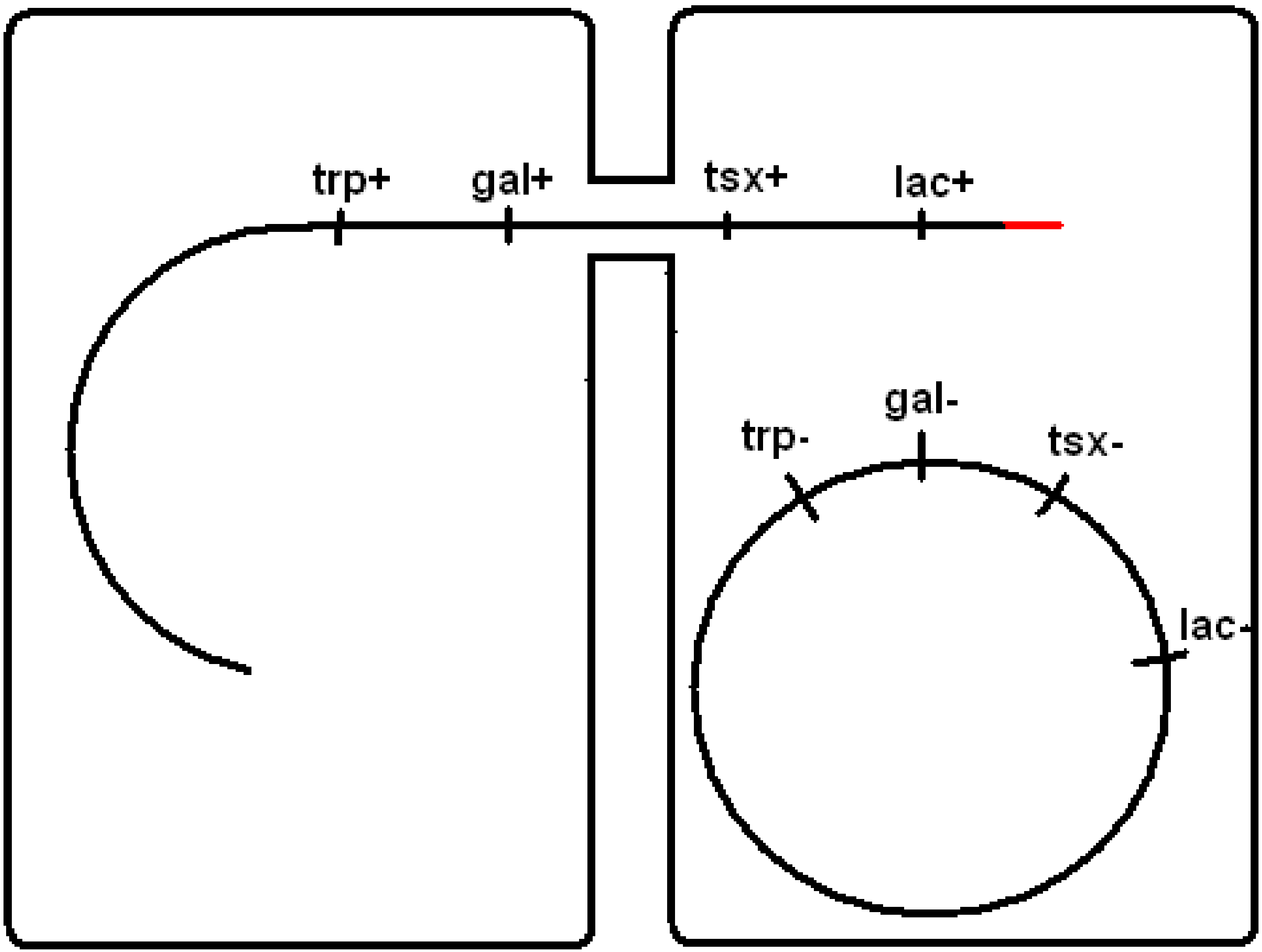
(c) When an Hfr donor passes a portion of its chromosome into an F⁻ recipient, a recombinant F⁻ cell results.

Gene Transfer in Bacteria

- Conjugation (cont.)
  - The order of genes near the F insertion site on the chromosome can be determined in an "interrupted mating" cross between Hfr x F^- strains.
    - Select an Hfr strain and an F^- strain that differ in specific phenotypes. For example, an Hfr with the phenotypes gal^+, trp^+, lac^+, tsx^+ could be mated to an F^- that is gal^-, trp^-, lac^-, tsx^-.
    - Mix together broth cultures of the Hfr & F^- cells. The two strains will begin the conjugation process. This is "time 0" of the interrupted mating experiment.
    - At time intervals, remove a sample from the culture & gently shake it to break up the conjugating pair ("interrupted mating")

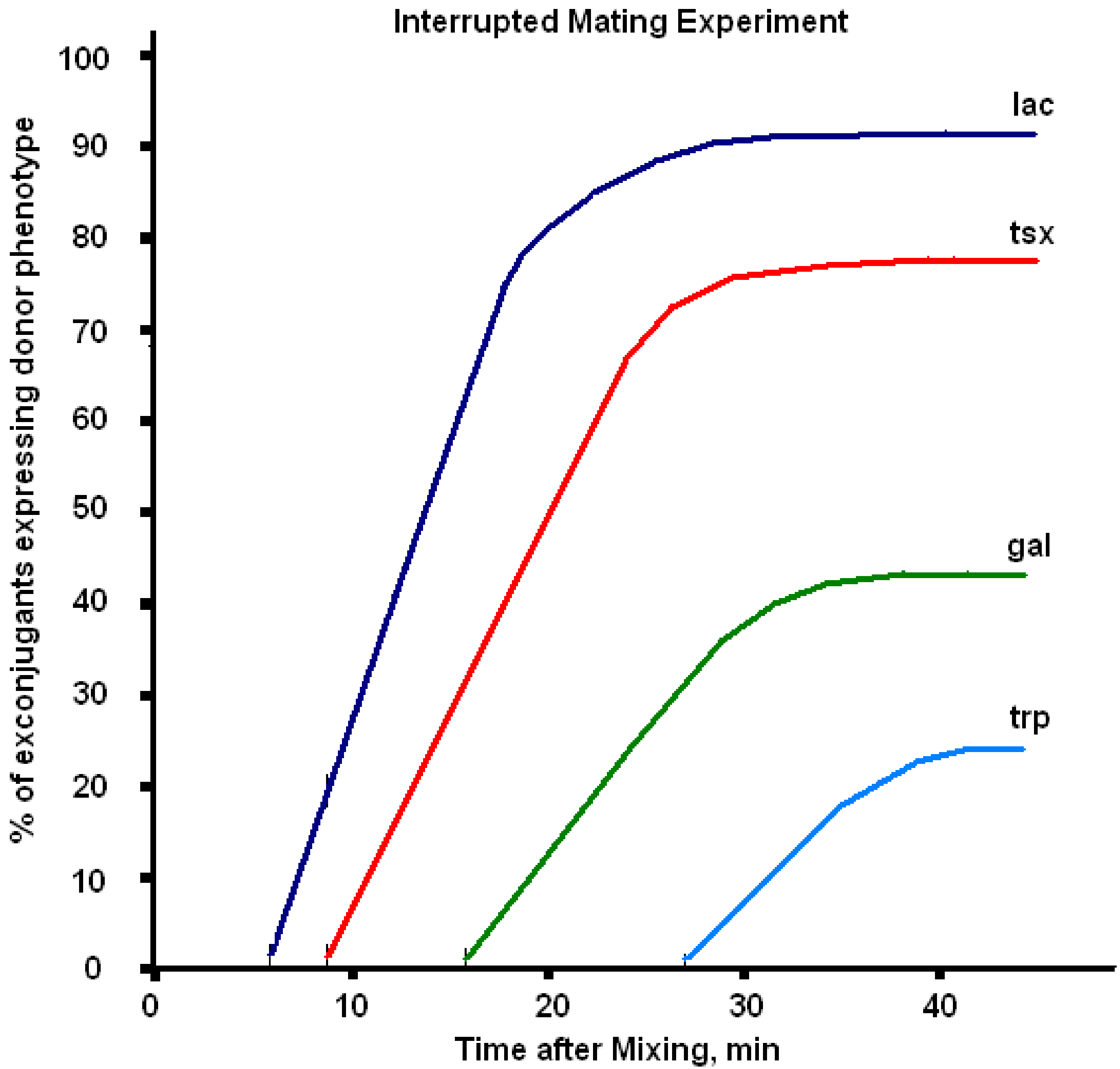
Gene Transfer in Bacteria

- Conjugation (cont.)
  - "Interrupted mating" cross (cont.)
    - Plate the sample onto selective media to determine the number of Hfr phenotypes found among the exconjugants.
    - The order that the genes are transferred from the Hfr to F^- strains reflects their order on the chromosome; in the example given the order would be lac, tsx, gal, trp



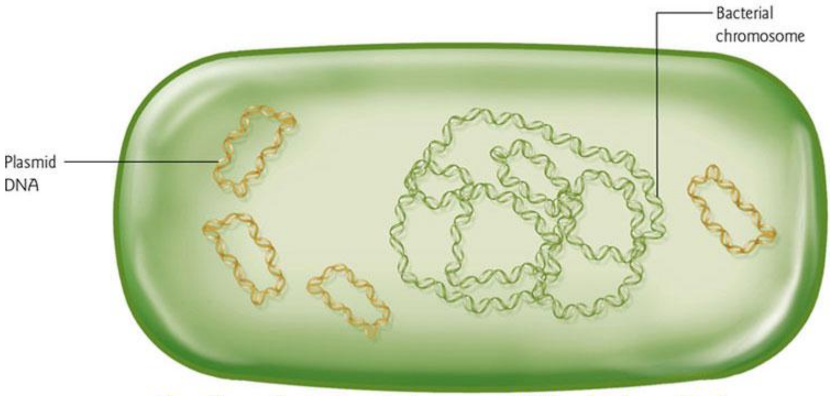
Hfr Strain

F- Strain


Interrupted Mating Experiment
% of exconjugants expressing donor phenotype
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
Time after Mixing, min
lac
tsx
gal
trp

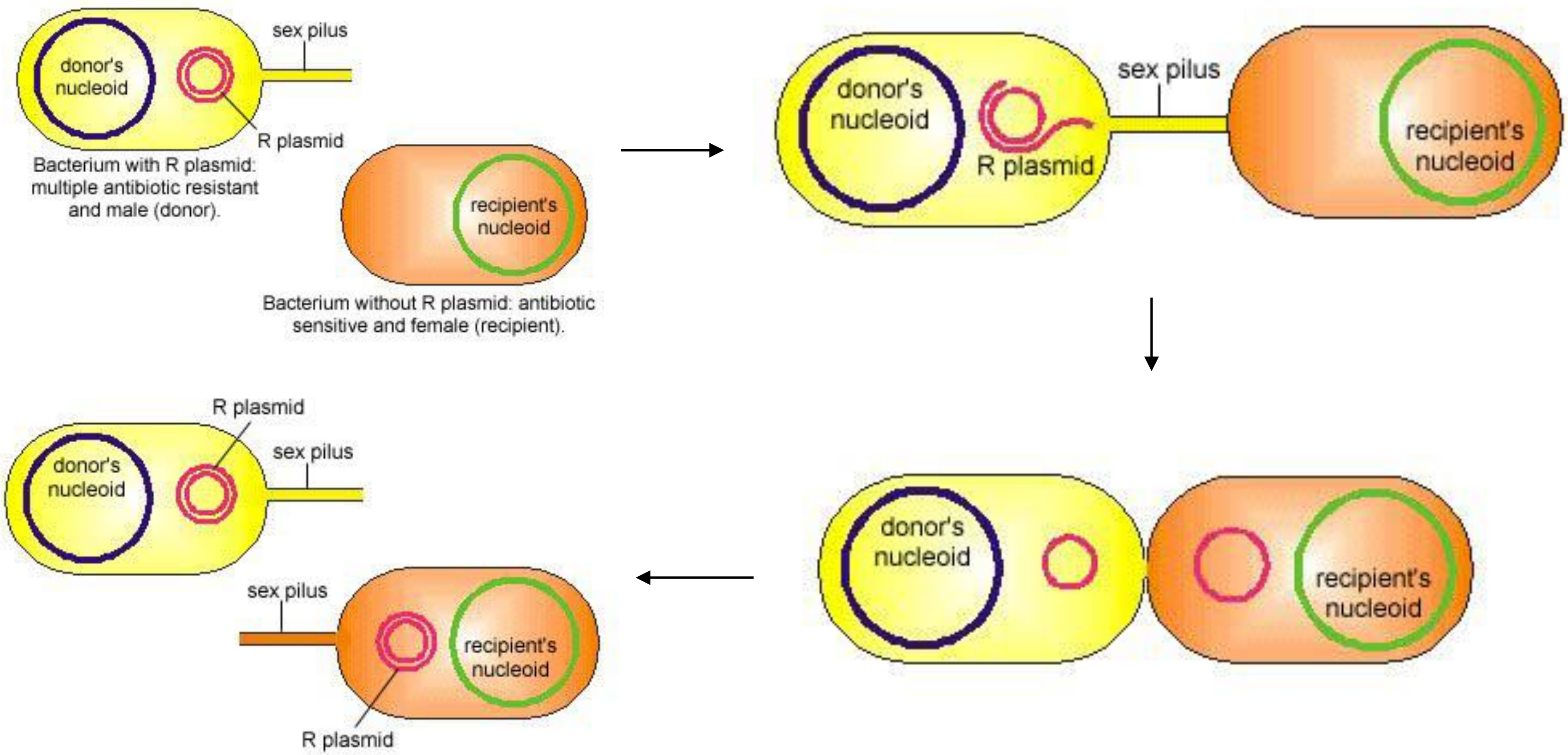
Other Plasmids

- R factors
  - Resistance to AB
  - ~10 genes
  - Different bacterial species share
- Bacteriocin factors
  - Specific protein toxins
  - Kill other cells of same or similar species
- Virulence
  - Pathogenicity
    - Structures
    - Enzymes
    - Toxins



Plasmid = small, circular extrachromosomal DNA molecule capable of autonomous replication within a bacterial cell; a commonly used cloning vector.

Conjugation: R Plasmid transfer



Gene Transfer in Bacteria

- Transduction
  - Transfer of genes from a donor cell to a recipient cell through a bacteriophage intermediate.
  - Bacteriophage: A bacterial virus
  - Virulent bacteriophage:
    - Has only a lytic stage in its developmental cycle
    - When a virulent bacteriophage infects its host bacterium, it does not integrate its DNA into the host chromsome. Instead, it replicates its own DNA and capsid protein within the infected host, reassembles thousands of new virus particles, and lyses the host cell to release the new viruses.
    - Example: T4 phage of *E. coli*

Transduction

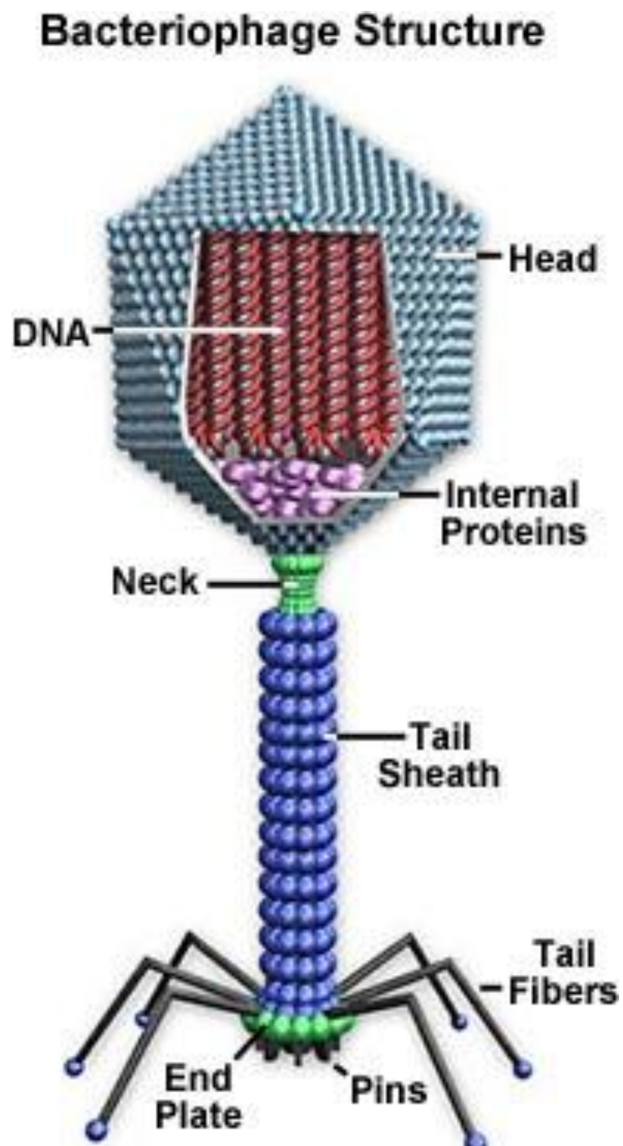


Figure 1

Gene Transfer in Bacteria

- Transduction (cont.)
  - Temperate bacteriophage:
    - A bacteriophage that has both lytic and lysogenic stages in its replication cycle
    - In the lysogenic stage, the DNA of a temperate phage is inserted into a specific region of the host chromosome, where it is replicated every time the bacterial cell replicates
    - During adverse growth conditions, the phage DNA comes out of the chromosome and begins a lytic stage, similar to that of a virulent phage. The virus replicates its DNA and protein, thousands of new virus particles are assembled, and the cell lyses to release the viruses.
    - Example: lambda (λ) phage of *E. coli*

Gene Transfer in Bacteria

- Transduction (cont.)
  - Random Generalized Transduction
    - In this process, any of the genes from the donor chromosome may be transferred to the recipient.
    - Random generalized transduction can be mediated by either virulent phages or certain temperate phages during their lytic stage. The virus must break down the host chromosome into fragments as part of its replication.
    - When the host chromosome is broken into fragments, a small number of host chromosome fragments become packaged into viral capsids. These are the transducing particles of random generalized transduction. Since the host chromosome has been randomly broken into fragments, any of the host genes can randomly packaged into the transducing particles.

Gene Transfer in Bacteria

- Transduction (cont.)
  - Random Generalized Transduction (cont.)
    - To map genes by random generalized transduction, a donor strain and recipient strain are selected that differ in specific phenotypes.
    - The bacteriophage is used to infect a culture of the donor strain, with the cells of the donor strain being lysed. The donor lysate contains mostly viral particles, with a small percentage of transducing particles.
    - The donor lysate is used to infect a culture of recipient cells. Most of the recipient cells are killed, but the cells that become infected with transducing particles instead of viral particles get donor DNA and survive.

Gene Transfer in Bacteria

- Transduction (cont.)
  - Random Generalized Transduction (cont.)
    - The donor DNA can recombine with the recipient chromosome to change the recipient to the donor phenotype. The map distance between two genes is calculated as the frequency of crossover between the genes.

Gene Transfer in Bacteria

- Transduction (cont.)
  - Specialized transduction
    - This process can only be mediated by temperate phages.
    - The only genes that can be transferred from the donor to the recipient are the genes that are immediately adjacent to the phage insertion site on the donor chromosome.
    - When the phage DNA is excised from the chromosome as the virus enters its lytic cycle, occasionally there is a mistake and some of the chromosomal DNA becomes packaged into the phage capsid along with the viral DNA. These are the transducing particles of specialized transduction. The host chromosome is not broken up; instead, only the genes that are adjacent (next to) the phage insertion site can be packaged into the transducing particles.

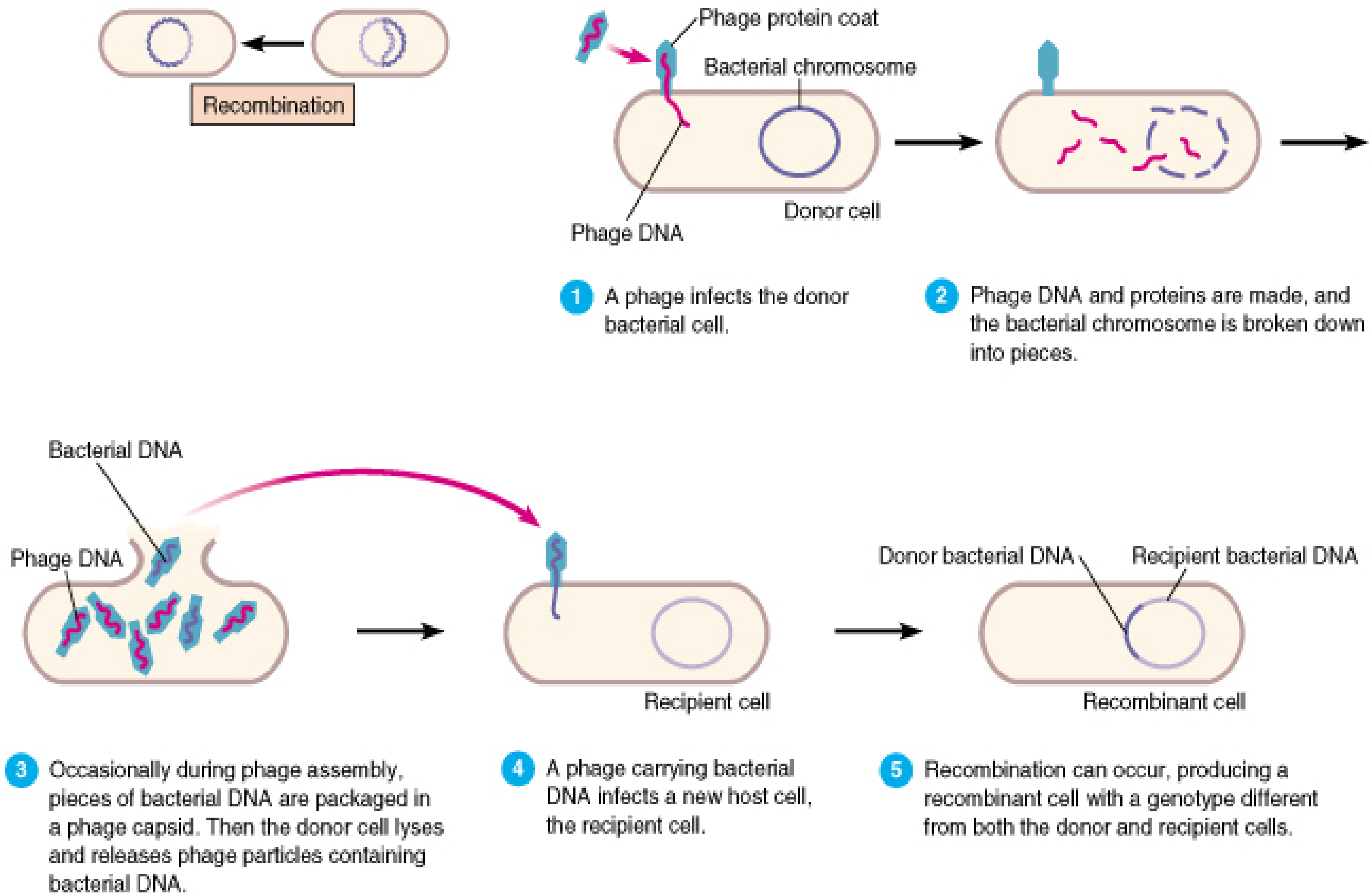
Gene Transfer in Bacteria

- Transduction (cont.)
  - Specialized transduction (cont.)
    - To map genes by specialized transduction, a donor strain and recipient strain are selected that differ in specific phenotypes.
    - The bacteriophage is used to infect a culture of the donor strain, with the cells of the donor strain being lysogenized.
    - The infected donor cells are treated with a chemical or ultraviolet radiation to induce the lytic stage.
    - The infected donor cells are lysed. The lysate contains mostly viral particles, with a small percentage of specialized transducing particles.
    - The donor lysate is used to infect a culture of recipient cells. Cells that become infected with transducing particles instead of viral particles get donor DNA.

Gene Transfer in Bacteria

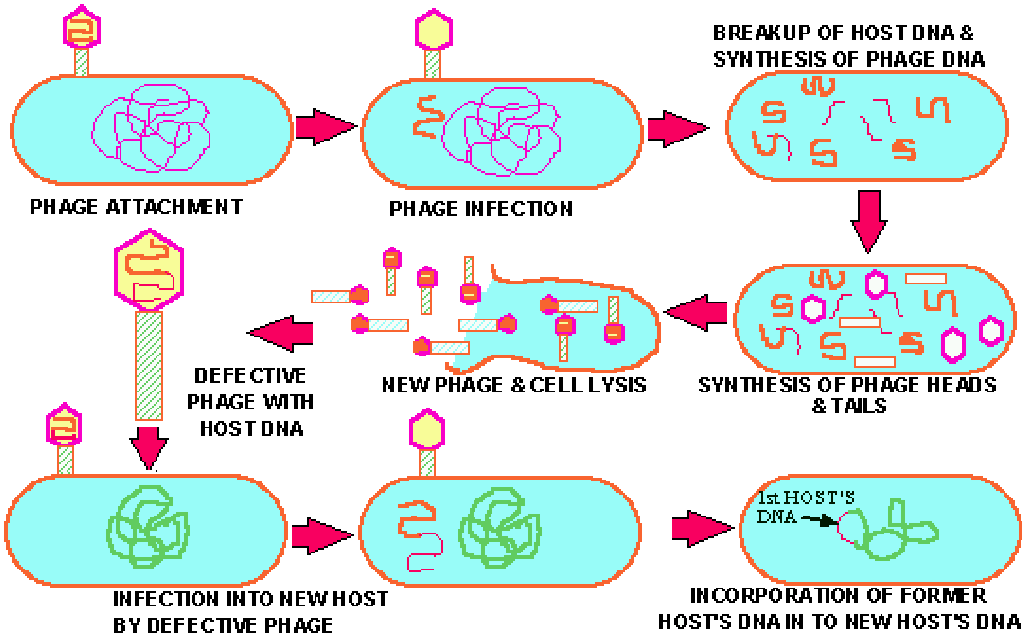
- Transduction (cont.)
  - Specialized transduction (cont.)
    - The donor DNA can recombine with the recipient chromosome to change the recipient to the donor phenotype. The map distance between two genes is calculated as the frequency of crossover between the genes.

Transduction by a Bacteriophage

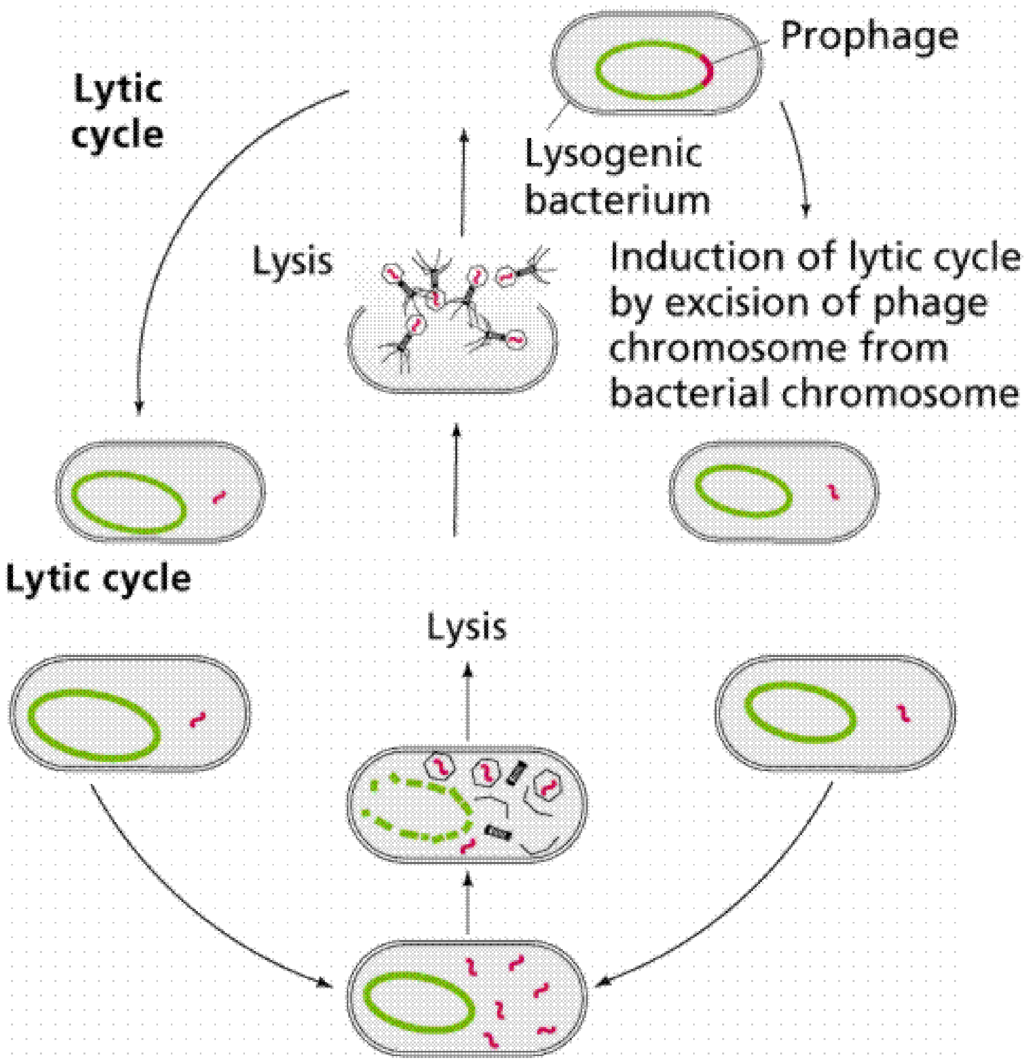


Generalized Lytic Cycle

- Random pieces of host cell DNA (any genes)
- Packaged with phage during lytic cycle
- Donor DNA combines with recipient

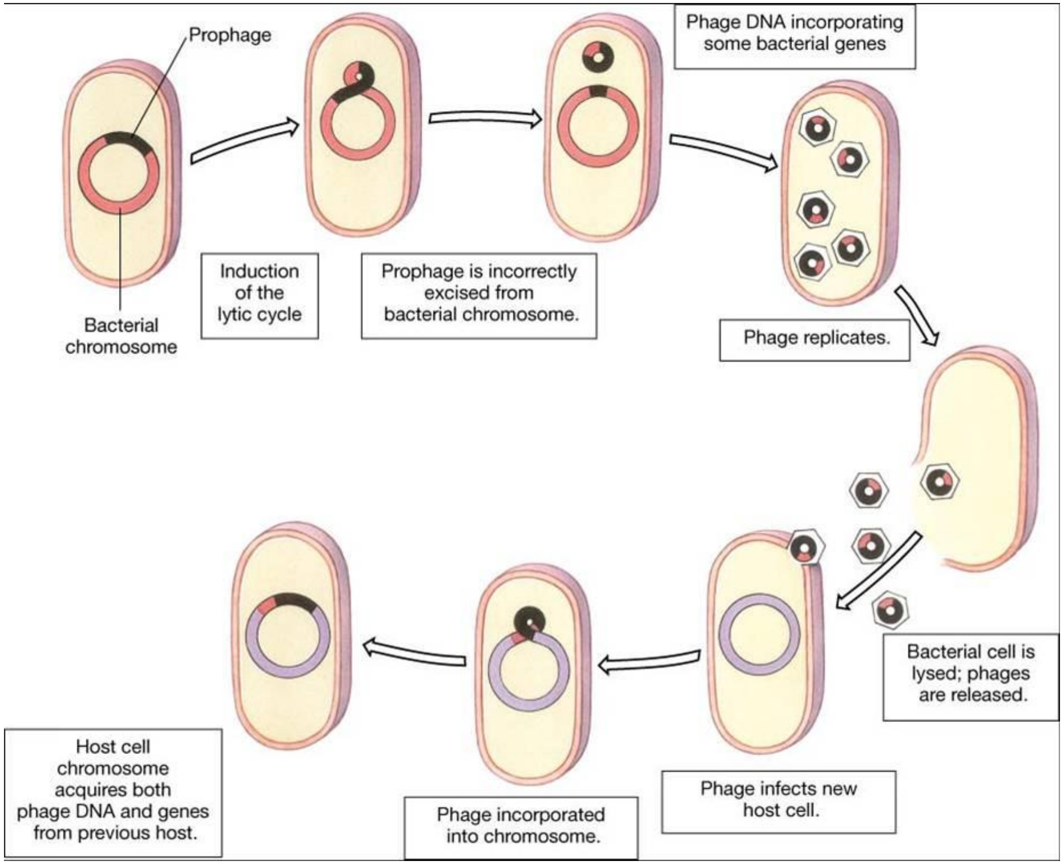


Lytic Cycle Summary

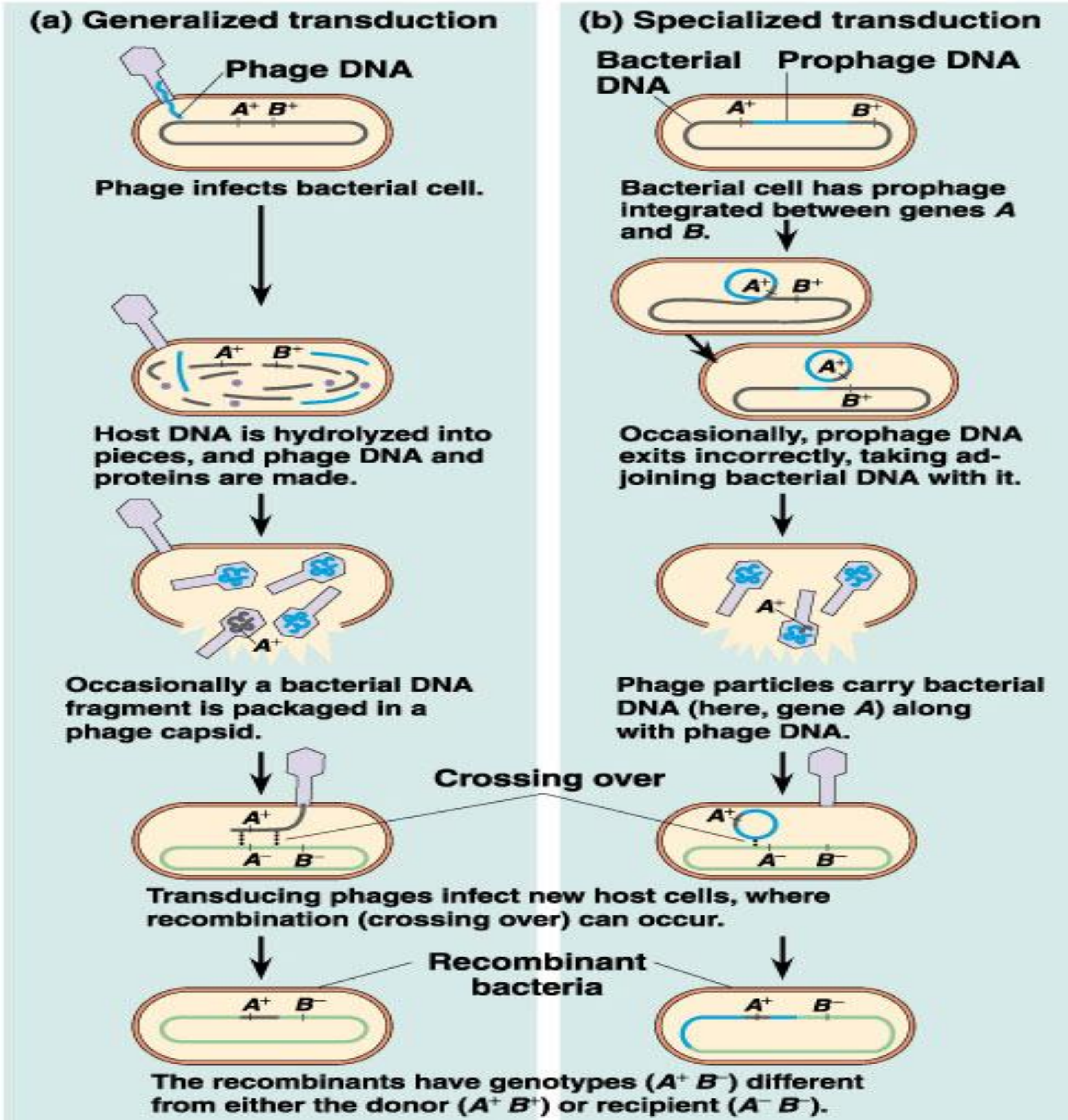


Specialized Transduction Cycle

- Only certain specific bacterial genes are transferred
- Example: Toxins
  - *Corynebacterium*
    - Diphtheria toxin
  - *Streptococcus pyogenes*
    - Erythrogenic toxin
  - *E. coli*
    - Shiga-like toxin

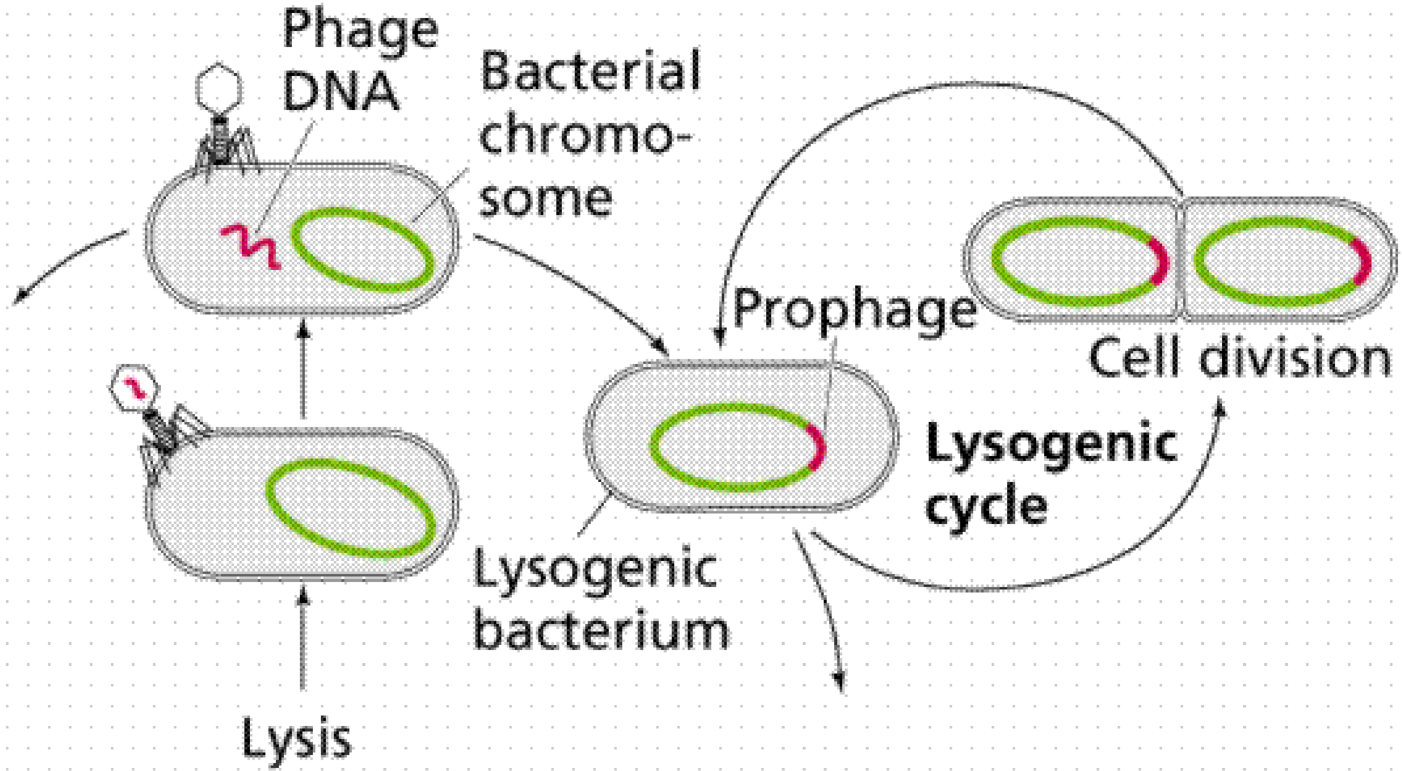


Random genes transferred



Specific Genes transferred

Lysogenic Cycle Summary



Gene Transfer in Bacteria

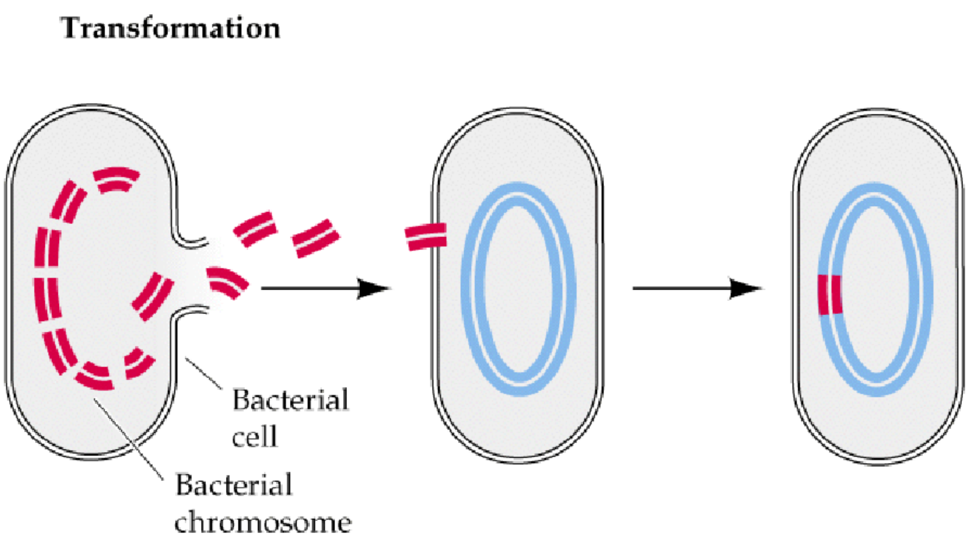
- Transformation
  - Transfer of isolated donor DNA (either chromosomal DNA fragments or plasmid DNA) to a recipient cell.
  - Successful transformation depends on the presence of double-stranded donor DNA molecules that are large enough, as well as cells that are competent for transformation

Transformation

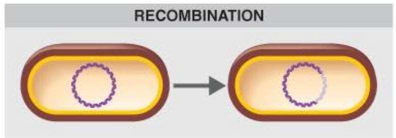
- Naked DNA is transferred from one bacteria to another.
- Was the first experiment that showed DNA was the genetic information

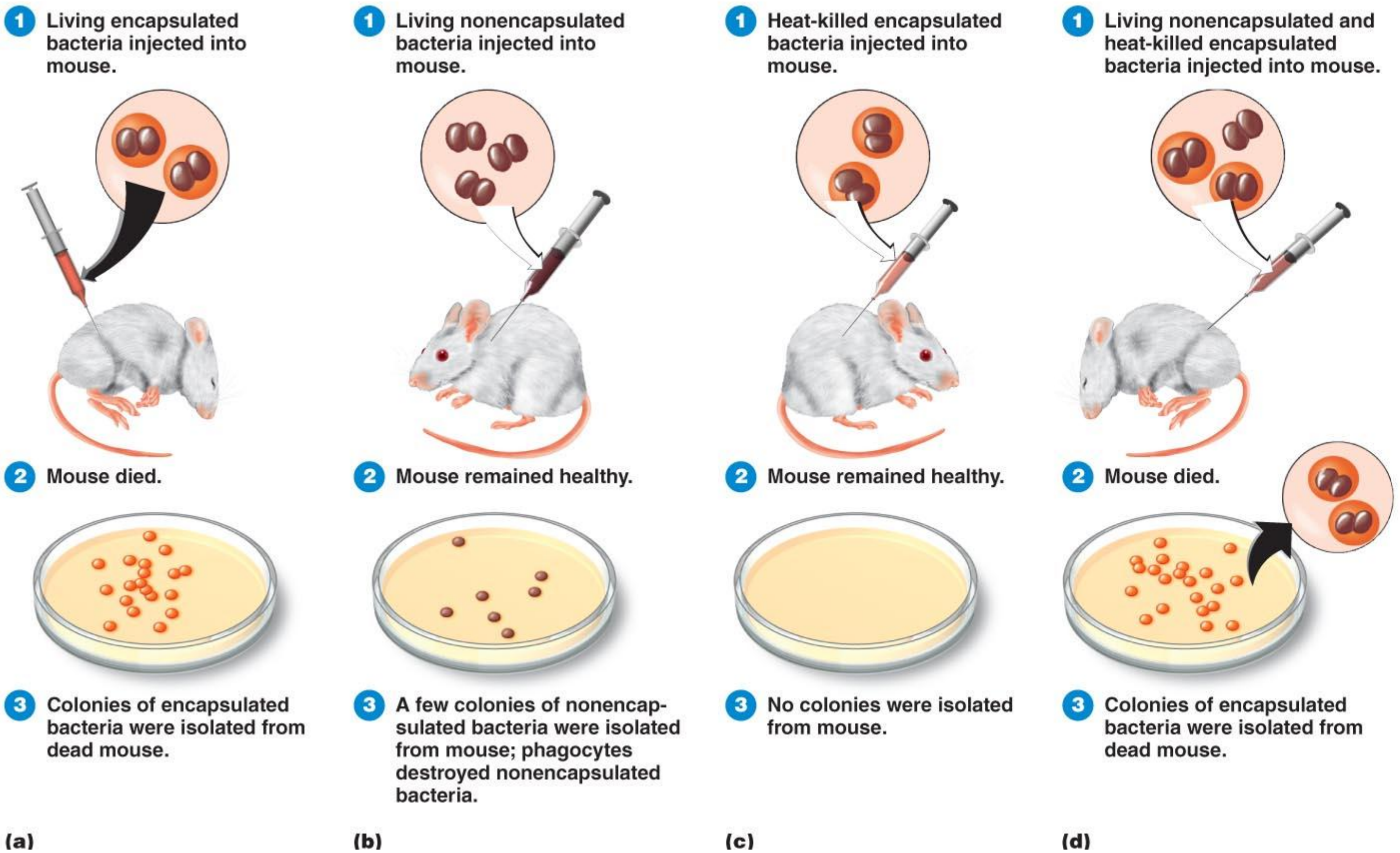
Transformation

- Occurence
  - 1%
  - Random
  - Naturally in certain species
    - *Haemophilus*
    - *Neisseria*
    - *Pseudomonas*
    - *Streptococcus*
    - *Staphylococcus*
- Griffith experiment
- Genetic transfer
  - Environmental surroundings
  - Naked DNA assimilated
  - Competent cells
    - Cell wall
    - Plasma membrane
  - Bacterial lysis
    - DNA
    - Plasmids

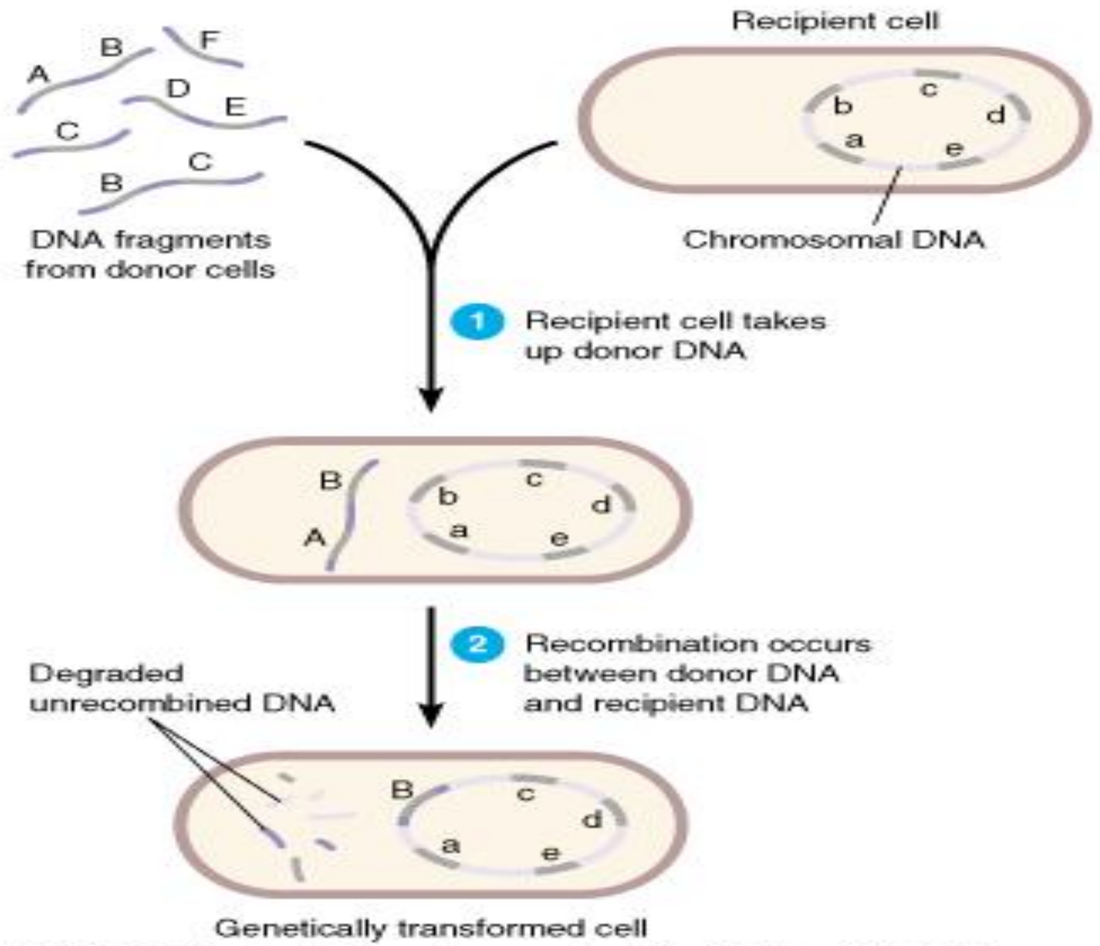


Genetic Transformation





Transformation



Gene Transfer in Bacteria

- Transformation
  - Competence
    - Competence is the ability of a bacterial species or strain to take up DNA from its environment.
    - Many species are naturally competent, such as *Streptococcus pneumioniae*, *Acinetobacter calcoaceticus*, *Neiserria gonorrheae*, and *Bacillus subtilis*
    - Naturally competent species possess a nucleic acid transporter that spans their cell wall & plasma membrane.
      - The transporter binds to double-stranded DNA, hydrolyzes one of the strands, and pulls the other strand into the recipient cell.
      - The donor DNA strand may then recombine with the recipient chromosome, possibly changing the phenotype of the recipient to the donor phenotype.

Gene Transfer in Bacteria

- Transformation
  - Competence
    - Competence can be induced in some species that are not naturally competent
      - In certain noncompetent gram-negative species (for example, *Escherichia coli*), competence can be induced by treating the cells with divalent calcium ions (Ca^{2+}), usually as a solution of calcium chloride.
      - In certain noncompetent gram-positive species (for example, *Geobacillus stearothermophilus*), competence can be induced by “protoplasting,” or removing the cell wall from the cells by lysozyme digestion.

Gene Transfer in Bacteria

- Transformation
  - To map genes by transformation:
    - A donor strain and recipient strain are selected that differ in specific phenotypes.
    - The donor cells are broken open by a combination of enzyme and detergent treatment, and the double-stranded donor DNA is isolated and purified.
    - If necessary, the recipient cells are treated to make them competent; this is not needed if one is using a naturally competent species.
    - The competent recipient cells are mixed with donor DNA.
    - The donor DNA can recombine with the recipient chromosome to change the recipient to the donor phenotype. The map distance between two genes is calculated as the frequency of crossover between the genes.

Gene Transfer in Bacteria

- Transformation
  - Transformation is also a major technique used to introduce recombinant DNA molecules into host cells. In this case, the DNA is usually recombinant plasmids.

Major Cell Components

- ***Nucleus*****:** approximately spherical membranebound organelle near the center of a cell; contains almost all of cell's genome; functions: gene expression (transcription of DNA to RNA to make proteins), DNA replication prior to cell division; surrounded by a double membrane called *nuclear envelope (membrane);* the outer membrane connected to rough ER;

- ***Endoplasmic Reticulum*** *(ER)*: is a network of folded membranes with large surface to facilitate processes; *rough ER* hosts ribosomes, where the synthesis of proteins occurs; *smooth ER* contains lipid vesicles and is involved in lipid and steroid synthesis; ER also involved in adding carbohydrates to proteins, splicing and folding peptides, and packaging proteins into lipid vesicles for transport to other parts of the cell;

- ***Golgi Apparatus***: similar to smooth ER (folded membrane); functions: processing and packaging of lipids and proteins, breakdown of carbohydrates and lipids;

- ***Vesicles*****:** small spherical bilayer containers, they fuse with or bud from the plasma membrane; *lysosomes*: vesicles with enzymes lysozymes to break down or digest larger molecules; *peroxisomes*: vesicles that break down long chain fatty acids;

Major Cell Compartments

- ***Vacuoles*****:** giant vesicles without a particular shape; functions: isolate harmful objects and waste products, help maintain correct hydrostatic pressure;

- ***Ribosomes*****:** large complex of proteins, enzymes, and ribosomal RNA (rRNA) found in both prokaryotes and eukaryotes; function: protein synthesis according to the sequence of messenger RNA (mRNA);

- ***Mitochondria***: membranebound organelles, also contain DNA (*mtDNA*); function: ATP (adenine triphosphate) synthesis, convert energy stored in food into high energy phosphate bonds of ATP;

- ***Chloroplasts*****:** organelles mostly found in plant cells (green parts), carry out photosynthesis (capture light and convert it into chemical bond energy of carbohydrates and ATP);

- ***Cytoskeleton*****:** interconnected tube or ropelike fibrous structures made of proteins; function: to support, transmit, or apply forces, to preserve the shape of the cell and anchor various organelles in place; three types: *microtubules, intermediate filaments, and microfilaments*;

Organelles of the Cell