Bacterial Reproduction

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- In a general sense, **recombination** is the process in which one or more nucleic acids molecules are rearranged or combined to produce a new nucleotide sequence.
- Most eucaryotes exhibit a complete sexual life cycle, including meiosis, a process of extreme importance in generating new combinations of alleles (alternate forms of a particular gene) through recombination.

Bacterial Conjugation

- 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.
- 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.
- Recombinant prototrophic colonies appeared on the minimal medium after incubation.
- Thus the chromosomes of the two auxotrophs were able to associate and undergo recombination.

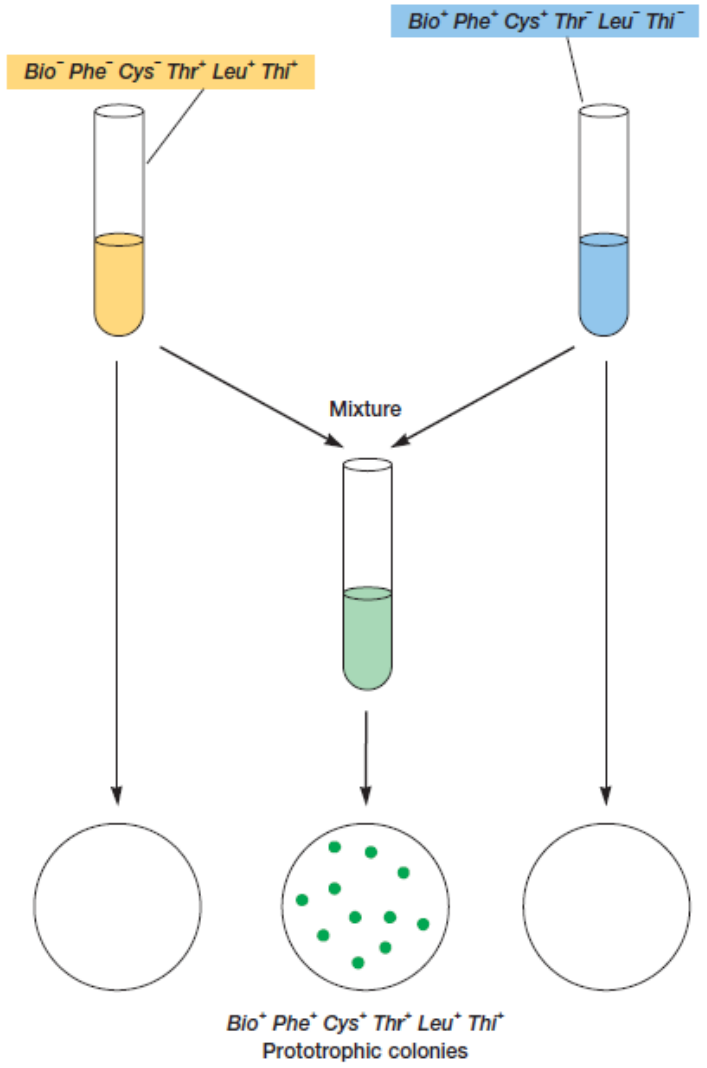
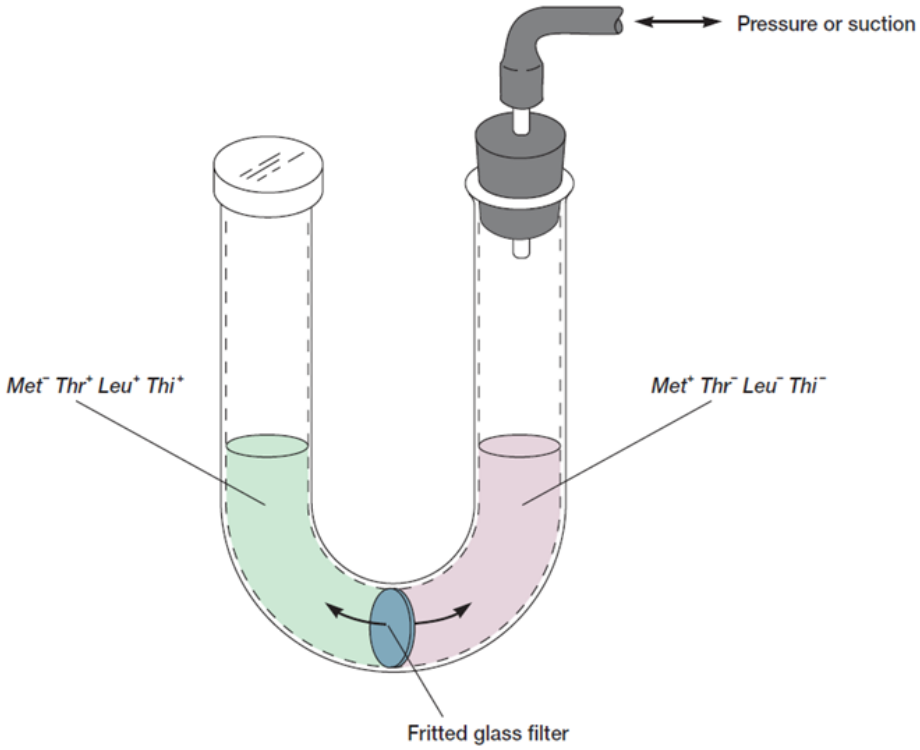


Figure 13.12 Evidence for Bacterial Conjugation. Lederberg and Tatum's demonstration of genetic recombination using triple auxotrophs. See text for details.

...Conjugation

- 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*.
- During incubation, the medium was pumped back and forth through the filter to ensure medium exchange between the halves.
- After a 4 hour incubation, the bacteria were plated on minimal medium.
- Davis discovered that when the two auxotrophic strains were separated from each other by the fine filter, gene transfer could not take place.
- Therefore direct contact was required for the recombination that Lederberg and Tatum had observed.



F^+ x F^- Mating

- In 1952 William Hayes demonstrated that the gene transfer observed by Lederberg and Tatum was polar.
- That is, there were definite donor (F^+) and recipient (F^-) strains, and gene transfer was nonreciprocal.
- He also found that in F^+ x F^- mating the progeny were only rarely changed with regard to auxotrophy (that is, bacterial genes were not often transferred), but F^- strains frequently became F^+.
- The F^+ strain contains an extrachromosomal F factor carrying the genes for pilus formation and plasmid transfer.
- During F^+ x F^- mating or conjugation, the F factor replicates by the rolling-circle mechanism, and a copy moves to the recipient (**figure 13.14*a***).
- The entering strand is copied to produce double-stranded DNA.
- Because bacterial chromosome genes are rarely transferred with the independent F factor, the recombination frequency is low.

F^+ x F^- Mating

- The **sex pilus** or F pilus joins the donor and recipient and may contract to draw them together.
- The channel for DNA transfer could be either the hollow F pilus or a special conjugation bridge formed upon contact (eg. *E. coli)*.
- Selftransmissible plasmids are present in gram-positive bacterial genera such as *Bacillus, Streptococcus, Enterococcus, Staphylococcus,* and *Streptomyces*.
- It appears that fewer transfer genes are involved, possibly because a sex pilus does not seem to be required for plasmid transfer.
- For example, *Enterococcus faecalis* recipient cells release short peptide chemical signals that activate transfer genes in donor cells containing the proper plasmid.
- Donor and recipient cells directly adhere to one another through special plasmid-encoded proteins released by the activated donor cell.
- Plasmid transfer then occurs.

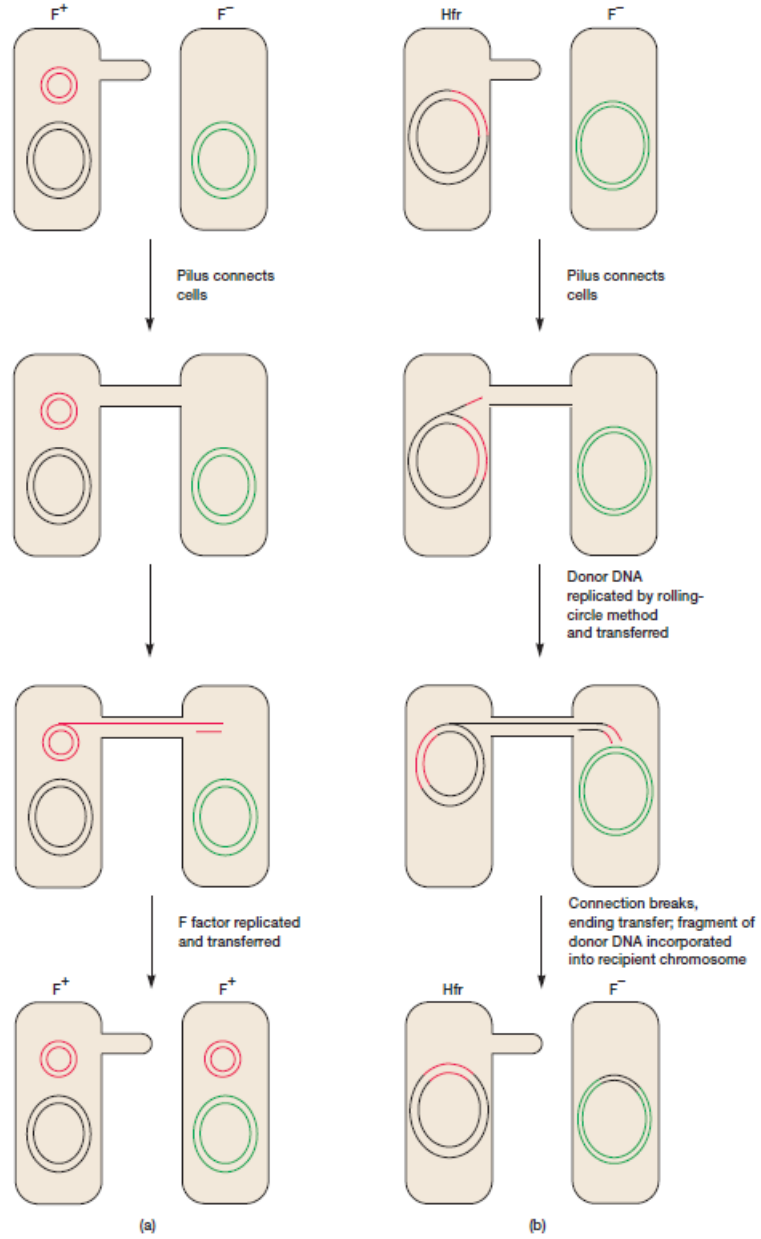


Figure 13.14 The Mechanism of Bacterial Conjugation. (a) $F^+ \times F^-$ mating. (b) Hfr $\times$ F^- mating (the integrated F factor is shown in red).

Hfr Conjugation

- Because certain donor strains transfer bacterial genes with great efficiency and do not usually change recipient bacteria to donors, a second type of conjugation must exist.
- The F factor is an episome and can integrate into the bacterial chromosome at several different locations by recombination between homologous insertion sesequences present on both the plasmid and host chromosomes.
- When integrated, the F plasmid's *tra* operon is still functional; the plasmid can direct the synthesis of pili, carry out rolling-circle replication, and transfer genetic material to an F recipient cell.
- Such a donor is called an **Hfr strain** (for high frequency of recombination) because it exhibits a very high efficiency of chromosomal gene transfer in comparison with F cells.
- DNA transfer begins when the integrated F factor is nicked at its site of transfer origin.
- As it is replicated, the chromosome moves through the pilus or conjugation bridge connecting the donor and recipient.
- Because only part of the F factor is transferred at the start (the initial break is within the F plasmid), the F^- recipient does not become F^+ unless the whole chromosome is transferred.
- Transfer is standardized at 100 minutes in *E. coli,* and the connection usually breaks before this process is finished.
- Thus a complete F factor usually is not transferred, and the recipient remains F^-.
- Gene transfer can be in either a clockwise or counterclockwise direction around the circular chromosome, depending on the orientation of the integrated F factor.
- After the replicated donor chromosome enters the recipient cell, it may be degraded or incorporated into the F^- genome by recombination.

F' Conjugation

- Because the F plasmid is an episome, it can leave the bacterial chromosome.
- Sometimes during this process the plasmid makes an error in excision and picks up a portion of the chromosomal material to form an **F' plasmid** (**figure 13.15*a***).
- The F' cell retains all of its genes, although some of them are on the plasmid, and still mates only with an F^- recipient.
- F' x F^- conjugation is virtually identical with F^+ x F^- mating.
- Bacterial genes on the F' plasmid are transferred with it and need not be incorporated into the recipient chromosome to be expressed.
- The recipient becomes F' and is a partially diploid merozygote since it has two sets of the genes carried by the plasmid.
- In this way specific bacterial genes may spread rapidly throughout a bacterial population.
- Such transfer of bacterial genes is often called sexduction.

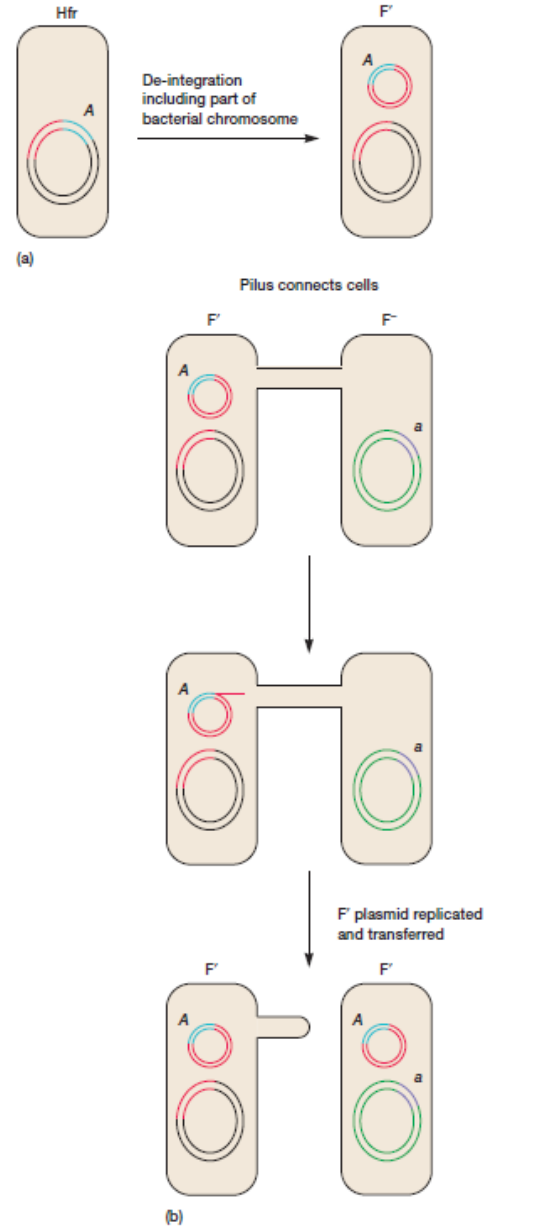


Figure 13.15 F′ Conjugation. (a) Due to an error in excision, the *A* gene of an Hfr cell is picked up by the F factor. (b) The *A* gene is then transferred to a recipient during conjugation.

DNA Transformation

- The second way in which DNA can move between bacteria is through transformation, discovered by Fred Griffith in 1928.
- **Transformation** is the uptake by a cell of a naked DNA molecule or fragment from the medium and the incorporation of this molecule into the recipient chromosome in a heritable form.
- In natural transformation the DNA comes from a donor bacterium.
- The process is random, and any portion of a genome may be transferred between bacteria.
- When bacteria lyse, they release considerable amounts of DNA into the surrounding environment.
- These fragments may be relatively large and contain several genes.
- If a fragment contacts a **competent** cell, one able to take up DNA and be transformed, it can be bound to the cell and taken inside (**figure 13.16*a***).
- Competency is a complex phenomenon and is dependent on several conditions.
- Bacteria need to be in a certain stage of growth; for example, *S. pneumoniae* becomes competent during the exponential phase when the population reaches about 10^7 to 10^8 cells per ml.
- When a population becomes competent, bacteria such as pneumococci secrete a small protein called the competence factor that stimulates the production of 8 to 10 new proteins required for transformation.

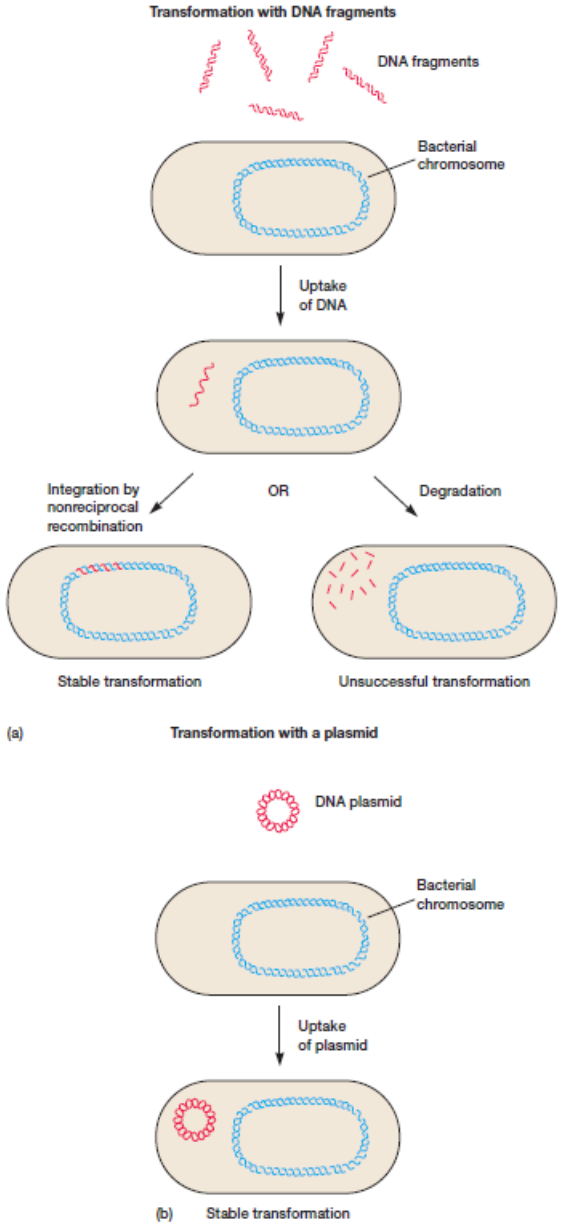
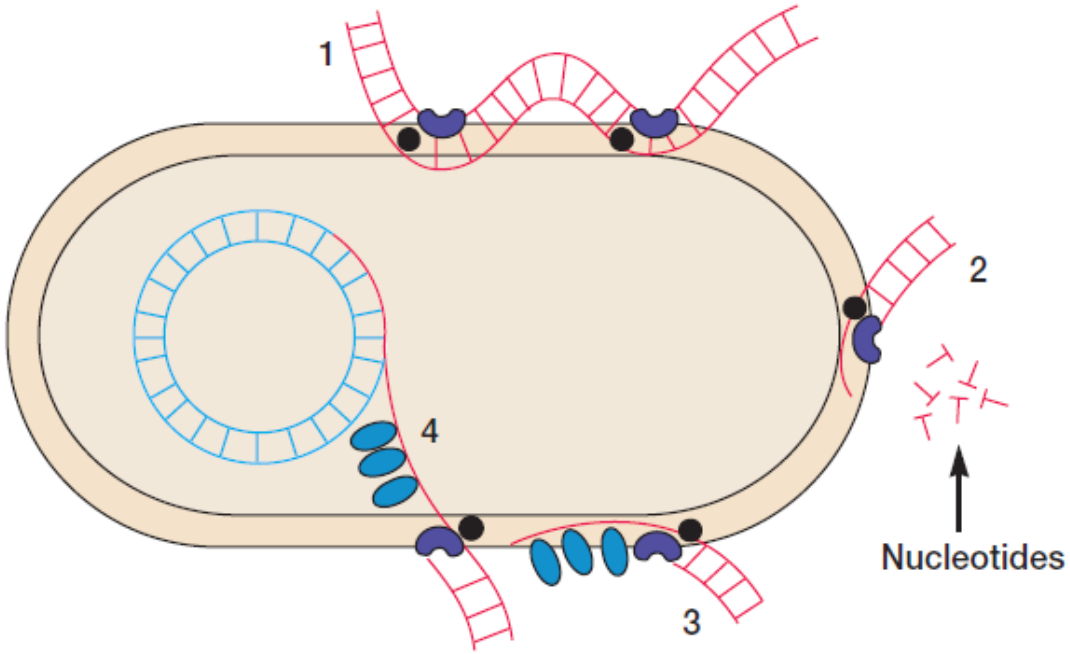


Figure 13.16 Bacterial Transformation. Transformation with (**a**) DNA fragments and (**b**) plasmids. Transformation with a plasmid often is induced artificially in the laboratory. The transforming DNA is in red and integration is at a homologous region of the genome. See text for details.

The Mechanism of Transformation

- A long double-stranded DNA molecule binds to the surface with the aid of aDNA-binding protein (●) and is nicked by a nuclease (◠).
- One strand is degraded by the nuclease.
- The undegraded strand associates with a competence-specific protein (⬭).
- The single strand enters the cell and is integrated into the host chromosome in place of the homologous region of the host DNA.
- DNA uptake requires energy expenditure.



…The Mechanism of Transformation

- Transformation in *Haemophilus influenzae,* a gram-negative bacterium, differs from that in *S. pneumoniae* in several respects.
- *Haemophilus* does not produce a competence factor to stimulate the development of competence, and it takes up DNA from only closely related species (*S. pneumoniae* is less particular about the source of its DNA).
- Double-stranded DNA, complexed with proteins, is taken in by membrane vesicles.
- The specificity of *Haemophilus* transformation is due to a special 11 base pair sequence (5'AAGTGCGGTCA3') that is repeated over 1,400 times in *H. influenzae* DNA.
- DNA must have this sequence to be bound by a competent cell.
- Artificial transformation is carried out in the laboratory by a variety of techniques, including treatment of the cells with calcium chloride, which renders their membranes more permeable to DNA.

Transduction

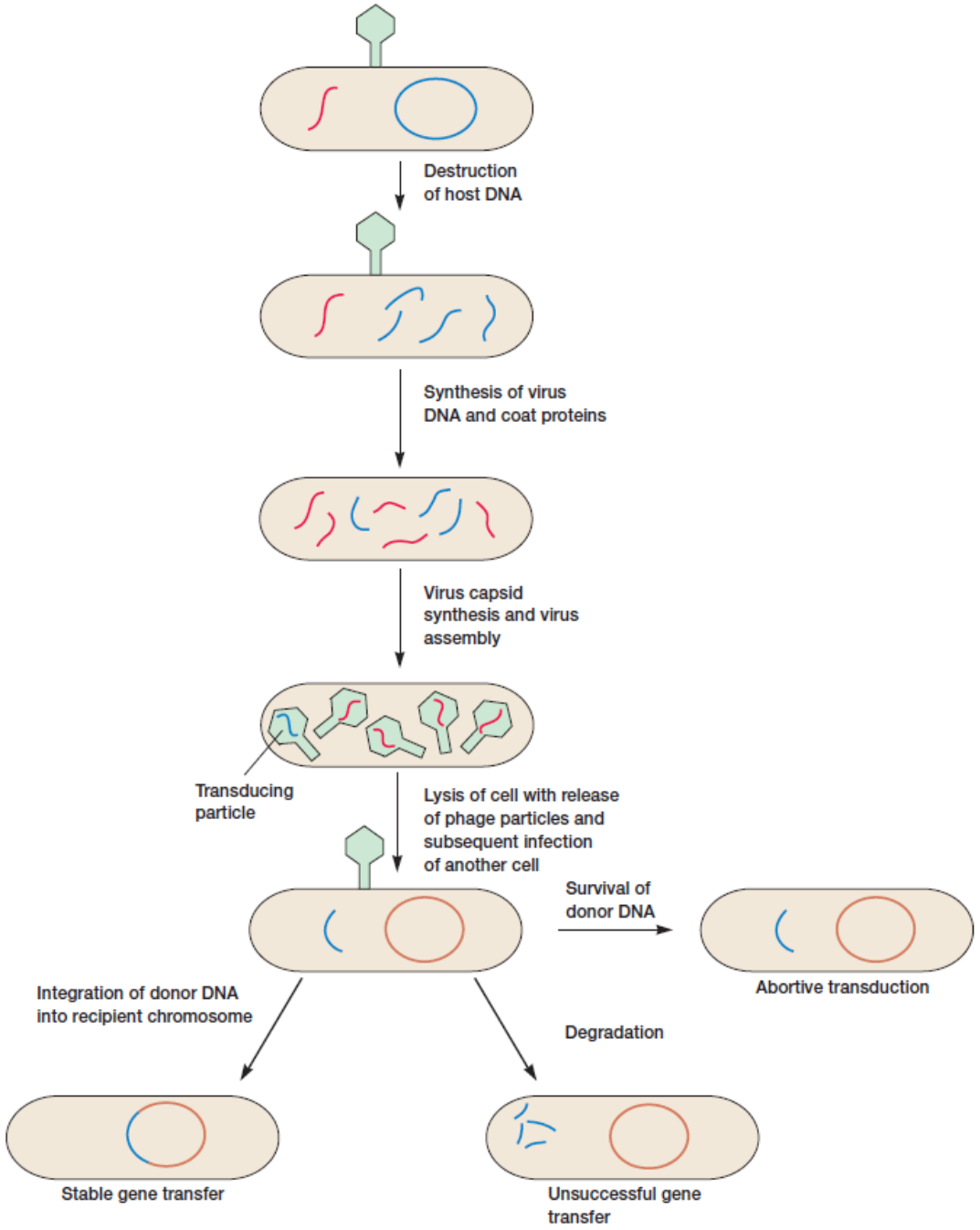
- Bacterial viruses that reproduce using a lytic cycle often are called virulent bacteriophages.
- In many DNA phages, such as the lambda phage, after adsorption and penetration, the viral genome does not take control of its host and destroy it while producing new phages.
- Instead the genome remains within the host cell and is reproduced along with the bacterial chromosome.
- A clone of infected cells arises and may grow for long periods while appearing perfectly normal.
- Each of these infected bacteria can produce phages and lyse under appropriate environmental conditions.
- This relationship between the phage and its host is called **lysogeny.**
- Bacteria that can produce phage particles under some conditions are said to be **lysogens** or **lysogenic,** and phages able to establish this relationship are **temperate phages.**
- The latent form of the virus genome that remains within the host without destroying it is called the **prophage.**
- The prophage usually is integrated into the bacterial genome.
- Sometimes phage reproduction is triggered in a lysogenized culture by exposure to UV radiation or other factors. The lysogens are then destroyed and new phages released. This phenomenon is called induction.
- **Transduction is the transfer of bacterial genes by viruses.**
- Bacterial genes are incorporated into a phage capsid because of errors made during the virus life cycle. The virus containing these genes then injects them into another bacterium, completing the transfer.
- There are two very different kinds of transduction: generalized and specialized

Generalized Transduction

- **Generalized transduction** (**figure 13.19**) occurs during the lytic cycle of virulent and temperate phages and can transfer any part of the bacterial genome.
- During the assembly stage, when the viral chromosomes are packaged into protein capsids, random fragments of the partially degraded bacterial chromosome also may be packaged by mistake.
- Because the capsid can contain only a limited quantity of DNA, the viral DNA is left behind.
- The quantity of bacterial DNA carried depends primarily on the size of the capsid.
- The P22 phage of *Salmonella typhimurium* usually carries about 1% of the bacterial genome; the P1 phage of *E. coli* and a variety of gram-negative bacteria carries about 2.0 to 2.5% of the genome.
- The resulting virus particle often injects the DNA into another bacterial cell but does not initiate a lytic cycle.
- This phage is known as a **generalized transducing particle** or phage and is simply a carrier of genetic information from the original bacterium to another cell.
- Once the DNA has been injected, it must be incorporated into the recipient cell's chromosome to preserve the transferred genes.
- The DNA remains double stranded during transfer, and both strands are integrated into the endogenote's genome.
- About 70 to 90% of the transferred DNA is not integrated but often is able to survive and express itself.
- **Abortive transductants** are bacteria that contain this nonintegrated, transduced DNA and are partial diploids.

Generalized Transduction

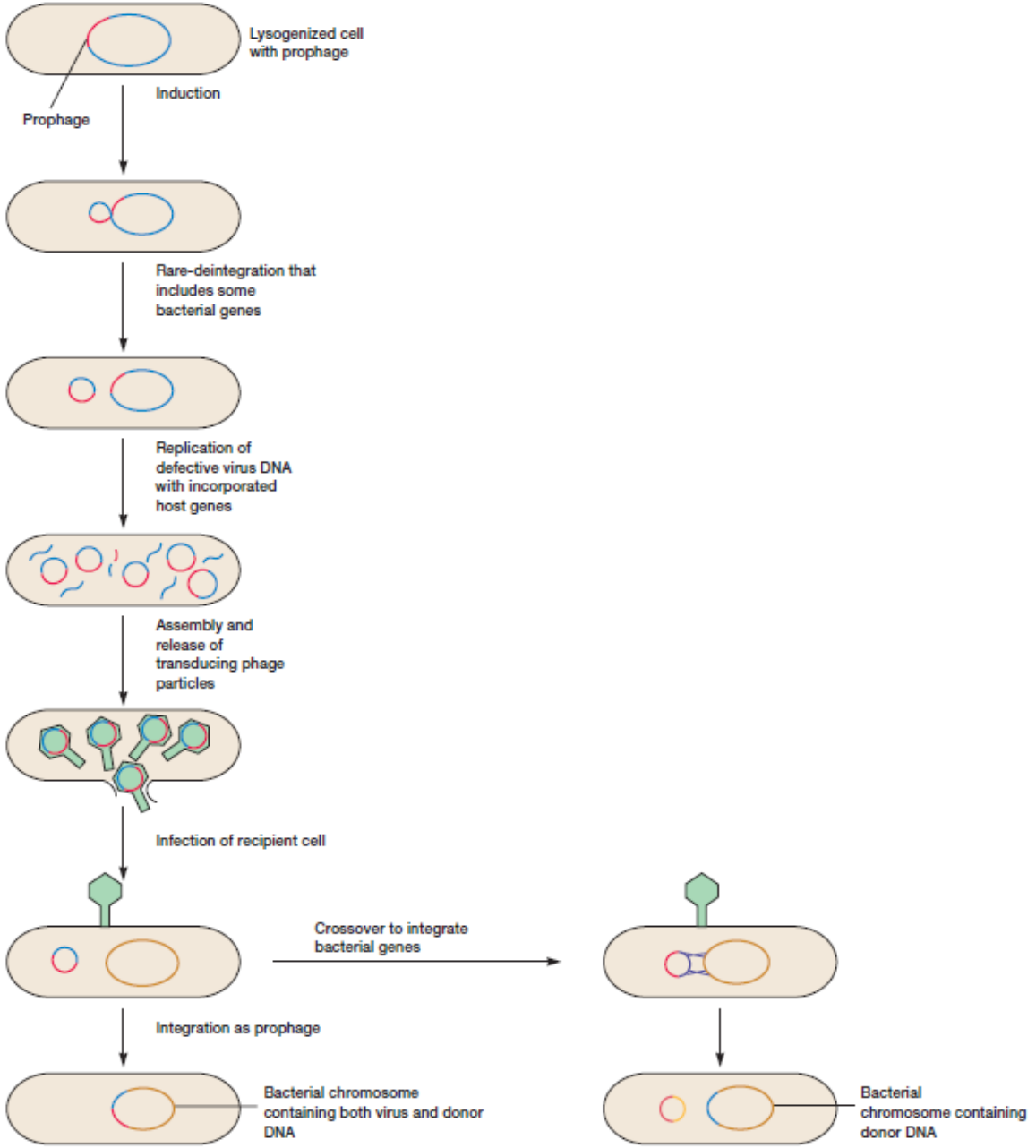
- Generalized transduction was discovered in 1951 by Joshua Lederberg and Norton Zinder during an attempt to show that conjugation, discovered several years earlier in *E. coli,* could occur in other bacterial species.
- Lederberg and Zinder were repeating the earlier experiments with *Salmonella typhimurium*.
- They found that incubation of a mixture of two multiply auxotrophic strains yielded prototrophs at the level of about one in 10^5.
- When these investigators performed the U-tube experiment with *Salmonella,* they still recovered prototrophs.
- The filter in the U tube had small enough pores to block the movement of bacteria between the two sides but allowed the phage P22 to pass.
- Lederberg and Zinder had intended to confirm that conjugation was present in another bacterial species and had instead discovered a completely new mechanism of bacterial gene transfer.



Specialized Transduction

- In **specialized** or **restricted transduction,** the transducing particle carries only specific portions of the bacterial genome.
- Specialized transduction is made possible by an error in the lysogenic life cycle.
- When a prophage is induced to leave the host chromosome, excision is sometimes carried out improperly.
- The resulting phage genome contains portions of the bacterial chromosome (about 5 to 10% of the bacterial DNA) next to the integration site, much like the situation with F' plasmids.
- A transducing phage genome usually is defective and lacks some part of its attachment site.
- The transducing particle will inject bacterial genes into another bacterium, even though the defective phage cannot reproduce without assistance.
- The bacterial genes may become stably incorporated under the proper circumstances.
- The best-studied example of specialized transduction is the lambda phage.

Specialized Transduction



…Specialized Transduction

- The lambda genome inserts into the host chromosome at specific locations known as attachment or *att* site.
- The phage *att* sites and bacterial *att* sites are similar and can complex with each other, although they are not identical.
- The *att* site for lambda is next to the *gal* and *bio* genes on the *E. coli* chromosome; consequently, specialized transducing lambda phages most often carry these bacterial genes.
- The lysate, or product of cell lysis, resulting from the induction of lysogenized *E. coli* contains normal phage and a few defective transducing particles.
- These particles are called lambda *dgal* because they carry the galactose utilization genes (figure 13.21).
- Because these lysates contain only a few transducing particles, they often are called **low-frequency transduction lysates (LFT lysates).**
- Whereas the normal phage has a complete *att* site, defective transducing particles have a nonfunctional hybrid integration site that is part bacterial and part phage in origin.
- Integration of the defective phage chromosome does not readily take place.
- Transducing phages also may have lost some genes essential for reproduction.
- Stable transductants can arise from recombination between the phage and the bacterial chromosome because of crossovers on both sides of the *gal* site (figure 13.21).

Figure 13.21 The Mechanism of Transduction for Phage Lambda and *E. coli*. Integrated lambda phage lies next to the *gal* genes. When it excises normally (top left), the new phage is complete and contains no bacterial genes. Rarely excision occurs asymmetrically (top right), and the *gal* genes are then picked up and some phage genes are lost. The result is a defective lambda phage that carries bacterial genes and can transfer them to a new recipient. See text for details.

…Specialized Transduction

- Defective lambda phages carrying the *gal* gene can integrate if there is a normal lambda phage in the same cell.
- The normal phage will integrate, yielding two bacterial/phage hybrid *att* sites here the defective lambda *dgal* phage can insert (figure 13.21).
- It also supplies the genes missing in the defective phage.
- The normal phage in this instance is termed the **helper phage** because it aids integration and reproduction of the defective phage.
- These transductants are unstable because the prophages can be inducedto excise by agents such as UV radiation.
- Excision, however, produces a lysate containing a fairly equal mixture of defectivelambda *dgal* phage and normal helper phage.
- Because it is very effective in transduction, the lysate is called a **high-frequency transduction lysate (HFT lysate).**