13.4 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. For example, one strain required biotin (Bio^-), phenylalanine (Phe^-), and cysteine (Cys^-) for growth, and another needed threonine (Thr^-), leucine (Leu^-), and thiamine (Thi^-). Recombinant prototrophic colonies appeared on the minimal medium after incubation (**figure 13.12**). Thus the chromosomes of the two auxotrophs were able to associate and undergo recombination.

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 13.13**). 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.

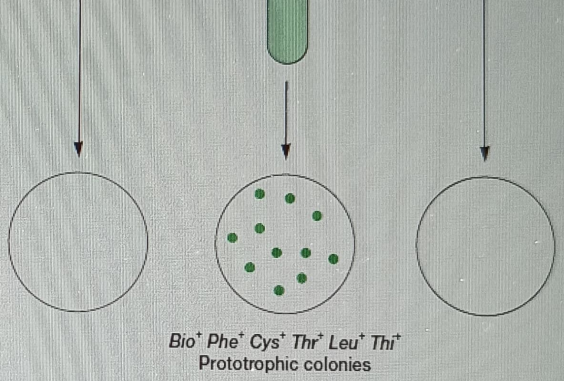


Figure 13.12 Evidence for Bacterial Conjugation. Lederberg and Tatum's demonstration of genetic recombination using triple auxotrophs. See text for details.

$F^+ \times F^-$ 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 $F^+ \times F^-$ 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^+.

These results are readily explained in terms of the F factor previously described (figure 13.5). The F^+ strain contains an extrachromosomal F factor carrying the genes for pilus formation and plasmid transfer. During $F^+ \times F^-$ mating or conjugation, the F factor replicates by the rolling-circle mechanism, and a copy

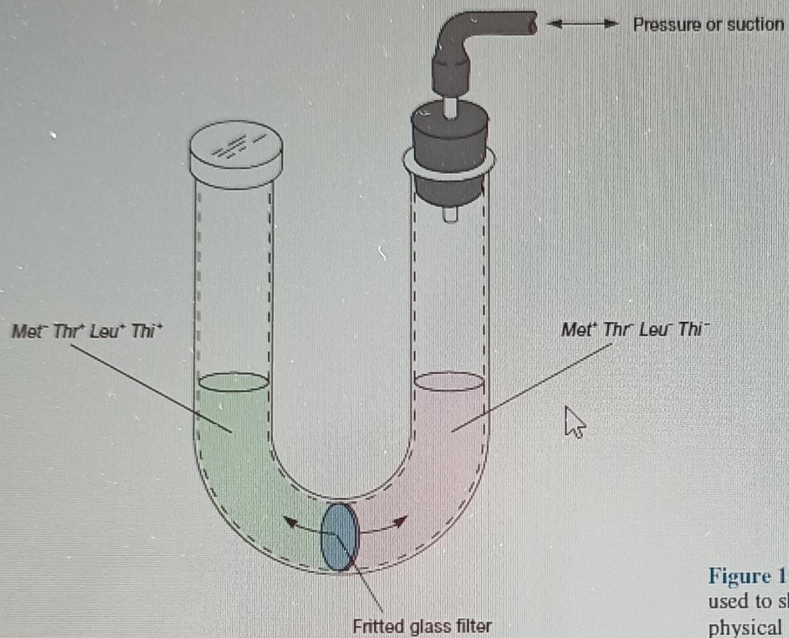


Figure 13.13 The U-Tube Experiment. The U-tube experiment used to show that genetic recombination by conjugation requires direct physical contact between bacteria. See text for details.

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. It is still not completely clear how the plasmid moves between bacteria. 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. The rolling-circle mechanism of DNA replication (p. 236)

Although most research on plasmids and conjugation has been done using *E. coli* and other gram-negative bacteria, self-transmissible plasmids are present in gram-positive bacterial genera such as *Bacillus, Streptococcus, Enterococcus, Staphylococcus,* and *Streptomyces.* Much less is known about these systems. 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.

Hfr Conjugation

Because certain donor strains transfer bacterial genes with great ef-

quences 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 (figure 13.14*b*). 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^-.

As mentioned earlier, when an Hfr strain participates in conjugation, bacterial genes are frequently transferred to the recipient. 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

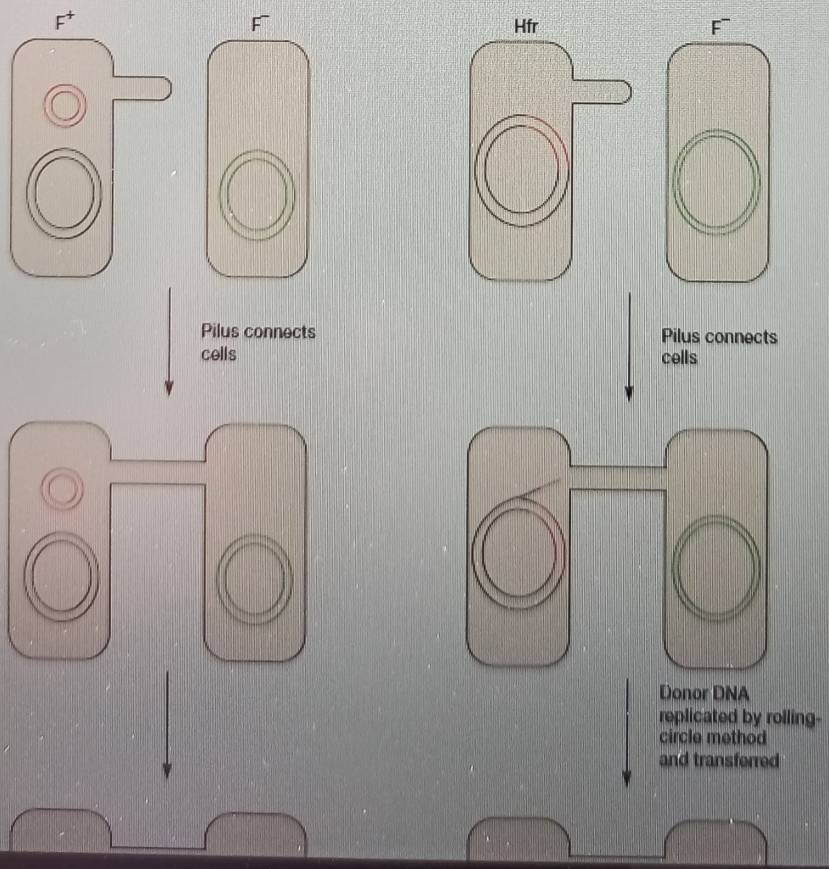
Hfr Conjugation

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 se-

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



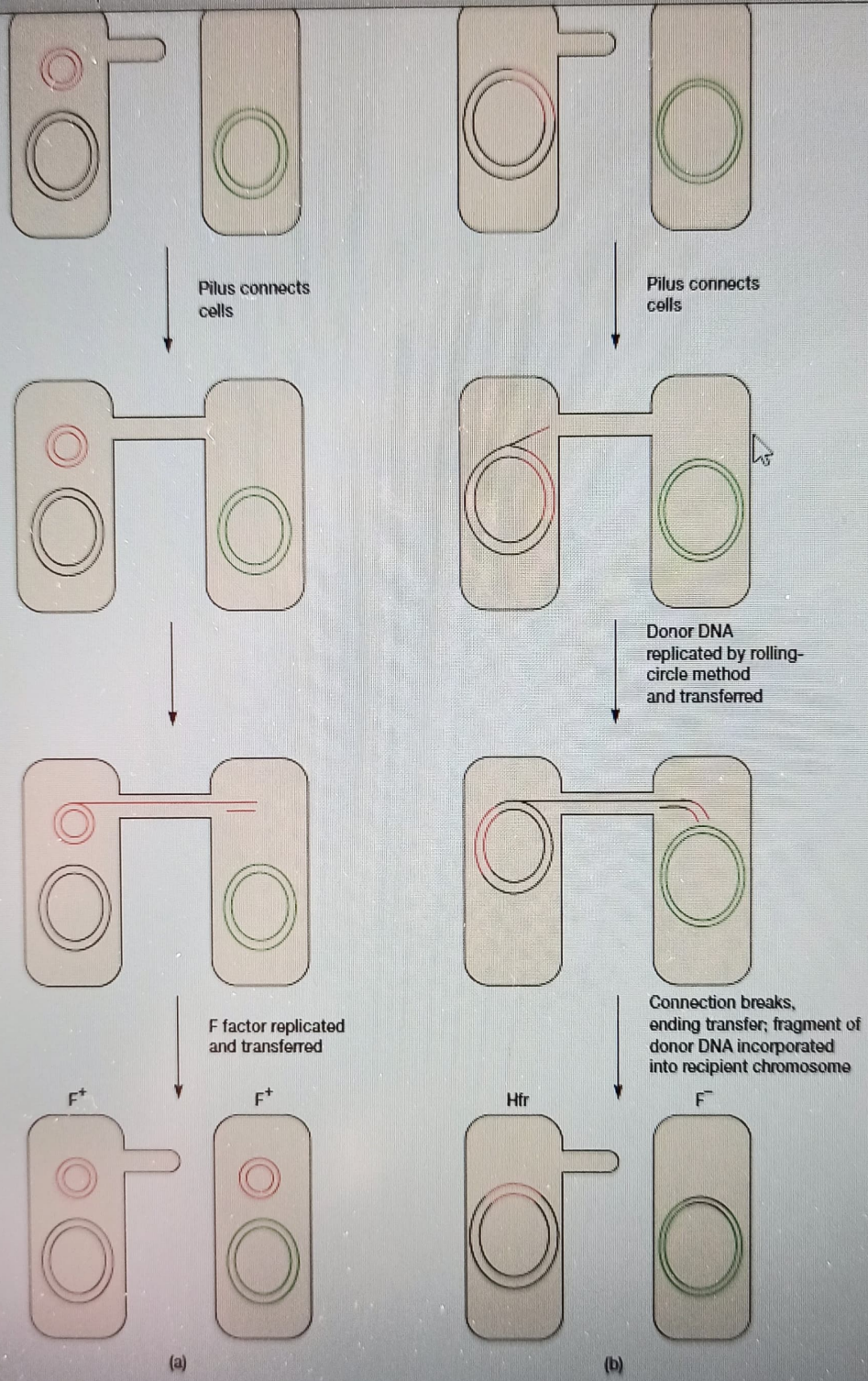


Figure 13.14 The Mechanism of Bacterial Conjugation. (a) $F^+ \times F^-$ mating. (b) Hfr $\times$ F^- mating (the integrated F factor is shown in red).

material to form an **F′ plasmid (figure 13.15*a*)**. It is not unusual to observe the inclusion of one or more genes in excised F plasmids. 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′ $\times$ F^- conjugation is virtually identical with $F^+ \times F^-$ mating. Once again, the plasmid is transferred, but usually bacterial genes on the chromosome are not (figure 13.15*b*). 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.

F′ conjugation is very important to the microbial geneticist. A partial diploid's behavior shows whether the allele carried by an F′ plasmid is dominant or recessive to the chromosomal gene. The formation of F′ plasmids also is useful in mapping the chromosome since if two genes are picked up by an F factor they must be neighbors.

1. What is bacterial conjugation and how was it discovered?
2. Distinguish between F^+, Hfr, and F^- strains of *E. coli* with respect to their physical nature and role in conjugation.
3. Describe in some detail how $F^+ \times F^-$ and Hfr conjugation processes proceed, and distinguish between the two in terms of mechanism and the final results.
4. What is F′ conjugation and why is it so useful to the microbial geneticist? How does the F′ plasmid differ from a regular F plasmid? What is sexduction?

13.5 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. The discovery of transformation (pp. 228–29)

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*)**. The transformation frequency of very competent cells is around 10^{-3} for most genera when an excess of DNA is used. That is, about one cell in every thousand will take up and integrate the gene. 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

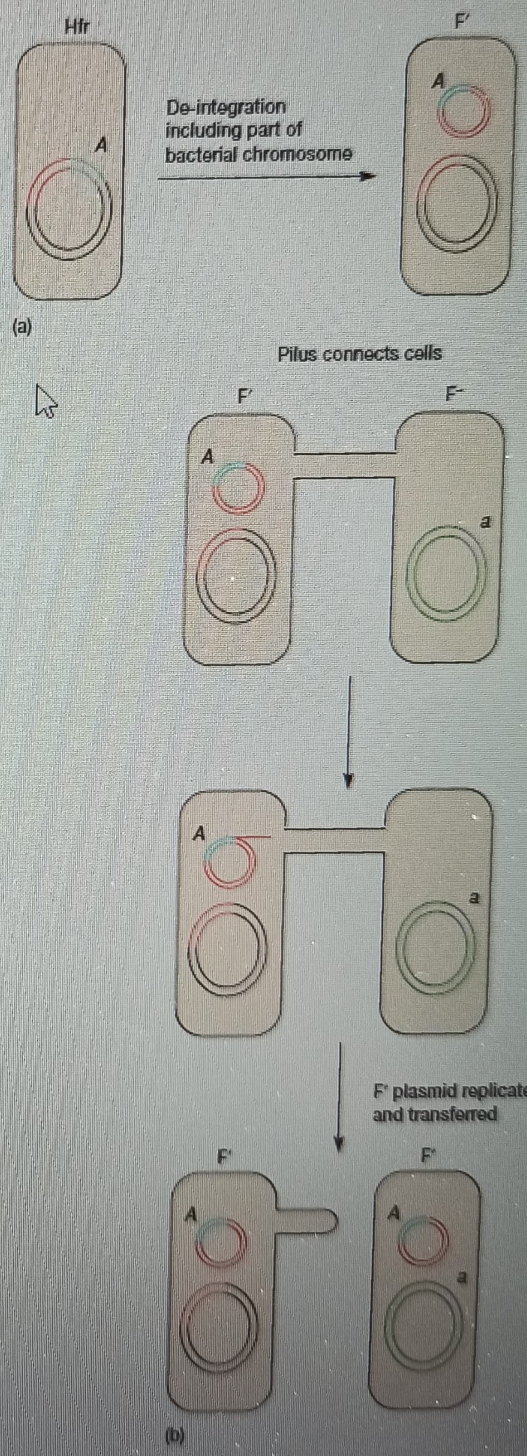
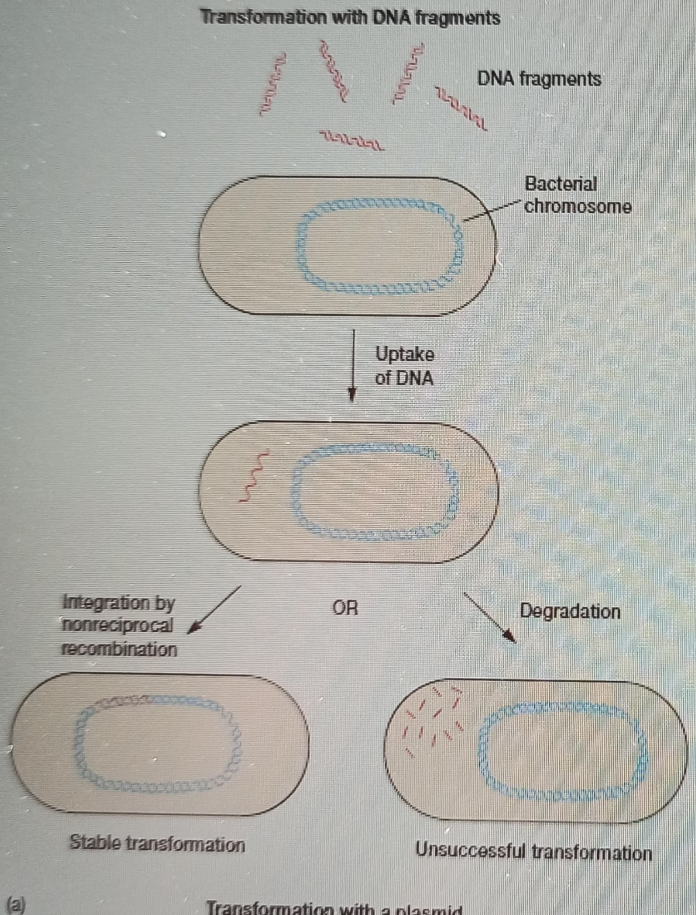


Figure 13.15 F′ Conjugation. (a) Due to an error in excision, the *A* gene of an Hfr cell is picked up by the F factor. (b) The *A* gene is then transferred to a recipient during conjugation.

of 8 to 10 new proteins required for transformation. Natural trans-



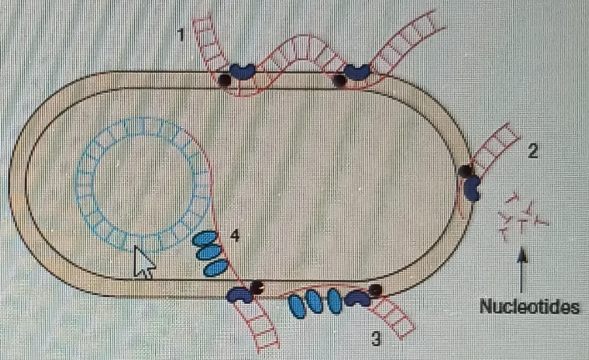
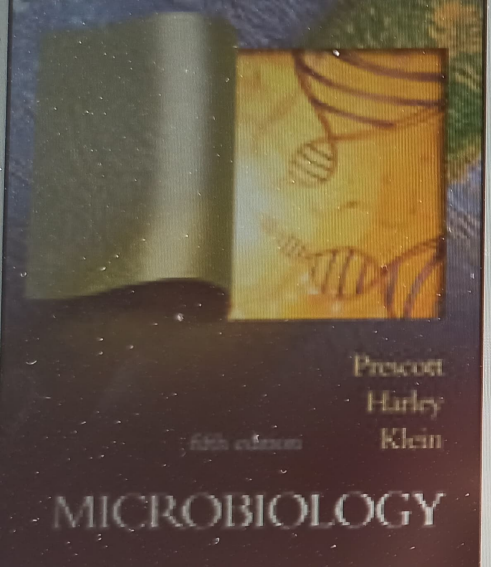


Figure 13.17 The Mechanism of Transformation. (*1*) A long double-stranded DNA molecule binds to the surface with the aid of a DNA-binding protein (●) and is nicked by a nuclease (◠). (*2*) One strand is degraded by the nuclease. (*3*) The undegraded strand associates with a competence-specific protein (⬭). (*4*) The single strand enters the cell and is integrated into the host chromosome in place of the homologous region of the host DNA.

Azotobacter, and *Pseudomonas.* Other genera also may be capable of transformation. Gene transfer by this process occurs in soil and marine environments and may be an important route of genetic exchange in nature.

The mechanism of transformation has been intensively studied in *S. pneumoniae* (**figure 13.17**). A competent cell binds a double-stranded DNA fragment if the fragment is moderately large; the process is random, and donor fragments compete with each other. The DNA then is cleaved by endonucleases to double-stranded fragments about 5 to 15 kilobases in size. DNA uptake requires energy expenditure. One strand is hydrolyzed by an envelope-associated exonuclease during uptake; the other strand associates with small proteins and moves through the plasma membrane. The single-stranded fragment can then align with a homologous region of the genome and be integrated, probably by a mechanism similar to that depicted in figure 13.3.

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



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