Bacteriophages

The discovery of bacterial viruses (bacteriophages or phages) in 1941 opened a new era in the study of the genetics of prokaryotic organisms. Although they were disappointing in the original hope that they could be used to fight bacterial infections, phages served during the 1950s as vehicles for genetic analysis of bacteria. Unlike viruses that infect plant or animal cells, phages can relatively easily be analyzed in their host cells. Names associated with phage analysis are Max Delbrück, Salvador Luria, and Alfred D. Hershey (the "phage group," see: Cairns et al., 1966).

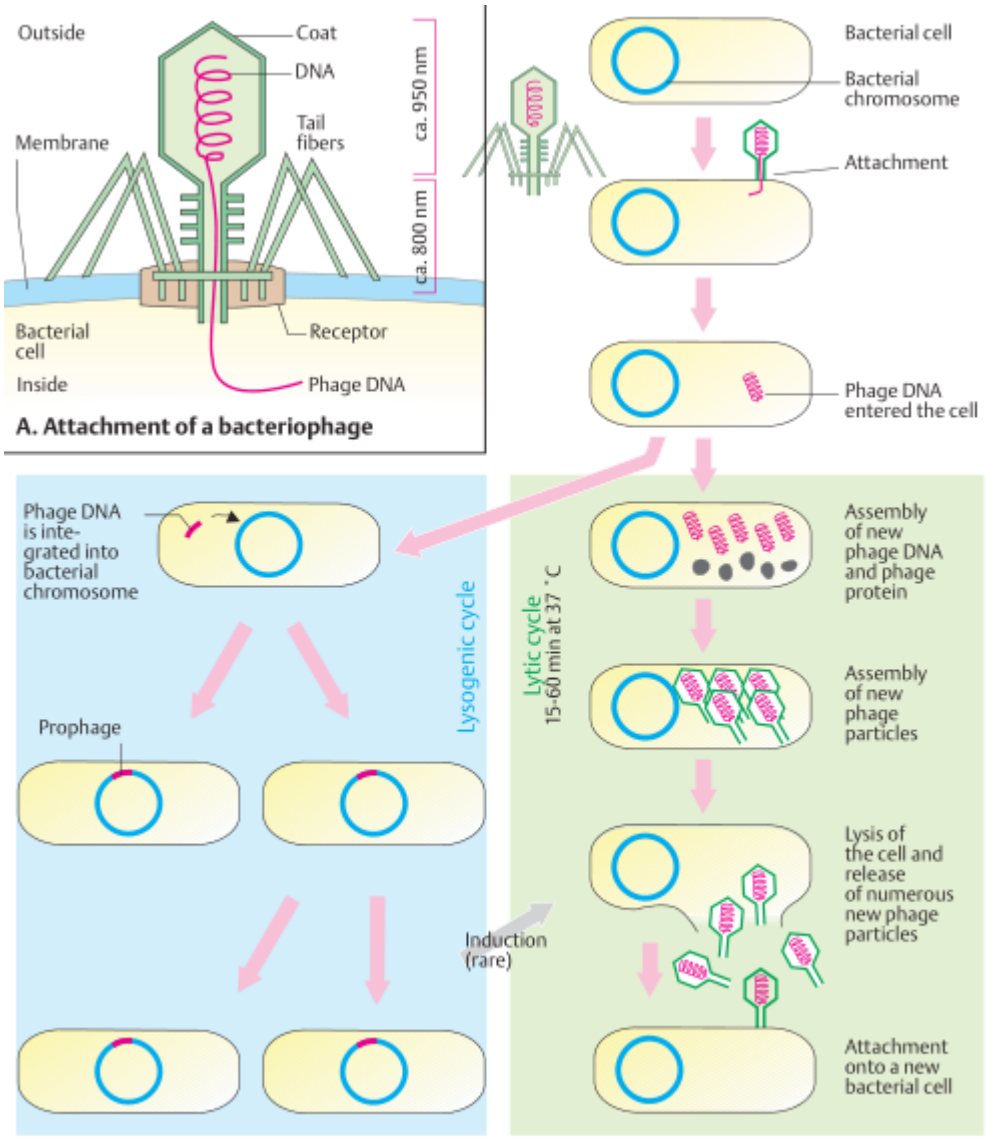
A. Attachment of a bacteriophage

Phages consist of DNA, a coat (coat protein) for protection, and a means of attachment (terminal filaments). Like other viruses, phages are basically nothing more than packaged DNA. One or more bacteriophages attach to a receptor on the surface of the outer cell membrane of a bacterium. The figure shows how an attached phage inserts its DNA into a bacterium. Numerous different phages are known,e.g., for Escherichia coli and Salmonella (phages T1, T2, P1, F1, lambda, T4, T7, phiX174 and others).

B. Lytic and lysogenic cycles of a bacteriophage

Phages do not reproduce by cell division like bacteria, but by intracellular formation and assembly of the different components. This begins with the attachment of a phage particle to a specific receptor on the surface of a sensitive bacterium. Different phages use different receptors, thus giving rise to specificity of interaction (restriction). The invading phage DNA contains the information for production of

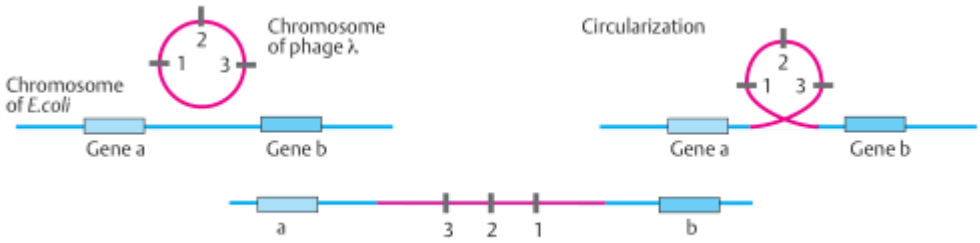
coat proteins for new phages and factors for DNA replication and transcription. Translation is provided for by cell enzymes. The phage DNA and phage protein synthesized in the cell are assembled into new phage particles. Finally, the cell disintegrates (lysis) and hundreds of phage particles are released. With attachment of a new phage to a new cell, the procedure is repeated (lytic cycle). Phage reproduction does not always occur after invasion of the cell. Occasionally, phage DNA is integrated into the bacterial chromosome and replicated with it (lysogenic cycle). Phage DNA that has been integrated into the bacterial chromosome is designated a prophage. Bacteria containing prophages are designated lysogenic bacteria; the corresponding phages are termed lysogenic phages. The change from a lysogenic to a lytic cycle is rare. It requires induction by external influences and complex genetic mechanisms.



C. Insertion of a lambda phage into the bacterial chromosome by crossing-over

A phage can be inserted into a bacterial chromosome by different mechanisms. With the lambda phage (λ), insertion results from crossing-over between the E. coli chromosome and the lambda chromosome. First, the lambda chromosome forms a ring. Then it attaches to a homologous section of the bacterial chromosome. Both the bacterial and the lambda chromosome are opened by a break and attach to each other. Since the homologies between the two chromosomes are limited

to very small regions, phage DNA is seldom integrated. The phage is released (and the lytic cycle is induced) by there verse procedure.(Figures adapted from Watson et al., 1987).



C. Insertion of phage lambda into the bacterial chromosome by crossing-over

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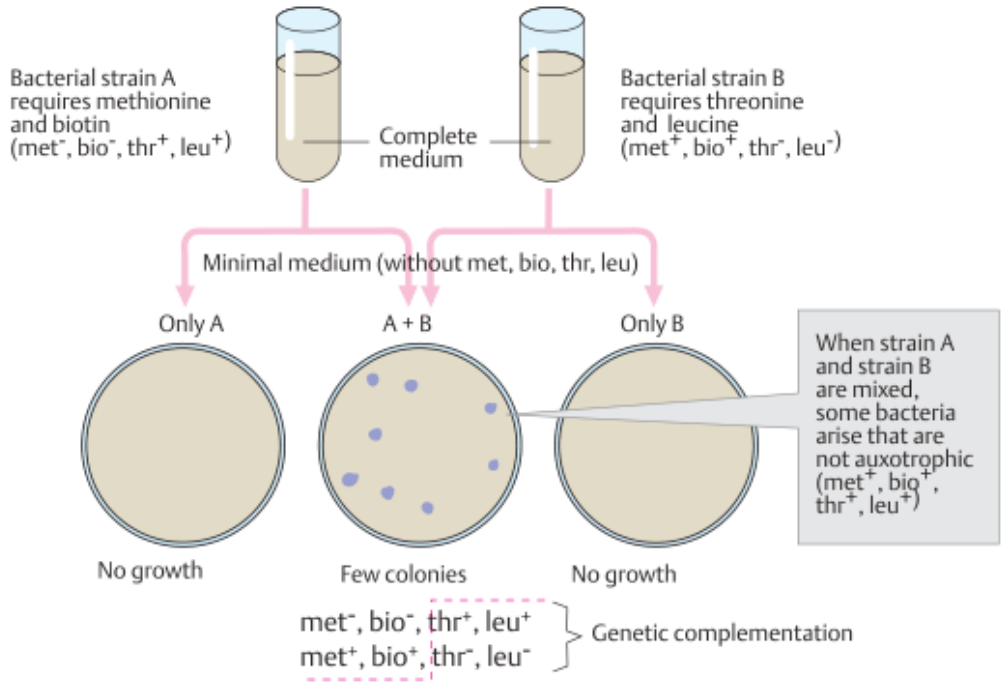
Recombination in Bacteria

In 1946, J. Lederberg and E. L. Tatum first demonstrated that genetic information can be exchanged between different mutant bacterial strains. This corresponds to a type of sexuality and leads to genetic recombination.

A. Genetic recombination in bacteria

In their classic experiment, Lederberg and Tatum used two different auxotrophic bacterial strains. One (A) was auxotrophic for methionine (Met –) and biotin (Bio –). This strain required methionine and biotin, but not threonine and leucine (Thr + , Leu +), to be added to the medium. The opposite was true for bacterial strain B, auxotrophic for threonine and leucine (Thr – , Leu –), but prototrophic for methionine and biotin (Met + , Bio +). When the cultures were mixed together without the addition of any of these four amino acids and then plated on an agar plate with minimal medium, a few single colonies (Met + , Bio + , Thr + , Leu +)

unexpectedly appeared. Although this occurred rarely (about 1 in 10 7 platedcells), a few colonies with altered genetic properties usually appeared owing to the large number of plated bacteria. The interpretation: genetic recombination between strain A and strain B. The genetic properties of the parent cells complemented each other (genetic complementation). (Figure adapted from Stent & Calendar, 1978).

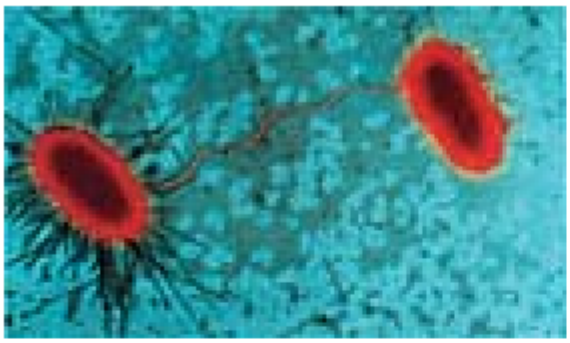


Genetic recombination in bacteria

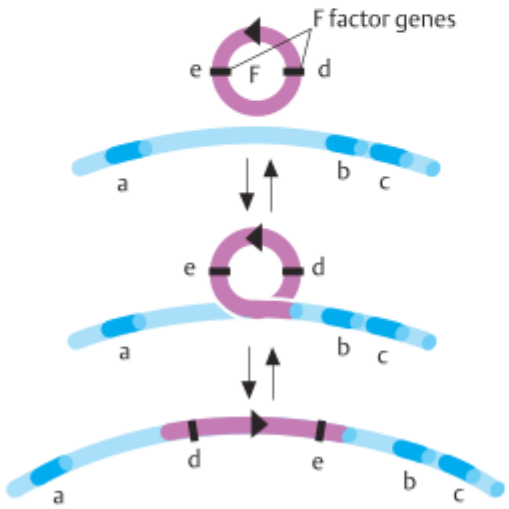
B. Conjugation in bacteria

Later, the genetic exchange between bacteria (conjugation) was demonstrated by light microscopy. Conjugation occurs with bacteria possessing a gene that enables frequent recombination. Bacterial DNA transfer occurs in one direction only. “Male” chromosomal material is introduced into a “female” cell. The so-called male and female cells of E.coli differ in the presence of a fertility factor (F). When F + and F – cells are mixed together, conjugal pairs can form with attachment of a male (F +) sex pilus to the surface of an F – cell. (Photograph from Science 257 :1037, 1992). C. Integration of the F factor into an Hfr – chromosome The F

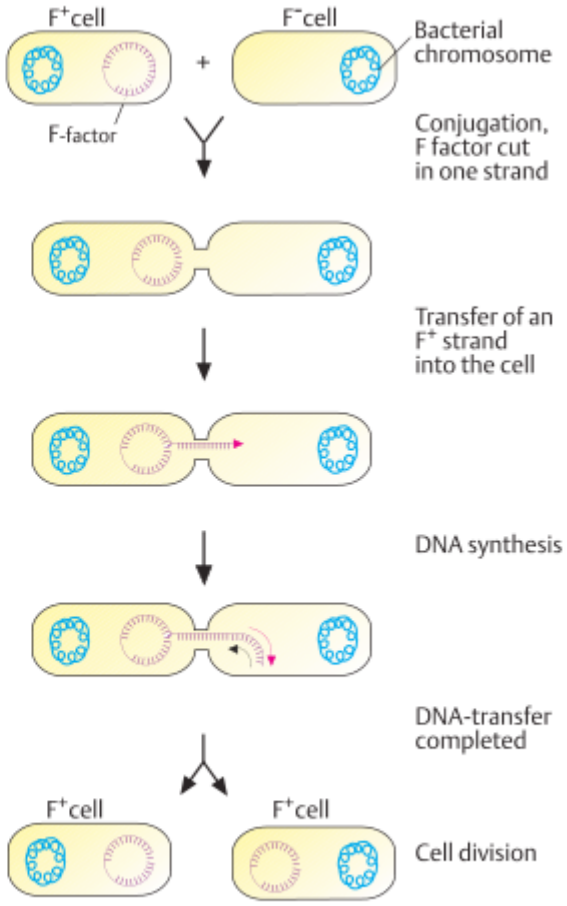
factor can be integrated into the bacterial chromosome by means of specific crossingover. After the factor is integrated, the original bacterial chromosome with the sections a, b, and c contains additional genes, the F factor genes(e,d). Such a chromosome is called an Hfr chromosome (Hfr, high frequency of recombination) owing to its high rate of recombination with genes of other cells as a result of conjugation. D. Transfer of F DNA from an F + to an F – cell Bacteria may contain the F factor(fertility) as an additional small chromosome, i.e., a small ring shaped DNA molecule (F plasmid) of about 94000 base pairs (not shown to scale). This corresponds to about 1/40th of the total genetic information of a bacterial chromosome. It occurs once per cell and can be transferred to other bacterial cells. About a third of the F + DNA consists of transfer genes, including genes for the formation of sex pili. The transfer of the F factor begins after a strand of the DNA double helix is opened. One strand is transferred to the acceptor cell. There it is replicated, so that it becomes double-stranded. The DNA strand remaining in the donor cell is likewise restored to a double strand by replication. Thus, DNA synthesis occurs in both the donor and the acceptor cell. When all is concluded, the acceptor cell is also an F + cell.(Figures in C and D adapted from Watson et al., 1987).



B. Conjugation in bacteria



C. Integration of the F factor into an Hfr⁻ chromosome



D. Transfer of F DNA from an F⁺ into an F⁻ cell