Milestones of bacterial genetic research:

1944 Avery's pneumococcal transformation experiment shows that DNA is the hereditary material
1946 Lederberg & Tatum describes bacterial conjugation using biochemical mutants
1952 Hersey & Chase shows that the hereditary material of the bacteriophage is DNA;
   Zinder & Lederberg achieves phage-mediated transduction in Salmonella
1953 Cavalli-Sforza et al show the F factor in bacteria (Cavalli-Sforza LL, Lederberg J,
   Lederberg M. J Gen Microbiol 1953;8:89)
1961 Jacob & Monad describes the operon structure
1977 Sanger sequences the phage phiX174 (identification of overlapping genes).

Properties of Bacteria

A bacterium has four types of genetic material: its single (haploid), covalently closed, circular
dsDNA chromosome (in a supercoiled state); a plasmid(s); a bacteriophage or prophage; and
transposon(s)/retrotrasposon(s).

Genetic exchange between bacteria can occur by transfection, transduction or conjugation. Conjugation involves F+ male bacterium and F- female bacterium. Bacteria are haploid, but following a gene transfer (such as conjugation), they can be partially diploid (merozygote). This may result in a double cross-over event between the circular DNA and the linear, newly introduced DNA if the two copies of the DNA are related. Sexual reproduction and meiosis do not occur in bacteria but genetic recombination to increase diversity is still possible by horizontal gene transfer.

While bacteria are haploid organisms, plasmids can be considered as additional mini-chromosomes. Plasmids can be 1 to 300 kb long and usually exist as multiple, free copies in a bacterium. As a rule, small plasmids occur in multiple copies per cell (high copy number), and large plasmids have a low copy number. Plasmids cannot replicate outside a bacterium. More than one types of plasmids can co-inhabit the same bacterium. Up to 10 kb (on average 3 kb) long DNA fragments can be inserted into a plasmid. They can enter the cells in two ways: vertical (via cell division - binary fission) or horizontal transmission (bacterial gene swapping). Most plasmids contain genes that confer an evolutionary advantage to their hosts. These can be anti-bacterial toxins, catabolic enzymes (to use unusual carbon sources), virulence factors (pathogenic toxins), enzymes to degrade toxic compounds (like crude oil, polychlorinated biphenyls, pesticides) and most importantly, antibiotic resistance (conferred by R plasmids). Sometimes, they may confer resistance up to five antibiotics at the same time. Plasmids can be exchanged between unrelated bacteria. This is the reason for speedy spread of antibiotic resistance among them.

Sometimes, a bacterium also contains a prophage as an inserted DNA fragment into its chromosome and this additional genetic material may be beneficial for it. For example, a prophage of the bacterium Corynbacterium diphtheriae carries a gene that encodes the diphtheria toxin causing the disease.

Temperate phages may exist in a bacteria in a non-replicating, latent state (prophage). Naturally, every time a bacteria divides (every 15 to 60 minutes), the prophage will also be replicated.
Transposable elements cannot exist as free particles in a bacteria. They are integrated in the bacterial genome or into the genetic material of a plasmid or a prophage. They have the ability to move between these sites using an enzyme called transposase. Transposons may also encode proteins that are useful for bacteria (such as antibiotic or heavy metal resistance factors).

There are hundreds of thousands of bacterial species in existence on Earth. They grow relatively quickly, and most reproduce by binary fission, the production of two identical daughter cells from one mother cell. Therefore, each replication cycle doubles the number of cells in a bacterial population. The bacterial chromosome is a long circle of deoxyribonucleic acid (DNA) that is attached to the membrane of the cell. During replication, the chromosome is copied, and the two copies are divided into the two daughter cells. Transfer of genetic information from the mother cell to offspring is called vertical transmission.

Beneficial mutations that develop in one bacterial cell can also be passed to related bacteria of different lineages through the process of horizontal transmission. There are three main forms of horizontal transmission used to spread genes between members of the same or different species: conjugation (bacteria-to-bacteria transfer), transduction (viral-mediated transfer), and transformation (free DNA transfer). These forms of genetic transfer can move plasmid, bacteriophage, or genomic DNA sequences. A plasmid is a small circle of DNA separate from the chromosome; a bacteriophage is a virus that reproduces in bacteria by injecting its DNA; the genome is the total DNA of the bacterial organism.

After transfer, the DNA molecules can exist in two forms, either as DNA molecules separate from the bacterial chromosome (an episome), or can become part of the bacterial chromosome. The study of basic mechanisms used by bacteria to exchange genes allowed scientists to develop many of the essential tools of modern molecular biology.

Transformation involves the uptake of DNA from the environment. Cells that are able to take up DNA are called competent. While some bacteria (H. influenzae, B. subtilis) are naturally competent owing to some surface proteins they possess, others can be made competent by various treatments (calcium chloride treatment or electroporation). This kind of transformation is an important method used in genetic engineering (also called in vitro recombinant DNA technology).

Bacteria can take up DNA from other bacteria in nature but the fate of such DNA is usually degradation. Historically, the principal application of transformation experiments was genetic mapping studies on naturally competent bacteria (co-transformation frequencies are inversely related to map distances).

When DNA from (dead) bacteria is introduced into living bacteria is called DNA transformation. Some bacteria have evolved systems that transport free DNA from the outside of the bacterial cell into the cytoplasm. These bacterial are called "naturally competent" for DNA transformation. Natural DNA transformation of Streptococcus pneumoniae provided the first proof that DNA encoded the genetic material in experiments by Oswald Avery and colleagues. Some other naturally competent bacteria include Bacillus subtilis, Haemophilus influenzae, and Neisseria gonorrhoeae. Other bacterial species such as E. coli are not naturally competent for DNA transformation. Scientists have devised many ways to
physically or chemically force non-competent bacteria to take up DNA. These methods of artificial DNA transformation form the basis of plasmid cloning in molecular biology.

Most naturally competent bacteria regulate transformation competence so that they only take up DNA into their cells when there is a high density of cells in the environment. The ability to sense how many other cells are in an area is called quorum sensing. Bacteria that are naturally competent for DNA transformation express ten to twenty proteins that form a structure that spans the bacterial cell envelope. In some bacteria this structure also is required to form a particular type of pilus different than the F factor pilus (sex pilus). Other bacteria express similar structures that are involved in secreting proteins into the exterior medium (Type II secretion). Therefore, it appears that DNA transformation and protein secretion have evolved together.

During natural DNA transformation, doubled-stranded DNA is bound to the recipient cell surface by a protein receptor. One strand of the DNA is transported through the cell envelope, where it can recombine with similar sequences present in the recipient cell. If the DNA taken up is not homologous to genes already present in the cell, the DNA is usually broken down and the nucleotides released are used to synthesize new DNA during normal replication. This observation has led to the speculation that DNA transformation competence may have originally evolved to allow the acquisition of nucleic acids for food.

The source of DNA for transformation is thought to be DNA released from other cells in the same population. Most naturally competent bacteria spontaneously break apart by expressing enzymes that break the cell wall. Autolysis will release the genomic DNA into the environment where it will be available for DNA transformation. Of course, this results in the death of some cells in the population, but usually not large numbers of cells. It appears that losing a few cells from the population is counterbalanced by having the possibility of gaining new traits by DNA transformation.

Transformation in living systems is not very efficient, but contributes to horizontal spread of genes.

**Conjugation**

Conjugation is the most effective way of horizontal gene transfer.

In conjugation, a direct contact between a male (carrying a fertility factor, or F+) and a female (F-) bacteria results in a one-way genetic material transfer (from male-to-female). Gram-negative bacteria (like E.coli) use a physical bridge called (sex) pilus (encoded by a conjugative plasmid) for gene transfer in conjugation, whereas, gram-positive bacteria (like pneumococcus) use a protein called clumping factor to get together. Some phage use the pili as receptors to attach to the bacteria. During conjugation in E.coli, the F factor (which is a conjugative plasmid) is not lost from the donor as it is only one of the strands of the plasmid that has been transferred. Subsequent replications of the bacteria restore the double-stranded state of the plasmid. When a conjugative plasmid initiates conjugation, other plasmids can be transferred (this is called mobilization). Conjugation is the exception to the rule that bacteria reproduce asexually.

Although conjugation resembles sexual reproduction, an important difference is that conjugation is a one-way process. The F factor may exist as a free plasmid or may be inserted into the bacterial genome. Some conjugative plasmids (like the F factor of E.coli) can achieve transfer of
chromosomal genes. An E. coli strain that has this property is called **Hfr strain** (for high frequency recombination). It is important to know that chromosomal genes are transferred before the plasmid itself. If the bridge is broken during transfer, the recipient will remain F-.

Controlled conjugation experiments can be used for gene mapping. Indeed, this approach was used to show the circularity of the E. coli chromosome and to determine the location of 1900 of its genes. Transformation, transduction and conjugation are means of horizontal gene transfer in nature.

Bacterial conjugation refers to the transfer of DNA between bacterial cells that requires cell-to-cell contact. Joshua Lederberg and Edward Tatum first described conjugation in 1946 when they discovered the F factor (an episome) that can move between *Escherichia coli* cells. The F factor is one of the most well studied conjugative plasmids (plasmids are circular episomes) and is the most well studied conjugative system. There are many different conjugal plasmids carried by members of most bacterial species. Conjugal plasmids that carry antibiotic resistance genes are called R factors. The F factor and R factors usually exist as episomes and each carries functions that allow it to replicate its DNA and thus be inherited by the daughter cells after binary fission. However, conjugative plasmids also express transfer functions that allow the movement of DNA from a donor to a recipient cell; this is the process of conjugation.

The steps of bacterial conjugation are: mating pair formation, conjugal DNA synthesis, DNA transfer, and maturation. The main structure of the F factor that allows mating pair formation is the F pilus or sex pilus (a long thin fiber that extends from the bacterial cell surface). There are one to three pili expressed on an *E. coli* cell that carries the F factor, and one pilus will specifically interact with several molecules on the recipient cell surface (attachment). About twenty genes on the F factor are required to produce a functional pilus, but the structure is mainly made up of one protein, pilin. To bring the donor and recipient cell into close proximity, the F pilus retracts into the donor cell by removing pilin protein monomers from the base of the pilus to draw the bacterial cells together.

Once a stable mating pair is formed, a specialized form of DNA replication starts. Conjugal DNA synthesis produces a single-stranded copy of the F factor DNA (as opposed to a double-stranded DNA that is formed by normal replication). This DNA strand is transferred into the recipient cell. Once in the recipient cell, the single-stranded copy of the F plasmid DNA is copied to make a double-stranded DNA molecule, which then forms a mature circular plasmid. At the end of conjugation the mating pair is broken and both the donor and the recipient cells carry an identical episomal copy of the F factor. All of the approximately one hundred genes carried on the F factor can now be expressed by the recipient cell and will be inherited by its offspring.

In addition to transferring itself, the F factor can also transfer chromosomal genes between a donor and recipient cell. The F factor can be found inserted (integrated) into the bacterial chromosome at many locations in a small fraction of bacterial cells. An integrated F factor is replicated along with the rest of the chromosome and inherited by offspring along with the rest of the chromosome. When a mating pair is formed between the donor cell carrying an integrated F factor and a recipient cell, DNA transfer occurs as it does for the episomal F factor, but now the chromosomal sequences adjacent to the integrated F factor are transferred into the recipient. Since these DNA sequences encode bacterial genes, they can recombine with the same genes in the recipient. If the donor gene has minor changes in DNA sequence from the recipient gene, the different sequence can be incorporated into the recipient gene and inherited by the recipient cell's offspring. Donor cells that have an integrated copy of the F factor are called Hfr strains (High frequency of recombination).
Properties of Bacteriophages

They have a simple structure, which consists of their double-stranded DNA and protein coat. Only the DNA enters into the bacteria. Bacterial RNA polymerase is composed of five individual polypeptide subunits (σ, σ', σ″, σ‴). The σ (sigma) factor is responsible for initiating transcription by recognizing bacterial promoter DNA sequences. Some phages supply their own σ factors to instruct the bacteria to transcribe phage genes preferentially. They are virtually viruses but can only infect bacteria. Bacteriophages usually infect only one species of bacteria but there are some who can infect several species even in different genera. Their life cycle may be either lytic (virulent phage) or lysogenic (temperate phage). Their DNA may be integrated into the host chromosome and remain as a prophage. Integration is achieved by recombination between a 15 bp sequence called att (for attachment) in the host chromosome and an identical sequence in the phage chromosome. This recombination requires an integrase (Int) enzyme encoded by the phage.

Bacteriophages are used for DNA cloning in molecular biology. DNA fragments can be inserted into a phage and following transfection of a competent bacteria, many copies of the desired DNA fragment can be obtained.

Transduction

Transduction involves the transfer of a bacterial DNA by means of a phage particle. In specialized transduction, the genome of a temperate phage (such as λ) integrates as a prophage into a bacterium's chromosome usually at a specific site. When the phage leaves the bacterium (to infect another bacterial cell) due to a recombination event it leaves part of the phage genome in the chromosome, while carrying part of the host genome. In specialized transduction the phage is carrying one of the neighboring genes with it, introducing into the next host cell.

In generalized transduction, it is not a specific DNA segment but whatever DNA has been loaded into the phage is transferred. Any piece of the host genome might replace part of the phage DNA, rendering the phage defective. Generalized transfection is a very rear event.

Transduction can also be used to establish gene order of bacteria and for mapping purposes (only closely spaced genes will show co-transduction). In nature, a phage may transfer parts of bacterial DNA from one bacterium to another.

The third way that DNA is transferred between bacterial cells is through a phage particle in the process of transduction. Joshua Lederberg and Norton Zinder first discovered transduction in 1956. When phage inject their DNA into a recipient cell, a process occurs that produces new bacteriophage particles and kills the host cell (lytic growth). Some phage do not always kill the host cell (temperate phage), but instead can be inherited by daughter host cells. Therefore acquisition of a so-called temperate "prophage" by a recipient cell is a form of transduction. Many phages also have the ability to transfer chromosomal or plasmid genes between bacterial cells. During generalized transduction any gene can be transferred from a donor cell to a recipient cell. Generalized transducing phages are produced when a phage packages random bacterial genes into its capsid (protein envelope) instead of its own DNA. When a phage particle
carrying bacterial chromosomal genes attaches to a recipient cell, the DNA is injected into the cytoplasm where it can recombine with a homologous DNA sequences. These phages are unable to reproduce, are called defective phages.

Some bacteriophages can pick up a gene very close to the attachment site (where phages are integrated into the host genome) and transfer it to other bacteria. This process is called specialized transduction.