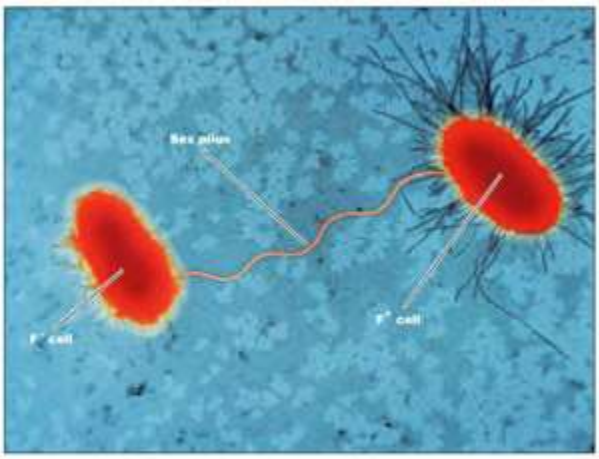
Horizontal Gene Transfer

Horizontal gene transfer , the transmission of DNA between different genomes , occur between different species . Acquisition of DNA through horizontal gene transfer is distinguished from the transmission of genetic material from parents to offspring during reproduction, which is known as vertical gene transfer. Horizontal gene transfer is made possible in large part by the existence of mobile genetic elements, such as plasmids (extrachromosomal genetic material), transposons ("jumping genes"), and bacteria-infecting viruses (bacteriophages). These elements are transferred between organisms through different mechanisms, which in prokaryotes include transformation, conjugation, and transduction. In transformation, prokaryotes take up free fragments of DNA, found in their environment. In conjugation, genetic material is exchanged during a temporary union between two cells, which may entail the transfer of a plasmid. In transduction, DNA is transmitted from one cell to another via a bacteriophage. In horizontal gene transfer, newly acquired DNA is incorporated into the genome of the recipient through either recombination or insertion. Recombination essentially is the regrouping of genes, such that native and foreign (new) DNA segments that are homologous are edited and combined. Insertion occurs when the foreign DNA introduced into a cell shares no homology with existing DNA. In this case, the new genetic material is embedded between existing genes in the recipient's genome.

1. Conjugation

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. Discovered in 1946 by Joshua Lederberg and Edward Tatum.

In most cases, this involves the transfer of plasmid DNA, although with some organisms chromosomal transfer can also occur. In the simplest of cases, conjugation is achieved in the laboratory by mixing the two strains together and after a period of incubation to allow conjugation to occur, plating the mixture onto a medium that does not allow either parent to grow, but on which a trans-conjugant that contains genes from both parents will grow. Plasmid transfer can be readily detected even if only, say, 1 in 10^6 recipients have received a copy of it. Conjugation is most easily demonstrated amongst members of the Enterobacteriaceae and other Gram-negative bacteria (such as Vibrios and Pseudomonads).

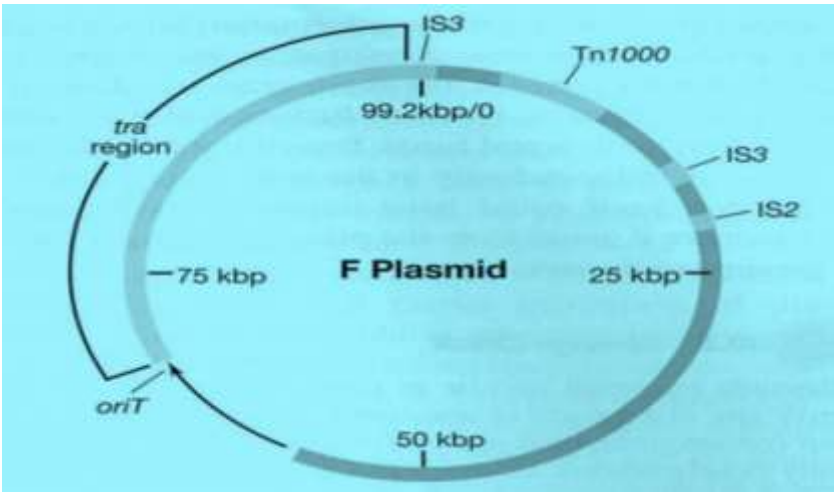


Direct contact between two conjugating bacteria is first made via a pilus.
[Czura, Microbial Genetics,Chapter 8)

F plasmid

The F plasmid was originally discovered during attempts to demonstrate genetic exchange in *E. coli* by mixed culture of two auxotrophic strains, so that plating onto minimal medium would only permit recombinants to grow. It was shown quite early on that the recombinants were all derived from one of the parental strains and that a one-way transfer of information was therefore involved, from the donor (‘male’) to the recipient (‘female’).

The donor strains carry the F plasmid (F) while the recipients are F^-. One feature of this system which must have seemed curious at the time is that co-cultivation of an F and an F^- strain resulted in the ‘females’ being converted into ‘males’. This is due to the transmission of the F plasmid itself which occurs at a high frequency.



Genetic map of the F plasmid of *Escherichia coli.*

Mechanism of conjugation

Formation of mating pairs

In the vast majority of cases, the occurrence of conjugation is dependent on the presence, in the donor strain, of a plasmid that carries the genes required for promoting DNA transfer. In *E. coli* and other Gram-negative bacteria, the donor cell carries appendages on the cell surface known as pili. These vary considerably in structure – for example the pilus specified by the F plasmid is long, thin and flexible, while the RP4 pilus is short, thicker and rigid. The pili make contact with receptors on the surface of the recipient cell, thus forming a mating pair.

The pili then contract to bring the cells into intimate contact and a channel or pore is made through which the DNA passes from the donor to the recipient

Interestingly, this mechanism has much in common with a protein secretion system which is used by some bacteria to deliver protein toxins directly into host cells.

Transfer of DNA

The transfer of plasmid DNA from the donor to the recipient is initiated by a protein which makes a single-strand break (nick) at a specific site in the DNA, known as the origin of transfer (oriT). A plasmid-encoded helicase unwinds the plasmid DNA and the single nicked strand is transferred to the recipient starting with the 5^- end generated by the nick. Concurrently, the free 3^- end of the nicked strand is extended to replace the DNA transferred, by a process known as rolling circle replication which is analogous to the replication of single stranded plasmids and bacteriophages.The nicking protein remains attached to the 5^- end of the transferred DNA.

DNA synthesis in the recipient converts the transferred single strand into a double stranded molecule. Note that this is a replicative process. Thus although there is said to be a transfer of the plasmid from one cell to another, what is really meant is a transfer of a copy of the plasmid. The donor strain still has a copy of the plasmid and can indulge in further mating with another recipient. It is also worth noting that after conjugation the recipient cell has a copy of the plasmid and it can transfer a copy to another recipient cell. The consequence can be an epidemic spread of the plasmid through the mixed population.

Mobilization and chromosomal transfer

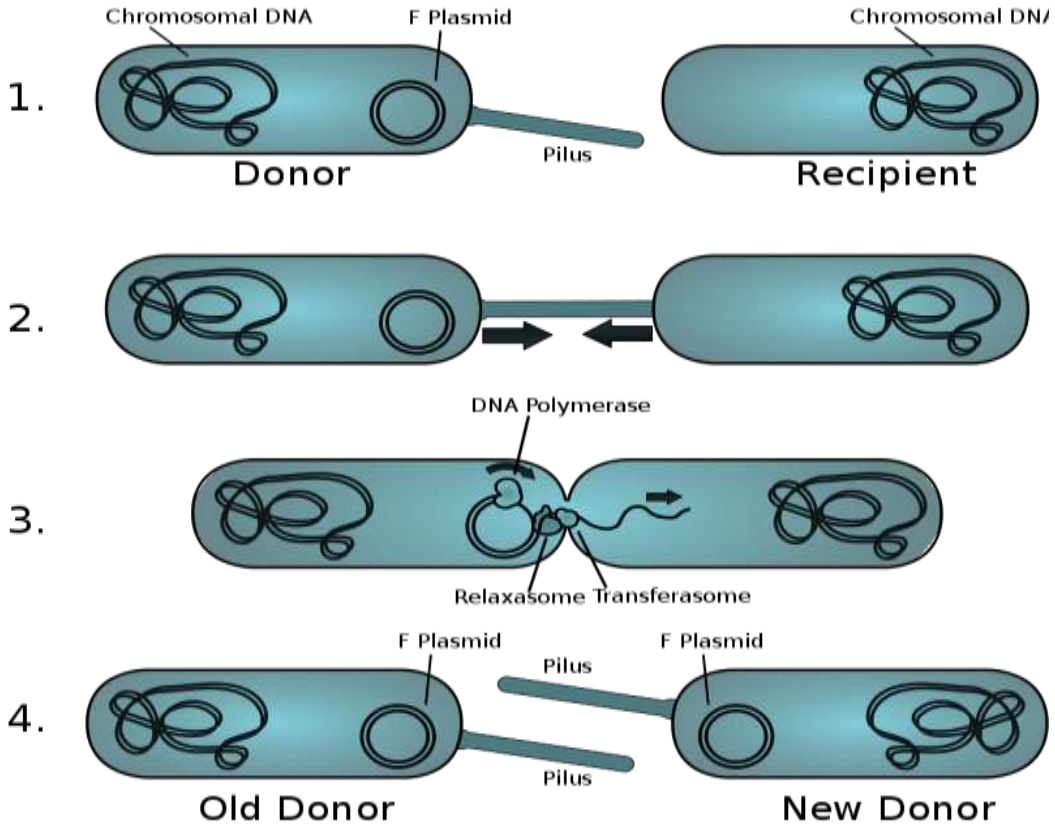
Not all plasmids are capable of achieving this transfer to another cell unaided; those that can are known as conjugative plasmids. In some cases a conjugative plasmid is able to promote the transfer of (mobilize) a second otherwise nonconjugative plasmid from the same donor cell. This does not happen by chance and not all non-conjugative plasmids can be mobilized.

In order to understand mobilization the plasmid ColEI can be taken as an example . Mobilization involves the mob gene, which encodes a specific nuclease, and the bom site (¼oriT, the origin of transfer), where the Mob nuclease makes a nick in the DNA. ColE1 has the genes needed for DNA transfer but it does not carry the genes required for mating-pair formation. The presence of another (conjugative) plasmid enables the donor to form mating pairs with the recipient cell and ColE1 can then use its own machinery to carry out the DNA transfer.

Some plasmids which can be mobilized do not carry a *mob* gene. Mobilization then depends on the ability of the Mob nuclease of the conjugative plasmid to recognize the bom site on the plasmid to be mobilized. This only works if the two plasmids are closely related. On the other hand, the bom site is essential for mobilization. This is an important factor in genetic modification as removal of the bom site from a plasmid vector ensures that the modified plasmids cannot be transferred to other bacterial strains . However, some types of plasmids can also promote transfer of chromosomal DNA. The first of these to be discovered, and the best known, is the F (fertility) plasmid of *E. coli*, but similar systems exist in other species, notably *Pseudomonas aeruginosa*. However, in many cases chromosomal transfer occurs without any stable association with the plasmid, possibly by a mechanism analogous to mobilization of a non-conjugative plasmid.

When a plasmid is transferred from one cell to another by conjugation, the complete plasmid is transferred. In contrast, chromosomal transfer does not involve a complete intact copy of the chromosome. One reason for this is the time required for transfer. The process is less efficient than normal DNA replication and transfer of the whole chromosome would take about 100 min (in *E. coli*). The mating pair very rarely remains together this long. In contrast, a plasmid of say 40 kb is equivalent to 1 per cent of the length of the chromosome, thus the transfer of the plasmid would be expected to be completed in 1 min.

DNA synthesis by the rolling circle mechanism replaces the transferred strand in the donor, while the complementary DNA strand is made in the recipient. Therefore, at the end of the process, both donor and recipient possess completely formed plasmids. For transfer of the F plasmid then, an F-containing cell, which is designated F+, can mate with a cell lacking the plasmid, designated F-, to yield two F+ cells.



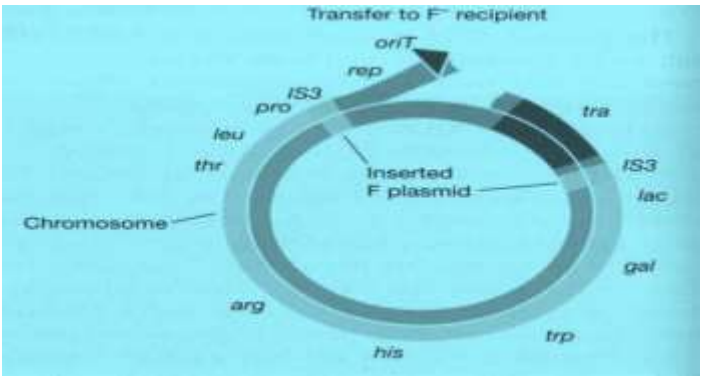
Schematic drawing of bacterial conjugation[Czura, Microbial Genetics,Chapter 8)

The F plasmid is an episome, a plasmid that can integrate into the host chromosome . When the F plasmid is integrated into the chromosome, the chromosome becomes mobilized and can lead to transfer of chromosomal genes. Following genetic recombination between donor and recipient, lateral gene transfer by this mechanism can be very extensive. Cells possessing an unintegrated F plasmid are called F+. Those that have a chromosome-integrated F plasmid are called Hfr (for high frequency of recombination).

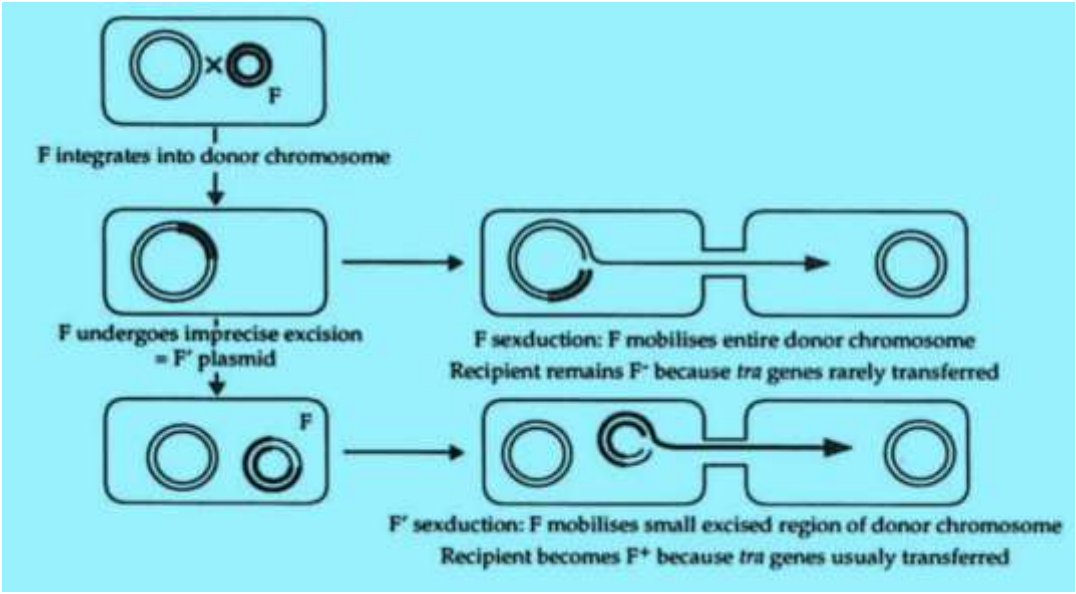
High-frequency recombination cell

A high-frequency recombination cell (also called an Hfr strain) is a bacterium with a conjugative plasmid (often the F-factor) integrated into its genomic DNA. The Hfr strain was first characterized by Luca Cavalli-Sforza. Unlike a normal F^+ cell, Hfr strains will, upon conjugation with a F^- cell, attempt to transfer their entire DNA through the mating bridge, not to be confused with the pilus. This occurs because the F factor has integrated itself via an insertion point in the bacterial chromosome. Due to the F factor's inherent tendency to transfer itself during conjugation, the rest of the bacterial genome is dragged along with it, thus making such cells very useful and interesting in terms of studying gene linkage and recombination. Because the genome's rate of transfer through the mating bridge is constant, molecular biologists and geneticists can use Hfr strain of bacteria (often *E. coli*) to study genetic linkage and map the chromosome. The procedure commonly used for this is called interrupted mating. An important breakthrough came when Luca Cavalli-Sforza discovered a derivative of an F^+ strain. On crossing with F^- strains this new strain produced 1000 times as many recombinants for genetic markers as did a normal F^+ strain. Cavalli-Sforza designated this derivative an Hfr strain to indicate a high frequency of recombination. In Hfr × F^- crosses, virtually none of the F^- parents were converted into F^+ or into Hfr. This result is in contrast with $F^+ \times F^-$ crosses, where infectious transfer of F results in a large proportion of the F^- parents being converted into F^+. It became apparent that an Hfr strain results from the integration of the F factor into the chromosome.

Now, during conjugation between an Hfr cell and a F^- cell a part of the chromosome is transferred with F. Random breakage interrupts the transfer before the entire chromosome is transferred. The chromosomal fragment can then recombine with the recipient chromosome.

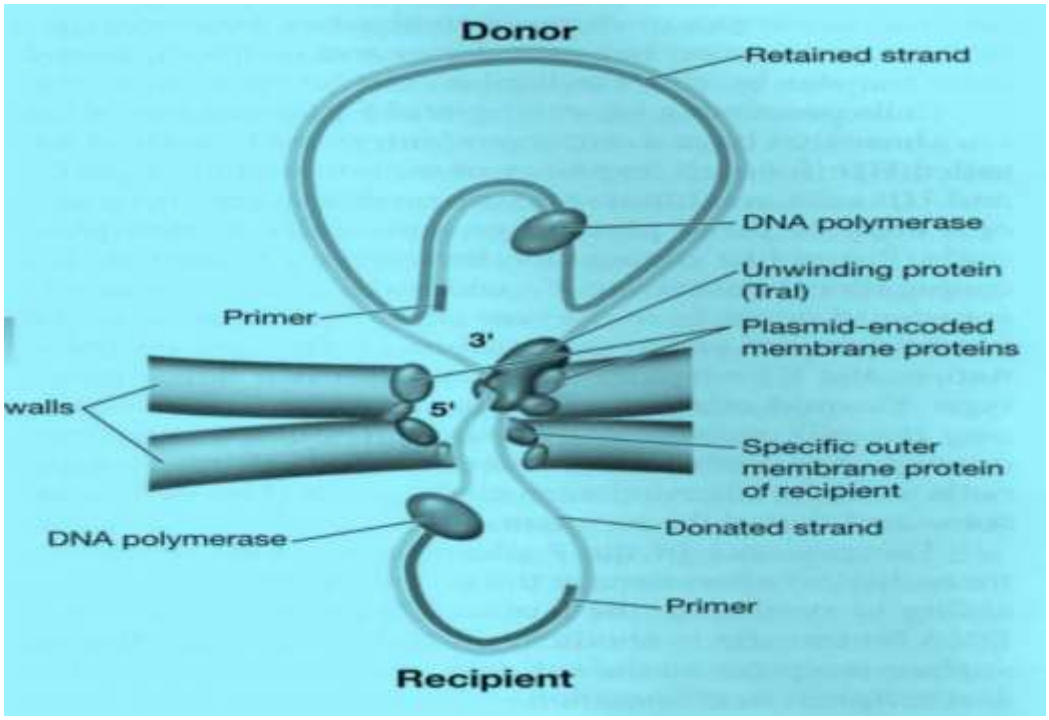


Breakage of the Hfr chromosome at the origin of transfer and the beginning of DNA transfer to the recipient.



F sexduction :F mobilizes small excised region of donor hromosome.

F plasmids containing chromosomal genes are called F' *(Fprime) plasmids.* F' plasmids differ from normal F plasmids in that they contain identifiable chromosomal genes, and they transfer these genes at high frequency to recipients. F' -mediated transfer resembles specialized transduction in that only a restricted group of chromosomal genes can be transferred. Transferring a known F' into a recipient allows one to establish diploids (two copies of each gene) for a limited region of the chromosome. Such partial diploids are called *merodiploids.*



Details of the replication and transfer process.

Conjugation in other bacteria

Many Gram-positive species, ranging from Streptomyces to Enterococcus, also possess plasmids that are transmissible by conjugation and in many cases the mechanism of DNA transfer is quite similar to that described above. In general, the number of genes required for conjugative transfer, in some cases as few as five genes, is very much less than in Gram-negative bacteria where 20 or more genes are needed. Conjugative plasmids in Gram-positive bacteria can therefore be considerably smaller. One reason for a smaller number of genes being required is that there seems to be no need for production of a pilus. This is probably, at least in part, a reflection of the different cell-wall architecture in Gram-positive bacteria which lack the outer membrane characteristic of the Gram-negatives. One group of Gram-positive bacteria where conjugation systems have been studied in detail are the enterococci, principally *Enterococcus faecalis*. Some strains of *E. faecalis* secrete diffusible peptides that have a pheromone-like action that can stimulate the expression of the transfer (*tra*) genes of a specific plasmid in a neighbouring cell. Note that, rather surprisingly, it is the recipient cell that produces the pheromones. The donor cell, carrying the plasmid, has a plasmid encoded receptor on the cell surface to which the pheromone binds. Different types of plasmid code for different receptors and are therefore stimulated by different pheromones. However the recipient produces a range of pheromones and is therefore capable of mating with cells carrying different plasmids.

After the pheromone has bound to the cell-surface receptor it is transported into the cytoplasm, by a specific transport protein, where it interacts with a protein called TraA. This protein is a repressor of the *tra* genes on the plasmid and the binding of the peptide to it relieves that repression, thus stimulating expression of the tra genes. One result is the formation of aggregation products which cause the formation of a mating aggregate containing donor and recipient cells bound together. A further consequence of expression of the tra genes is stimulation of the events needed for transfer of the plasmid which occurs by a mechanism similar to that described previously.

One advantage of this system is that the cells containing the plasmid do not express the genes needed for plasmid transfer unless there is a suitable recipient in the vicinity. Not only does this reduce the metabolic load on the cell but it also means that they are not expressing surface antigens (such as conjugative pili) that could be recognized by the host immune system.