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, conjugation is a mechanism of horizontal gene transfer as are transformation and transduction although these two other mechanisms do not involve cell-to-cell contact.

Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating since it involves the exchange of genetic material. During conjugation the *donor* cell provides a conjugative or mobilizable genetic element that is most often a plasmid or transposon. Most conjugative plasmids have systems ensuring that the *recipient* cell does not already contain a similar element.

The genetic information transferred is often beneficial to the recipient. Benefits may include antibiotic resistance, xenobiotic tolerance or the ability to use new metabolites. Such beneficial plasmids may be considered bacterial endosymbionts. Other elements, however, may be viewed as bacterial parasites and conjugation as a mechanism evolved by them to allow for their spread.

Mechanism

The prototypical conjugative plasmid is the **F-plasmid**, or F-factor. The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome by homologous recombination) with a length of about 100 kb. It carries its own origin of replication, the *oriV*, and an origin of transfer, or *oriT*. There can only be one copy of the F-plasmid in a given bacterium, either free or integrated, and bacteria that possess a copy are called *F-positive* or *F-plus* (denoted F^+). Cells that lack F plasmids are called *F-negative* or *F-minus* (F^-) and as such can function as recipient cells.

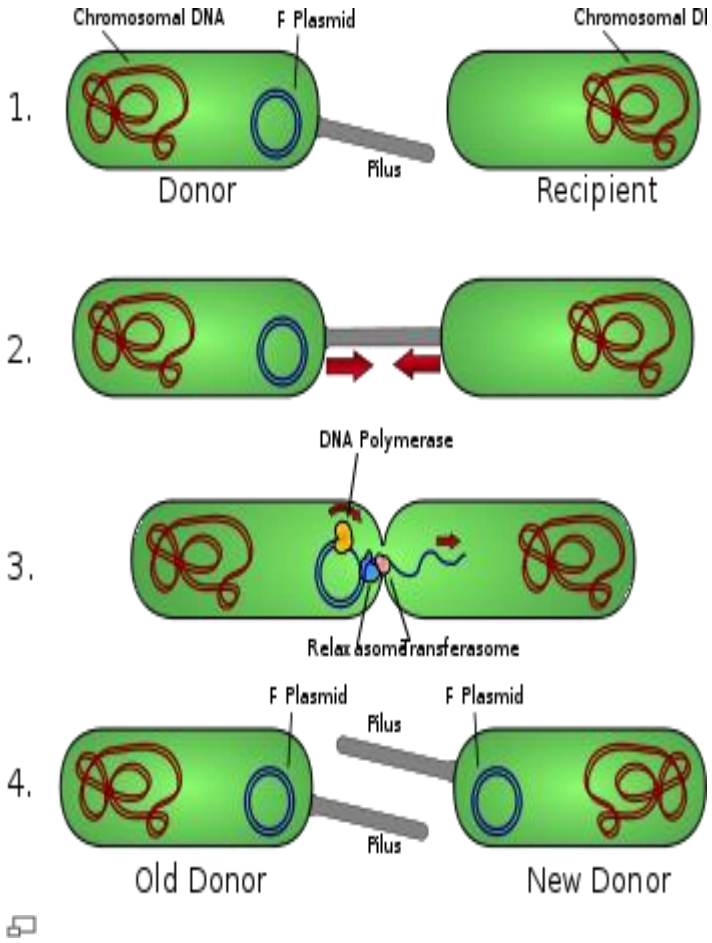


Fig.1. Bacterial conjugation. Conjugation diagram 1- Donor cell produces pilus. 2- Pilus attaches to recipient cell and brings the two cells together. 3- The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell. 4- Both cells synthesize a complementary strand to produce a double stranded circular plasmid and also reproduce pili; both cells are now viable donors.

Among other genetic information the F-plasmid carries a *tra* and *trb* locus, which together are about 33 kb long and consist of about 40 genes. The *tra* locus includes the *pilin* gene and regulatory genes, which together form pili on the cell surface. The locus also includes the genes for the proteins that attach themselves to the surface of F^- bacteria and initiate conjugation. Though there is some debate on the exact mechanism of conjugation it seems that the pili are not the structures through which DNA exchange occurs. This has been shown in experiments where the pilus are allowed to make contact, but then are denatured with SDS and yet DNA transformation still proceeds. Several proteins coded for in the *tra* or *trb* locus seem to open a channel between the bacteria and it is thought that the traD enzyme, located at the base of the pilus, initiates membrane fusion.

When conjugation is initiated by a signal the **relaxase** enzyme creates a nick in one of the strands of the conjugative plasmid at the *oriT*. Relaxase may work alone or in a complex of over a dozen proteins known collectively as a **relaxosome**. In the F-plasmid system the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM and the integrated host factor IHF. The nicked strand, or *T-strand*, is then unwound from the unbroken strand and transferred to the recipient cell in a 5'-terminus to 3'-terminus direction. The remaining strand is replicated either independent of conjugative action (vegetative replication beginning at the *oriV*) or in concert with conjugation (conjugative replication similar to the rolling circle replication of lambda phage). Conjugative replication may require a second nick before successful transfer can occur. A recent report claims to have inhibited conjugation with chemicals that mimic an intermediate step of this second nicking event.

If the F-plasmid that is transferred has previously been integrated into the donor's genome (producing an Hfr strain ["High Frequency of Recombination"]) some of the donor's chromosomal DNA may also be transferred with the plasmid DNA. The amount of chromosomal DNA that is transferred depends on how long the two conjugating bacteria remain in contact. In common laboratory strains of *E. coli* the transfer of the entire bacterial chromosome takes about 100 minutes. The transferred DNA can then be integrated into the recipient genome via homologous recombination.

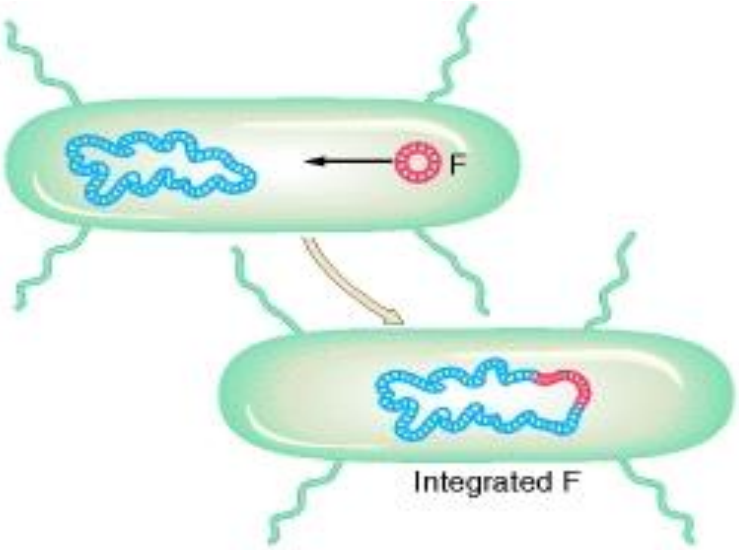
A cell culture that contains in its population cells with non-integrated F-plasmids usually also contains a few cells that have accidentally integrated their

plasmids. It is these cells that are responsible for the low-frequency chromosomal gene transfers that occur in such cultures. Some strains of bacteria with an integrated F-plasmid can be isolated and grown in pure culture. Because such strains transfer chromosomal genes very efficiently they are called **Hfr** (**h**igh **f**requency of **r**ecombination). The *E. coli* genome was originally mapped by interrupted mating experiments in which various Hfr cells in the process of conjugation were sheared from recipients after less than 100 minutes (initially using a Waring blender). The genes that were transferred were then investigated.

Genetic Engineering Applications

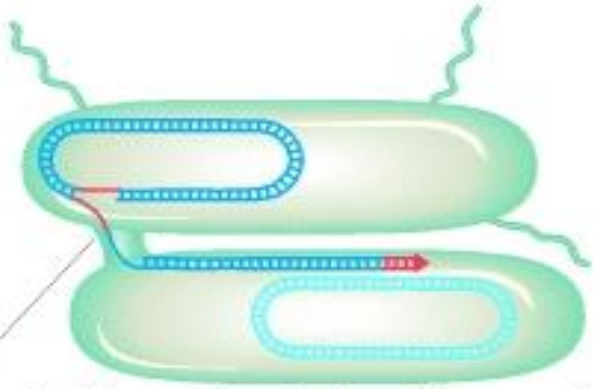
Conjugation is a convenient means for transferring genetic material to a variety of targets. In laboratories successful transfers have been reported from bacteria to yeast, plants, mammalian cells and isolated mammalian mitochondria. Conjugation has advantages over other forms of genetic transfer including minimal disruption of the target's cellular envelope and the ability to transfer relatively large amounts of genetic material.

Hfr:

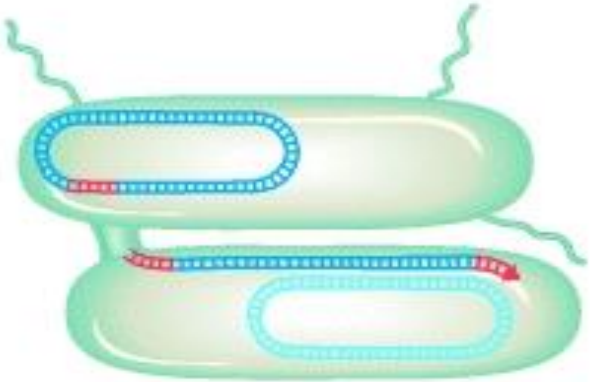


F is integrated into the host chromosome

(a)



A single strand of F is transferred, along with a copy of part of the host chromosome, to a recipient cell, where a second strand is synthesized.



A copy of the host chromosome with F integrated (both generated by replication) remains in the donor cell after replication of the remaining single strand.

(b)

Fig.2 The transfer of *E. coli* chromosomal markers mediated by F. (a) Occasionally, the independent F factor combines with the *E. coli* chromosome. (b) When the integrated F transfers to another *E. coli* cell during conjugation, it carries along any *E. coli* DNA

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^+ × F^- crosses, where infectious transfer of F results in a large proportion of the F^- parents being converted into F^+. Figure 2 portrays this concept. It became apparent that an Hfr strain results from the integration of the F factor into the chromosome, as pictured in Figure 2a.

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. Clearly, the low level of chromosomal marker transfer observed by Lederberg and Tatum (see Figure 2) in an F^+ × F^- cross can be explained by the presence of rare Hfr cells in the population. When these cells are isolated and purified, as first done by Cavalli, they now transfer chromosomal markers at a high frequency, because every cell is an Hfr.

Discovery of the fertility factor (F)

In 1953, William Hayes determined that genetic transfer occurred in one direction in the above types of crosses. Therefore, the transfer of genetic material in *E. coli* is not reciprocal. One cell acts as donor, and the other cell acts as the recipient. This kind of unidirectional transfer of genes was originally compared to a sexual difference, with the donor being termed “male” and the recipient “female.” However, this type of gene transfer is not true sexual reproduction. In **bacterial gene transfer,** one organism receives genetic information from a **donor;** the **recipient** is changed by that information. In **sexual reproduction,** two organisms donate equally (or nearly so) to the

formation of a new organism, but only in exceptional cases is either of the donors changed.