Gene Transfer

We now know that bacteria do **exchange genetic information**, not only in the **laboratory** but also in **nature**. There are **three fundamentally distinct mechanisms (Horizontal Gene Transfer**) (HGT) by which such genetic transfer can occur.

(1) Transformation, in which a cell takes up isolated DNA molecules from the medium surrounding it.

(2) Conjugation, which involves the direct transfer of DNA from one cell to another.

(3) **Transduction** in which the transfer is mediated by bacterial viruses (bacteriophages).

Not all bacterial species exhibit all of these modes of genetic transfer.

Conjugation Process

- Is most readily demonstrated in **Gram-negative** bacteria but does occur in some **Gram-positive** genera such as ***Streptomyces*** and ***Streptococcus***.
- **These mechanisms** differ from true sexual reproduction in two **main respects**: there is **no link with reproduction** and the **genetic contributio**n from the parents is **unequal**. The **parents** are thus referred to as **donor** and **recipient cells**; the **recombinant progeny** resemble the original recipient strain in most characteristics.
- **Conjugation** is the **direct transmission of DNA** from one bacterial cell to another. In most cases, this involves the **transfer of plasmid DNA**, although with some organisms **chromosomal transfer** can also occur.

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 (Figure 1). 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** is used by some bacteria to deliver protein toxins directly into host cells.

Other mechanisms of conjugation that are important in Gram-positive bacteria.

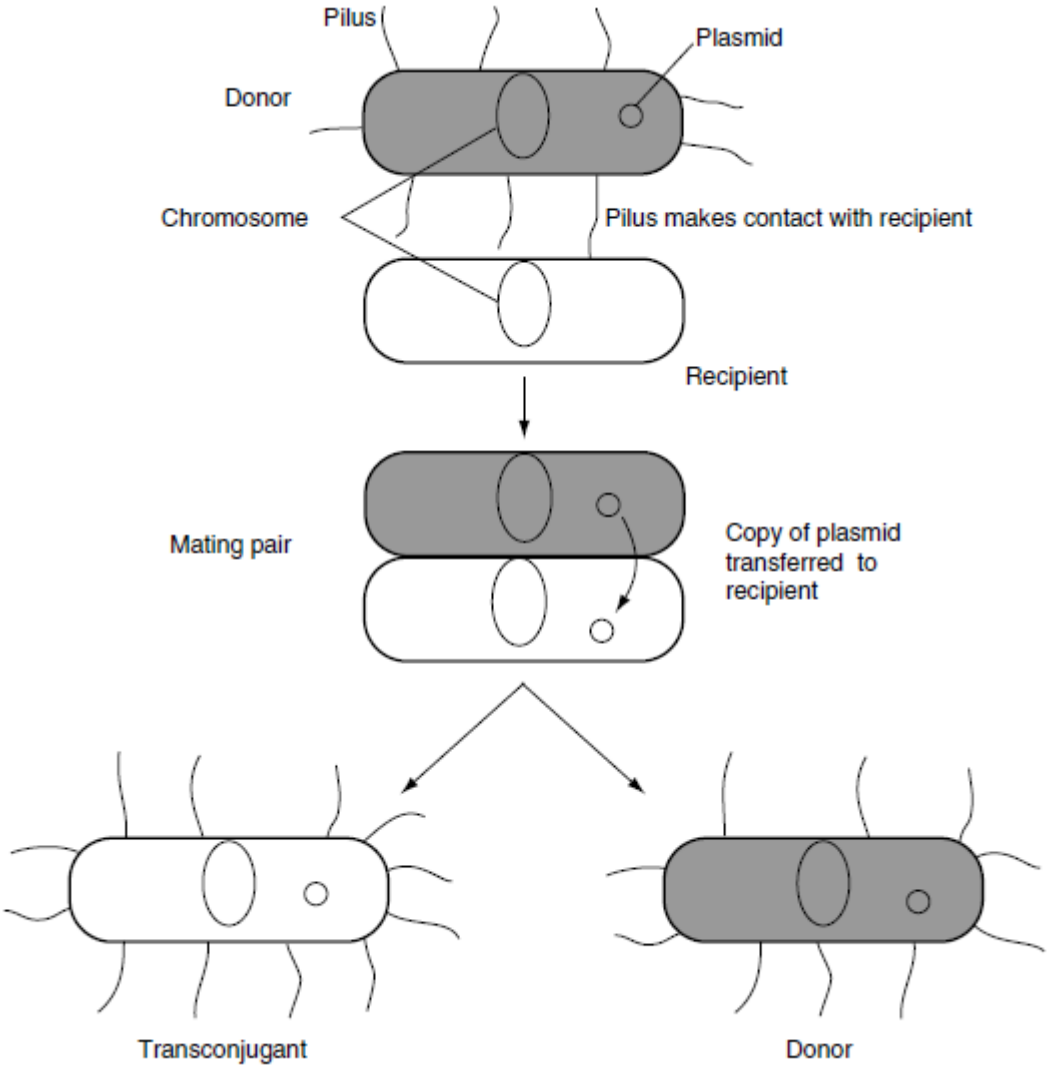

Figure 1. Transfer of DNA by conjugation

Transfer of DNA

- The transfer of plasmid DNA from the donor to the recipient (Figur2) is initiated by a protein that makes a single-strand break (specific site nick) 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 t analogous to replicating single-stranded plasmids 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.

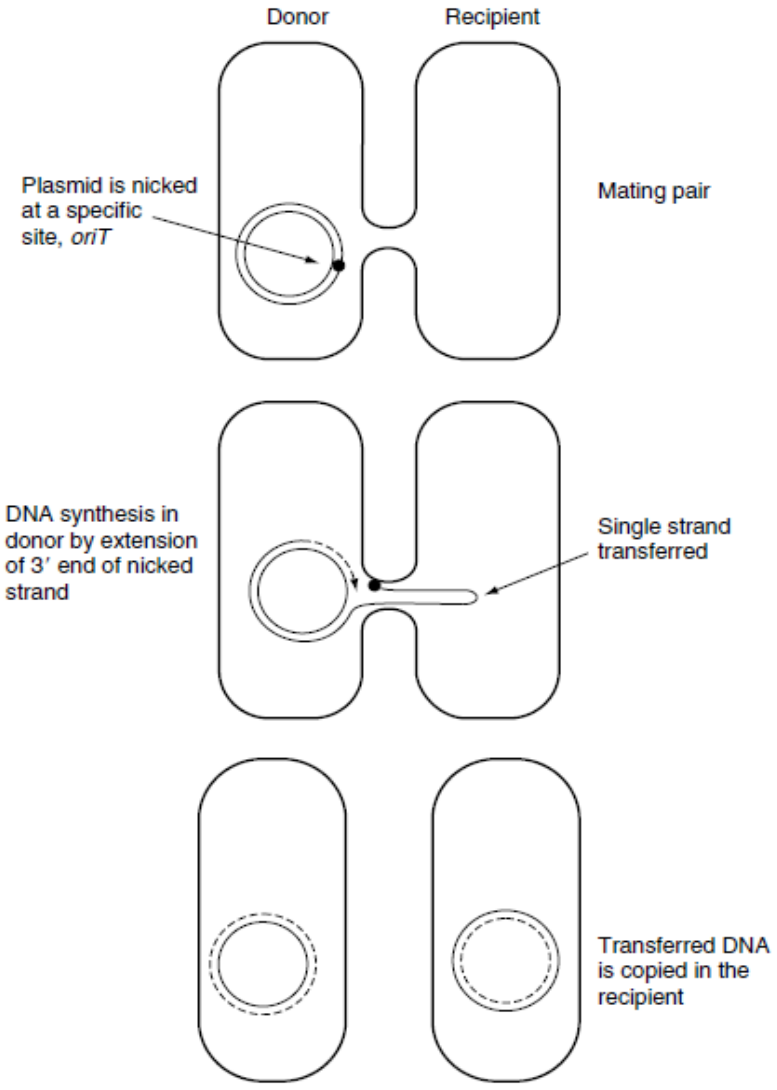

Figure 2. Mechanism of plasmid DNA transfer by conjugation. For clarity, only the plasmid is shown

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 **non-conjugative 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** (oriT, 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.

The F plasmid

- The donor strains carry the **F plasmid** (F)**('male')** while the recipients are **F^-**('**female'**). 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'.**

Hfr strains

- These **Hfr (High Frequency of Recombination**) strains arise by integration of the F plasmid into the bacterial chromosome.
- An additional characteristic of an Hfr strain is that chromosomal transfer starts from a defined point and proceeds in a specific direction.
- The origin of transfer is determined by the **site of insertion of the F plasmid** and the direction is governed by the orientation of the inserted plasmid.
- Transfer is thus initiated from the **oriT site** on the integrated plasmid but now results in the transfer of a copy of the bacterial chromosome rather than just the plasmid.
- **An F donor**, in contrast, transfers genes in a more or less random manner, since transfer does not start from a defined point on the chromosome. The combination of the partial transfer of chromosomal DNA with the ordered transfer of genes made conjugation an important tool in the mapping of bacterial chromosomes.

Integration and excision of F:

- Formation of F' plasmids Integration of the F plasmid occurs by recombination between a sequence on the plasmid and a chromosomal site.
- Integration is reversible since recombination between sites at the ends of the integrated plasmid will lead to its excision from the chromosome as an independent circular molecule.
- However, it is possible for this **excision to occur inaccurately**, i.e. recombination occurs at a different site. If this happens, the resulting plasmid will have incorporated a small amount of bacterial DNA. This forms what is known as an **F' (F-prime)** plasmid.
- Formation of an **F'lac plasmid**, where the recombination event leading to excision has occurred at a site beyond the lac operon, rather than between the sites flanking the **integrated F plasmid**.
- **Before** the advent of **gene cloning, F' plasmids** were useful in a number of ways, including the isolation of specific genes and their transfer to other host strains. This enabled the creation of **partial diploids**, i.e. strains with one copy of a specific gene on the plasmid in addition to the chromosomal copy.

Conjugation in other bacteria

- The above description of conjugation applies mainly to **Gram-negative bacteria** such as ***E. coli*** and *Pseudomonas*. 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.
- However, there are substantial differences in other respects. 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 the **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 **Tra A.**
- 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 the transfer of the plasmid which occurs by a mechanism similar to that described previously.