**Lecture (7) Microbial Genetics المرحلة الثالثة / احياء مجهرية**

**Conjugation in other bacteria**

**1)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.

The enterococci and their plasmids are of special importance, because these organisms are important hospital- acquired (nosocomial) pathogens. Their diverse plasmids carry both genes that enhance virulence and genes that confer resistance to multiple antibiotics. The enterococcal plasmids can also transfer their genes to other gram-positive bacteria, including the very dangerous pathogen *Staphylococcus aureus*. This is because *S.* *aureus* can produce pheromones to attract enterococcal plasmids .

This type of transfer is of particular medical importance because enterococcal plasmids can confer resistance to vancomycin, which is often a “last-resort” antibiotic in the treatment of *S. aureus* infections.



**Simplified model of regulation of a conjugative plasmid in *Enterococcus*. In**

**the absence of a recipient, the transfer genes are switched off by TraA. The recipient**

**produces a peptide, cAD1, which inactivates TraA, allowing conjugation to proceed.**

The donorcell contains a plasmid-encoded membrane protein, TraC. Binding of cAD1

to TraC is followed by transport of cAD1 across the cell wall. Once inside

the donor cell, cAD1 will bind to the TraA repressor and inactivate it, thus

allowing expression of the transfer genes. One of the products, aggregation

substance (Asa) coats the outside of the donor cell, resulting in the formation

of a mating aggregate with donor and recipient cells bound together. Plasmid

transfer then ensues, by a mechanism similar to that described previously.

Conjugative transposons *E. faecalis* also provides an example of an exception to the general rule that conjugation is plasmid-mediated. Some strains of *E. faecalis* contain a transposon known as Tn916. Transposons are able to move from one DNA site to another. What sets Tn916 apart from other transposons is its ability to transfer from one cell to another by conjugation.

Conjugative transposons such as Tn916 differ from plasmids in that they are

replicated and inherited as part of the chromosome. There is no stable independently replicating form as there is with a plasmid. However, closer inspection of the method of transfer

 In particular, Tn916 contains a origin of transfer (oriT) which is quite similar to that found in many plasmids. The first step in transfer is the excision of the transposon from the chromosome, using transposon-encoded

enzymes (Int and Xis) which are related to those responsible for the integration and excision of bacteriophage lambda . This produces a circular molecule that resembles a plasmid in all but one vital feature – it does not have an origin of replication so is unable to be copied in the normal way. However since it does have an oriT site and carries the *tra* genes needed for conjugal transfer, it can be transferred to a recipient cell.

 transfer of Tn916 involves single-stranded DNA synthesis initiated at oriT and transfer of the displaced strand to the recipient. The transferred single strand is then circularized and converted to a double-stranded circular form which is inserted randomly into the recipient chromosome by the action of the integrase.

Transfer would start from oriT and would have to work right round the chromosome before reaching the rest of the transposon. This does not seem to happen. The reason is that the promoter for expression of the tra genes is found towards the left-hand end of the transposon and faces away from the *tra* genes.

In the integrated linear form the tra genes will not be expressed. However, when the transposon is excised from the chromosome and circularized, this brings the promoter into the correct position and orientation for transcription of the *tra* genes. They will therefore be expressed from the circular intermediate, but not from the integrated form. This ensures that the transfer system will only be activated after excision has occurred.

Tn916 is the prototype of a family of related conjugative transposons that are especially widespread in Gram-positive cocci, although related elements also occur in Gram-negative bacteria (e.g. Bacteroides). For many of these elements, including Tn916, conjugative transmission is promiscuous in that they can transfer to other species or genera. It can be assumed therefore that conjugative transposons have played a significant role in the dissemination of genetic material, including antibiotic resistance genes, throughout the bacterial kingdom. In particular. many of these transposons, including Tn916, carry a tetracycline resistance gene (*tet*M) which is found in a wide range of bacterial species, suggesting that they have played a role in the dispersal of this particular gene. ****

**Transfer of the conjugative transposon Tn*916*. The transposon is excised from**

**the chromosome, using the Int and Xis enzymes, and circularized. This enables the *tra***

**genes to be expressed, and conjugative transfer is initiated from *oriT*. In the recipient,**

**the transferred DNA is circularized, the second strand is made and the transposon is**

**integrated into the chromosome.**

**2) Conjugation in *Mycobacteria smegmatis***, like conjugation in *E. coli*, requires stable and extended contact between a donor and a recipient strain, is DNase resistant, and the transferred DNA is incorporated into the recipient chromosome by homologous recombination. However, unlike *E. coli* Hfr conjugation, mycobacterial conjugation is chromosome rather than plasmid based. Furthermore, in contrast to *E. coli* Hfr conjugation, in *M. smegmatis* all regions of the chromosome are transferred with comparable efficiencies. The lengths of the donor segments vary widely, but have an average length of 44.2kb. Since a mean of 13 tracts are transferred, the average total of transferred DNA per genome is 575kb. This process is referred to as Distributive conjugal transfer. Gray et al. found substantial blending of the parental genomes as a result of conjugation and regarded this blending as reminiscent of that seen in the meiotic products of sexual reproduction.

**3) Bacteria related to the** [**nitrogen fixing**](https://en.wikipedia.org/wiki/Diazotroph)[***Rhizobia***](https://en.wikipedia.org/wiki/Rhizobia) are an interesting case of inter-[kingdom](https://en.wikipedia.org/wiki/Kingdom_%28biology%29) conjugation. For example, the tumor-inducing (Ti) plasmid of [*Agrobacterium*](https://en.wikipedia.org/wiki/Agrobacterium) and the root-tumor inducing (Ri) of *A. rhizogenes* contain genes that are capable of transferring to plant cells. The expression of these genes effectively transforms the plant cells into [opine](https://en.wikipedia.org/wiki/Opines)-producing factories. Opines are used by the bacteria as sources of nitrogen and energy. Infected cells form [crown gall](https://en.wikipedia.org/wiki/Agrobacterium_tumefaciens) or [root tumors](https://en.wikipedia.org/wiki/Agrobacterium_rhizogenes), respectively. The Ti and Ri plasmids are thus [endosymbionts](https://en.wikipedia.org/wiki/Endosymbiont) of the bacteria, which are in turn endosymbionts (or parasites) of the infected plant.

The Ti and Ri plasmids can also be transferred between bacteria using a system (the *tra*, or [transfer, operon](https://en.wikipedia.org/wiki/Transfer_gene)) that is different and independent of the system used for inter-kingdom transfer (the *vir*, or [virulence](https://en.wikipedia.org/wiki/Virulence), operon). Such transfers create virulent strains from previously a virulent strains.

4) Several conjugative plasmids have also been found in *Sulfolobus,* a genus of ***Archaea****.* Little is known about conjugation in *Sulfolobus,*although it is known that cell pairing occurs before plasmid transfer and that transfer is unidirectional. However, with one exception, the genes involved seem to have little similarity to those in gram-negative *Bacteria.* The exception is a gene similar to *traG,* whose protein product in F plasmid-mediated conjugation seems to be involved in stabilizing mating pairs. It thus seems likely that the mechanism of conjugation in *Archaea* is quite different from that in *Bacteria.*

* General Note

The plasmid genes whose products are involved in transfer are called the *tra* genes. The site on the plasmid DNA at which transfer initiates is called the origin of transfer (*oriT*). The *tra* genes can be divided into two groups: those whose products are involved in mating pair formation (Mpf) and those whose products are involved in processing the plasmid DNA for transfer (Dtr).

* The Mpf component includes a sex pilus that extrudes from the cell and holds mating cells together. The Mpf system also includes the channel in the membrane through which DNA and proteins pass, as well as a coupling protein that lies on the channel, docks the relaxase of the Dtr component, and translocates DNA through the channel.
* The Dtr component includes the relaxase, which makes a nick within the *oriT* sequence and rejoins the ends of the plasmid in the recipient cell. The relaxase also often contains a helicase activity, which separates the strands of DNA during transfer. The Dtr component also includes proteins that bind to the *oriT* sequence to form the multiprotein complex called the relaxosome and a primase that primes

replication in the recipient cell and is sometimes transferred along with the DNA.