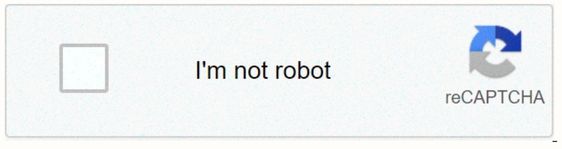

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Bacterial conjugation notes pdf

4 March 2018 Gaurab Karki Genetics, Source of Microbial Genetics 0;sci.sdsu.edu Conjugation in bacteria is a process where plasmids are transmitted alone or in combination with other DNA elements from one cell to another through a conjugation tube. Conjugation is done by bringing physical contact between cells. The cell that transfers plasmid is called a donor and a cell that receives plasmid is called the recipient. A cell that has received plasmid from a donor cell is called a trans conjugal. The phenomenon of conjugation bacteria was discovered in laderburg and Tatum in 1946. Self-transferable (F-plasmid) plasmid: This plasmid encodes all the functions necessary for their transfer, as well as other DNA elements and mobilized plasmid into the recipient cell. A cell that holds a self-transferable plasmid is called donor cells and other cells, which usually do not own a self-transferable plasmid called a recipient cell. These plasmid contains both the Tra gene and The Ori T sites These plasmid are known as the F-factor or F-plasmid or conjugal plasmid occurring in Pseudomonas, E. coli, Bacillus, streptococcus, Staphyloccus, Streptomyces, etc. 2. Mobilisable plasmid: These plasmid encodes only the function of this transfer to the recipient's cell. the mobiliser's plasmid does not carry on itself. It requires help with self-transferring plasmid to its delivery. Conjugation mechanism: conjugation is caused by 2 genes in self-transmissible plasmid, namely the transmissible gene (Tra gene) and the origin of the transfer (Ori T). I. Tra gene: The Tra gene consists of 2 components-DTR and mpf i. DTR(DNA transfer and replication) component: DTR component to prepare plasmid for transfer. It contains components such as = relaxases, relaxosome complex and primate. Relaxase: Relaxase is a site specific to endonuclease that acts as plasmid on your OriT site. Relaxase also recuclizes plasmid after it is transferred to the recipient cell. Relaxase is transcribed with plasmid into the recipient Relaxosome complex: It consists of a group of proteins clustered around the Slave T site Relaxosome leads to three basic functions–it helps relaxase bind the slave to the site and initiates plasmid transfer, relaxosome interacts with the coupling protein mpf component, which signals to relax when cut plasmid slave t in place, Helicase is a component relaxosome that helps to separate plasmid DNA strands during the displacement and transfer of plasmid. Primase: Primase has no role in replication of replication of the donor plasmid donor cell free 3-OH end created by the nic site acts as a primer for the donor cell. Primase is a trnasfered recipient cell and synthesizes the plot to complete replication of another branch of plasmidd DNA in the recipient cell. ii. Mpf (Mating pair formation) component: The Mpf component holds the donor and recipient cell together, forming the channel through which the DNA is transmitted and the signal DTR component initiate It has 3 components- slit, duct and coupling protein slit: Slit holds the donor and recipient cell together It is a 10nm diameter tubular structure with a central channel projecting out of the cell surface. Pili can be structurally long, thin and flexible and is encoded with F-plasmid in these cells Incompatibility f-plasmid (Inc F) is a long and rigid bilge, which is encoded in pKM101 (Inc N) Short, thick and rigid plasmid is encoded in RP4 (Inc P) Long, thin and flexible pili mediates the conjugation of a cell in a liquid environment Short, thick and rigid pili mediates the conjugation of the cell with fixed solid support (Agar medium) Inc I plasmid (col 1BP9) encodes as long , thin, flexible pili and short, thick and rigid instrument, thus can mediate in both liquid and solid media Channel: Channel is also encoded in the Tra Gene Channel mediating the transfer of DNA from the donor to the recipient cell Lukewarm protein: The connection protein is bound to the channel It signals relaxase, which then initiates the process of DNA transmission The Coupling Protein determines which proteins must be transported to the recipient cell (relaxase and primates) II. A: This is where plasmiddNA transfer initiates donor cells and at the site of rectization of the cell. This is a site that is specifically recognized as relaxase. Any plasmid that owns the Ori T site can be transferred with the help of a self-transferable plasmid Slave T site on a cis-functioning site known as the Ori T site F-plasmid is about 300 bp and contains inverted repeats and AT rich sites. Chromosome transfer by plasmid: Plasmid can also mediate chromosome transfusion because the Ori T site and tra gene are present in plasmid. To transfer the chromosome, plasmid must be integrated with the chromosome. The integrated form of plasmid is called Hfr or High frequency recombination. Plasmid can be integrated into the chromosome through the 2 mechanism. Recombination: plasmid can be re-combined with a chromosome when plasmid and chromosome share common sequences (homologos sequences). Although the sequences of plasmid are unique to the chromosome they share homology in certain insertion sequences. Takeover: plasmid can be added to the end of the chromosome when they are taken over and results in Hfr-levels of bacterial conjugation: The donor cell (F+ cell) produces a sex slot, which is the structure that projects out of the cell and begins contact with the F-(recipient) cell. The slit allows direct contact between the donor and the recipient cells to form a conjugation tube with the F-factor opening of the replication origin (Slave T site). one branch of the F factor is cut off at origin and then enters the 5'end of the 5'end of the 5'end of the recipient's cell. In the final stage of the donor cell and recipient cell, both contain single-stranded DNA F-plasmid Additional strand then synthesized in both donor and recipient cell, now the recipient cell also contains a copy of the F-plasmid and obtain the donor cell. source;sci.sdsu.edu Method Gene transfer Bacterial conjugation is the transfer of genetic material between bacterial cells through direct cell-cell contact or bridge-like connection between two cells. [1] It takes place through the slot. [2] This is a form of reproduction of parasexual bacteria. It is a horizontal gene transfer mechanism, as well as transformation and transductions, although these two other mechanisms do not involve cell and cell contact. [3] Classical E. coli bacterial conjugation is often considered to be the bacterial equivalent of sexual reproduction or ma mate, as it involves the exchange of genetic material. However, this is not sexual breeding, because sexual intercourse does not take place and indeed no new organism has been generated: instead, the existing organism is changed. During classical E. coli conjugation, the donor cell provides a conjugator or mobilized genetic element that is most commonly plasmid or transposon. [4] Most conjugal torpedolasm has systems to ensure that the recipient cell does not already contain a similar element. The genetic information transmitted is often beneficial to the recipient. The benefits may be antibiotic resistance, xenobiotic tolerance or the ability to use new metabolites. [5] Other elements may be harmful and can be seen as bacterial parasites. Conjugation in Escherichia coli by spontaneous zygogenesis[6] and Mycobacterium smegbraat by distributionive conjugal transmission[7] [8] differs from the more thoroughly studied conjugation of classical E. coli, as these cases involve significant mixing of older genomes. History The Process was discovered by Joshua Lederberg and Edward Tatum in 1946. Mechanism Schematic drawing of bacterial conjugation. Conjugation scheme Donor cell produces slit. The slot attaches to the recipient cell and brings the two cells together. Mobile plasmit is nicked and one branch of DNA is then transferred to the recipient's cell. Both cells synthesize an additional branch to produce double stranded circular plasmid and also reproduce the pili; both cells are now a viable donor F-factor. [1] F-plasmid is an episi (plasmid that can integrate itself with a homologosation recombination) of approximately 100 kb. It carries its origin in replication, oriV and origin of transfer, oriT. [4] This bacteria may contain only one copy of the F-plasmid, whether free or integrated, and the bacteria with a copy are called F-positive or F-plus (known as F+). F-plasmid-free cells are called F-negative or F-negative (F−) and can therefore act as recipient cells. Among other genetic information, F-plasmid carries a tra and trb locus, which together are about 33 kb long and consists of about 40 genes. The Tra locus contains the pilin gene and the regulatory genes that together form a bilge on the cell surface. The locus also contains genes of these proteins, which attach to the surface of the F-bacteria and start conjugation. Although there is some discussion of the precise conjugation mechanism, it seems that pili are not the structures through which DNA is exchanged. This is shown in tests where contact is allowed in the slit, but then the SDS is denatured and the DNA transformation continues. Several proteins encoded in the tra or trb locus appear to open the channel between bacteria and it is thought that the traD enzyme, located at the base of the slot, initiates membrane fusion. When conjugation is initiated signal relaxase enzyme creates a nickname in one direction conjugal plasmid slave. Relaxase can work alone or complex with over a dozen proteins known as collectively relaxing. In the F-plasmid system, relaxase enzyme TraI is called and the relaxing part is TraI, TraY, TraM and the integrated host factor IHF. The stranded branch or T branch is then scrolled out of the unbroken branch and moved to the 5'-term recipient chamber in a 3'-terminus direction. The rest of the strands are copied either regardless of the conjugal action (vegetative replication begins with oriV) or in accordance with conjugation (conjugation similar to rolling circle replication lambda phage). Conjugal replication may require another nickname before a successful transfer may occur. A recent report claims to have inhibited conjugation with chemicals that mimic the intermediate step of this second nicking event. [10] 1.Both factor F plasmid and chromosome insertion sequences (yellow) have similar sequences that allow the F factor to insert itself into the cell genome. This is called a homologoher recombination and it creates an Hfr cell (high frequency of recombination). 2.The Hfr cell forms the slots and binds the recipient to the F cell. 3.Nick in one branch of the Hfr cell chromosome is created. 4.DNA begins to be transferred to the Hfr cell's recipient cell while another branch of your chromosome is copied. 5.The slot detaches from the recipient cell and withdraws. The Hfr cell ideally wants to transfer its entire genome to the recipient cell. But because of its great size and inability to maintain contact with the recipient's cell, he can't do that. 6.a. Article 6(a) and Article 6(a) The F-element remains F because the entire F-factor jad couldn't be accepted. Since there was no homologoologist recombination, the transferred DNA enzymes are degraded. [11] (b). In very rare cases, the f-factor is fully transferred and the F cell becomes an Hfr element. [12] If the transferred F-plasmid is previously integrated into the donor genome (producing hfr strain [Recombin high frequency]), some donor chromosome DNA may also be transferred by plasmidd-DNA. [3] The amount of DNA of the spillable chromosome depends on how long these two conjugate bacteria are exposed. In the usual laboratory strains of E. coli, the chromosome for about 100 minutes. The transferred DNA can then be integrated into the recipient's genome through a homologosan recombination. A cell culture that contains its own population cells without integrating F-plasmids also contains some cells that are inadvertently integrated into their plasmids. These are the cells that are responsible for low-frequency chromosomal gene transmissions that occur in such cultures. Some strains of bacteria integrated with F-plasmid can be isolated and grown in a clean culture. Because such strains carry chromosomal genes very effectively, they are called Hfr (high frequency of recombination). The E. coli genome was initially mapped by interrupted mating experiments, during which different Hfr cells were in the conjugation process after less than 100 minutes (initially using a waring blender). The genes transferred were then examined. Since the integration of F-plasmid into the E. coli chromosome is a rare spontaneous occurrence, and because numerous genes that promote DNA transfer are in the plasmid genome rather than in the bacterial genome, it has been argued that conjugal bacterial gene transfer, as it occurs in the E. coli Hfr system, is not an evolutionary adaptation of the bacteriological gene - nor is it likely that ancestral eukaryotic sex is unlikely. [13] Spontaneous zygogenesis in E. coli. [6] Z-mating has complete genetic mixing and unstable diploids are formed, throwing phenotypic haploid cells away, some of which indicate the older phenotype and some are true recombinants. Conjugal transmission in the conjugation of mycobacterials in mycobacterial segmatis, such as conjugation in E. coli, requires stable and extended contact between the donor and the recipient strain, is DNa resistant and transferred DNA is added by the homologoological recombination of the recipientkromosoma. However, unlike Hfr conjugation of E. coli, mycobacterial conjugation is based on chromosome rather than plasmid. [7] [8] Unlike the conjugation of E. coli Hfr, all chromosome regions are transferred to M. smegaatis with a comparable efficiency. Donor segments have very different lengths, but have an average length of 44.2 kb. Since an average of 13 cells are transferred, the average amount of DNA transferred per genome is 575kb. [8] This process is called addingable transfer. [7] Gray et al. [7] as a result of conjugation, the parental genome was found to be a significant mixing and considered this mixing to resemble sexual reproduction in meiotic products. Inter-kingdom transfer of Agrobacterium tumefaciens bile root Carya illinoensis. Bacteria that are involved in nitrogen fixing rhizobia are an interesting case of inter kingdom conjugation. [14] For example, (Ti) Agrobacterium plasmid and A. rhizomes containing root tumour-inducing (Ri) plasmid containing genes capable of being transferred to plant cells. The expression of these genes effectively transforms plant cells into factories producing opisin. In opines, bacteria are used as sources of nitrogen and energy. Infected cells form a crown of bile or root tumors. Ti and Ri plasmids are therefore endosymbiondid bacteria, which is in turn an endosymbione (or parasites) infected plant. Ti and Ri plasmids can also be transferred between bacteria using a system (tra, or transfer, operon), which is a different and independent system used for interbank transmission (vir, or virulence, operon). Such transfers create virulent tensions from previously avirulent strains. Gene technology applications Conjugation is a convenient tool for transferring genetic material to different targets. Successful transfer of bacteria from yeast[15] from plants, mammalian cells[16] from diatoms[18] and isolated mammalian mitochondria have been reported in laboratories. Conjugation has advantages over other forms of genetic transmission, including minimal interference in the target cell envelope and the ability to transfer relatively large amounts of genetic material (see discussion above on E. coli chromosome transfusion). In plant construction, agrobacterium-like conjugation complements other standard vehicles, such as tobacco mosaic virus (TMV). Although TMV is able to infect many plant families these are mainly herbaceous dicots. Agrobacterial conjugation is mainly used for dikotsts, but monocosal recips are not uncommon. 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