MOOC 4, Module 20

Sexual Reproduction in Bacteria-I

Main Body:

The bacteria is represented by a group of prokaryotic organisms which do not reproduce by true sexual means through the production of haploid gametes, followed by their fusion to produce a diploid cell, but they have a unique way sexual reproduction where the genetic material in some form is exchanged between the donor and the recipient cell that results in the increase in the vigour of the recipient cell. The sexual reproduction is of the following types, viz. Conjugation, transformation, transduction and lysogeny.

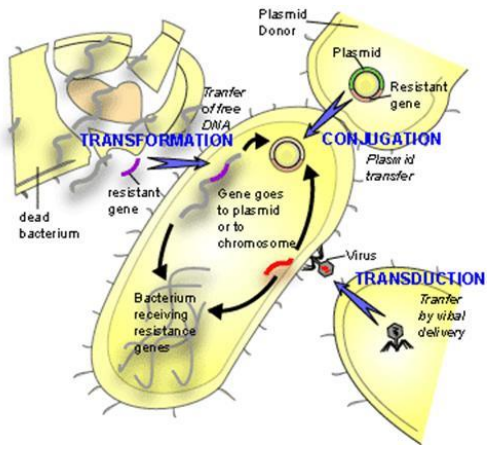

Fig.1: Different types of sexual reproduction in bacteria

Conjugation

Some bacteria are capable of transferring pieces of their genes to other bacteria by coming close to each other, they get interconnected with the help of a conjugation bridge, formed by the pili of the bacteria. This process usually involves a donor strain and a recipient strain. The donor is often called the F+ strain, because it has the F plasmid, while the recipient is called F- strain because it is devoid of the F plasmid or the fertility factor. By this method the F- is converted to F+ and the process is called complete conjugation, it is also called legitimate conjugation, since it involves the male strain or the donor or the F+ strain and the female strain or the recipient strain or the F- strain. If this process is always true, then all the F- will be converted to F+ and there will be no free existing natural F- strain. But in nature, the existence of free living F- strain is possible because the conjugation may be sometimes incomplete in nature, that is before the F plasmid is donated, the two bacterial cells separate. Sometimes the process of conjugation is

illegitimate in nature that is there is attachment of two F- strain, which is a non-productive conjugation and the two cells separate without the exchange of any gene fragment.

Discovery: It wasdiscovered in 1946 by Joshua Lederberg and Edward Tatum. It is considered as a mechanism of horizontal gene transfer. In that way, it is similar to transformation and transduction, only in case of conjugation, there is direct 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.

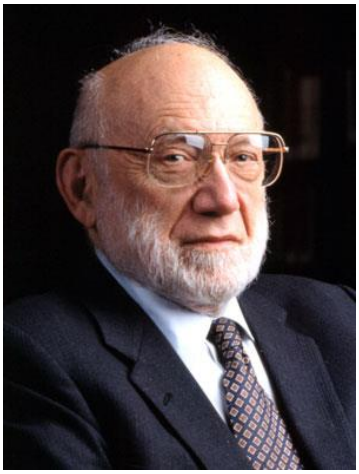

Fig.2: Joshua Lederberg(1925-2008)

Fig.3: Edward Tatum (1909-1975)

Original Experiment: The two strains of *Escherichia coli* with different nutritional requirements were taken. Strain A would grow on a minimal medium only if the medium were

supplemented with methionine and biotin; strain B would grow on a minimal medium only if it were supplemented with threonine, leucine, and thiamine. Thus the strain A was designated as *met*$^{-}$ *bio*$^{-}$ *thr*$^{+}$ *leu*$^{+}$ *thi*$^{+}$ and strain B as *met*$^{+}$ *bio*$^{+}$ *thr*$^{-}$ *leu*$^{-}$ *thi*$^{-}$. The strains A and B are mixed together, and the following observations were made:

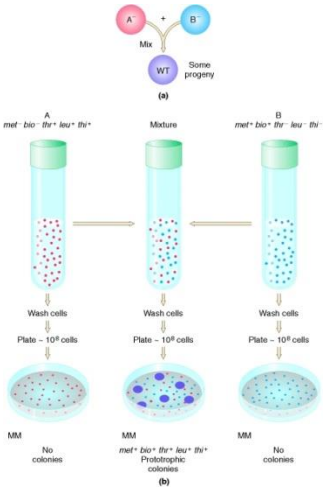

Fig.4: Experimental demonstration by Lederberg and Tatum of genetic recombination between bacterial cells.

Lederberg and Tatum plated bacteria into dishes containing only unsupplemented minimal medium. Some of the dishes were plated only with strain A bacteria, some only with strain B bacteria, and some with a mixture of strain A and strain B bacteria that had been incubated together for several hours in a liquid medium containing all the supplements. No colonies arose on plates containing either strain A or strain B alone, showing that there was no back mutations which restored the ability to grow on unsupplemented minimal medium. However, the plates that received the mixture of the two strains produced growing colonies at a frequency of 1 in every 10,000,000 cells plated (in scientific notation, 1×10^{-7}). This observation suggested that some form of recombination of genes had taken place between the genomes of the two strains to produce prototrophs.

Demonstration of physical contact:

It could be suggested that the cells of the two strains do not really exchange genes but instead leak substances that the other cells can absorb and use for growing. This possibility of "cross feeding" was ruled out by Bernard Davis (1950).

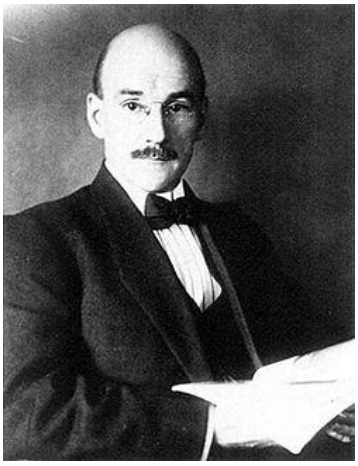

Fig.5: Bernard Davis (1916-1994)

He constructed a U-tube in which the two arms were separated by a fine filter. The pores of the filter were too small to allow bacteria to pass through but large enough to allow easy passage of the fluid medium and any dissolved substances. Strain A was put in one arm; strain B in the other. After the strains had been incubated for a while, Davis tested the content of each arm to see if cells had become able to grow on a minimal medium, and none were found. In other words, physical contact between the two strains was needed for wild-type cells to form. It looked as though some kind of gene transfer had taken place, and genetic recombinants were indeed produced.

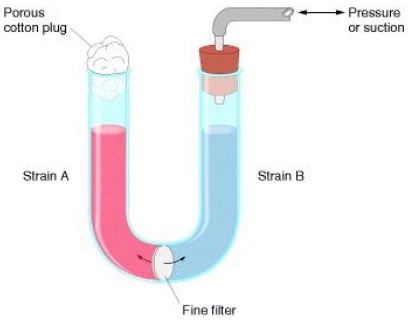

Fig.6: Experiment demonstrating that physical contact between bacterial cells is needed for genetic recombination to take place (Davis, 1950).

Discovery of fertility factor:

In 1953, William Hayes determined that genetic transfer occurred in one direction in bacteria. 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. Thus it is different from eukaryotic reproduction, where both the partners are

equally responsible for the constituent of the zygote.Hayes suggested that donor ability is itself a hereditary state imposed by a **fertility factor (F).**

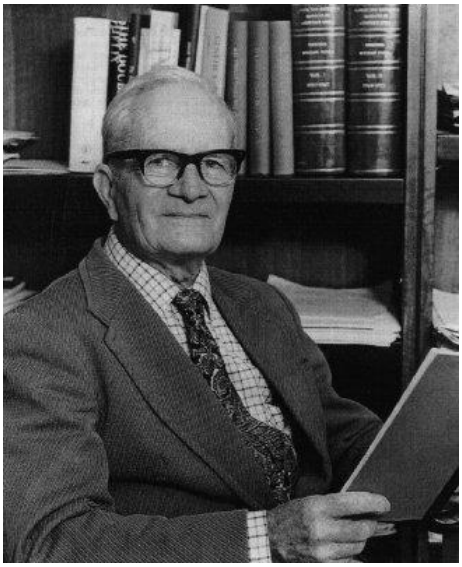

Fig.7: William Hayes (1913-1994)

Description of the F plasmid: 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.

Among other genetic information the F-plasmid carries a *tra* and *trb* locus, which together is about 33 kb long and consist of about 40 genes. The *tra* locus includes the pilin gene and regulatory genes, which together form pilion the cell surface. The locus also includes the genes for the proteins that attach themselves to the surface of F^-bacteria and initiate conjugation. 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.

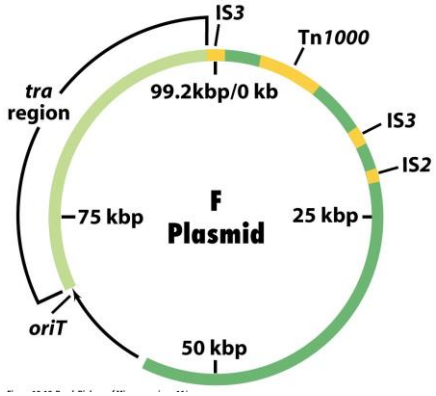

Fig.8: Structure of F plasmid

Steps of conjugation:

1. 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.
2. 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 (like the rolling circle model)
3. Conjugative replication may require a second nick before successful transfer can occur.
4. The pili of the donor draw the recipient closer, allowing the F DNA to pass through a pore into the recipient cell.
5. One strand of the double-stranded F DNA is transferred and then DNAreplication restores the complementary strand in both the donor and the recipient.
6. In common laboratory strains of *E. coli* the transfer of the entire bacterial chromosome takes about 100 minutes

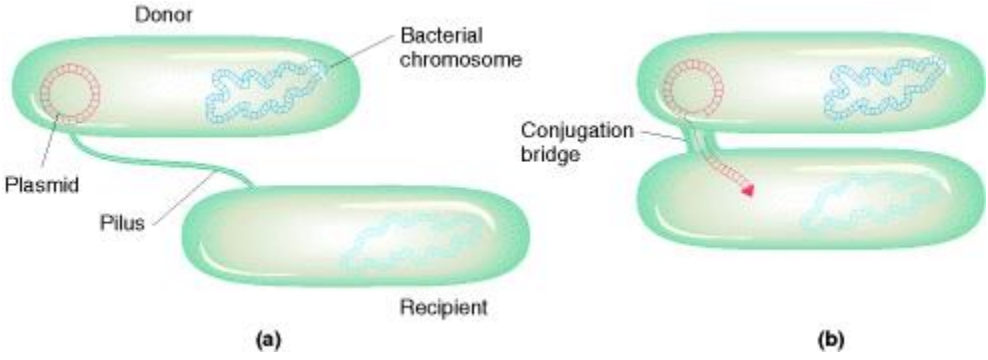

Fig.9: Stages of conjugation in bacteria(a) The pilus pulls two bacteria together. (b) The bridge (essentially a pore) is formed between the two cells and one strand of plasmid DNA passes into the recipient bacterium, finally each single strand becomes double stranded again.

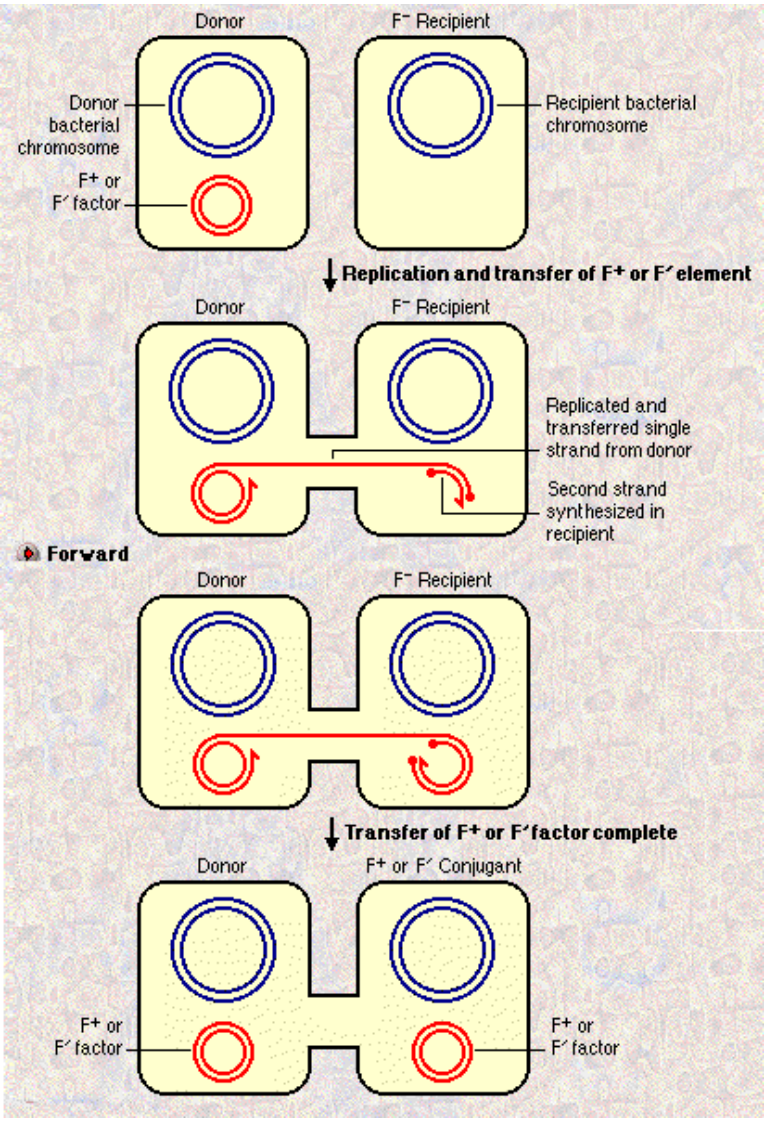

Fig.10: Diagrammatic representation of conjugation

Formation of the Hfr strain:

Luca Cavalli-Sforza (1952) discovered a derivative of an F^+ strain. On crossing with F^- strains this new strain produced 1000 times more recombinantsthan normal F^+ strain. Cavalli-Sforza designated this derivative an Hfr strain to designate high frequency of recombination.

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. This results in the transfer of selectable marker from the Hfr donor to F- recipient. The transferred strand is replicated in the recipient cell, and donor genes may become incorporated in the recipient's chromosome through crossovers, creating a recombinant cell. At a later stage, the transferred fragments of DNA in the recipient are lost in the course of cell division.

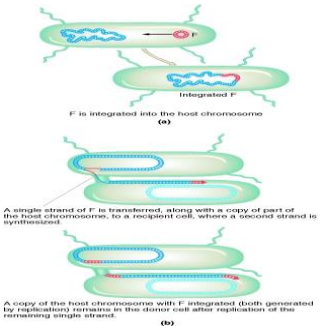

Fig.11: Stages of Hfr conjugation

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 the *E. coli* DNA.

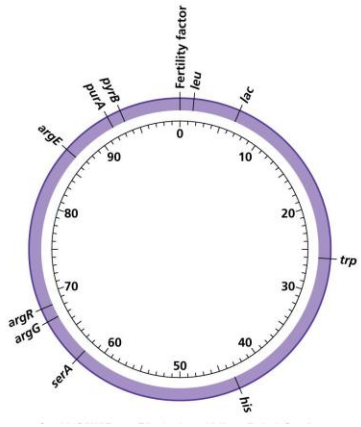

Fig.12: The circular Hfr genome of *E. coli* and divided into individual operons (outside labels) and the number of minutes (inside labels) it takes for each to be transferred during Hfr conjugation.

***E.coli* conjugation cycle:**

The E. coli conjugation cycle may include the following steps:

1. F+a+ produces other F+a+ strain by conjugation.
2. The F+a+ can convert other F+a- strains.
3. It can also produce the Hfr a+ strain.
4. The Hfr a+ strain can conjugate with F-a- strain to produce both F-a+ and F-a- strain.

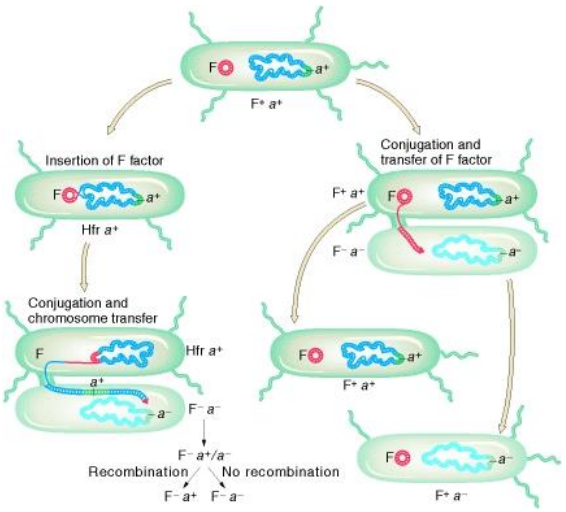

Fig.13: Summary of the various events that take place in the conjugational cycle of *E. coli.*

Discovery of R factors:

Bacterial dysentery is caused by bacteria of the genus *Shigella.* This bacterium initially proved sensitive to a wide array of antibiotics that were used to control the disease. In the Japanese hospitals, however, *Shigella* isolated from patients with dysentery proved to be simultaneously resistant to many of these drugs, including penicillin, tetracycline, sulfanilamide, streptomycin, and chloramphenicol. This multiple-drug-resistance phenotype was inherited as a single genetic package, and it could be transmitted in an infectious manner—not only to other sensitive *Shigella* strains, but also to other related species of bacteria. The vector carrying these resistances from one cell to another proved to be a self-replicating element similar to the F factor. These R factors (for "resistance") are transferred rapidly on cell conjugation, much like the F particle in *E. coli.*

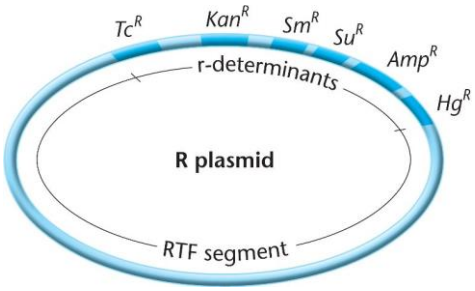

Fig.14: R plasmid, showing resistance transfer factor (RTF), which enables conjugation, and one or more r-determinants like Tc, tetracycline; Kan, kanamycin; Sm, streptomycin; Su, sulfonamide; Amp, ampicillin; and Hg, mercury.

Genetic Determinants Borne by Plasmids:

Characteristic	Plasmid examples
Fertility	F, R1, Col
Bacteriocin production	Col E1
Heavy-metal resistance	R6
Enterotoxin production	Ent
Metabolism of camphor	Cam
Tumorigenicity in plants	Ti (in *Agrobacterium tumefaciens)*

Inter generic transfer of genetic elements:

It is observed in the tumor-inducing (Ti) plasmid of *Agrobacterium* and the root-tumor inducing (Ri) plasmid of *A. rhizogenes* contains genes that are capable of transferring to plant cells. The expression of these genes effectively transforms the plant cells into opine-producing cells. Opines are used by the bacteria as sources of nitrogen and energy. The Ti and Ri plasmids are expressed in the plants via the endosymbiotic bacteria. The Ti and Ri plasmids can also be transferred between bacteria using a system (the *tra*, or transfer, operon) that is different and independent of the system used for inter-kingdom transfer.

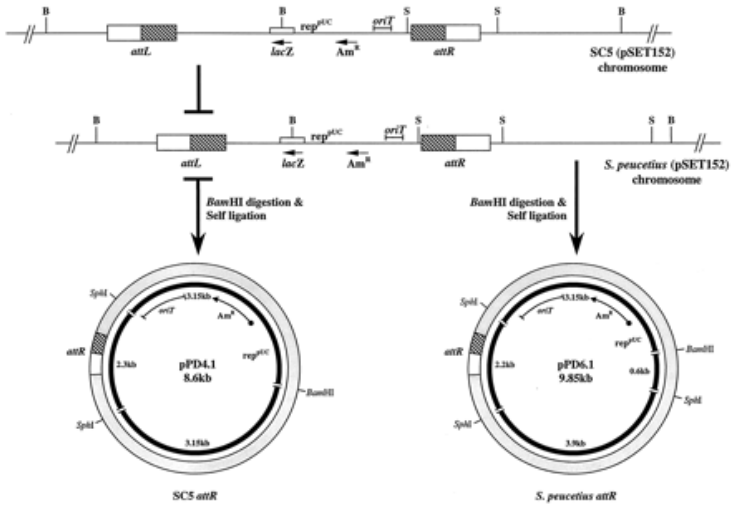

Fig.15: Schematic representation of cloning of the *attR* locus from the exconjugants of C5 and *S. peucetius*. The recombinant plasmids obtained by *Bam*HI-digested total DNA from the exconjugant were self-ligated and transformed into *E. coli*. The resulting transformants were selected with apramycin. The restriction sites for the *Bam*HI (B) and *Sph*I (S) enzymes are shown. Am^R, apramycin resistance. Open box, chromosomal DNA; hatched box, phage DNA.

Applications of conjugation in bacteria:

1. Transfer of genes from bacteria to yeast, plants and even to mammalian mitochondria.
2. 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.
3. In plant engineering via the Ti plasmid.

Transformation

Definition: The process by which the bacteria take up DNA fragments from the environment is called transformation. These DNA remnants most commonly come from dead bacterial cells. During transformation, the bacterium binds the DNA and transports it across the bacterial cell membrane. The new DNA is then incorporated into the bacterial cell's DNA.

History:

1. **Griffith's Effect**: Transformation was first demonstrated in 1928 by British bacteriologist Frederick Griffith.

Fig.16: Frederick Griffith (1879-1941)

2. Griffith discovered that a harmless strain of *Streptococcus pneumoniae* (Type IIR) could be made virulent (Type IIIS) after being exposed to heat-killed virulent strains. Griffith hypothesized that some "transforming principle" from the heat-killed strain was responsible for making the harmless strain virulent.He could not explain the phenomenon and this was called Griffith's effect.

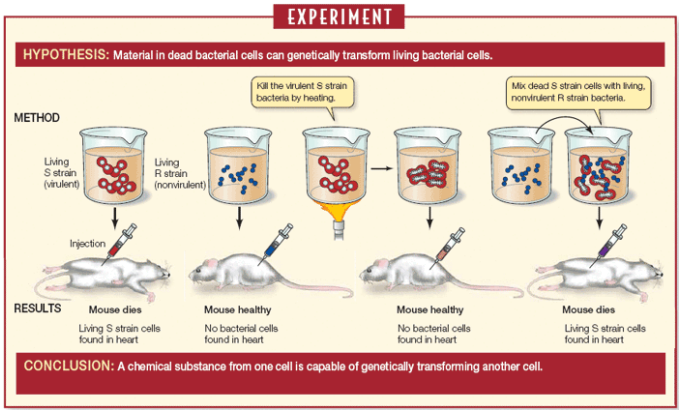

Fig.17: Demonstration of Griffith's experiment

3. **Scientific Explanation of Griffith's Effect**: In 1944 this "transforming principle" was identified and explained by Oswald Avery, Colin MacLeod, and Maclyn McCarty. They isolated DNA from a virulent strain of *S. pneumoniae* and using just this DNA, they were able to make a harmless strain virulent. They called this uptake and incorporation of DNA by bacteria "transformation". The results of Avery et al.'s experiments were well received by the scientific community in 1947.

Fig.18: Oswald Avery (1877-1955)

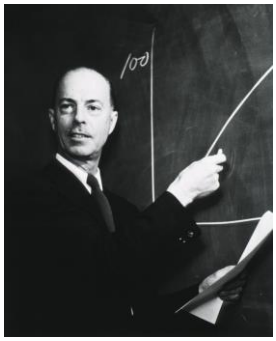

Fig.19: Colin MacLeod (1909-1972)

Fig.20. Maclyn McCarty (1911-2005)

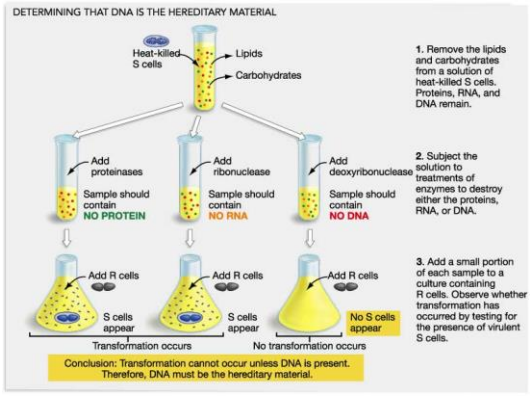

Fig.21: Experimental Demonstration of Avery, MacLeod and McCarty

4. **Transformation of *E.coli***: It was originally thought that *Escherichia coli*, a commonly-used laboratory organism, were resistant to transformation. However, in 1970, Morton Mandel and Akiko Higa showed that *E. coli* may be induced to take up DNA from bacteriophage λ without the use of helper phage after treatment with calcium chloride solution.

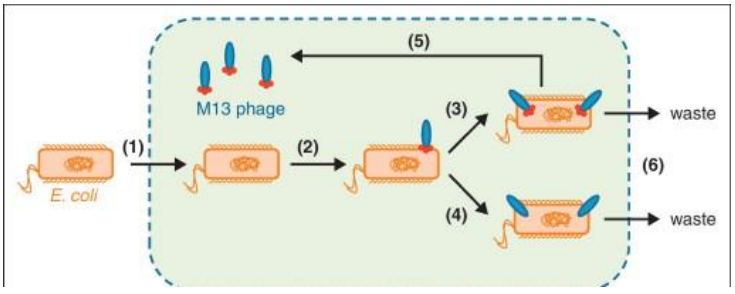

Fig.22: Stages of *E.coli* transformation **(1)** Phage infection of an *E. coli* cell **(2)** The target protein is linked to production of pIII, a protein that the phage requires to produce infectious progeny. **(3)** When a phage encoding a protein with the desired function infects *E. coli*, pIII can be synthesized; further infectious phages are produced harboring the functional target gene **(3)**. **(4)**When a phage encoding a nonfunctional target protein infects *E. coli*, pIII is not synthesized,

and so that cell does not produce any infectious phage with the nonfunctional target gene. **(5)** The result is a phage population in the lagoon encoding functional target genes that can re-infect host cells. **(6)** The non-functional genes are washed out with the noninfectious progeny.

5. **Use of $CaCl_2$:** In 1972, Stanley Cohen, Annie Chang and Leslie Hsu showed that $CaCl_2$ treatment is also effective for transformation of plasmid DNA. The method of transformation by Mandel and Higa was later improved upon by Douglas Hanahan. The discovery of artificially-induced competence in *E. coli* created an efficient and convenient procedure for transforming bacteria which allows for simpler molecular cloning methods in biotechnology and research,

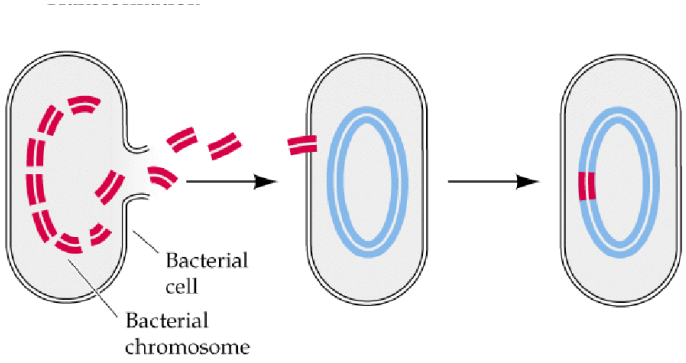

Fig.23: Gene entry in naturally competent bacterial cell.

Competence in Bacterial cell:

Bacterial transformation may be referred to as a stable genetic change brought about by the uptake of naked DNA (DNA without associated cells or proteins) and competence refers to the state of being able to take up exogenous DNA from the environment.

There are two forms of competence: natural and artificial.

Natural competence

Only 1% of bacterial species are capable of naturally taking up DNA under laboratory conditions; more may be able to take it up in their natural environments. DNA material can be transferred between different strains of bacteria, in a process that is called horizontal gene transfer. Some species upon cell death release their DNA to be taken up by other cells, however transformation works best with DNA from closely related species.

i) These naturally competent bacteria carry sets of genes that provide the protein machinery to bring DNA across the cell membrane(s).

ii) The transport of the exogenous DNA into the cells may require proteins that are involved in the assembly of type IV pili and type II secretion system, as well as DNA translocase complex at the cytoplasmic membrane.

iii) The DNA first binds to the surface of the competent cells on a DNA receptor, and passes through the cytoplasmic membrane via DNA translocase.

iv) Only single-stranded DNA may pass through, one strand is therefore degraded by nucleases in the process, and the translocated single-stranded DNA may then be integrated into the bacterial chromosomes by a RecA-dependent process.

v) In Gram-negative cells, due to the presence of an extra membrane, the DNA requires the presence of a channel formed by secretins on the outer membrane.

vi) The role of pilin in transformation is useful but its role is not defined. The uptake of DNA is generally non-sequence specific, although in some species the presence of specific DNA uptake sequences may facilitate efficient DNA uptake.

Artificial competence

Artificial competence can be induced in laboratory procedures that involve making the cell passively permeable to DNA by exposing it to conditions that do not normally occur in nature. Typically the cells are incubated in a solution containing divalent cations, most commonly calcium chloride solution under cold condition, and then exposed to a pulse of heat shock.

The surface of bacteria such as *E. coli* is negatively charged due to the presence of phospholipids and lipopolysaccharides on its cell surface, and the DNA is also negatively-charged. One function of the divalent cation therefore would be to shield the charges by coordinating the phosphate groups and other negative charges, thereby allowing a DNA molecule to adhere to the cell surface. It also weakens the cell membrane, making it more permeable to DNA. The heat-pulse is thought to create a thermal imbalance on either side of the cell membrane, which forces the DNA to enter the cells through either cell pores or the damaged cell wall.

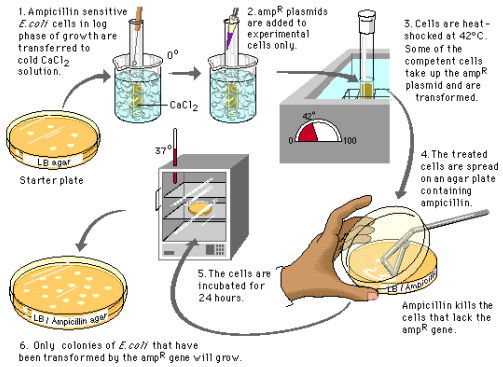

Fig.24: Creation of artificial competence in bacterial cell by heat shock

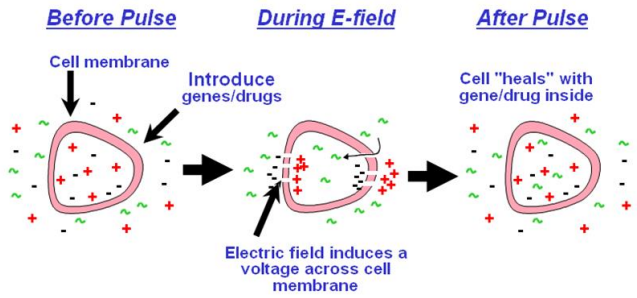

Fig.25: Creation of artificial competence by electrical pulse

Artificial Transformation of higher eukaryotes using bacterial genes:

Use of Electroporation in Plants:

Transformation using electroporation was developed in the late 1980s, increasing the efficiency of in-vitro transformation and increasing the number of bacterial strains that could be transformed.

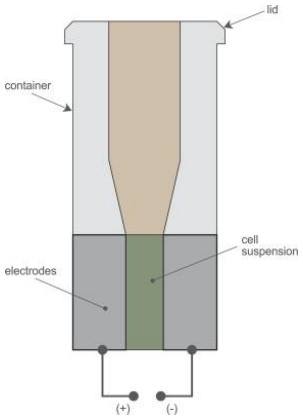

Fig.26: Diagrammatic representation of electroporation unit

In 1907 a bacterium that caused plant tumors, *Agrobacterium tumefaciens*, was discovered and in the early 1970s the tumor inducing agent was found to be a DNA plasmid called the Ti plasmid.

By removing the genes in the plasmid that caused the tumor and adding in novel genes researchers were able to infect plants with *A. tumefaciens* and let the bacteria insert their chosen DNA into the genomes of the plants. These methods were developed including electroporation and micro-injection. Particle bombardment was made possible with the invention of the Biolistic Particle Delivery System (gene gun) by John Sanford in 1990.

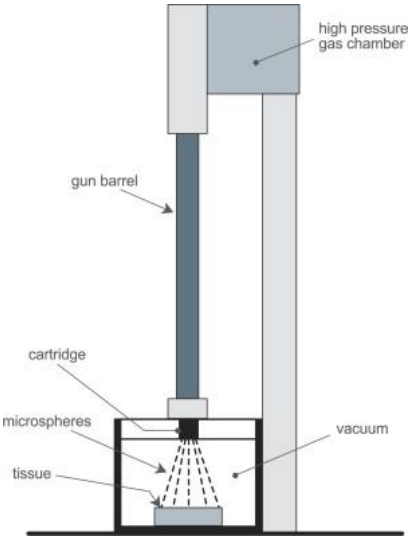

Fig.27: Mechanism of gene gun used in artificial transformation of plant cells

Procedure:

i) Plant tissue (often leaves) are cut into small pieces, e.g. 10x10mm, and soaked for 10 minutes in a fluid containing suspended *Agrobacterium*.
ii) Some cells along the cut will be transformed by the bacterium, which inserts its DNA into the cell.
iii) The plants will regrows in proper media.
iv) Some plants species can be transformed just by dipping the flowers into suspension of *Agrobacterium* and then planting the seeds in a selective medium.
v) Particle bombardment: Particles of gold or tungsten are coated with DNA and then shot into young plant cells or plant embryos. Some genetic material will stay in the cells and transform them. This method also allows transformation of plant plastids.
The transformation efficiency is lower than in *Agrobacterium* mediated transformation, but most plants can be transformed with this method.
vi) Electroporation at the rate of 10-20 kV/cm makes transient holes in cell membranes using electric shock; this allows DNA to enter as described above for Bacteria.

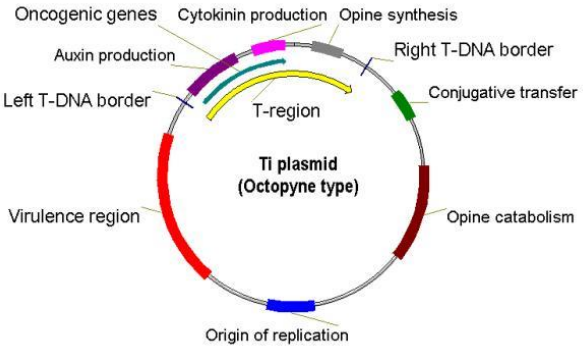

Fig.28: Structure of Ti plasmid

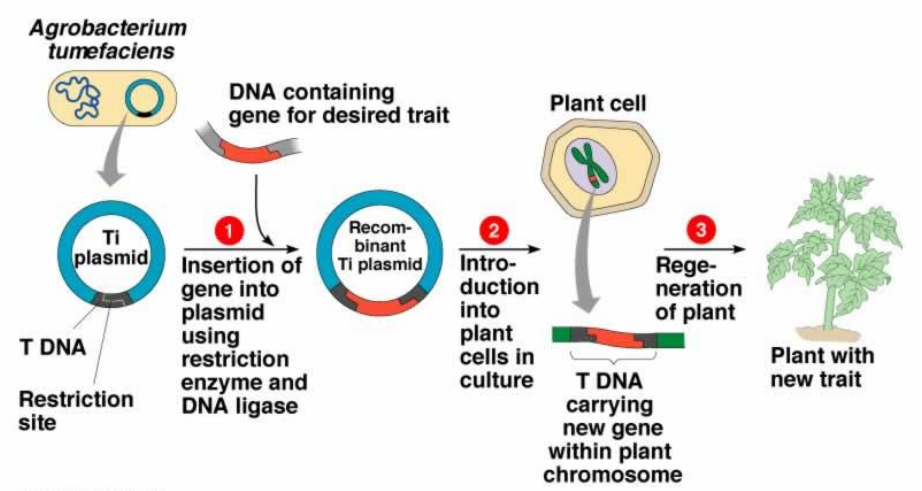

Fig.29: Stages of transformation in plant cell using Ti plasmid.

Transformation in yeast:

Saccharomyces cerevisiae may be transformed by exogenous DNA in the environment or under laboratory conditions.

i) The yeast cells are exposed to alkali cations such as those of cesium or lithium allows the cells to take up plasmid DNA.

ii) In these protocols, the single-stranded DNA preferentially binds to the yeast cell wall, preventing plasmid DNA from binding and thus it is available for the transformation process.

iii) Mechanical injury using glass beads or enzymatic degradation may also help in yeast transformation.

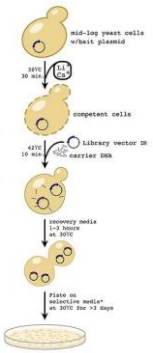

Fig.30: Stages of transformation in yeast