BACTERIAL GENETICS

Genetics is the study of genes including the structure of genetic materials, what information is stored in the genes, how the genes are expressed and how the genetic information is transferred. Genetics is also the study of heredity and variation. The arrangement of genes within organisms is its genotype and the physical characteristics an organism based on its genotype and the interaction with its environment, make up its phenotype. The order of DNA bases constitutes the bacterium's genotype. A particular organism may possess alternate forms of some genes. Such alternate forms of genes are referred to as alleles. The cell's genome is stored in chromosomes, which are chains of double stranded DNA. Genes are sequences of nucleotides within DNA that code for functional proteins. The genetic material of bacteria and plasmids is DNA. The two essential functions of genetic material are replication and expression.

Structure of DNA
The DNA molecule is composed of two chains of nucleotides wound around each other in the form of "double helix". Double-stranded DNA is helical, and the two strands in the helix are antiparallel. The backbone of each strand comprises of repeating units of deoxyribose and phosphate residue. Attached to the deoxyribose is purine (AG) or pyrimidine (CT) base. Nucleic acids are large polymers consisting of repeating nucleotide units. Each nucleotide contains one phosphate group, one deoxyribose sugar, and one purine or pyrimidine base. In DNA the sugar is deoxyribose; in RNA the sugar is ribose. The double helix is stabilized by hydrogen bonds between purine and pyrimidine bases on the opposite strands. A on one strand pairs by two hydrogen bonds with T on the opposite strand, or G pairs by three hydrogen bonds with C. The two strands of double-helical DNA are, therefore complementary. Because of complementarity, double-stranded DNA contains equimolar amounts of purines (A + G) and pyrimidines (T + C), with A equal to T and G equal to C, but the mole fraction of G + C in DNA varies widely among different bacteria. One of the differences between DNA and RNA is that RNA contains uracil instead of the base thymine.

Structure of chromosome
In contrast to the linear chromosomes found in eukaryotic cells, most bacteria have single, covalently closed, circular chromosomes. Not all bacteria have a single circular chromosome: some bacteria have multiple circular chromosomes, and many bacteria have linear chromosomes and linear plasmids. Multiple chromosomes have also been found in many other bacteria, including Brucella, *Leptospira interrogans*, Burkholderia and *Vibrio cholerae*. Borrelia and Streptomyces have linear chromosomes and most strains contain both linear and circular plasmids. The chromosome of E coli has a length of approximately 1.35 mm, several hundred times longer than the bacterial cell, but the circular DNA is then looped and supercoiled to allow the chromosome to fit into the small space inside the cell.

Codon
A set of three base pairs constitutes a codon, which codes for a single amino acid. The "triplet code" is said to be degenerate or redundant because more than codon may exist for the same amino acid. For example, the codons AGA, AGG, CGU, CGC, CGA and CGG all code for arginine. There are 64 codons, of which 3 (UAA, UAG and UGA) are nonsense codons. They don't code for any amino acid, but act as stop codons. There are specific codons which code for start and stop sequences. The start codon (AUG) indicates the beginning of the sequence to be translated, and the stop codons (UAA, UGA, UAG) terminate the protein synthesis. With the exception of methionine, all amino acids are coded for by more than one codon. The DNA in a gene that are expressed into the protein product are called exons and the non-coding DNA segments are called introns. There are no introns in bacterial chromosome. A segment of DNA carrying codons specifying a particular polypeptide is called a cistron or a gene.

Flow of genetic information
The central dogma of molecular biology is that DNA carries all genetic information. The flow of genetic information includes the replication of DNA to make more DNA, the transcription of the DNA into mRNA and the translation of mRNA into proteins. Replication of DNA first involves the separation of the two strands of DNA followed by

synthesis of new identical DNA strand by enzymes called DNA polymerases. The RNA strand is synthesized by enzymes called RNA polymerases. The RNA sequence will be complementary to the DNA sequence. The mRNA strands are then guided to the ribosomes for protein translation. Amino acid residues are brought to the mRNA strand on the ribosomes by transfer RNA (tRNA).

Operon concept

Some proteins (or enzymes) are always required by a bacterium, genes coding for such proteins are constitutively expressed. These genes are usually needed for the cell to survive. Other genes that may not be needed at all times are regulated to conserve energy and cellular materials. Some proteins (or enzymes) are produced only when the need arises or when stimulated by certain environmental conditions. Such genes are normally repressed and are induced whenever required. Repression is a method of inhibiting or decreasing the expression of specific genetic products. This inhibition is controlled by proteins called repressors, which usually block the binding of RNA polymerase to the template DNA. Induction is the opposite of repression, inducers act to "turn on" genes that are not constitutive. The operon concept was first demonstrated by Jacob and Monad.

Bacteria utilize a special energy saving system of genetic control called operons. The operon is a sequence of DNA that contains multiple genes used to produce multiple proteins for a single purpose. An example of an operon is the lac operon in E. coli. In order to break down lactose, E. coli must use a series of enzymes (beta-galactosidase, galactoside permease and transacetylase). The genes for these three enzymes are located in a row on the DNA and share a single promoter. Genes determining structure of a particular protein are called structural genes and the activity of structural genes are controlled by regulator genes, which lie adjacent to them. The genes *lac*Z, *lac*Y and *lac*A which code for the three enzymes are the structural genes. *lac*I gene codes for the repressor protein, hence is the regulator gene. Between the *lac*I gene and the structural genes lie promoter and operator genes. For transcription of the structural genes, the enzyme RNA polymerase first has to bind to promoter region. The operator region lies in between the promoter and structural genes and the RNA polymerase has to go through the operator region. Under normal circumstances, when the structural genes are not transcribed, the repressor protein is bound to the operator region thus preventing the passage of RNA polymerase from the operator region towards the operon. When lactose is available in the environment, the repressor protein leaves the operator region and binds to lactose because it has high affinity for lactose. This frees the operator region and the RNA polymerase enzyme moves towards the operon and transcribes the structural genes. The products of structural genes result in the metabolism of lactose. When lactose is no more available, the repressor protein goes back and binds to the operator region, thus stopping further transcription of structural genes. This way lactose acts both as inducer as well as a substrate for beta galactosidase.
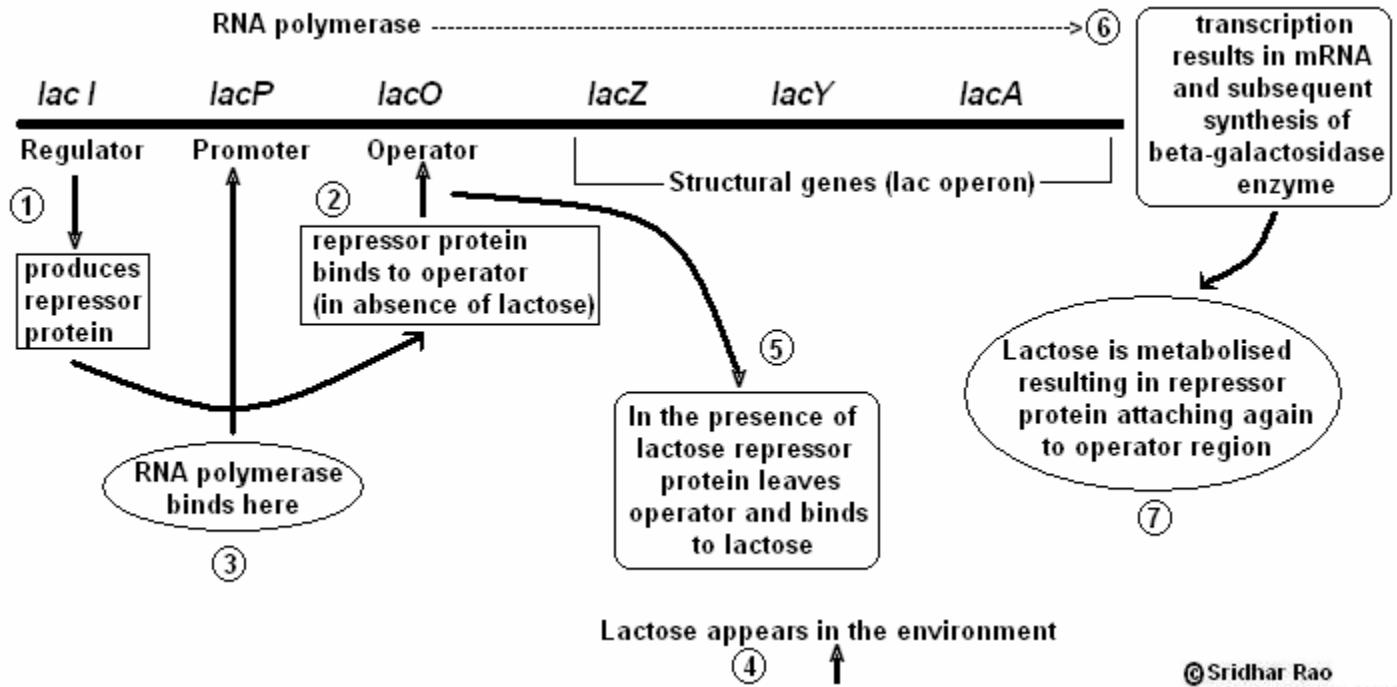
Mutations

The term "mutation" was coined by Hugo de Vries, which is derived from Latin word meaning "to change". Mutations are heritable changes in genotype that can occur spontaneously or be induced by chemical or physical treatments. (Organisms selected as reference strains are called wild type, and their progeny with mutations are called mutants.) The process of mutation is called mutagenesis and the agent inducing mutations is called mutagen. Changes in the sequence of template DNA (mutations) can drastically affect the type of protein end product produced. For a particular bacterial strain under defined growth conditions, the mutation rate for any specific gene is constant and is expressed as the probability of mutation per cell division. Spontaneous mutation occurs naturally about one in every million to one in every billion divisions. Mutation rates of individual genes in bacteria range from 10^{-2} to 10^{-10} per bacterium per division. Most spontaneous mutations occur during DNA replication.

Mechanisms of mutation

a. Substitution of a nucleotide: Base substitution, also called point mutation, involves the changing of single base in the DNA sequence. This mistake is copied during replication to produce a permanent change. If one purine [A or G] or pyrimidine [C or T] is replaced by the other, the substitution is called a transition. If a purine is replaced by a pyrimidine or vice-versa, the substitution is called a transversion. This is the most common mechanism of mutation.
b. Deletion or addition of a nucleotide: deletion or addition of a nucleotide during DNA replication. When a transposon (jumping gene) inserts itself into a gene, it leads to disruption of gene and is called insertional mutation.

Results of mutation

a. **Missense mutation:** Missense mutations are DNA mutations which lead to changes in the amino acid sequence (one wrong codon and one wrong amino acid) of the protein product. This could be caused by a single point mutation or a series of mutations.
b. **Nonsense mutation:** A mutation that leads to the formation of a stop codon is called a nonsense mutation. Since these codon cause the termination of protein synthesis, a nonsense mutation leads to incomplete protein products.
c. **Silent mutation:** Sometimes a single substitution mutation change in the DNA base sequence results in a new codon still coding for the same amino acid. Since there is no change in the product, such mutations are called silent.
d. **Frameshift mutation:** Frameshift mutations involve the addition or deletion of base pairs causing a shift in the "reading frame" of the gene. This causes a reading frame shift and all of the codons and all of the amino acids after that mutation are usually wrong. Since the addition of amino acids to the protein chain is determined by the three base codons, when the overall sequence of the gene is altered, the amino acid sequence may be altered as well.
e. **Lethal mutation:** Sometimes some mutations affect vital functions and the bacterial cell become nonviable. Hence those mutations that can kill the cell are called lethal mutation.
f. **Suppressor mutation:** It is a reversal of a mutant phenotype by another mutation at a position on the DNA distinct from that of original mutation. True reversion or back mutation results in reversion of a mutant to original form, which occurs as a result of mutation occurring at the same spot once again.
g. **Conditional lethal mutation:** Sometimes a mutation may affect an organism in such a way that the mutant can survive only in certain environmental condition. Example; a temperature sensitive mutant can survive at permissive temperature of $35^{o}C$ but not at restrictive temperature of $39^{o}C$.
h. **Inversion mutation**: If a segment of DNA is removed and reinserted in a reverse direction, it is called inversion mutation.

Based on extent of base pair changes, mutations can be of two types; microlesion and macrolesion. Microlesions are basically point mutations (affecting single base pairs) whereas macrolesions involve addition, deletion, inversion or duplication of several base pairs.

The mutations in DNA can occur spontaneously or can be caused by an external force or substance called a mutagen. Mutagens can be chemicals such as nitrous acid, which alters adenine to pair with cytosine instead of thymine. Other chemical mutagens include acridine dyes, nucleoside analogs that are similar in structure to nitrogenous bases, benzpyrene (from smoke and soot) and aflatoxin. Radiation can also be a cause of DNA mutations. High energy light waves such as X-rays, gamma rays, and ultraviolet light have been shown to damage DNA. UV light is responsible for the formation of thymine dimers in which covalent links are established between the thymine molecules. These links change the physical shape of the DNA preventing transcription and replication.

Significance of mutation:

- Discovery of a mutation in a gene can help in identifying the function of that gene.
- Mutations can be induced at a desired region to create a suitable mutant, especially to produce vaccines.
- Spontaneous mutations can result in emergence of antibiotic resistance in bacteria.

- Mutations can result in change in phenotype such as appearance of novel surface antigen, alternation in physiological properties, change in colony morphology, nutritional requirements, biochemical reactions, growth characteristics, virulence and host range.

Tests to detect or select mutations:
- Replica plating
- Penicilin enrichment
- Fluctuation test
- Ames test

TRANSFER OF GENETIC MATERIAL

Sometimes when two pieces of DNA come into contact with each other, sections of each DNA strand will be exchanged. This is usually done through a process called crossing over in which the DNA breaks and is attached on the other DNA strand leading to the transfer of genes and possibly the formation of new genes. Genetic recombination is the transfer of DNA from one organism to another. The transferred donor DNA may then be integrated into the recipient's nucleoid by various mechanisms. In the case of homologous recombination, homologous DNA sequences having nearly the same nucleotide sequences are exchanged by means of breakage and reunion of paired DNA segments. Genetic information can be transferred from organism to organism through vertical transfer (from a parent to offspring) or through horizontal transfer methods such as conjugation, transformation or transduction. Bacterial genes are usually transferred to members of the same species but occasionally transfer to other species can also occur.

When two DNA strands having homologous regions come close, one strand from each crosses over to the other side and attaches to the new strand. Thus new genes may be transferred to the other strand. This is homologous recombination.
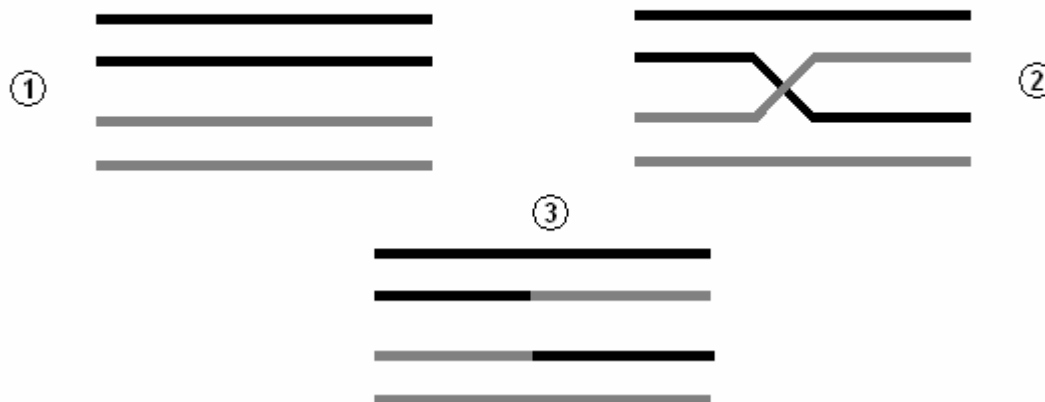

General or homologous recombination requires extensive homology and is mediated by an enzyme, RecA protein.
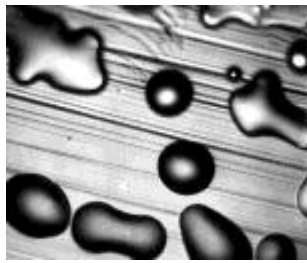
TRANSFORMATION:

Transformation involves the uptake of free or naked DNA released by donor by a recipient. It was the first example of genetic exchange in bacteria to have been discovered. This was first demonstrated in an experiment conducted by Griffith in 1928. The presence of a capsule around some strains of pneumococci gives the colonies a glistening, smooth (S) appearance while pneumococci lacking capsules have produce rough (R) colonies. Strains of pneumococci with a capsule (type I) are virulent and can kill a mouse whereas strains lacking it (type II) are harmless. Griffith found that mice died when they were injected with a mixture of live non capsulated (R, type II) strains and heat killed capsulated (S, type I) strains. Neither of these two when injected alone could kill the mice, only the mixture of two proved fatal. Live S strains with capsule were isolated from the blood of the animal suggesting that some factor from the dead S cells converted the R strains into S type. The factor that transformed the other strain was found to be DNA by Avery, McLeod and McCarty in 1944.

Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell. Certain bacteria (e.g. Bacillus, Haemophilus, Neisseria, Pneumococcus) can take up DNA from the environment and the DNA that is taken up can be incorporated into the recipient's chromosome.
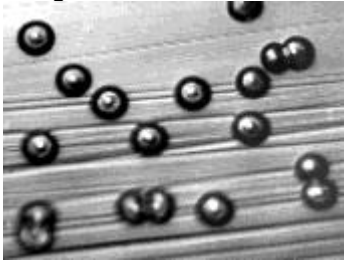
Rough colonies   Smooth colonies

The steps involved in transformation are:
1. A donor bacterium dies and is degraded.
2. A fragment of DNA (usually about 20 genes long) from the dead donor bacterium binds to DNA binding proteins on the cell wall of a competent, living recipient bacterium.
3. Nuclease enzymes then cut the bound DNA into fragments.
4. One strand is destroyed and the other penetrates the recipient bacterium.
3. The Rec A protein promotes genetic exchange (recombination) between a fragment of the donor's DNA and the recipient's DNA.

Some bacteria are able to take up DNA naturally. However, these bacteria only take up DNA a particular time in their growth cycle (log phase) when they produce a specific protein called a competence factor. Uptake of DNA by Gram positive and Gram negative bacteria differs. In Gram positive bacteria the DNA is taken up as a single stranded molecule and the complementary strand is made in the recipient. In contrast, Gram negative bacteria take up double stranded DNA.
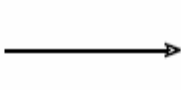
Rough (R) non capsulated Type II strain → Mouse survives
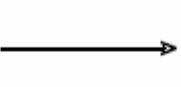
Smooth (S) capsulated Type I strain → Mouse dies
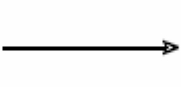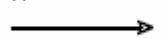
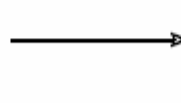Heat killed Smooth (S) capsulated Type I strain → Mouse survives

Rough (R) non capsulated Type II strain / Heat killed Smooth (S) capsulated Type I strain → Mouse dies → Smooth (S) capsulated Type I strain isolated from blood

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Significance: Transformation occurs in nature and it can lead to increased virulence. In addition transformation is widely used in recombinant DNA technology.
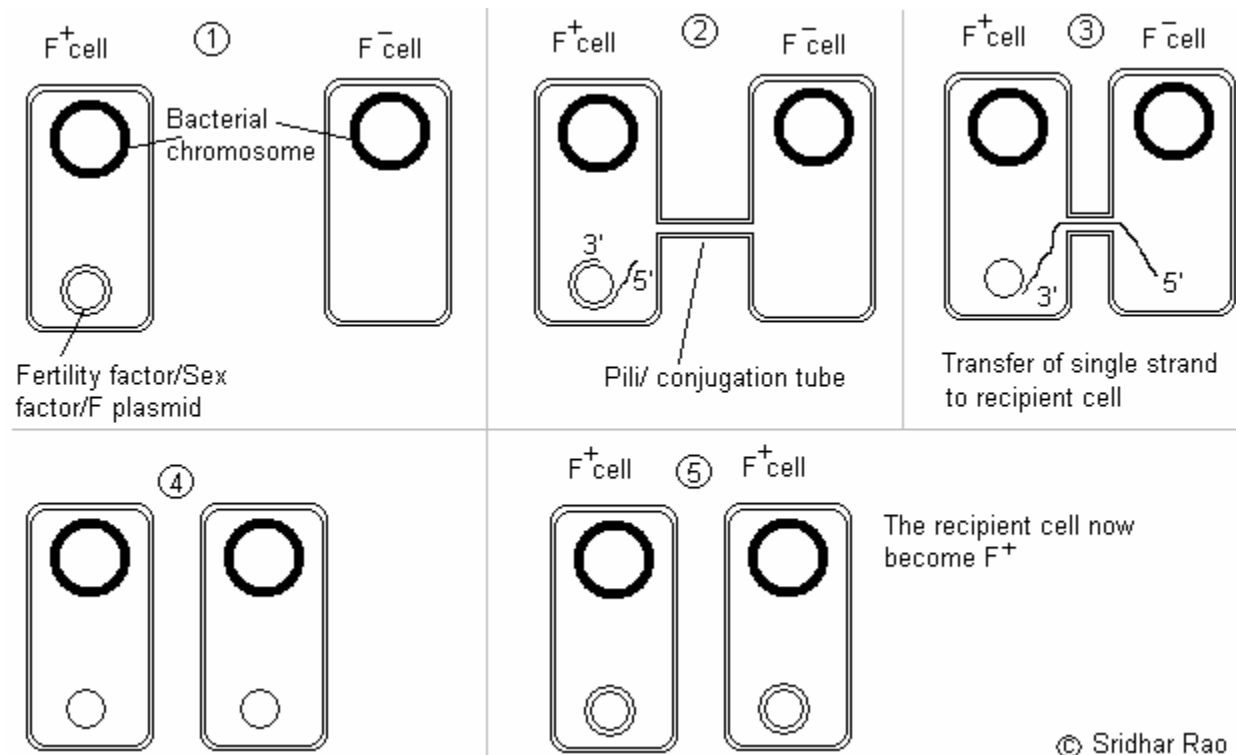
CONJUGATION:

In 1946 Joshua Lederberg and Tatum discovered that some bacteria can transfer genetic information to other bacteria through a process known as conjugation. Bacterial conjugation is the transfer of DNA from a living donor bacterium to a recipient bacterium.

Plasmids are small autonomously replicating circular pieces of double-stranded circular DNA. Conjugation involves the transfer of plasmids from donor bacterium to recipient bacterium. Plasmid transfer in Gram-negative bacteria occurs only between strains of the same species or closely related species. Some plasmids are designated as F factor (F plasmid, fertility factor or sex factor) because they carry genes that mediate their own transfer. The F factor can replicate autonomously in the cell. These genes code for the production of the sex pilus and enzymes necessary for conjugation. Cells possessing F plasmids are F^+ (male) and act as donors. Those cells lacking this plasmid are F^- (female) and act as recipient. All those plasmids, which confer on their host cells to act as donors in conjugation are called transfer factor.

Each Gram negative F^+ bacterium has 1 to 3 sex pili that bind to a specific outer membrane protein on recipient bacteria to initiate mating. The sex pilus then retracts, bringing the two bacteria in contact and the two cells become bound together at a point of direct envelope-to-envelope contact. In Gram-positive bacteria sticky surface molecules are produced which bring the two bacteria into contact. Gram-positive donor bacteria produce adhesins that cause them to aggregate with recipient cells, but sex pili are not involved. DNA is then transferred from the donor to the recipient. Plasmid-mediated conjugation occurs in *Bacillus subtilis*, *Streptococcus lactis*, and *Enterococcus faecalis* but is not found as commonly in the Gram-positive bacteria as compared to the Gram-negative bacteria.

1. F+ conjugation:

This results in the transfer of an F^+ plasmid (coding only for a sex pilus) but not chromosomal DNA from a male donor bacterium to a female recipient bacterium. The two strands of the plasmid separate. One strand enters the recipient bacterium progressing in the 5' to 3' direction while one strand remains in the donor. The complementary strands are synthesized in both donor and recipient cells. The recipient then becomes an F^+ male and can make a sex pilus. During conjugation, no cytoplasm or cell material except DNA passes from donor to recipient. The mating pairs can be separated by shear forces and conjugation can be interrupted. Consequently, the mating pairs remain associated for only a short time. After conjugation, the cells break apart. Following successful conjugation the recipient becomes F^+ and the donor remains F^+.
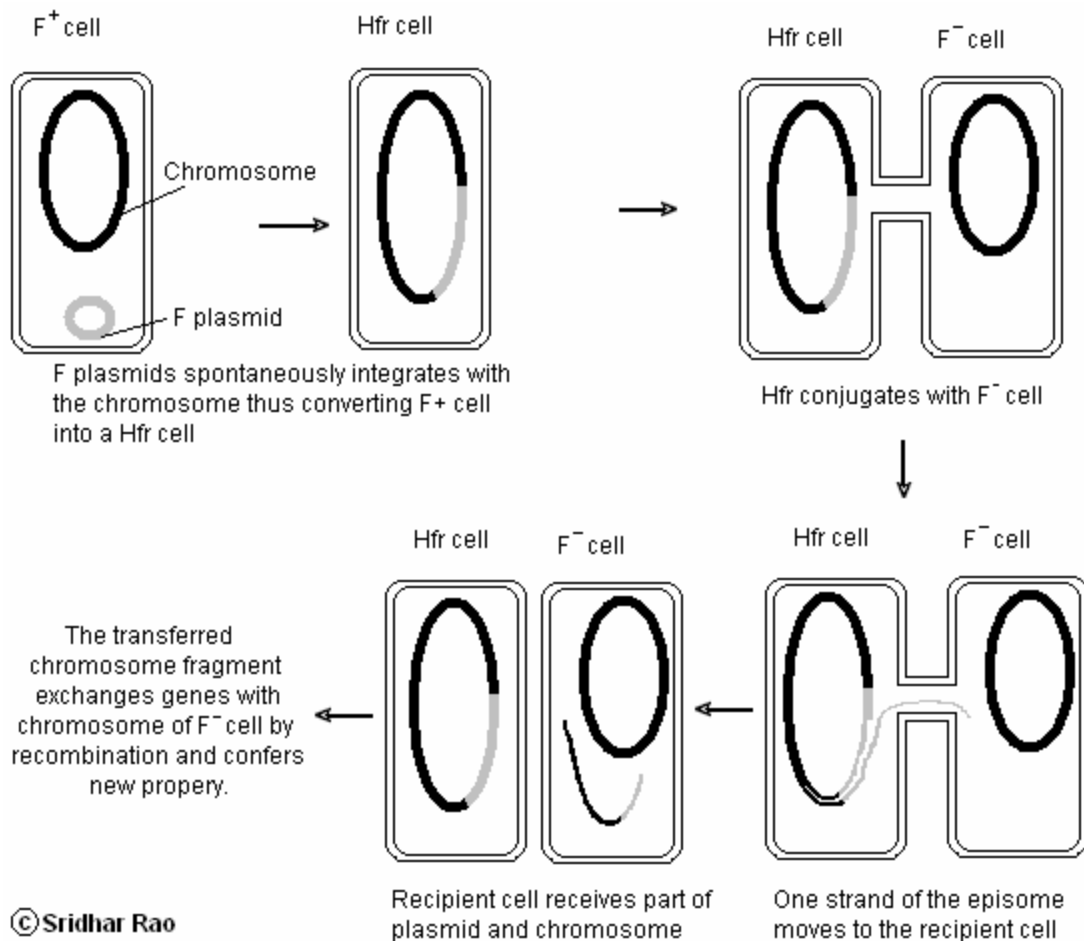

2. Resistance plasmid conjugation:

Some Gram-negative bacteria harbor plasmids that contain antibiotic resistance genes, such plasmids are called R factors. The R factor has two components, one that codes for self transfer (like F factor) called RTF (resistance
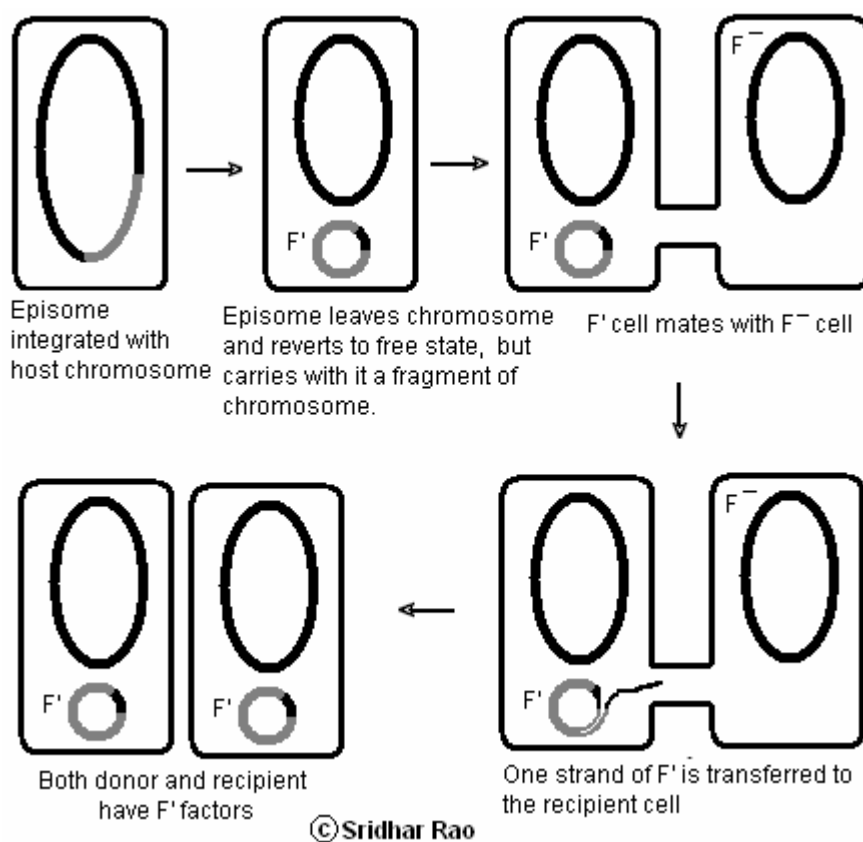
transfer factor) and the other R determinant that contains genes coding for antibiotic resistance. R plasmids may confer resistance to as many as five different antibiotics at once upon the cell and by conjugation; they can be rapidly disseminated through the bacterial population. The difference between F factor and R factor is that the latter has additional genes coding for drug resistance. During conjugation there is transfer of resistance plasmid (R-plasmid) from a donor bacterium to a recipient. One plasmid strand enters the recipient bacterium while one strand remains in the donor. Each strand then makes a complementary copy. R-plasmid has genes coding for multiple antibiotic resistance as well as sex pilus formation. The recipient becomes multiple antibiotic resistant and male, and is now able to transfer R-plasmids to other bacteria. When the recipient cells acquire entire R factor, it too expresses antibiotic resistance. Sometimes RTF may disassociate from the R determinant and the two components may exist as separate entities. In such cases though the host cell remains resistant to antibiotics, it can not transfer this resistance to other cells. Sometimes RTF can have other genes (such as those coding for hemolysin, enterotoxin) apart from R determinants attached to it.

3. Hfr (high frequency recombinant) conjugation:

Plasmids may integrate into the bacterial chromosome by a recombination event depending upon the extent of DNA homology between the two. After integration, both plasmid and chromosome will replicate as a single unit. A plasmid that is capable of integrating into the chromosome is called an episome. If the F plasmid is integrated into the chromosome it is called an Hfr cell. After integration, both chromosome and plasmid can be conjugally transferred to a recipient cell. Hfr cells are called so because they are able to transfer chromosomal genes to recipient cells with high frequency.

The DNA is nicked at the origin of transfer and is replicated. One DNA strand begins to passes through a cytoplasmic bridge to the F- cell, where its complementary strand is synthesized. Along with the portion of integrated plasmid, the chromosome is also transmitted to the F⁻ cell. The bacterial connection usually breaks before the transfer of the entire chromosome is completed so the remainder of the F⁺ plasmid rarely enters the recipient. Usually only a part of the Hfr chromosome as well as the plasmid is transferred during conjugation and the recipient cell does not receive complete F factor. After conjugation the Hfr cell remains Hfr but the F⁻ cell does not become F+ and continues to remain F⁻. However the transferred chromosome fragment recombines with the chromosome of F⁻ cell thereby transferring some new property to the recipient cell.

F⁺ cell   Hfr cell

Chromosome

F plasmid

F plasmids spontaneously integrates with the chromosome thus converting F+ cell into a Hfr cell

Hfr cell   F⁻ cell

Hfr conjugates with F⁻ cell

The transferred chromosome fragment exchanges genes with chromosome of F⁻ cell by recombination and confers new propery.

Hfr cell   F⁻cell

Hfr cell   F⁻ cell

Recipient cell receives part of plasmid and chromosome

One strand of the episome moves to the recipient cell

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Episode integrated with host chromosome

Episode leaves chromosome and reverts to free state, but carries with it a fragment of chromosome.

F' cell mates with F⁻ cell

Both donor and recipient have F' factors

One strand of F' is transferred to the recipient cell

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The integration of episome into the chromosome is not stable and the episomes are known to revert back to free state. While doing so, the episomes sometimes carry fragments of chromosomal genes along with it. Such an F factor that incorporates some chromosomal genes is called F prime (F') factor. When such a F' cell mates with F- recipient cell, it not only transfers the F factor but also the host genes that it carried with it. This process of transfer of chromosomal genes along with F factor is known is **sexduction**.

Significance: Among the Gram negative bacteria this is the major way that bacterial genes are transferred. Transfer can occur between different species of bacteria. Transfer of multiple antibiotic resistance by conjugation has become a major problem in the treatment of certain bacterial diseases. Since the recipient cell becomes a donor after transfer of a plasmid, an antibiotic resistance gene carried on a plasmid can quickly convert a sensitive population of cells to a resistant one.
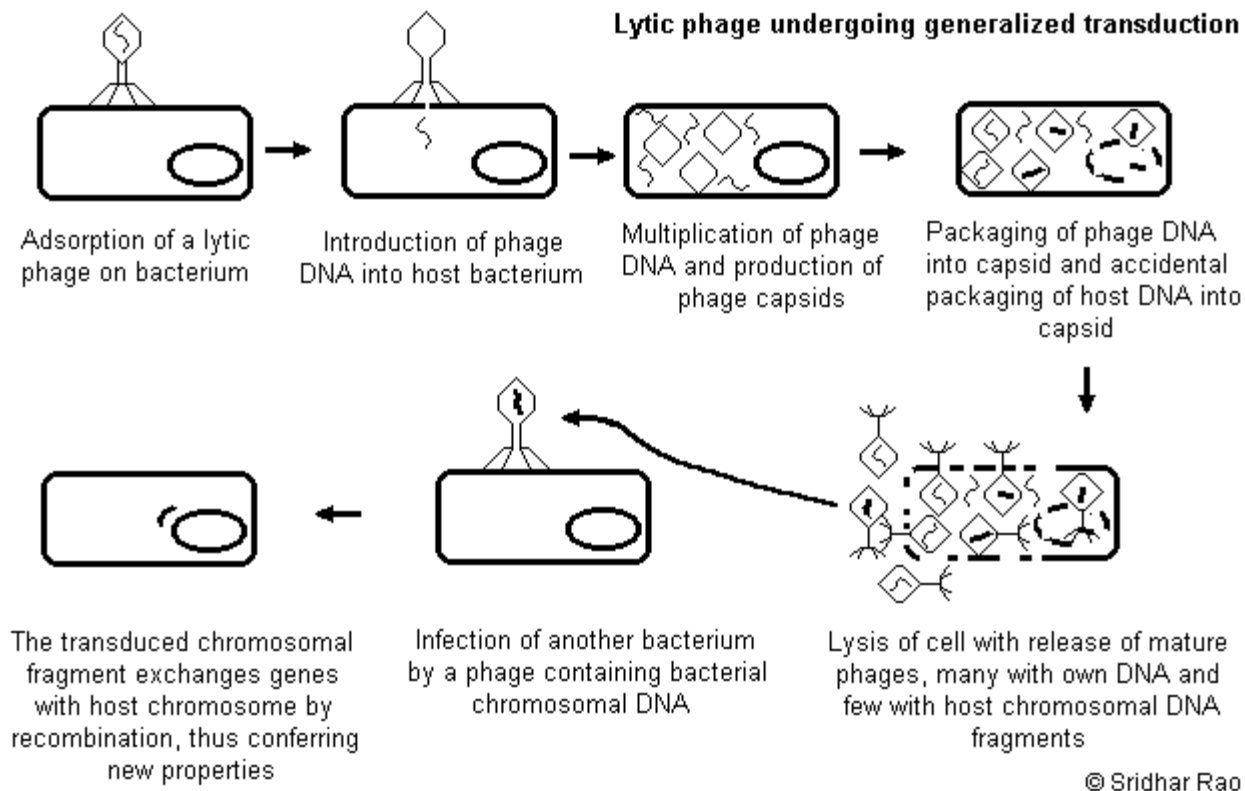
TRANSDUCTION:

Bacteriophage are viruses that parasitize bacteria and use their machinery for their own replication. During the process of replication inside the host bacteria the bacterial chromosome or plasmid is erroneously packaged into the bacteriophage capsid. Thus newer progeny of phages may contain fragments of host chromosome along with their own DNA or entirely host chromosome. When such phage infects another bacterium, the bacterial chromosome in the phage also gets transferred to the new bacterium. This fragment may undergo recombination with the host chromosome and confer new property to the bacterium.

Life cycle of bacteriophage may either by lytic or lysogenic. In the former, the parasitized bacterial cell is killed with the release of mature phages while in the latter the phage DNA gets incorporated into the bacterial chromosome as prophage.

Following are the stages of transduction involving a lytic phage:
1. A lytic bacteriophage adsorbs to a susceptible bacterium.
2. The bacteriophage genome enters the bacterium. The phage DNA directs the bacterium's metabolic machinery to manufacture bacteriophage components and enzymes.
3. Occasionally during maturation, a bacteriophage capsid incorporates a fragment of donor bacterium's chromosome or a plasmid instead of a phage genome by mistake.
4. The bacteriophages are released with the lysis of bacterium.
5. The bacteriophage carrying the donor bacterium's DNA adsorbs to another recipient bacterium.
6. The bacteriophage inserts the donor bacterium's DNA it is carrying into the recipient bacterium.
7. The donor bacterium's DNA is exchanged by recombination for some of the recipient's DNA.

Lytic phage undergoing generalized transduction

Adsorption of a lytic phage on bacterium

Introduction of phage DNA into host bacterium

Multiplication of phage DNA and production of phage capsids

Packaging of phage DNA into capsid and accidental packaging of host DNA into capsid

The transduced chromosomal fragment exchanges genes with host chromosome by recombination, thus conferring new properties

Infection of another bacterium by a phage containing bacterial chromosomal DNA

Lysis of cell with release of mature phages, many with own DNA and few with host chromosomal DNA fragments

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In case of temperate phages that undergo lysogenic cycle, the phage DNA gets incorporated into the bacterium chromosome. This is called a prophage and it behaves as if it were a part of bacterial chromosome. This process is known as lysogenic conversion and the bacteria are called lysogenic bacteria. The genes present in the phage DNA also get expressed in the bacterium. Only those strains of *Corynebacterium diphtheriae* that have been lysogenised with beta prophage produce the diphtheria toxin. The prophage sometimes disassociates itself from the host chromosome during multiplication of lysogenic bacteria, and in doing so; it sometimes carries along with itself fragments of bacterial chromosome. The separated prophage then initiates lytic cycle and the subsequent phage progeny may have a piece of chromosomal DNA. When such phage infects another bacterium, newer characteristics coded by that chromosomal gene are conferred.

Two types of transduction are known; restricted transduction and generalized transduction. Generalized transduction can transfer any bacterial gene to the recipient. This process may occur with phages (lytic phages) that degrade their host DNA into pieces the size of viral genomes. If these pieces are erroneously packaged into phage particles, they can be delivered to another bacterium in the next phage infection cycle. Phages P22 of *Salmonella typhimurium* and P1 and μ of *E. coli* carry out generalized transduction. In restricted transduction only those chromosomal genes that lie adjacent to the prophage are transmitted. The lambda phage that infects *E.coli* always transfers gal+ gene (responsible for galactose fermentation). Specialized transduction is only effective in transducing a few special bacterial genes while generalized transduction can transduce any bacterial gene.

PLASMIDS:

Plasmids are extrachromosomal elements found inside a bacterium. These are not essential for the survival of the bacterium but they confer certain extra advantages to the cell.
Number and size: A bacterium can have no plasmids at all or have many plasmids (20-30) or multiple copies of a plasmid. Usually they are closed circular molecules; however they occur as linear molecule in *Borrelia burgdorferi*. Their size can vary from 1 Kb to 400 Kb.
Multiplication: Plasmids multiply independently of the chromosome and are inherited regularly by the daughter cells.
Types of plasmids: R factor, Col factor, RTF and F factor.
F factor: This is also known as fertility factor or sex factor. Most plasmids are unable to mediate their own transfer to other cells. Vertical (inheritance) or horizontal (transfer) transmissions maintain plasmids. F factor is a plasmid that codes for sex pili and its transfer to other cells. Those bacteria that possess transfer factor are called F+, such bacteria have sex pili on their surface. Those cells lacking this factor are designated F-. The F factor plasmid is

transferred to other cells through conjugation. An F- cell will become F+ when it receives the fertility factor from another F+ cell.

R factor: Those plasmids that code for the transmissible drug resistance are called R factor. These plasmids contain genes that code for resistance to many antibiotics. R factors may be transferred by conjugation and its transfer to other bacteria is independent of the F factor. Bacteria possessing such plasmids are resistant to many antibiotics and this drug resistance is transferred to closely related species. R factors may simultaneously confer resistance to five antibiotics. They are usually transferred to related species along with RTF.

Significance of plasmids:
1.   Codes for resistance to several antibiotics. Gram-negative bacteria carry plasmids that give resistance to antibiotics such as neomycin, kanamycin, streptomycin, chloramphenicol, tetracycline, penicillins and sulfonamides.
2.   Codes for the production of bacteriocines.
3.   Codes for the production of toxins (such as Enterotoxins by *Escherichia coli*, *Vibrio cholerae*, exfoliative toxin by *Staphylococcus aureus* and neurotoxin of *Clostridium tetani*).
4.   Codes for resistance to heavy metals (such as Hg, Ag, Cd, Pb etc.).
5.   Plasmids carry virulence determinant genes. Eg, the plasmid Col V of *Escherichia coli* contains genes for iron sequestering compounds.
6.   Codes resistance to uv light (DNA repair enzymes are coded in the plasmid).
7.   Codes for colonization factors that is necessary for their attachment. Eg, as produced by the plasmids of *Yersinia enterocolitica*, *Shigella flexneri*, Enteroinvasive *Escherichia coli*.
8.   Contains genes coding for enzymes that allow bacteria unique or unusual materials for carbon or energy sources. Some strains are used for clearing oil spillage.

Application of plasmids:
1.   Used in genetic engineering as vectors.
2.   Plasmid profiling is a useful genotyping method.

Episomes: Jacob and Wollman coined the term episome. Previously, it was considered synonymous with plasmids. F factors are those plasmids that can code for self transfer to other bacteria. Occasionally such plasmids get spontaneously integrated into chromosome. Plasmids with this capability are called episomes and such bacterial cells are called Hfr cells i.e. high frequency of recombination.

TRANSPOSABLE GENETIC ELEMENTS:

Transposable genetic elements are segments of DNA that have the capacity to move from one location to another (i.e. jumping genes).

Properties of Transposable Genetic Elements:
1. Random movement: Transposable genetic elements can move from any DNA molecule to any DNA other molecule or even to another location on the same molecule. The movement is not totally random; there are preferred sites in a DNA molecule at which the transposable genetic element will insert.
2. Not capable of self replication: The transposable genetic elements do not exist autonomously and thus, to be replicated they must be a part of some other replicon.
3. Transposition mediated by site-specific recombination: Transposition requires little or no homology between the current location and the new site. The transposition event is mediated by an enzyme transposase that is coded by the transposable genetic element. Recombination that does not require homology between the recombining molecules is called illegitimate or nonhomologous recombination.
4. Transposition can be accompanied by duplication: In many instances transposition of the transposable genetic element results in removal of the element from the original site and insertion at a new site. However, in some cases the transposition event is accompanied by the duplication of the transposable genetic element. One copy remains at the original site and the other is transposed to the new site.

Types of Transposable Genetic Elements:
A. Insertion sequences (IS): Insertion sequences are transposable genetic elements that carry no known genes except those that are required for transposition. Insertion sequences are small stretches of DNA that have at their ends repeated sequences, which are involved in transposition. In between the terminal repeated sequences there are genes involved in transposition and sequences that can control the expression of the genes but no other nonessential genes are present.
Importance of IS:
i) Mutation - The introduction of an insertion sequence into a bacterial gene will result in the inactivation of the gene.

ii) The sites at which plasmids insert into the bacterial chromosome are at or near insertion sequence in the chromosome.

iii) Phase Variation: In Salmonella there are two genes, which code for two antigenically different flagellar antigens. The expression of these genes is regulated by an insertion sequences.

B. Transposons: Transposons are transposable genetic elements that carry one or more other genes in addition to those, which are essential for transposition. The structure of a transposon is similar to that of an insertion sequence. The extra genes are located between the terminal repeated sequences.

Importance of transposons: Many antibiotic resistance genes are located on transposons. Since transposons can jump from one DNA molecule to another, these antibiotic resistance transposons are a major factor in the development of plasmids, which can confer multiple drug resistance on a bacterium harboring such a plasmid. These multiple drug resistance plasmids have become a major medical problem.

GENETIC MECHANISM OF DRUG RESISTANCE:

Antibiotic resistance in bacteria may either be intrinsic or acquired. Intrinsic resistance means that the bacteria were resistant to the antibiotic even before the antibiotic was introduced. Acquired resistance means that a bacterium that was previously sensitive to an antibiotic has now turned resistant. It is the acquired resistance that is of great importance because it would result in treatment failure as well as potential dissemination of resistance to other bacteria.

The physiological mechanisms of antibiotic resistance include:
- Inactivation of the antibiotic by enzymes produced by the bacteria
- Alteration of target proteins such that the antibiotic doesn't bind or binds with decreased affinity
- Alteration of the membrane which decreases the permeability of the antibiotic
- Active efflux of the antibiotic
- Development of alternate metabolic pathway to bypass the action of antibiotic

The genetic mechanisms of antibiotic resistance include:

Mutations:
Mutations can alter the protein to which the antibiotic must bind resulting in a protein with little or no affinity for the drug. Mutations can be either step-wise, as seen with Penicillin, where high levels of resistance are achieved by a series of small-step mutations. Multiple-drug resistance in Mycobacteria is apparently the result of the step-wise accumulation of resistance to individual drugs. Mutations can also be one-step where single mutation is sufficient to bring about resistance in the bacteria as in Streptomycin resistance in Mycobacterium tuberculosis. In case of tuberculosis, Mycobacteria are known to mutate during the course of treatment. Initially the antitubercular drug kills the bacteria but soon resistant mutants develop which would eventually replace the sensitive ones resulting in treatment failure. It is for this reason that multiple drugs are included in the treatment of tuberculosis.

Examples:
- o   Methicillin resistance in *S.aureus* due to mutation in Penicillin Binding Protein
- o   Mutations in genes are associated with isoniazid and rifampin resistance in *Mycobacterium tuberculosis*.

Transferable drug resistance:
Drug resistance can be transferred between related bacteria or different taxonomic groups by the process of conjugation and transduction. Drug resistance mediated by R plasmids is the most important method of drug resistance. Acquisition of R factor confers resistance to several antibiotics.

Examples:
- o   Resistance to penicillins in Gram negative bacteria due to beta-lantanas enzymes coded by plasmids
- o   Resistance to penicillin in Staphylococci due to beta-lactamase transferred by transduction
- o   Resistance to Chloramphenicol in Salmonella due to chloramphenicol acetyl transferase coded by a plasmid

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