Mechanisms of Genetic exchange

As explained earlier, the **genome** of an organism and therefore the **genotype** is not necessarily static over time. Within populations, organisms can appear with novel characteristics, encoded by genes not previously present. There are two basic mechanisms involved in bringing about changes in genetic information within individuals and within populations; these are **mutations** and **genetic exchange mechanisms** (sexual reproduction). Although genetic exchange associated with prokaryotic organisms is unlike that common to eukaryotes, it does occur, and provides prokaryotes with the same advantages it affords eukaryotic organisms.

Sexual reproduction involves combining genetic information from two different "parent" individuals resulting in the formation of one or more genetically unique "offspring" individuals. It is advantageous to living organisms because it increases the genetic variation present within populations and improves the potential for survival when environmental conditions change. For many years, researchers believed bacteria were incapable of sexual reproduction because bacteria do not form **haploid gametes** (sex cells) able to fuse together forming **diploid zygotes**. However, bacteria do recombine their genetic material forming unique individuals, and in this sense they do engage in sexual reproduction.

There are three types of genetic exchange occurring naturally in association with prokaryotic organisms; these are **transformation**, **conjugation** and **transduction**. Before examining the differences between these mechanisms, it is useful to consider the ways in which they are all similar to one another.

Transformation, conjugation and transduction are all similar in that:

1. The cells involved **do not fuse** their cytoplasm and nuclear regions to form diploid zygotes. Recall that when eukaryotic organisms engage in sexual reproduction, haploid cells (sometimes called gametes) fuse their cytoplasm (plasmogamy) and nuclei (karyogamy) forming diploid zygotes. These then undergo **meiosis** (reduction division) resulting in the formation of new haploid cells. Prokaryotes typically have only one chromosome, so are haploid always. They do not undergo meiosis and their cells do not fuse.
2. The genetic **exchange is one-way**, i.e., from a donor to a recipient. There is no reciprocal exchange as sometimes occurs in eukaryotes such as protozoa.
3. The DNA transferred is typically a relatively small segment containing **only a few genes**. Although under experimental conditions, *E. coli* cells can be forced to transfer a complete copy of their chromosome from a donor to a recipient, this is highly unlikely under natural conditions.
4. The DNA transferred will usually replace **homologous** genes already present within the recipient cell. Homologous DNA is that coding for the same characteristic in general, but is not identical. For example, different bacteria produce flagellin proteins with different amino acid sequences. The genes coding for these flagellins are examples of homologous DNA. They are also called **alleles** (alternate forms of a gene).

Human genes influencing blood type are also alleles, and will be described in greater detail during a laboratory activity occurring later in the semester.

5. The exchange is most likely to be successful if the cells involved are closely related (within the same species). This is because bacteria form enzymes called **restriction endonucleases** (restriction enzymes) that recognize and chop up foreign DNA. Restriction enzymes will also be described more fully during a laboratory activity.

All genetic exchange mechanisms have the potential of generating **recombinant DNA**. Although this term can have various meanings, for our purposes here it will be defined as a **combination of chromosomal DNA from more than one source**. Under experimental conditions, DNA from various types of organisms (both eukaryotic and prokaryotic) can be combined with plasmids (or alternate cloning vectors) and transferred into bacteria, fungi or other types of cells. Techniques involving the manipulation of DNA in vitro are called **Recombinant DNA Techniques**, and the resulting molecules are also recombinant DNA. More information about recombinant DNA prepared in vitro will be provided in the laboratory.

Transformation:

Transformation, sometimes called **DNA mediated transformation**, is the transfer of DNA from a dead donor cell to a live recipient. Since the donor cells do not have to be present, transformation can also be considered as a mechanism allowing bacteria to pick up "naked" DNA from their environment. Naked DNA is non-viral DNA, i.e., DNA not contained within a protein coat.

Transformation was first observed to occur in *Streptococcus pneumoniae* by Frederick Griffith (1928) while he was attempting to develop a vaccine against pneumonia. Griffith recognized that capsule (glycocalyx) formation was a factor influencing the virulence (disease-causing ability) of Streptococci. Cells capable of capsule formation are much more likely to cause serious disease and death than are non-capsule-forming organisms because capsules render bacteria resistant to phagocytosis by white blood cells. During his experiments, Griffith injected mice with bacterial preparations and obtained the following unexpected results.

1. When live, capsule-forming *Streptococcus* cells were injected into mice, the mice developed pneumonia and died.
2. When live, non-capsule-forming *Streptococcus* cells were injected into mice, the mice did not become sick and survived nicely (or until their next injection).
3. When dead, capsule-forming *Streptococcus* cells were injected into mice, the mice did not develop pneumonia, and survived nicely.
4. When a combination of live, non-capsule-forming *Streptococcus* and dead, capsule-forming *Streptococcus* cells were injected into mice, the mice developed pneumonia and died. Since neither of these two preparations alone caused disease, this was an unexpected finding.

By applying Koch's Postulates, Griffith was able to determine that live, capsule-forming *Streptococcus* were responsible for killing the mice injected with the cell combination described (#4) above. The non-virulent organisms initially injected had been **transformed** into virulent pathogens. From this he concluded correctly that bacteria in the genus *Streptococcus* were capable of transferring genes from dead donor cells to living recipients.

DNA-mediated transformation occurs naturally in a number of different types of bacteria including organisms in the genera *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Haemophilus* and *Neisseria*. Cells not naturally able to exchange DNA in this manner can be made **competent**, i.e., capable of taking up DNA by a variety of methods applied under laboratory conditions. In our laboratory we rendered *E. coli* cells competent by growing them to the mid-log phase and treating them with ice-cold calcium chloride ($CaCl_2$). Because *E. coli* cells can be made to take up DNA in this manner, transformation is frequently used to introduce recombinant DNA (plasmids carrying genes from other organisms) into *E. coli* cells. This type of activity, commonly called **genetic engineering** has generated populations of *E. coli* cells capable of producing a variety of proteins originally encoded by genes from other organisms, e.g., human insulin, human interferon and fluorescent green protein from the jellyfish *Aequorea victoria*.

Conjugation:

Conjugation is somewhat more similar to sexual reproduction as it occurs in eukaryotic organisms in that it involves the transfer of DNA from a live donor to a live recipient; however, references to donor cells as "male" and recipient cells as "female" is potentially misleading. Bacteria do not have different sexes. Conjugation involves thin, hair-like structures called **sex pili**. These are produced by donor cells and attach to recipients by their "sticky" ends (receptors at the ends of pili bind to specific molecules called ligands found on recipient cell walls). After contacting the recipient cell, the pilus shortens and pulls the two cells together. In *E. coli*, the genes encoding sex pilus formation and the initiation of gene transfer are carried on a plasmid called **F plasmid** or **F factor** (F = fertility).

The F factor or F plasmid is a relatively large, double-stranded loop (ccc-DNA) about 94,500bp in size. It has two origins of replication (*ori*V and *ori*S) associated with bidirectional and unidirectional replication, respectively. It also carries genes encoding proteins used to form sex pili, to initiate gene transfer and to control plasmid replication keeping the copy number low. F factor is an **episome**, i.e., a segment of extrachromosomal DNA that can become incorporated into the chromosome of a host cell (note – the term episome can be applied to both plasmids and viral genomes). When outside the chromosome, F factor replicates as does a bacterial chromosome, i.e., replication begins at an origin of replication (in this case, *ori*V) and proceeds in both directions around the closed loop. When integrated into the chromosome, F factor replicates in a unidirectional manner with replication initiated at *ori*S. F factor also includes a transfer origin (*ori*T) located midway along its length when the plasmid is integrated in the host chromosome.

Conjugation can only occur between donor cells (those carrying F factor and producing sex pili) and recipient cells (those lacking F factor and not capable of forming pili), because certain proteins encoded by F factor genes prevent the binding of pili with donor cell surfaces. For this reason (and because humans tend toward anthropomorphism), the donor cells are called "males" and the recipients "females". Given this, E. coli cells can be divided into "mating types" as follow:

a) (F^-) = **F-minus**, female, a cell lacking F factor, can serve as a recipient cell.
b) (F^+) = **F-plus**, male, a cell carrying F factor as an autonomous plasmid.
c) (Hfr) = **High frequency recombinant**, male, a cell carrying F-factor integrated into its chromosome.

d) (F') = **F-prime**, male, a cell carrying an F factor that has exited the chromosome and is carrying a few chromosomal genes along with its own. Since F factor can enter the chromosome at various locations (at least eight different sites), the genes carried on **prime plasmids** are variable.

The results of conjugation vary depending on mating types (males) involved in the process. Possible outcomes are listed in the order of "male" type cells described above.

a) When conjugation occurs between an F-minus cell and an F-plus, the F factor is transferred into the F-minus cell and it becomes F-plus, but is not recombinant.
b) When mating occurs between an F-minus and an Hfr, the DNA transferred from donor to recipient is single-stranded. Transfer is initiated at *ori*T (origin of transfer), in the middle of F factor. One nucleotide strand is "nicked" (a phosphodiester bond is broken) and "unwound" (hydrogen bonds are broken) from the double helix. This strand begins with a portion of F factor (about half of it) and continues into the host chromosome for one or more genes (usually). When the strand is transferred into the recipient, it aligns itself with homologous DNA present there, and then through a cut and splice process, replaces the host sequence. It is then replicated to form double-stranded DNA. Since genes from the donor chromosome are combined with those of the recipient, the **recipient chromosome/cell is recombinant**. The recipient cell does not become male because only half of the F factor was transferred.
c) When mating occurs between an F-minus and and F-prime, the F factor transferred is carrying a portion of chromosomal DNA from the donor, so again the recipient cell becomes recombinant; however, in this case it also becomes male. If the F-prime plasmid remains outside the host chromosome, the recipient will also be **partially diploid**, i.e., it will retain two copies of the chromosomal genes present on the plasmid. The term **sexduction** is sometimes applied to the mating of a F-minus cell with an F-prime, but some references also apply this term to matings involving Hfr.

Experiments involving gene transfers from Hfr cells to F-minus cells were used for mapping the locations of genes on the *E. coli* chromosome. Interrupting mating cycles at various time intervals and then testing for the presence of various characteristics known to be associated with specific genes, allowed researchers to determine the order of genes on the chromosome. Consider the following example:

Conjugation is initiated between F-minus cells and Hfr cells at time zero, and then the process is interrupted by placing a sample of the culture into a blender (this breaks the connections between cells). Testing for characteristics present in the F-minus cells indicates genes present at each progressively longer time interval.

Genes present after 10 minutes = G
Genes present after 15 minutes = B and G
Genes present after 20 minutes = A, B and G
Genes present after 25 minutes = A, B, E and G
Genes present after 30 minutes = A, B, C, E and G
Genes present after 35 minutes = A, B, C, E, F and G
Genes present after 40 minutes = A, B, C, D, E. F and G

What is the order of gene transfer, and therefore the order of the genes on the chromosome? This is a highly simplified version of the actual process, but serves to demonstrate the principle. The gene sequence on this chromosome is G, B, A, E, C, F, D.

Transduction:

Transduction is the transfer of DNA from a donor cell to a recipient cell by means of a **virus**. Viruses are non-cellular entities known to infect all types of cells; those infecting bacteria are called **bacteriophages**. Viruses vary considerably in form and exact composition, but typically have a genome composed of either DNA or RNA (not both) surrounded by a protein coat. Though in most instances, the donor cells involved in transduction are dead, this is not a requirement of the process. Some viruses reproduce within and are shed more or less continually from living cells, so the donors involved in transduction may be alive. Transduction can be divided into two categories based on the condition of the virus when the process is initiated, and what portion of the donor cell chromosome is transferred. The two categories are **generalized** or **general transduction** and **specialized** or **specific transduction.**

During **generalized transduction**, any segment of the donor chromosome may be transferred because the virus involved is **initially cytolytic**. A **cytolytic virus** typically chops up the chromosome of its host shortly after entering, and then uses the components released (nucleotides) to synthesize copies of its own genome. During transduction, some of the host cell's chromosomal DNA becomes attached to virus DNA and is then "packaged" within the viral **capsid** (the protein coat). When the virus is released and infects a new cell, it carries this cellular DNA with it. The final step in transduction involves integration of viral DNA into the chromosome of the new host. When a bacteriophage genome has become integrated into the chromosome of a host cell, the virus is called a **prophage**. During transduction, the virus carries with it a segment of donor cell DNA and incorporating this into the chromosome the recipient forms recombinant DNA. The recipient cell is also recombinant.

During **specialized or specific transduction**, only specific sections of DNA can be transferred, and the virus involved is **initially a prophage** (is integrated into the chromosome). In this case, the virus exits the chromosome, but takes a small portion of the chromosome with it. This can be chromosomal DNA adjacent to the virus at either end, but not from other regions (i.e., only specific segments can be involved). Once again, the donor DNA is "packaged" within the viral capsid, the virus exits the donor and infects a new host. Upon entering the recipient cell, the viral DNA and the chromosomal DNA it carries with it, enters the recipient chromosome forming a recombinant molecule. Again the recipient cell becomes recombinant.

Although the examples described above suggest that the virus genome is intact within transducing particles, this is not always the case. Sometimes chromosomal DNA alone is packaged within viral capsid proteins and is "injected" into host cells. This occurs commonly during generalized transduction. In other instances, a portion of the viral genome is packaged along with chromosomal DNA, but the virus is "defective" because some phage genes are missing. If the virus genome is complete, and carries chromosomal DNA along with it into a new host, but remains cytolytic, the genetic exchange is of no consequence because the virus will kill the new host (the recipient cell). Dead cells cannot reproduce, so any genetic recombination that might have occurred is lost.

Why is Genetic Exchange Significant?

Under natural conditions, genetic exchange mechanisms are responsible for the **horizontal transfer** of genetic information, i.e., from one cell to another in the same generation. Since genes encoding resistance to various antimicrobial agents are often located on plasmids, these can be passed from cell to cell within a population, or even between populations through genetic exchange. This is occurring at an alarming rate. Penicillin, a β-lactam antibiotic, was first used extensively during World War II. At that time it effectively controlled infections involving common pathogens such as *Staphylococcus aureus* and *Streptococcus pyogenes* (as well as many others). Currently, although penicillin still provides a means of effectively controlling *Streptococcus pyogenes*, as many as 80% of all strains of *Staphylococcus aureus* have become resistant to the drug. Furthermore, the ability to produce β-lactamase, an enzyme capable of degrading penicillin and other β-lactam antibiotics, has become wide spread within many populations of Gram-positive and Gram-negative bacteria.

By studying genetic exchange mechanisms as they occur in nature, biologists have significantly increased their understanding of and appreciation for microbial genetics and the potential applications available for this knowledge. For example:

1. Plasmids that typically replicate themselves numerous times within a host cell, i.e., plasmids with high copy numbers, can be used as **cloning vectors**. Any piece of DNA can be attached to a plasmid in vitro (though the use of restriction enzymes, ligase and modification enzymes as explained in the laboratory), and can then be returned to a cell (either through **conjugation** or **transformation**). When the cell reproduces itself, i.e., grows to form millions of cells in a broth culture, the DNA of interest is replicated billions of times. This can provide an extensive supply of DNA for use in nucleotide sequencing protocols, RFLP analysis, and other applications. Segments of DNA that can replicate themselves when placed into host cells are sometimes called **replicons**, and can be either plasmids or viruses (or modifications of these). Cloning vectors typically carry one or more **marker genes** (genes allowing them to be recognized and selected for, e.g., antibiotic resistance genes), and recognition sequences for one or more **restriction enzymes** (allowing for **ligation** of a gene of interest at a specific location).

2. Virus particles (virions) can enter cells through **transduction** (also called **transfection**), and can also be modified to carry genes from other organisms. Bacteriophage lambda DNA can be converted into a cloning vector called a **cosmid** by removing its lytic genes and inserting genes from some other organism. The viral DNA will still reproduce itself (along with the extra genes), become packaged within a capsid, and infect a new host, but cannot initiate cell lysis. Cosmids can carry more genes than can typical plasmids (up to 44 thousand bp as opposed to 10-15 thousand), and are used extensively to construct "genomic libraries", i.e., collections of DNA from various types of organisms.

3. Expression vectors can carry DNA into cells and reproduce it there, but will also allow the genes carried to be expressed, i.e., their gene products formed. These have insertion sites associated with **constitutive promoters**, so that the genetic information added can be transcribed into m-RNA and translated into proteins. Although bacteria are commonly used to produce eukaryotic proteins now, this technology requires

extensive knowledge of microbial genetics and how eukaryotic cells differ from prokaryotes.

Recall that eukaryotic m-RNA is extensively modified before it is used to encode proteins (**post-transcriptional modification**). Most eukaryotic genes contain numerous introns, and these must be removed and exons spliced together before the m-RNA can be translated. Most prokaryotic cells used in cloning experiments do not form **s-RNA** and do not make **spliceosomes**, so if eukaryotic genes are transferred directly into these cells, they cannot be expressed. To overcome this problem, researchers extract mature, functional m-RNA molecules from various types of tissue. This involves the use of filters equipped with poly-T sequences able to bind with poly-A "tails" on m-RNA molecules. The mature m-RNA lacks introns because it has already undergone modification. DNA can then be synthesized using these m-RNA templates and **reverse transcriptase** enzymes (**RNA-dependent DNA polymerase**). DNA formed by this process is called **complimentary-DNA** or **c-DNA** and can be expressed by prokaryotic cells.

Some human proteins currently produced by *E. coli* cells include human insulin, human interferon, human somatomedin C (an insulin-like growth factor I), human growth hormone and several interleukins. Gene transfer is not restricted to bacteria, and current technology includes placing genes from various types of organisms into alternate organisms for a variety of reasons. Genes from *Bacillus thurengiensis* encoding proteins toxic to butterfly and moth larvae have been placed into plants used in agricultural to prevent insect damage to crops. Unfortunately, this has caused extensive damage to certain butterfly populations (e.g. monarch butterflies) not associated with crop damage. Genes coding for proteins associated with spider webbing have been introduced into milk-producing goats, genes encoding human hemoglobin have been placed into pigs, and many different genes encoding a variety of proteins are currently grown in tissue cultures. Though generally accepted as beneficial, genetic engineering has potentially threatening aspects, and requires careful consideration prior to application.