TRANSFER OF GENETIC INFORMATION IN PROKARYOTES

Genetic recombination is the production of new combinations of genes derived from two different parental cells. Several processes have been described by which prokaryotic cells can exchange genetic information to yield recombinants. They are conjugation, transformation, and transduction. Conjugation requires physical contact between two cells of opposite mating type, resulting in the transfer of DNA from the donor cell to the recipient cell. The transfer of cell-free DNA into a recipient cell is called transformation, whereas transduction involves bacteriophage-mediated transfer of genetic information from a donor cell to a recipient cell.

CONJUGATION: Conjugation involves direct cell-to-cell contact to achieve DNA transfer (Fig. 3-3). For this process, certain types of extrachromosomal elements called plasmids are usually required. The prototype conjugative plasmid is the F, or fertility, factor of E. coli.

F Factor: F factor is an E. coli plasmid (100 kb) with genes coding for autonomous replication, sex pili formation, and conjugal transfer functions. In addition, there are several insertion sequences situated at various sites. F is considered an episome, since it replicates either independently of the host chromosome or as part of the host genome. Cells containing an autonomous F are referred to as F+ cells. Replication of the F factor in this situation requires host proteins but is independent of the dnaA gene product (see Chapter 2, initiation of DNA replication). There are only one to three copies of this plasmid per cell; thus, F is an example of a plasmid whose replication is stringently controlled.

Conjugal Transfer Process: A large portion of F-factor DNA is dedicated to the transfer process. (Figure 3-4 presents a portion of the genetic map for F encompassing the tra region. It is useful to refer to this diagram during the following discussion of F conjugation. In the Enterobacteriaceae, the presence of specific structural appendages (sex pili) on the cell surface is correlated with the ability of the cell to serve as a donor of genetic material. Of the 25 known transfer genes, 14 are involved with F-pilus formation (traA, -L,-E,-K,-B,-V ,-W,-C,-U,-F,-H,-G, Q, X). TraA is the F-pilin protein subunit while all the other genes are involved in the complex process of assembling the sex pilus (see Chapter 7). Sex pilus assembly occurs in the membranous layers of the cell envelope. F-donor cells possess only one to three sex pili. The tip of the pilus is involved in stable mating pair formation (TraN and TraG involvement), interacting with the OmpA protein on the outer membrane of the recipient. Once initial contact between the donor pilus and the recipient is established, the pilus is thought to contract, bringing the cell surfaces of the donor and recipient cells into close proximity. This wall-to-wall contact causes a fusion of cell envelopes and a conjugation bridge that includes a pore formed by TraD. Mating mixtures of E. coli actually involve aggregates of from 2 to 20 cells each rather than only mating pairs. Following mating-aggregate formation, a signal that a mating pair has formed is relayed from the TraD pore protein to TraM, a protein that binds the TraD pore, and ultimately to TraY, a site-specific DNA-binding protein that binds oriT (origin of conjugal transfer). The transfer of F+ DNA initiates from oriT as opposed to oriV, the vegetative replication origin used for plasmid maintenance. TraY and integration host factor (IHF, an E. coli DNA-binding protein) change the architecture of oriT to a structure suitable for binding the bifunctional TraI endonuclease/helicase. The DNA transfer machinery, known as a transferosome or relaxosome, is then assembled at this origin. The plasmid-encoded TraI endonuclease/helicase nicks the F plasmid at oriT, and the 5′ end of the DNA is attached to the protein. The nicked DNA, containing TraI as a pilot protein, travels through the TraD pore at the membrane bridge, not through the pilus itself, as originally believed. The 5′ -ended strand is transferred to the recipient via a rolling circle type of replication with the intact strand serving as a template. The traI gene product (DNA helicase I) unwinds the plasmid duplex (1000 bp/s), pumping one strand into the recipient cell. DNA pol III synthesizes a replacement strand in the donor. The 5′ end of the strand, upon entering the recipient, becomes anchored to the membrane. As the donor strand is transferred into the recipient cell, it, too, will undergo replication and become circularized. The circularization process does not rely on the host RecA synaptase (see "Recombination") but may require a plasmid-encoded recombination system. At this point, the recipient becomes a donor cell capable of transferring F to another cell. Figure 3-5 illustrates the overall process of conjugation while Figure 3-6 presents a more detailed analysis of the proteins involved.

Hfr Formation: The F factor is an example of a plasmid that can exist autonomously in a cell or can integrate into the bacterial chromosome. A cell that contains an integrated F factor is referred to as an Hfr cell (high frequency of recombination). The frequency of insertion occurs at about 10–5 to 10–7 per generation — that is, among a population of 107 F+ cells, 1 to 100 cells will have an integrated F. The mechanism of integration is illustrated in Figure 3-7. Integration involves homologous recombination between two covalently closed circular DNA molecules forming one circular molecule that contains both of the original DNA structures. It is thought that the insertion sequences (IS) present in the F genome (F carries two IS3 and one IS2 sequence) and those in the host chromosome (E. coli contains five each of IS2 and IS3) serve as regions of homology for the insertional event. F integration is predominantly RecA dependent (see "Recombination"), but rare RecA-independent Hfr's can be formed based on the transposition functions of the insertion elements. Consequently, Hfr formation is mainly a nonrandom event, primarily occurring in regions of the chromosome containing an insertion sequence element. Once integrated, the F DNA is replicated along with the host chromosome. However, in situations where the host dnaA gene is inactive, replication of the entire chromosome can initiate from an integrated F in a process called integrative suppression. An integrated F factor still has active transfer functions such that an Hfr to F– cross will transfer host DNA to the recipient where the donor DNA can recombine with the recipient DNA. In Figure 3-7, note that the direction of transfer from oriT is such that the tra genes are always transferred last. Thus, there is directionality to conjugal DNA transfer. In this illustration, the proximally transferred host gene is B while the distally transferred gene is A (the tip of the arrowhead represents the 5′ leading end of the transferred strand). If the orientation of the chromosomal IS element was in the opposite direction, then the resulting Hfr would transfer gene A as a proximal marker and gene B would be one of the last genes transferred. Since conjugal transfer of the host chromosome in an Hfr cell is time dependent (it takes approximately 100 minutes to transfer the entire E. coli chromosome), a gene can be mapped relative to the position of the integrated F factor simply by determining how long it takes for the gene to be transferred to a recipient. The map position assignments for genes are often given in minutes; an assignment is based on the 100 minutes required for conjugal transfer.

F' Formation: As indicated in Figure 3-7, integration of F factor is a reversible process. Normally, excision of the F factor restores the host chromosome to its original state. However, improper or aberrant excision can occur at a low frequency, forming a plasmid containing both F and bacterial DNA. This type of plasmid is called an F-prime (F′). There are two types of faulty genetic exchanges that can result in F′ formation. The first involves recombination between a region on the bacterial chromosome and one within the integrated F factor. The resulting F′ has lost some F sequences but now carries some host DNA originally located at one or the other side of the integrated F. This is a type I F′ . As an alternative, host sequences located on both sides of the integrated F can undergo genetic exchange. The F′ formed in this situation (type II) contains all of F plus some host DNA from both sides of the point where F was integrated. In both situations the host DNA contained in the F′ is deleted from the host chromosome. However, when F′ (type I or type II) is transferred to a new host, a partially diploid (merodiploid) situation occurs for the host genes contained on the F′ . By constructing merodiploids, information can be derived regarding dominance of certain mutations over wild-type alleles of specific genes.

TRANSFORMATION: Not all bacteria are capable of conjugation, but that does not stop them from exchanging genetic information. The first demonstrated system of gene transfer actually did not require cell-to-cell contact. Griffith first discovered the phenomenon, called transformation, in 1928 in the course of his investigations of Streptococcus pneumoniae (pneumococcus). Capsule-producing pneumococci were shown to be virulent for mice, while nonencapsulated strains were avirulent. Griffith discovered that if mice were injected with mixtures of heat-killed encapsulated (smooth = S) and live uncapsulated (rough = R) cells, a curious phenomenon occurred:

Living R cells + Heat-killed S cells -- – (Injected into mice)---→ Dead mice (recovered living, virulent S cells)

The R cells recovered the ability to produce capsules and regained the capacity of virulence! There are different antigenic types of capsular material produced by different strains of pneumococci. Consequently, Griffith showed that if the avirulent R cells injected into the mice were derived from capsular type II cells and the heat-killed cells were of capsular type III, the viable, encapsulated cells recovered from the dead mice were of capsular type III. This indicated a

transformation took place, so type II R cells now produced capsules of antigenic type III. Later, in 1944, Avery, MacLeod, and McCarty demonstrated that it was DNA from the heat-killed encapsulated strain that was responsible for the transformation. They found that if living, rough type II cells were exposed to DNA isolated from type III encapsulated pneumococci, viable type III encapsulated organisms that were virulent for mice could be recovered. These findings were of exceptional importance because they showed that DNA had the ability to carry hereditary information. Conventional wisdom prior to this time held that hereditary traits were more likely to be borne by protein molecules. Transformation occurs in many bacterial genera including Haemophilus, Neisseria, Xanthomonas, Rhizobium, Bacillus, and Staphylococcus. Considerable effort has been exerted to elucidate the nature of competence for transformation on the part of recipient cells. Competence is defined as a physiological state that permits a cell to take up transforming DNA and be genetically changed by it. Organisms that undergo natural transformation can be divided into two groups based on development of the competent state. Some organisms become transiently competent in late exponential phase — for example, Streptococcus pneumoniae. Others, such as Neisseria, are always competent. These different patterns of competence development belie a complex series of regulatory processes required to control this process. The specific physiological and genetic factors involved are discussed in relation to specific groups of organisms. Transformation processes in different microorganisms can be categorized into two main DNA uptake routes known as the Streptococcus–Bacillus model (gram-positive bacteria) and the Haemophilus–Neisseria model (gram-negative bacteria). Keep in mind that separation into these two basic models is artificial, since some of the bacteria in one group share features of the transformation process typically associated with the other group.

Transfection and Forced Competence: Transfection involves the transformation of bacterial cells with purified bacteriophage DNA. The transformed viral DNA will replicate and ultimately produce complete virus particles. Transfection has been demonstrated in a number of bacteria, including B. subtilis, H. influenzae, Streptococcus, Staphylococcus aureus, E. coli, and Salmonella typhimurium. It should also be noted that organisms not considered naturally transformable (e.g., E. coli and S. typhimurium) can be transformed under special laboratory conditions. Alterations made in the outer membrane with $CaCl_2$ or through an electrical shock (electroporation) can be used to transfer DNA such as plasmids into cells. This has been an important factor in the success of recombinant DNA research.

TRANSDUCTION: Transduction is the transfer of bacterial genetic markers from one cell to another mediated by a bacteriophage. There are two types of transduction: generalized and specialized.

Generalized transduction is the phage-mediated transfer of any portion of a donor cell's genome into a second cell. The transducing viral particle contains only bacterial DNA, without phage DNA. During normal loading of nucleic acid into virus protein heads (see Chapter 6), the packaging apparatus occasionally makes a mistake, packaging chromosomal DNA into the phage rather than phage DNA. When a transducing bacteriophage binds to a bacterial cell, the donor DNA is injected into the bacterium by the phage and becomes integrated into the genome of the new cell through generalized recombination. Thus, all genes from a donor cell can potentially be transduced to a recipient cell population. Phages that can mediate generalized transduction include P1 (E. coli) and P22 (Salmonella). A well-studied example of generalized transduction is that of Salmonella phage P22. To understand how generalized transduction occurs, you must have some understanding of how this phage replicates and packages DNA into its head. Phage DNA in P22 is linear but circularly permuted and terminally redundant. Thus, the termini of a P22 DNA molecule contain duplicate DNA sequences, but different P22 molecules within a population contain different terminal sequences. This seemingly odd chromosome structure is due to the packaging method P22 uses to insert DNA into its head. After infecting a cell, the linear P22 DNA circularizes through recombination between the terminal redundant ends. The circular molecule replicates via a rolling circle type of DNA synthesis, forming a long concatemer. A concatemer is a long DNA molecule containing multiple copies of the genome. Packaging of P22 DNA into empty heads initiates at a specific region in P22 DNA called the pac site, where the DNA is first cut, and then proceeds along the concatemer. Once the P22 head is full (headful packaging), a second, nonsequence-specific cut is made. This second cut also defines the start of packing for the next phage head. However, to assure that each head receives a complete genome, the P22 system packs a little more than one genome length of P22 DNA. This is the source of the terminal redundancy of the P22 genome. One

consequence of packing extra DNA is that the packing start site changes for each subsequent phage DNA molecule packaged from the concatemer. Each duplicated sequence at the ends of the P22 genome is different for each packed P22 molecule. When the packaging system encounters a sequence in the bacterial chromosome that is similar to a pac site, it does not distinguish this site from P22 pac sites. It will use this homologous site to package chromosomal DNA such that progressive packaging will generate a series of phage particles that carry different parts of the chromosome. The size of packaged DNA is approximately 44 kb, which is about oneone hundredth the size of the Salmonella chromosome. Therefore, a given P22 will package the equivalent of one conjugation minute worth of chromosomal DNA.

Cotransduction: is the simultaneous transfer of two or more traits during the same transduction event, which enables the mapping of genes relative to each other. Cotransduction of two or more genes requires that the genes be close enough to each other on the host chromosome such that both genes can be packaged into the same phage head. The closer the two genes are to each other, the higher the probability they will be cotransduced. The only thing that could separate the two genes would be a recombinational event occurring between them. Thus, the closer the two genes are to each other, the smaller the recombinational target that could separate them (Fig. 3-11).

In abortive transduction, DNA that is transferred to the recipient cell does not become integrated into the genome of the recipient cell. Since this DNA is not replicated, it is transmitted unilaterally from the original cell to only one of the daughter cells and only transiently expresses the function of the genetic information in these cells. It is not stably inherited, since passage of this DNA eventually is diluted out through subsequent cell divisions.

Specialized transduction is mediated by bacteriophage that integrate (lysogenize) into a specific site (att) on the bacterial chromosome. This specificity limits transfer of genetic material to host markers that lie within the immediate vicinity of this site. Lambda (λ) phage is the most actively studied phage of this type. λ phage almost invariably integrates at an att site near the galactose (gal) region of the chromosome and therefore can mediate specialized transduction of genes in that area to recipient cells (see Lambda Phage in Chapter 6). Both low-frequency and high-frequency transduction have been described for λ (Fig. 3-12). An infecting λ DNA integrates into the bacterial chromosome (A). In lowfrequency transduction (LFT), the integrated phage (prophage) may be induced through some forms of stress (e.g., DNA damage; see "SOS-Inducible Repair") to enter a cycle of lytic infection. As a relatively rare event (10−5 −10−6 per cell), a portion of the genome of the phage is replaced by a specific segment of the host chromosome (B). This occurs due to improper excision of integrated prophage DNA in a manner similar to the formation of type I F′ factors (see Fig. 3-7). These are defective phages because they lack some portion of the phage genome, but they are capable of transducing and integrating into a new host, carrying the original host genes with them (C). This establishes a merodiploid situation. At this point, the defective λ cannot produce more phage particles on its own. Nevertheless, a high-frequency transducing lysate can be made following an LFT event. This happens because a cell doubly lysogenized with a defective λ (carrying donor DNA) and a normal λ can be induced to yield new phage progeny (D). The normal copy of λ provides replication proteins missing from the defective λ. Thus, an HFT lysate is produced in which approximately half of the particles will be specialized transducing particles. As with conjugation, transduction usually occurs most readily between closely related species of the same bacterial genus (intrageneric). This is due to the need for specific cell surface receptors for the phage. However, intergeneric transduction has been demonstrated between closely related members of the enteric group of organisms — for example, between E. coli and Salmonella or Shigella species. Various genetic traits such as fermentation capabilities, antigenic structure, and resistance to chemotherapeutic agents are transducible. Keep in mind that transduction is not limited to the bacterial chromosome. Genetic information residing on plasmids may also be transferred by transduction.