

**COURSE:** Medical Microbiology, MBIM 650/720 - Fall 2009  
**TOPIC:** Exchange of Genetic Information I & II Lectures 38 & 39  
**FACULTY:** Dr. Buckhaults  
Richland MP14, Suite 500  
Phone: 434-1333  
Email: [phillip.buckhaults@uscmcd.sc.edu](mailto:phillip.buckhaults@uscmcd.sc.edu)

**TEACHING OBJECTIVES:**

1. To explain the mechanisms of gene transfer in bacteria.
2. To describe the nature of transposable genetic elements and plasmids.
3. To discuss the significance of gene transfer, transposable genetic elements and plasmids.

**SUPPLEMENTAL READING:**

Murray *et al.* Medical Microbiology, 6<sup>th</sup> Ed., p26-38

**KEY WORDS:** Merozygote, Transformation, Competence, Homologous recombination, Transduction, Generalized transduction, Specialized transduction, Lysogenic conversion, Conjugation, F/sex pilus, Replicon, F+, F-, Hfr, F', Transposable genetic element, Insertion sequence, Transposon, Site-specific recombination, Phase variation, Plasmid, Conjugative plasmid, Nonconjugative plasmid, R factor, RTF, R determinant, Bacteriophage, Capsid, Tail, Contractile sheath, Base plate, Tail fibers, Virulent phage, Lysogeny, Temperate phage, Prophage, Lysogen, Cohesive ends, Site-specific recombination, Repression, Induction

## EXCHANGE OF GENETIC INFORMATION

### I. INTRODUCTION

In bacterial populations mutations are constantly arising due to errors made during replication. If there is any selective advantage for a particular mutation (e.g. antibiotic resistance), the mutant will quickly become the major component of the population due to the rapid growth rate of bacteria. In addition, since bacteria are haploid organisms, even mutations that might normally be recessive will be expressed. Thus, mutations in bacterial populations can pose a problem in the treatment of bacterial infections. Not only are mutations a problem, bacteria have mechanisms by which genes can be transferred to other bacteria. Thus, a mutation arising in one cell can be passed on to other cells.

Gene transfer in bacteria is unidirectional from a donor cell to a recipient cell and the donor usually gives only a small part of its DNA to the recipient. Thus, complete zygotes are not formed; rather, partial zygotes (**merozygotes**) are formed. Bacterial genes are usually transferred to members of the same species but occasionally transfer to other species can also occur.

## II. GENE TRANSFER MECHANISMS IN BACTERIA

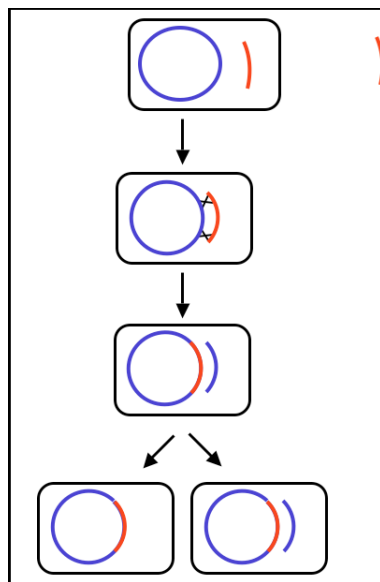
A. Transformation - Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell. Certain bacteria (e.g. Bacillus, Haemophilus, Neisseria, Pneumococcus) can take up DNA from the environment and the DNA that is taken up can be incorporated into the recipient's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

### 1. Factors affecting transformation

- DNA size state - Double stranded DNA of at least  $5 \times 10^5$  daltons works best. Thus, transformation is sensitive to nucleases in the environment.
- Competence of the recipient - Some bacteria are able to take up DNA naturally. However, these bacteria only take up DNA a particular time in their growth cycle when they produce a specific protein called a competence factor. At this stage the bacteria are said to be competent. Other bacteria are not able to take up DNA naturally. However, in these bacteria competence can be induced in vitro by treatment with chemicals (e.g.  $\text{CaCl}_2$ ).

### 2. Steps in transformation

- Uptake of DNA - Uptake of DNA by Gram+ and Gram- bacteria differs. In Gram + bacteria the DNA is taken up as a single stranded molecule and the complementary strand is made in the recipient. In contrast, Gram- bacteria take up double stranded DNA.



- Legitimate/Homologous/General Recombination - After the donor DNA is taken up, a reciprocal recombination event occurs between the chromosome and the donor DNA. This recombination requires homology between the donor DNA and the chromosome and results in the substitution of DNA between the recipient and the donor as illustrated in Figure 1. Recombination requires the bacterial recombination genes (recA, B and C) and homology between the DNA's involved. This type of recombination is called legitimate or homologous or general recombination. Because of the requirement for homology between the donor and host DNA, only DNA from closely related bacteria would be expected to successfully transform, although in rare instances gene transfer between distantly related bacteria has been shown to occur.

Figure 1. Homologous recombination during transformation. Donor DNA is red

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

B. Transduction - Transduction is the transfer of genetic information from a donor to a recipient by way of a bacteriophage. The phage coat protects the DNA in the environment so that transduction, unlike transformation, is not affected by nucleases in the environment. Not all phages can mediate transduction. In most cases gene transfer is between members of the same bacterial species. However, if a particular phage has a wide host range then transfer between species can occur. The ability of a phage to mediate transduction is related to the life cycle of the phage.

1. Bacteriophage

a. Composition - Although different bacteriophages may contain different materials they all contain nucleic acid and protein. Depending upon the phage, the nucleic acid can be either DNA or RNA but not both and it can exist in various forms. The nucleic acids of phages often contain unusual or modified bases. These modified bases protect phage nucleic acid from nucleases that break down host nucleic acids during phage infection. The size of the nucleic acid varies depending upon the phage. The simplest phages only have enough nucleic acid to code for 3-5 average size gene products while the more complex phages may code for over 100 gene products. The number of different kinds of protein and the amount of

each kind of protein in the phage particle will vary depending upon the phage. The simplest phage have many copies of only one or two different proteins while more complex phages may have many different kinds. The proteins function in infection and to protect the nucleic acid from nucleases in the environment .

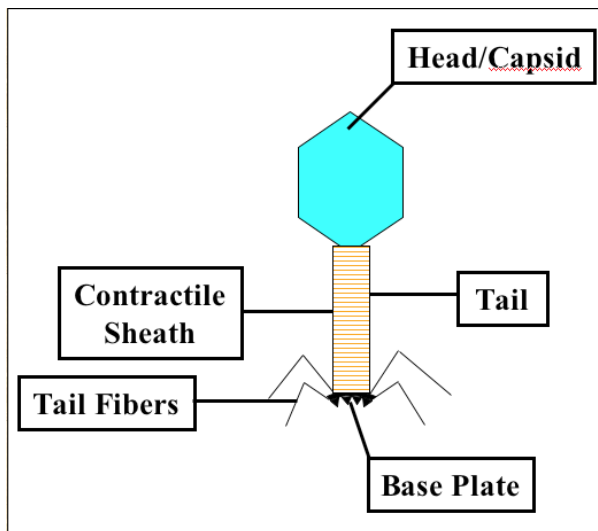


Figure 2. Structure of Bacteriophage T4

b. Structure - Bacteriophage come in many different sizes and shapes. The basic structural features of bacteriophages are illustrated in Figure 2, which depicts the phage called T4.

1. Size - T4 is among the largest phages; it is approximately 200 nm long and 80-100 nm wide. Other phages are smaller. Most phages range in size from 24-200 nm in length.
2. Head or Capsid - All phages contain a head structure which can vary in size and shape. Some are icosahedral (20 sides) others are filamentous. The head or capsid is composed of many copies of one or more different proteins. Inside the head is found the nucleic acid. The head acts as the protective covering for the nucleic acid.
3. Tail - Many but not all phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. The size of the tail can vary and some phages do not even have a tail structure. In the more complex phages like T4 the tail is surrounded by a contractile sheath which contracts during infection of the bacterium. At the end of the tail the more complex phages like T4 have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers. In these instances other structures are involved in binding of the phage particle to the bacterium.

c. Infection of host cells

1. Adsorption - The first step in the infection process is the adsorption of the phage to the bacterial cell. This step is mediated by the tail fibers or by some analogous structure on those phages that lack tail fibers. The tail fibers attach to specific receptors on the bacterial cell and the host range of the phage (i.e. the bacteria that it is able to infect) is usually determined by the type of tail fibers that a phage has. The nature of the bacterial receptor varies for different bacteria. Examples include proteins on the outer surface of the bacterium, LPS, pili, and lipoprotein. These receptors are on the bacteria for other purposes and phage have evolved to use these receptors for infection.
2. Irreversible attachment - The attachment of the phage to the bacterium via the tail fibers is a weak one and is reversible. Irreversible binding of phage to a bacterium is mediated by one or more of the components of the base plate. Phages lacking base plates have other ways of becoming tightly bound to the bacterial cell.
4. Sheath Contraction - The irreversible binding of the phage to the bacterium results in the contraction of the sheath (for those phages which have a sheath) and the hollow tail tube is pushed through the outer regions of the bacterial envelope

(Figure 3). However, the tail tube does not penetrate the cytoplasmic membrane. Phages that don't have contractile sheaths use other mechanisms to get the phage particle through the bacterial envelope. Some phages have enzymes that digest various components of the bacterial envelope.

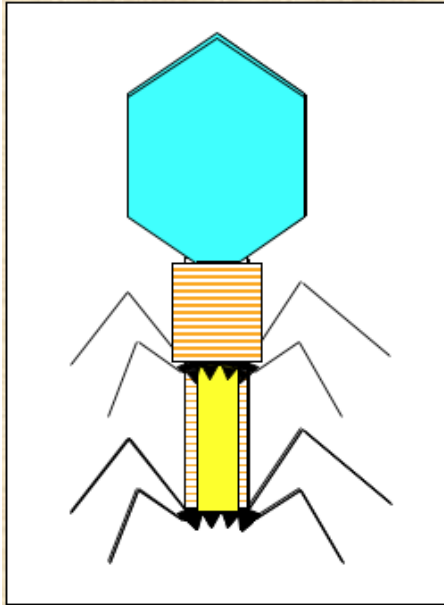


Figure 3. Sheath contraction during infection.

5. Nucleic Acid Injection - When the phage has gotten through the bacterial envelope the nucleic acid from the head passes through the hollow tail and enters the bacterial cell. Usually, the only phage component that actually enters the cell is the nucleic acid. The remainder of the phage remains on the outside of the bacterium. There are some exceptions to this rule. This is different from animal cell viruses in which most of the virus particle usually gets into the cell. This difference is probably due to the inability of

bacteria to engulf materials.

#### d. Phage multiplication cycle

1. Lytic or Virulent Phages - Lytic or virulent phages are phages which can only multiply on bacteria and kill the cell by lysis at the end of the life cycle.
2. Lysogenic or Temperate Phage - Lysogenic or temperate phages are those that can either multiply via the lytic cycle or enter a quiescent state in the cell. In this quiescent state most of the phage genes are not transcribed; the phage genome exists in a repressed state. The phage DNA in this repressed state is called a prophage because it is not a phage but it has the potential to produce phage. In most cases the phage DNA actually integrates into the host chromosome and is replicated along with the host chromosome and passed on to the daughter cells. The cell harboring a prophage is not adversely affected by the presence of the prophage and the lysogenic state may persist indefinitely. The cell harboring a prophage is termed a lysogen.

#### a) Events Leading to Lysogeny - The Prototype Phage: Lambda

- 1) Circularization of the phage chromosome - Lambda DNA is a double stranded linear molecule with small single stranded regions at the 5' ends. These single stranded ends are complementary (cohesive ends) so that they can base pair and

produce a circular molecule. In the cell the free ends of the circle can be ligated to form a covalently closed circle as illustrated in Figure 4.

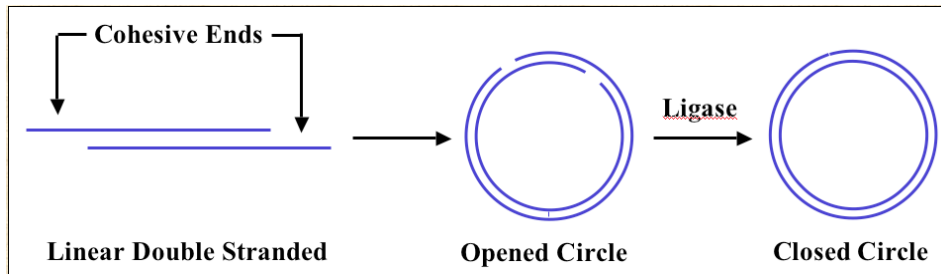


Figure 4. Circularization of the lambda chromosome during the establishment of lysogeny.

2) Site-specific recombination - A recombination event, catalyzed by a phage coded enzyme, occurs between a particular site on the circularized phage DNA and a particular site on the host chromosome. The result is the integration of the phage DNA into the host chromosome as illustrated in Figure 5.

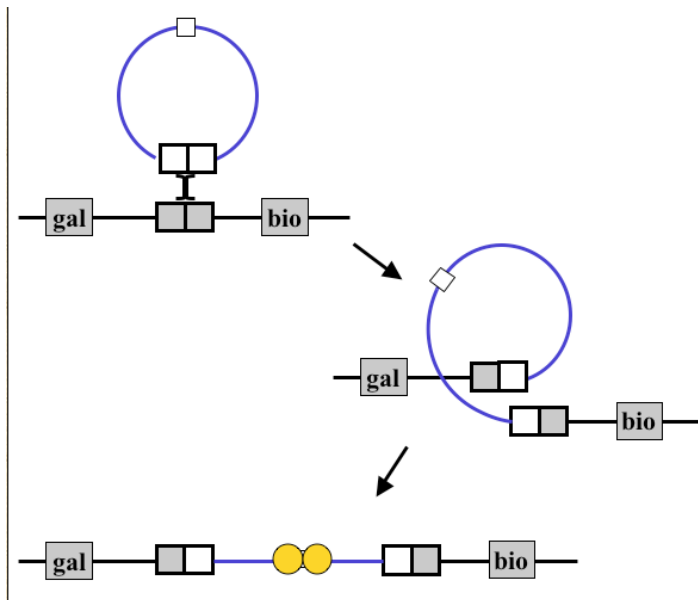


Figure 5. Site-specific recombination during establishment of lysogeny in bacteriophage lambda.

3) Repression of the phage genome - A phage coded protein, called a repressor, is made which binds to a particular site on the phage DNA, called the operator, and shuts off transcription of most phage genes EXCEPT the repressor gene. The result is a stable repressed phage genome which is integrated into the host chromosome. Each temperate phage will only repress its own DNA and not that from other phage, so that repression is very specific (immunity to superinfection with the same phage).

b. Events Leading to Termination of Lysogeny

Anytime a lysogenic bacterium is exposed to adverse conditions, the lysogenic state can be terminated. This process is called induction (Figure 6). Conditions which favor the termination of the lysogenic state include: desiccation, exposure

to UV or ionizing radiation, exposure to mutagenic chemicals, etc. Adverse conditions lead to the production of proteases (rec A protein) which destroy the repressor protein. This in turn leads to the expression of the phage genes, reversal of the integration process and lytic multiplication.

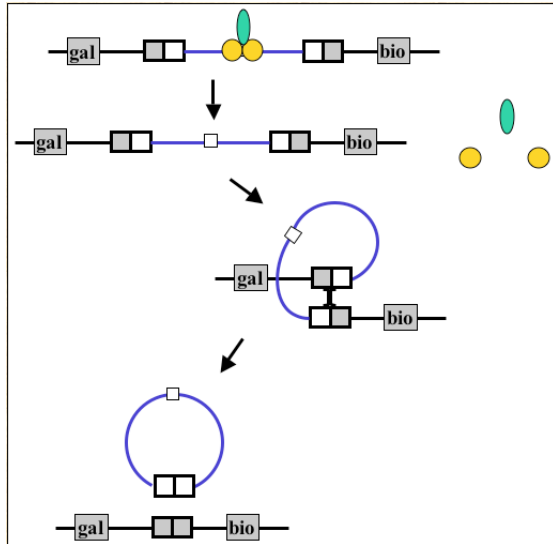


Figure 6. Termination of lysogeny in bacteriophage lambda by inactivation of the repressor and excision of the prophage

## 2. Types of Transduction

- a. Generalized Transduction - Generalized transduction is transduction in which potentially any bacterial gene from the donor can be transferred to the recipient. The mechanism of generalized transduction is illustrated in Figure 7.

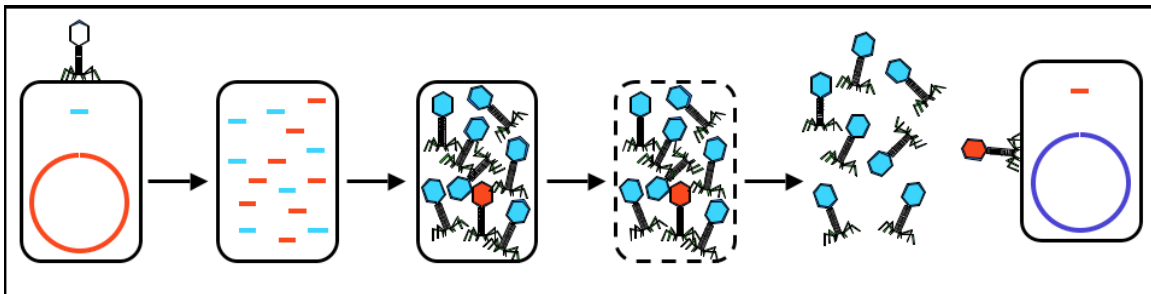


Figure 7. Mechanism of generalized transduction of bacterial genes by phage.

Phages that mediate generalized transduction generally breakdown host DNA into smaller pieces and package their DNA into the phage particle by a "head-full" mechanism. Occasionally one of the pieces of host DNA is randomly packaged into a phage coat. Thus, any donor gene can be potentially transferred but only enough DNA as can fit into a phage head can be transferred. If a recipient cell is infected by a phage that

contains donor DNA, donor DNA enters the recipient. In the recipient a generalized recombination event can occur which substitutes the donor DNA and recipient DNA (See Figure 1).

b. Specialized transduction - Specialized transduction is transduction in which only certain donor genes can be transferred to the recipient. Different phages may transfer different genes but an individual phage can only transfer certain genes. Specialized transduction is mediated by lysogenic or temperate phage and the genes that get transferred will depend on where the prophage has inserted in the chromosome. The mechanism of specialized transduction is illustrated in Figure 8. During excision of the prophage, occasionally an error occurs where some of the host DNA is excised with the phage DNA. Only host DNA on either side of where the prophage has inserted can be transferred (i.e. specialized transduction). After replication and release of phage and infection of a recipient, lysogenization of recipient can occur resulting in the stable transfer of donor genes. The recipient will now have two copies of the gene(s) that were transferred. Legitimate recombination between the donor and recipient genes is also possible.

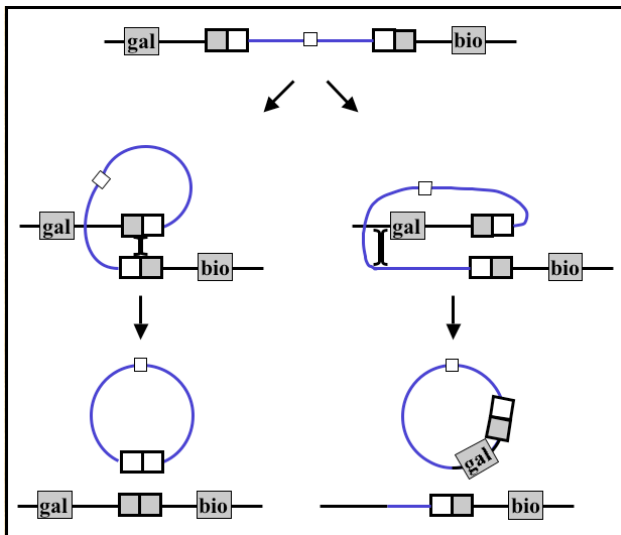


Figure 8. Generation of a specialized transducing phage; left normal excision of the prophage, right abnormal excision resulting in a specialized transducing phage.

3. Significance - Lysogenic (phage) conversion occurs in nature and is the source of virulent strains of bacteria.

C. Conjugation - Transfer of DNA from a donor to a recipient by direct physical contact between the cells. In bacteria there are two mating types a donor (male) and a recipient (female) and the direction of transfer of genetic material is one way; DNA is transferred from a donor to a recipient.

1. Mating types in bacteria

a. Donor - The ability of a bacterium to be a donor is a consequence of the presence in the cell of an extra piece of DNA called the F factor or fertility factor or sex factor. The F factor is a circular piece of DNA that can replicate autonomously in the cell; it is an independent replicon. Extrachromosomal pieces of DNA that can replicate autonomously are given the general name of plasmids. The F factor has genes on it that are needed for its replication and for its ability to transfer DNA to a recipient. One of the things the F factor codes for is the ability to produce a sex pilus (F pilus) on the surface of the bacterium. This pilus is important in the conjugation process. The F factor is not the only plasmid that can mediated conjugation but it is generally used as the model.

b. Recipient - The ability to act as a recipient is a consequence of the lack of the F factor.

2. Physiological states of the F factor

a. Autonomous (F<sup>+</sup>) - In this state the F factor carries only those genes necessary for its replication and for DNA transfer. There are no chromosomal genes associated with the F factor in F<sup>+</sup> strains.

In crosses of the type F<sup>+</sup> X F<sup>-</sup> the F<sup>+</sup> becomes F<sup>+</sup> while F<sup>-</sup> remains F<sup>-</sup>. Thus, the F factor is infectious. In addition, there is only low level transfer of chromosomal genes.

b. Integrated (Hfr) - In this state the F factor has integrated into the bacterial chromosome via a recombination event as illustrated in the Figure 9.

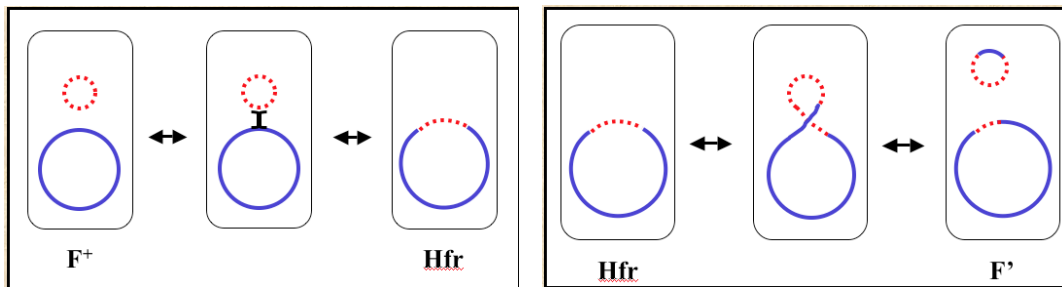


Figure 9. Physiological states of the F factor; F<sup>+</sup> -autonomous, Hfr - integrated into the chromosome, and F' - autonomous and carrying some chromosomal genes.

In crosses of the type Hfr X F<sup>-</sup> the F<sup>-</sup> rarely becomes Hfr and Hfr remains Hfr. In addition, there is a high frequency of transfer of donor chromosomal genes.

- c. Autonomous with chromosomal genes (F') - In this state the F factor is autonomous but it now carries some chromosomal genes. F' factors are produced by excision of the F factor from an Hfr, as illustrated in Figure 9. Occasionally, when the F factor is excising from the Hfr chromosome, donor genes on either side of the F factor can be excised with the F factor generating an F'. F' factors are named depending on the chromosomal genes that they carry.

In crosses of the type F' X F<sup>-</sup> the F<sup>-</sup> becomes F' while F' remains F'. In addition there is high frequency of transfer of those chromosomal genes on the F' and low frequency transfer of other donor chromosomal genes.

### 3. Mechanism of conjugation

- a. F<sup>+</sup> X F<sup>-</sup> crosses (Figure 10)

- 1) **Pair formation** - The tip of the sex pilus comes in contact with the recipient and a conjugation bridge is formed between the two cells. It is through this bridge that the DNA will pass from the donor to the recipient. Thus, the DNA is protected from environmental nucleases. 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.

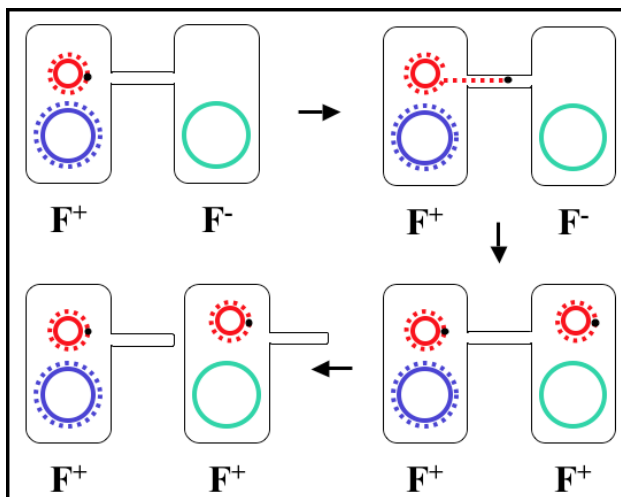


Figure 10. Mechanism of F<sup>+</sup> X F<sup>-</sup> conjugation

- 2) **DNA transfer** - The plasmid DNA is nicked at a specific site called the origin of transfer and is replicated by a rolling circle mechanism. A single strand of DNA passes through the conjugation bridge and enters the recipient where the second strand is replicated. This process explains the characteristics of F<sup>+</sup> X F<sup>-</sup> crosses. The recipient becomes F<sup>+</sup>, the donor remains F<sup>+</sup> and there is low frequency of transfer of donor chromosomal genes. Indeed, as depicted in Figure 10 there is no transfer of donor chromosomal genes. In practice however, there is a low level of transfer of donor

chromosomal genes in such crosses.

b. Hfr X F<sup>-</sup> crosses (Figure 11)

1) Pair Formation

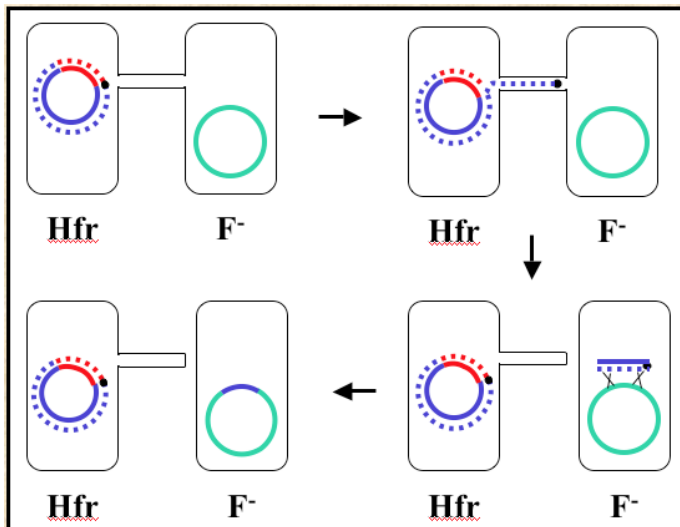


Figure 11. Mechanism of Hfr X F<sup>-</sup> conjugation

- 2) DNA transfer - The DNA is nicked at the origin of transfer and is replicated by a rolling circle mechanism. But the DNA that is transferred first is the chromosome. Depending upon where in the chromosome the F factor has integrated and in what orientation, different chromosomal genes will be transferred at different times. However, the relative order and distances of the genes will always remain the same. Only when the entire chromosome is transferred will the F factor be transferred. Since shearing forces separate the mating pairs it is rare that the entire chromosome will be transferred. Thus, the recipient does not receive the F factor in a Hfr X F<sup>-</sup> cross.
- 3) Legitimate recombination - Recombination between the transferred DNA and the chromosome results in the exchange of genetic material between the donor and recipient.
- 4) This mechanism explains the characteristics of Hfr X F<sup>-</sup> crosses. The recipient remains F<sup>-</sup>, the donor remains Hfr and there is a high frequency of transfer of donor chromosomal genes.

c. F' X F<sup>-</sup> crosses (Figure 12)

1) Pair Formation.

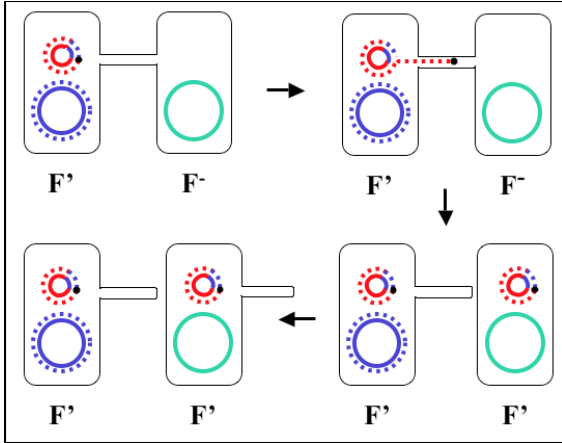


Figure 12. Mechanism of  $F' \times F^-$  conjugation

- 2) DNA transfer - This process is similar to  $F^+ \times F^-$  crosses. However, since the  $F'$  has some chromosomal genes on it these will also be transferred.
- 3) Homologous recombination is not necessary although it may occur.

This mechanism explains the characteristics of  $F' \times F^-$  crosses. The  $F^-$  becomes  $F'$ , the  $F'$  remains  $F'$  and there is high frequency transfer of donor genes on the  $F'$  but low frequency transfer of other donor chromosomal genes.

4. 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 it is easy to see why an antibiotic resistance gene carried on a plasmid can quickly convert a sensitive population of cells to a resistant one. Gram positive bacteria also have plasmids that carry multiple antibiotic resistance genes, in some cases these plasmids are transferred by conjugation while in others they are transferred by transduction. The mechanism of conjugation in Gram + bacteria is different than that for Gram -. In Gram + bacteria the donor makes an adhesive material which causes aggregation with the recipient and the DNA is transferred.

### III. TRANSPOSABLE GENETIC ELEMENTS

A. 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).

B. 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 (exception - some transposable phages) 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 a transposase coded for by the transposable genetic element. Recombination that does not require homology between the recombining molecules is called site-specific or 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.

C. Types of Transposable Genetic Elements

1. Insertion sequences (IS)- Insertion sequences are transposable genetic elements that carry no known genes except those that are required for transposition.
  - a. Nomenclature - Insertion sequences are given the designation IS followed by a number. e.g. IS1
  - b. Structure (Figure 13)

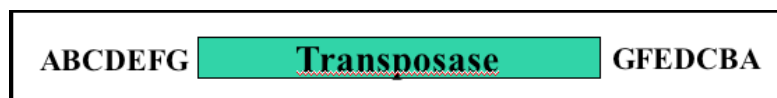


Figure 13. Structure of an IS with inverted repeats at the ends.

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.

c. Importance

- 1) Mutation - The introduction of an insertion sequence into a bacterial gene will result in the inactivation of the gene.

2) Plasmid insertion into chromosomes - The sites at which plasmids insert into the bacterial chromosome are at or near insertion sequence in the chromosome.

Phase Variation - The flagellar antigens are one of the main antigens to which the immune response is directed in our attempt to fight off a bacterial infection. 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. In one orientation one of the genes is active while in the other orientation the other flagellar gene is active. Thus, Salmonella can change their flagella in response to the immune systems' attack. Phase variation is not unique to Salmonella flagellar antigens. It is also seen with other bacterial surface antigens. Also the mechanism of phase variation may differ in different species of bacteria (e.g. Neisseria; transformation).

2. Transposons (Tn) - Transposons are transposable genetic elements that carry one or more other genes in addition to those which are essential for transposition.

a. Nomenclature - Transposons are given the designation Tn followed by a number.

b. Structure - The structure of a transposon is similar to that of an insertion sequence. The extra genes are located between the terminal repeated sequences. In some instances (composite transposons) the terminal repeated sequences are actually insertion sequences. (See Figure 14).

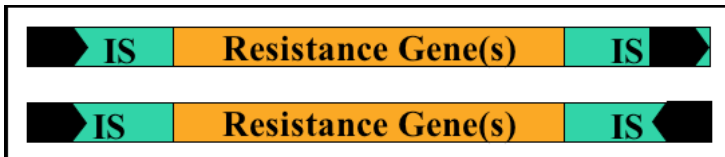


Figure 14. Structure of a composite Tn with direct or inverted IS at the ends.

c. Importance - 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 because the indiscriminate use of antibiotics have provided a selective advantage for bacteria harboring these plasmids.

#### IV. PLASMIDS

A. Definition - Plasmids are extrachromosomal genetic elements capable of autonomous replication. An episome is a plasmid that can integrate into the bacterial chromosome.

B. Classification of Plasmids

1. Transfer properties

a. Conjugative plasmids - Conjugative plasmids are those that mediated conjugation. These plasmids are usually large and have all the genes necessary for autonomous replication and for transfer of DNA to a recipient (e.g. genes for sex pilus).

b. Nonconjugative plasmids - Nonconjugative plasmids are those that cannot mediate conjugation. They are usually smaller than conjugative plasmids and they lack one or more of the genes needed for transfer of DNA. A nonconjugative plasmid can be transferred by conjugation if the cell also harbors a conjugative plasmid.

2. Phenotypic effects

a. Fertility plasmid (F factor)

b. Bacteriocinogenic plasmids - These plasmids have genes which code for substances that kill other bacteria. These substances are called bacteriocins or colicins.

c. Resistance plasmids R factors) - These plasmids carry antibiotic resistance genes. Origin - The origin of the R factors is not known. It is likely that they evolved for other purposes and the advent of the antibiotic age provided a selective advantage for their wide-spread dissemination.

3. Structure - R plasmids are conjugative plasmids in which the genes for replication and transfer are located on one part of the R factor and the resistance genes are located on another part as illustrated in Figure 15.

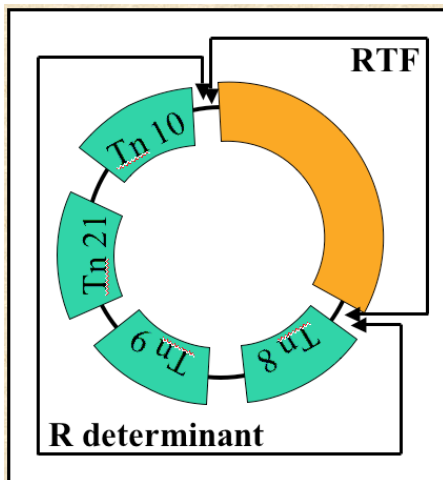


Figure 15. Structure of an R plasmid showing the RTF which mediates transfer and the R determinant which carry the antibiotic resistance genes

a) RTF (Resistance Transfer Factor) - carries the transfer genes.

b) R determinant - carries the resistance genes. The resistance genes are often parts of transposons.