BACTERIOLOGY - CHAPTER EIGHT

EXCHANGE OF GENETIC INFORMATION

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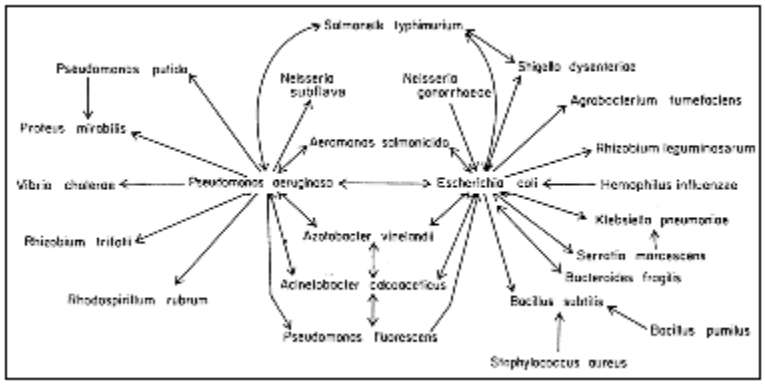
INTRODUCTION

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. Figure 1 illustrates gene transfers that have been shown to occur between different species of bacteria.

Figure 1



GENE TRANSFER MECHANISMS IN BACTERIA

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.

Factors affecting transformation

a. DNA size state
Double stranded DNA of at least 5 X 10^5 daltons works best. Thus, transformation is sensitive to nucleases in the environment.

b. 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.* $CaCl_2$).

Steps in transformation

a. 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.

b. 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 2.

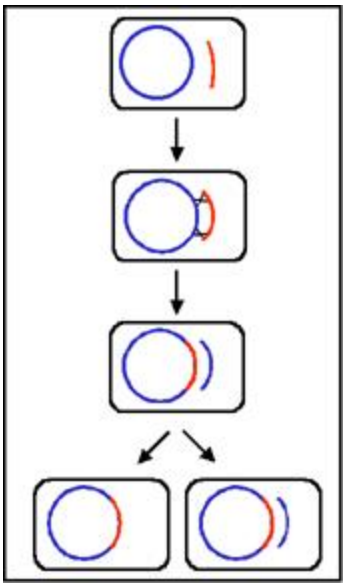


Figure 2

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.

Significance

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

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 mediated transduction is related to the life cycle of the phage.

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 3.

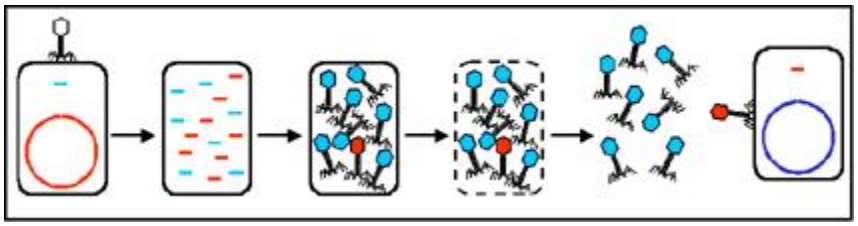


Figure 3

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 2).

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 4.

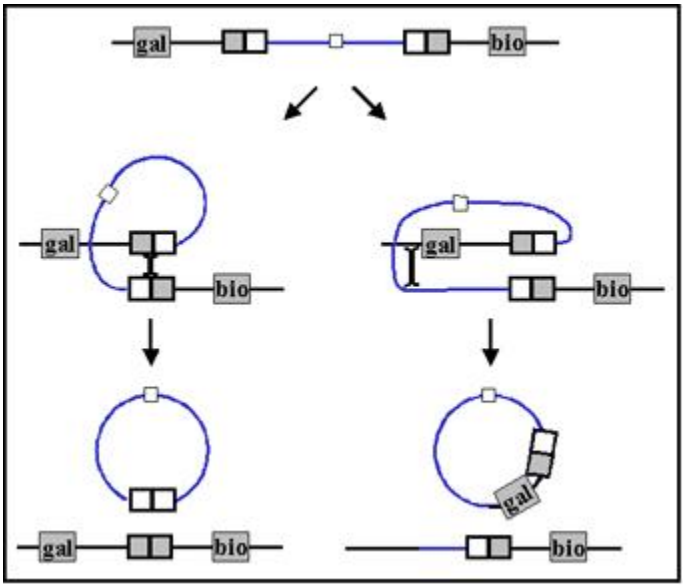


Figure 4

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.

Significance

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

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.

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.

Physiological states of the F factor

a. Autonomous (F^+)
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^+strains.

In crosses of the type F^+ X F^- the F^- becomes F^+ while F^+ remains F^+. 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 5a

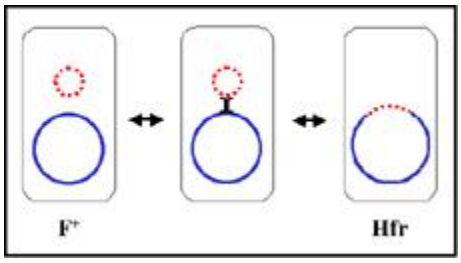


Figure 5a

In crosses of the type Hfr X F^- the F^- 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 5b. 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.

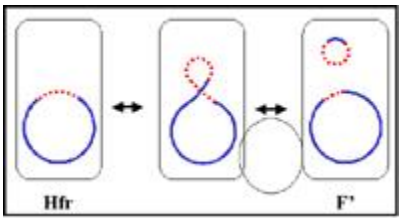


Figure 5b

In crosses of the type F' X F^- the F^- 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.

Mechanism of conjugation

a. F^+ X F^- crosses (Figure 6)

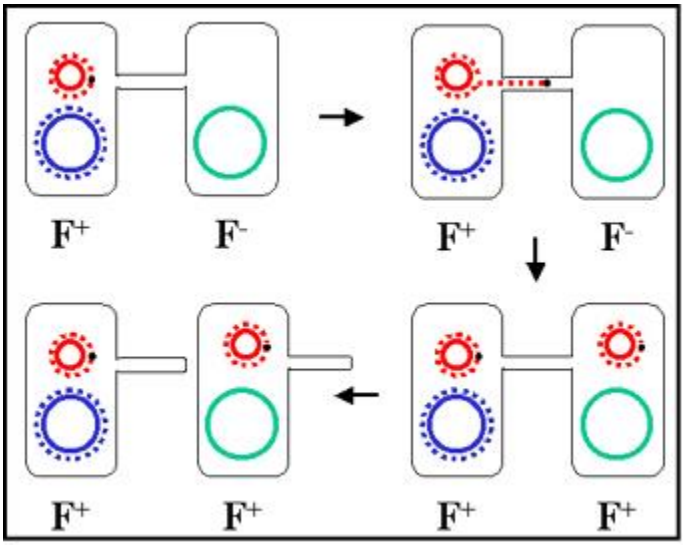


Figure 6

i) 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.

ii) 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.

iii) This process explains the characteristics of F^+ X F^- crosses. The recipient becomes F^+, the donor remains F^+ and there is low frequency of transfer of donor chromosomal genes. Indeed, as depicted in Figure 7 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.

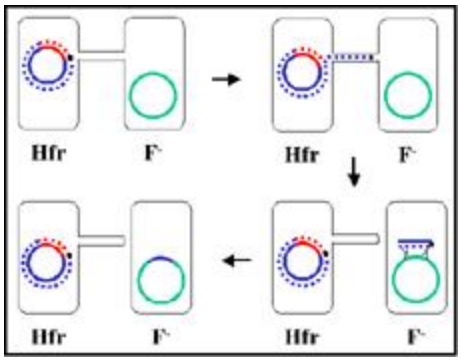


Figure 7

b. Hfr X F^- crosses (Figure 7)

i) Pair Formation

ii) 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^- cross.

iii) Legitimate recombination
Recombination between the transferred DNA and the chromosome results in the exchange of genetic material between the donor and recipient.

iv) This mechanism explains the characteristics of Hfr X F^- crosses. The recipient remains F-, the donor remains Hfr and there is a high frequency of transfer of donor chromosomal genes.

c. F' X F^- crosses (Figure 8)

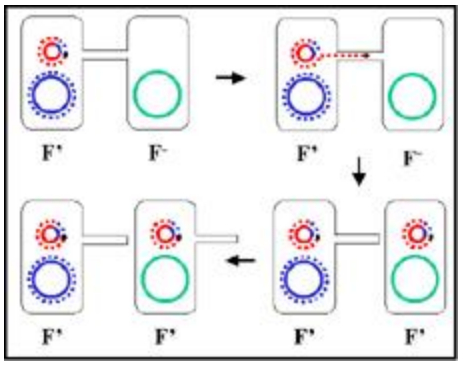


Figure 8

i) Pair formation

ii) DNA transfer
This process is similar to F^+ X F^- crosses. However, since the F' has some chromosomal genes on it these will also be transferred.

iii) Homologous recombination is not necessary although it may occur.

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

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.

TRANSPOSABLE GENETIC ELEMENTS

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

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.

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.

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.

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

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 9)
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.

Figure 9

c. Importance

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

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

iii) 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).

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 10).

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.

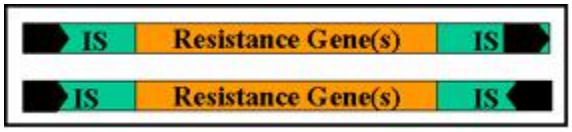


Figure 10

PLASMIDS

Definition

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

Classification of Plasmids

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. Non-conjugative plasmids
Non-conjugative 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 non-conjugative plasmid can be transferred by conjugation if the cell also harbors a conjugative plasmid.

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 7 factors)
These plasmids carry antibiotic resistance genes.

i) 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.

ii) 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 11.

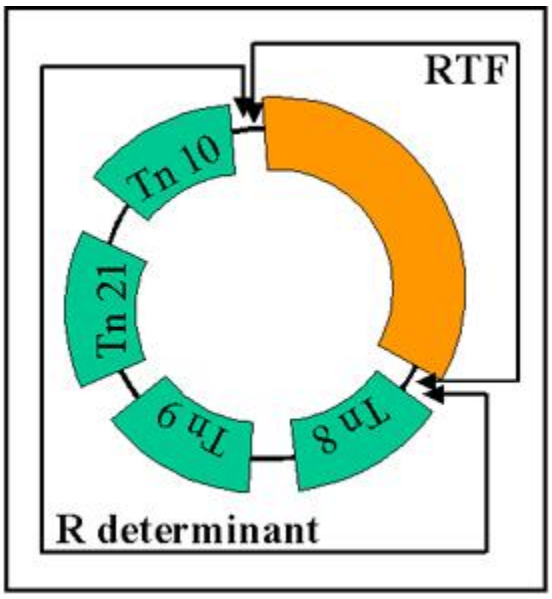


Figure 11

RTF (Resistance Transfer Factor)
Carries the transfer genes.

R determinant
Carries the resistance genes. The resistance genes are often parts of transposons.

Mode of action of resistance genes

a) Modification (detoxification) of antibiotic - e.g. β-lactamase

b) Alteration of target site - e.g. Streptomycin resistance

c) Alteration of uptake - Tetracycline resistance

d) Replacement of sensitive pathway - e.g. new folic acid pathway for resistance to sulfa drugs