

Introduction

The result of the effect of a mutation introduced into the genes of higher animals or plants will require tens of years, because their reproduction speed is measured in years and their number is relatively low.

Conversely, the effects of a new mutation on a bacterium can be observed within a few hours across the millions of cells originating from the original cell.

If the environment changes beyond their capacity to respond through the expression of genes existing in their genome, their only alternative is for one of them to mutate in such a way that a new trait compatible with or adapted to the new environment appears.

The new traits that a bacterium can acquire through genetic exchange allow it to adapt to new environments much more rapidly than through mutations. This adaptability is important in circumstances where the newly acquired genetic information allows the bacterium to survive in an environment that was previously lethal.

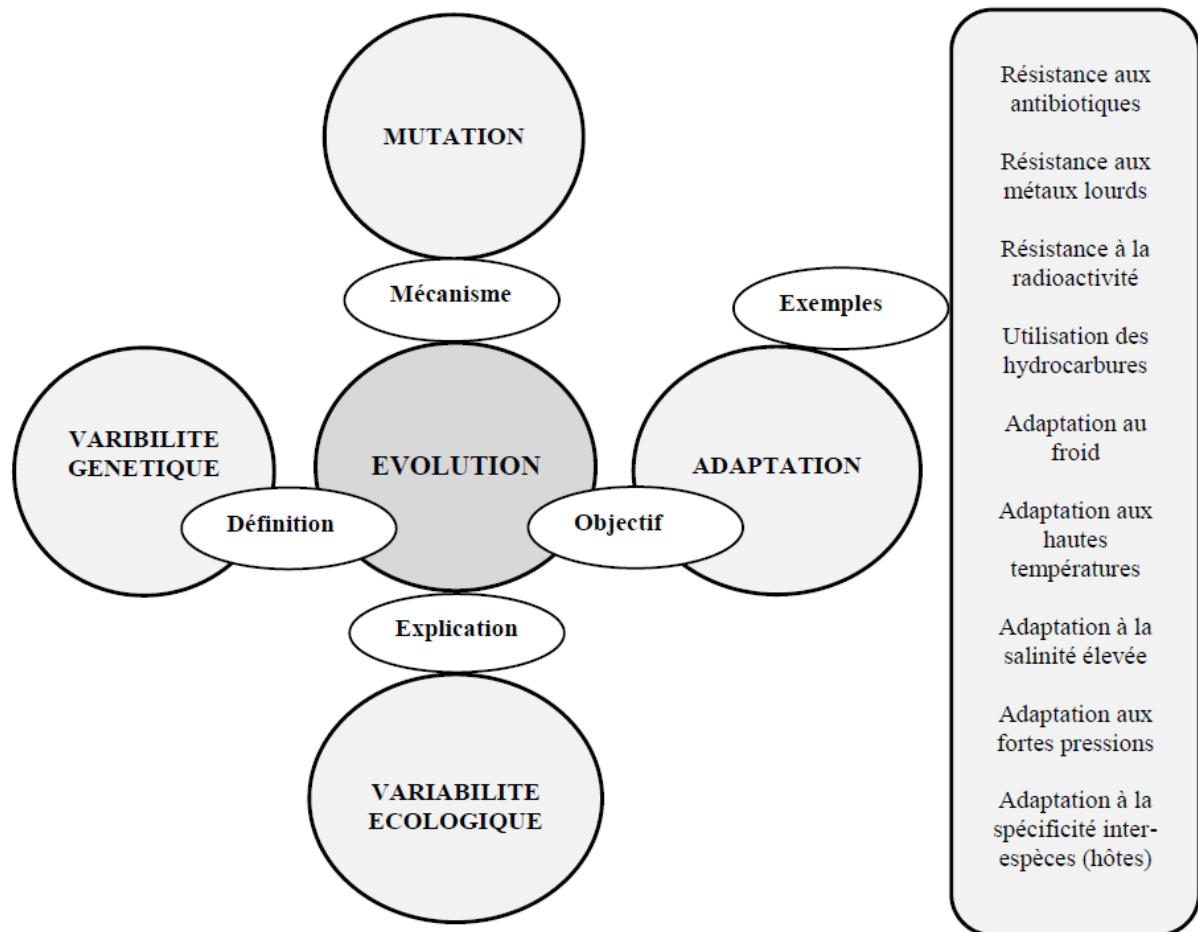


Figure 01. Adaptive power and living plasticity (Boubendir A., 2024).

- Antibiotic resistance
- Heavy metal resistance
- Radioactivity resistance
- Utilization of hydrocarbons
- Adaptation to cold
- Adaptation to high temperatures
- Adaptation to high salinity
- Adaptation to high pressures
- Adaptation to interspecies specificity (hosts)

PLASMIDS

The plasmids present in bacteria are generally circular double-stranded DNA molecules. Since a bacterial cell can be devoid of plasmids, many plasmids exist in a single-copy state and some are present in multiple copies, which can go up to 20 or 30 copies per cell.

The majority, but not all plasmids, can be transferred from one bacterium to another by a process called conjugation. Generally, only very close members of a bacterial genus can exchange plasmids, but some plasmids qualified as ubiquitous can be transferred to phylogenetically distant bacteria.

Plasmids carrying antibiotic resistance: R Plasmids

A very important group of plasmids consists of R factors (or R plasmids) which code for antibiotic resistance determinants. There are many different mechanisms of antibiotic resistance coded by plasmids, the most well-known are:

- The synthesis of enzymes that destroy the antibiotic
- Alteration of the membrane structure so that the antibiotic cannot enter the cell and reach its target.
- Modification of the molecular target of the antibiotic which makes the cell resistant.
- Action of efflux pumps that expel antibiotic molecules from the inside to the outside of the bacterial cell, etc.

The prevalence of R plasmids among pathogenic bacteria (responsible for diseases) is a serious problem for doctors treating infectious diseases because the presence of these plasmids can limit the choice of effective antibiotics for the treatment of the infection.

Furthermore, a plasmid can acquire additional resistance determinants from other R factors; thus, plasmids carrying multi-resistance to antibiotics are common (Figure 02).

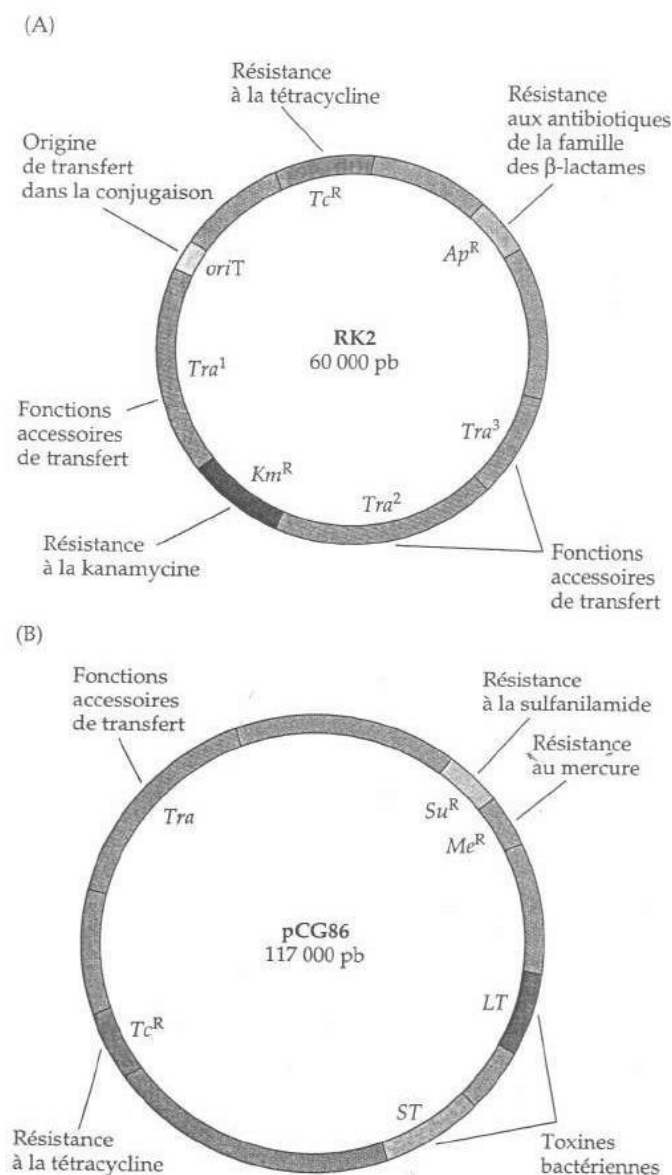


Figure 02. Plasmids carrying multi-resistance to antibiotics and other functions

Virulence plasmids

Plasmids of pathogenic bacteria can carry genes necessary for virulence which confer pathogenicity.

In addition to antibiotic resistance genes, the plasmid can also carry genes coding for adhesins, surface molecules necessary for the colonization of mucous membranes (Figure 02).

Also, plasmids can code for hemolytic factors, proteins that destroy the membranes of many cells, including red blood cells.

In Gram-positive bacteria, they can also code for the synthesis of toxins as in *Bacillus anthracis*. A group of insecticidal toxins is coded by a plasmid present in *Bacillus thuringiensis*, similarly neurotoxins are coded by a plasmid coded by *Clostridium*.

The infection of certain plants by the pathogenic agent *Agrobacterium tumefaciens* carrying the Ti plasmid, a tumor inducer, leads to the formation of crown gall.

Another group of plasmids can code for proteins toxic to other bacteria. These bactericidal proteins called bacteriocins are coded by specific plasmids called bacteriocinogenic plasmids.

Plasmids coding for proteins involved in particular metabolisms

Certain metabolic functions can also be coded by plasmids, such as the biodegradation of complex organic molecules in some species of *Pseudomonas*.

Indeed, plasmids can code for metabolic pathways for the degradation of aliphatic or aromatic compounds such as toluene, benzene, naphthalene, octanes, decanes, etc. Since many environmental pollutants are complex organic molecules, carrier bacteria are candidates of choice for bioremediation. Furthermore, some plasmids can code for functions of resistance to heavy metals.

GENETIC EXCHANGES IN BACTERIA

The bacterium can acquire new genetic information, namely new DNA segments, by three different mechanisms:

- transformation, in which the bacterial cell "absorbs" free DNA present in the environment.
- conjugation, where DNA is transferred via a plasmid from one bacterial cell to another.
- transduction, where a bacteriophage carries bacterial DNA from one cell to another.

TRANSFORMATION

The discovery of the possibility of transforming a bacterium to make it acquire hereditary traits by exposure to a DNA solution, constituted one of the landmark events in the history of genetics. The demonstration was conducted in an experiment by Avery et al. (1944).

They described the ability of an avirulent strain of *Streptococcus pneumoniae* (not causing disease) lacking a protective capsule, to synthesize a capsule again and become virulent, after exposure to free DNA from virulent strains.

The authors then showed that the possibility of transforming an avirulent strain of *S. pneumoniae* into a virulent strain, by a solution of DNA from a virulent strain, was lost when this solution was subjected to the action of DNases, but not after exposure to proteases or RNases. These results confirm that the genetic information transmitted by transformation is carried by DNA and not by proteins or RNA.

All bacteria are potentially capable of being transformed from the moment they are competent. Transformation competence is characterized by a particular membrane state during which the wall, usually relatively rigid, can allow the transport of relatively large DNA macromolecules (this phenomenon is particularly rare in bacteria).

In some bacteria like *Haemophilus*, *Streptococcus* or *Neisseria* competence is expressed during a particular stage of cell division, during which the cell wall allows the passage of DNA. These bacterial genera possess natural competence because their cells do not require any special treatment to increase their DNA absorption capacity.

However, not all bacteria are naturally competent, but treating cells with calcium chloride or rubidium chloride or by heat shock can lead to an alteration of their envelope, making them competent; this form of competence is called artificial competence.

During bacterial transformation, linear DNA is then a privileged target for hydrolysis by specific intracellular enzymes that degrade linear DNA.

This DNA can escape the action of these enzymes by integrating into the chromosome of the recipient cell, generally by recombination via a single crossover event.

CONJUGATION

Conjugation is a mechanism of DNA exchange via a plasmid. The majority, but not all plasmids are transferable, capable of promoting their own transfer as well as that of other plasmids, and even fragments of chromosomal DNA.

The bacteria that contain these transferable plasmids are called donor bacteria; the cells that receive the plasmid are the recipient cells. By analogy with sexual transfer in higher eukaryotic organisms, the donor bacterium is often called male and the recipient female (Figure 03).

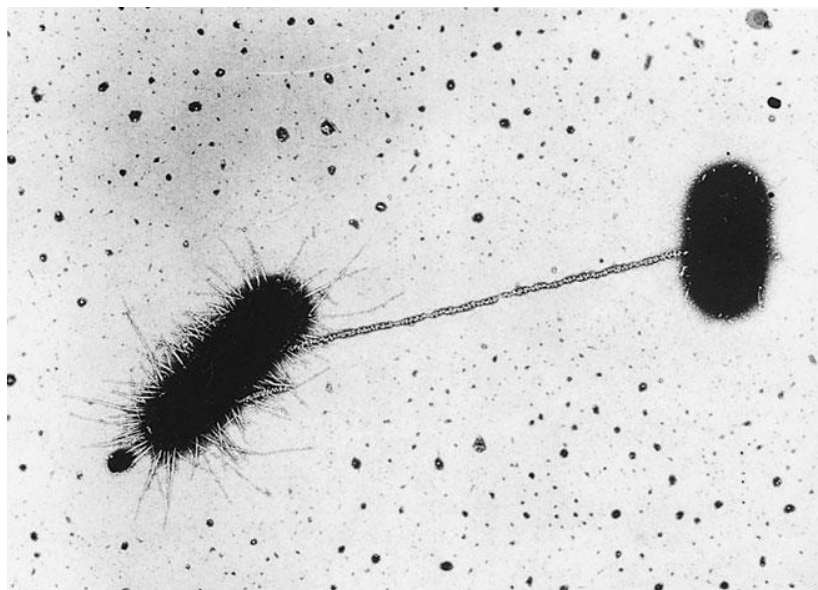


Figure 03. Electron microscopy of bacterial conjugation.

Many cellular genes are dedicated to promoting DNA transfer by conjugation. These transfer functions involve different genes, such as those coding for the components of the pilus and proteins involved in the regulation of expression and biogenesis of the pilus. The pilus is an organelle formed on the surface of the donor cell, which recognizes and initiates the first contact with the recipient cell.

The conjugation process can be divided into several steps (Figure 04).

- initial contact between the donor and recipient cell via pili.
- stabilization of the contact and close association between the two cells.
- formation of a channel in the bacterial cell walls.
- linearization of the plasmid DNA by a break at a specific site called the origin of transfer *oriT*.
- transfer of the 5' end of the DNA strand into the recipient cell. This strand is converted into a circular double-stranded DNA molecule. The synthesis of the complementary DNA strand is very likely initiated even before the end of the F plasmid transfer.

Transfer by conjugation, described for F plasmids or R plasmids, requires that the plasmid contains all the information necessary for its own transfer. These plasmids are called self-transmissible. Thus, the transfer by conjugation of a self-transmissible plasmid into a recipient cell converts the latter into a donor cell which fully acquires the capacity to conjugate with other recipient cells.

However, not all plasmids are self-transmissible. Some plasmids lack certain genes coding for proteins necessary for transfer functions, and are therefore unable to transfer themselves into other cells. These

plasmids are not condemned to reside permanently in a given bacterial cell.

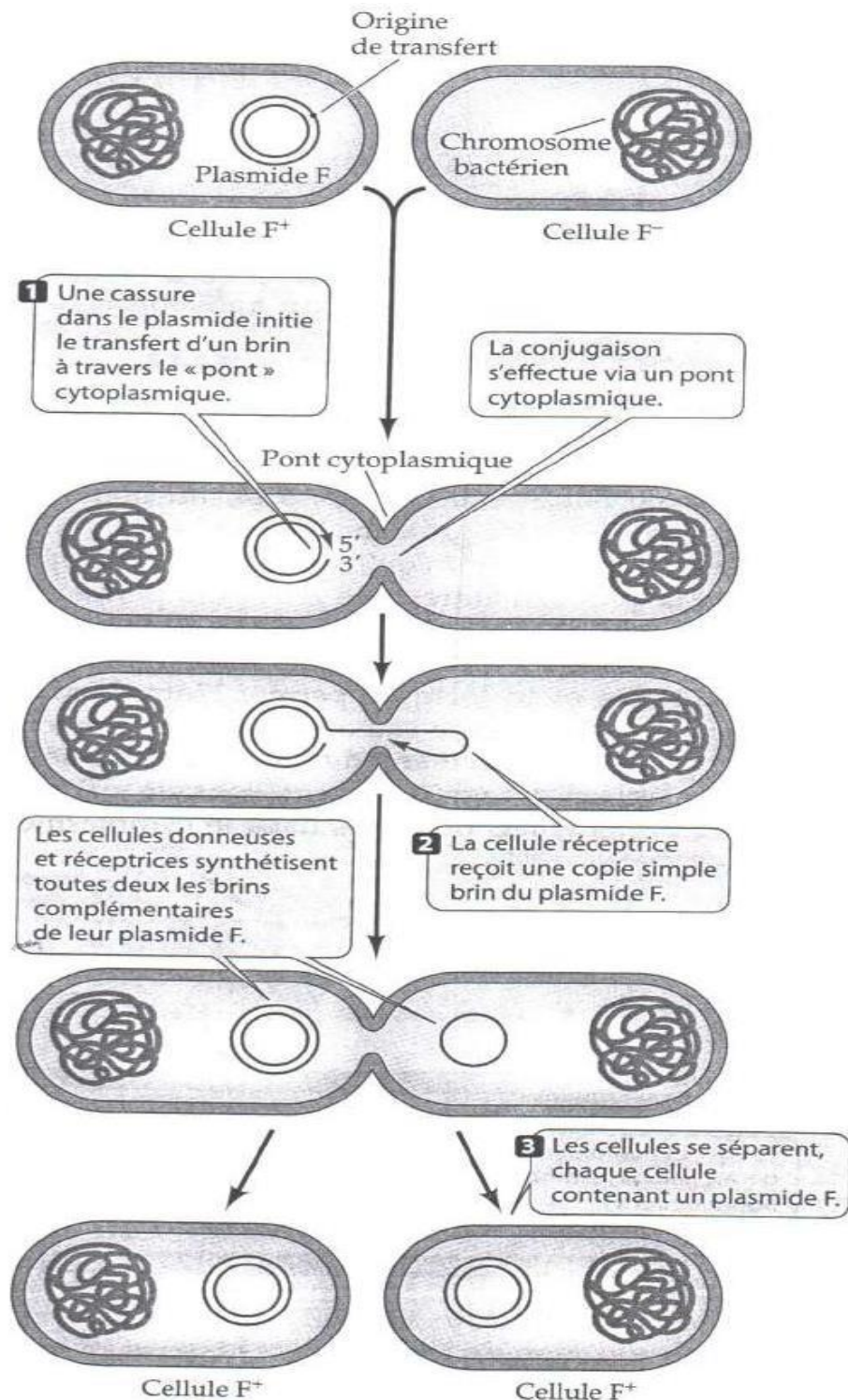


Figure 04. Bacterial conjugation. Transfer of the F plasmid from a donor cell to a recipient cell by conjugation. This transfer completed, both cells possess an intact copy of the F plasmid and behave as donor cells.

A bacterium can contain more than one type of plasmid; non-self-transmissible plasmids have the possibility of acquiring various conjugation functions from other plasmids.

A plasmid lacking, for example, the genes coding for proteins involved in the formation of pili, can be transferred into a recipient cell, if the donor cell harbors another plasmid containing these genes. This auxiliary (helper) plasmid is thus capable of mobilizing another plasmid for its transfer. It should be noted that, unlike self-transmissible plasmids, the cell receiving such a plasmid incomplete in its transfer functions does not become a donor cell, unless it already harbors an auxiliary plasmid.

Plasmids lacking an *oriT* are not transmissible, even in the presence of auxiliary plasmids. However, these plasmids may possess transfer functions and act as auxiliary plasmids. Plasmids lacking an *oriT* or a transfer function can be transmitted from one cell to another by hitchhiking on a transmissible element. This can happen if the two plasmids share a homologous sequence leading to the fusion of the two elements into a chimeric plasmid. The transfer of the chimeric plasmid is initiated by the *oriT* of the component from the transmissible plasmid.

Exchanges of chromosomal genes via a plasmid

In bacteria, gene transfers via self-transmissible plasmids are not limited to genes located on these plasmids. Many conjugative plasmids (transferable by conjugation) can also transfer chromosomal genes. In the F plasmid and several R plasmids, this property of also transferring chromosomal genes is defined as the ability to mobilize the chromosome.

The F plasmid can lead to the transfer of chromosomal genes by two related mechanisms which both require the formation of high-frequency recombination cells (Hfr cells) where the F plasmid is integrated into the chromosome (Figure 05).

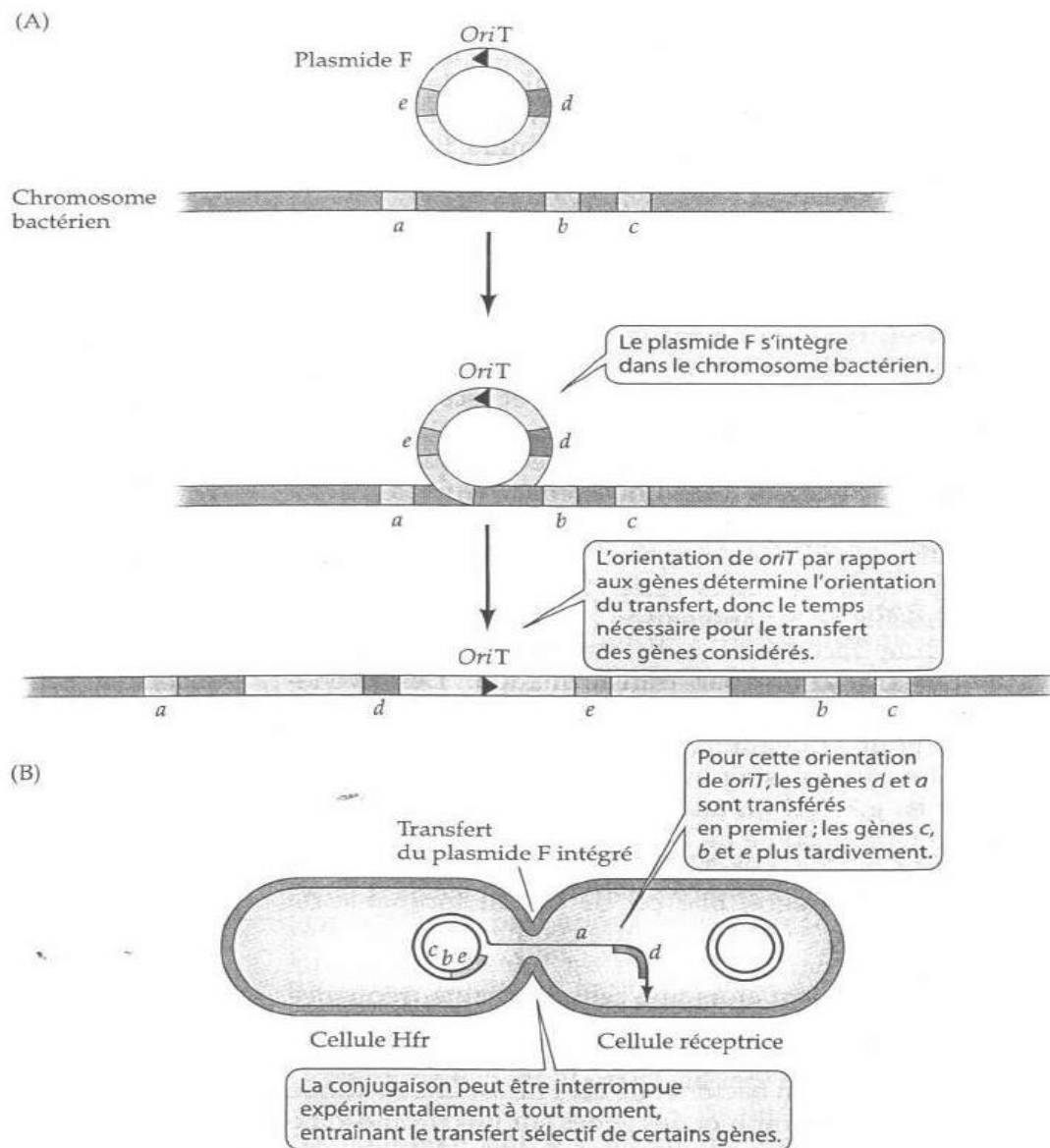


Figure 05. High-frequency recombination cells.

(A) Formation of an Hfr cell and (B) transfer of chromosomal genes into a recipient bacterium. The relative positions of genes on the bacterial chromosome can be determined by mixing donor and recipient bacteria, interrupting conjugation at different times, and placing the bacteria on an appropriate medium to identify the transferred genes.

The integration of the F plasmid does not occur randomly; it integrates at a small number of preferential sites on the chromosome. All these integration sites share a short common DNA sequence. After integration of the F plasmid, the cells can initiate a conjugation process identical to that described for the extra-chromosomal F plasmid.

The entire chromosome of a donor cell can be transferred into a recipient, leading to the formation of a diploid cell, containing an Hfr chromosome. However, the association between donor and recipient cells is generally not very strong and the amount of donor chromosomal DNA transferred is directly proportional to the contact time between the two cells.

Bacterial conjugation via Hfr is a useful method for mapping genes on a bacterial chromosome. Conjugation is controlled experimentally where it is interrupted at variable times, usually by very vigorous shaking (the suspension can be placed in a blender), which breaks the cell contacts. The recipient cells that have integrated DNA from the donor cell are then selected by growth on a specific medium (auxotrophic mutant, resistance / sensitivity to antibiotics).

However, imperfect excision sometimes occurs, taking along a part of the chromosomal DNA. The genes immediately adjacent to the recombination site then become an integral part of the excised plasmid. These plasmids are called F' or R' plasmids. The transfer of DNA from an F' or R' donor cell to a recipient cell is carried out by the same mechanism as that of the F or R plasmid (Figure 06).

The recipient cells, possessing the same genes as those transferred with the F' plasmid, then become partially diploid for these genes or merodiploid, that is, containing two copies of the same gene in one cell.

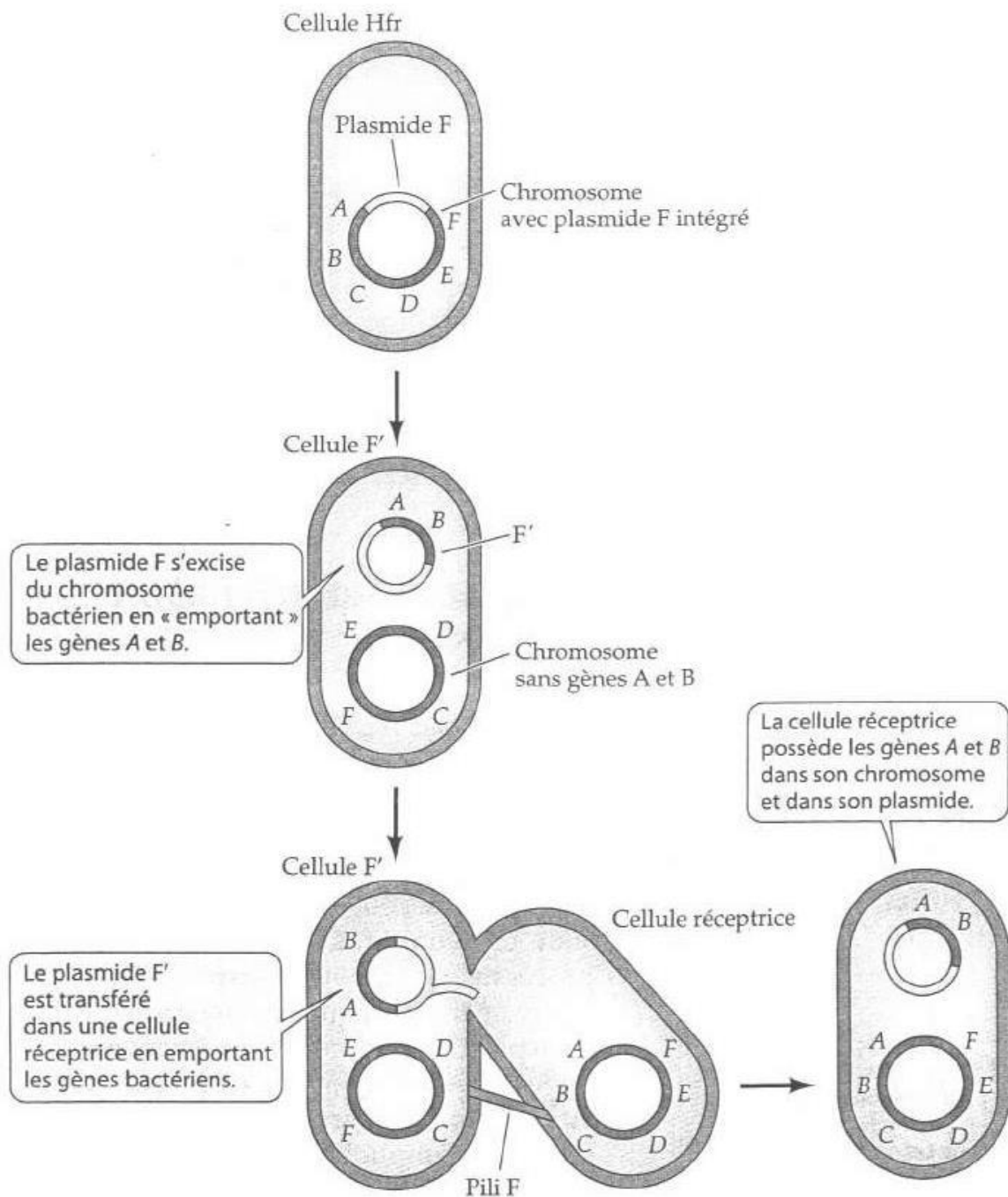


Figure 06. Formation of an F' cell from an Hfr cell, and transfer of a chromosomal DNA sequence to a recipient cell.

TRANSDUCTION

Transduction is the process by which genes are transferred from cell to cell via bacteriophage particles. Two fundamentally distinct transduction mechanisms are carried out by bacteriophages.

Generalized transduction allows the transfer of all bacterial genes at a low but constant frequency. In specialized transduction, on the contrary, some genes are transferred at a very high frequency and other genes are transferred at a low frequency or not transferred.

Generalized transduction

Generalized transduction is carried out by certain virulent bacteriophages (Figure 07). After infecting a cell, these bacteriophages undergo a complete replication cycle. During the infection process, the host's chromosome is degraded and the DNA is used as a source of nucleotide blocks to synthesize the phage genome.

Occasionally, relatively large fragments of genomic DNA remain in the cytoplasm when the phages form their capsid. Infrequent packaging errors lead to the incorporation of bacterial DNA instead of phage DNA. At the end of the infection cycle, the cell lyses and releases the produced phages.

Although the vast majority of capsids contain phage DNA and are therefore fully capable of binding to host cells to carry out new infectious cycles, the rare capsids containing bacterial DNA will also bind to new host cells and inject their genomic content into the cytoplasm.

This interaction between cell and phage does not lead to cell death because the introduced DNA does not code for the components of viral particles. However, the host cell then has a fragment of genomic DNA from another cell. This DNA fragment behaves like any DNA fragment introduced by a gene exchange mechanism such as transformation.

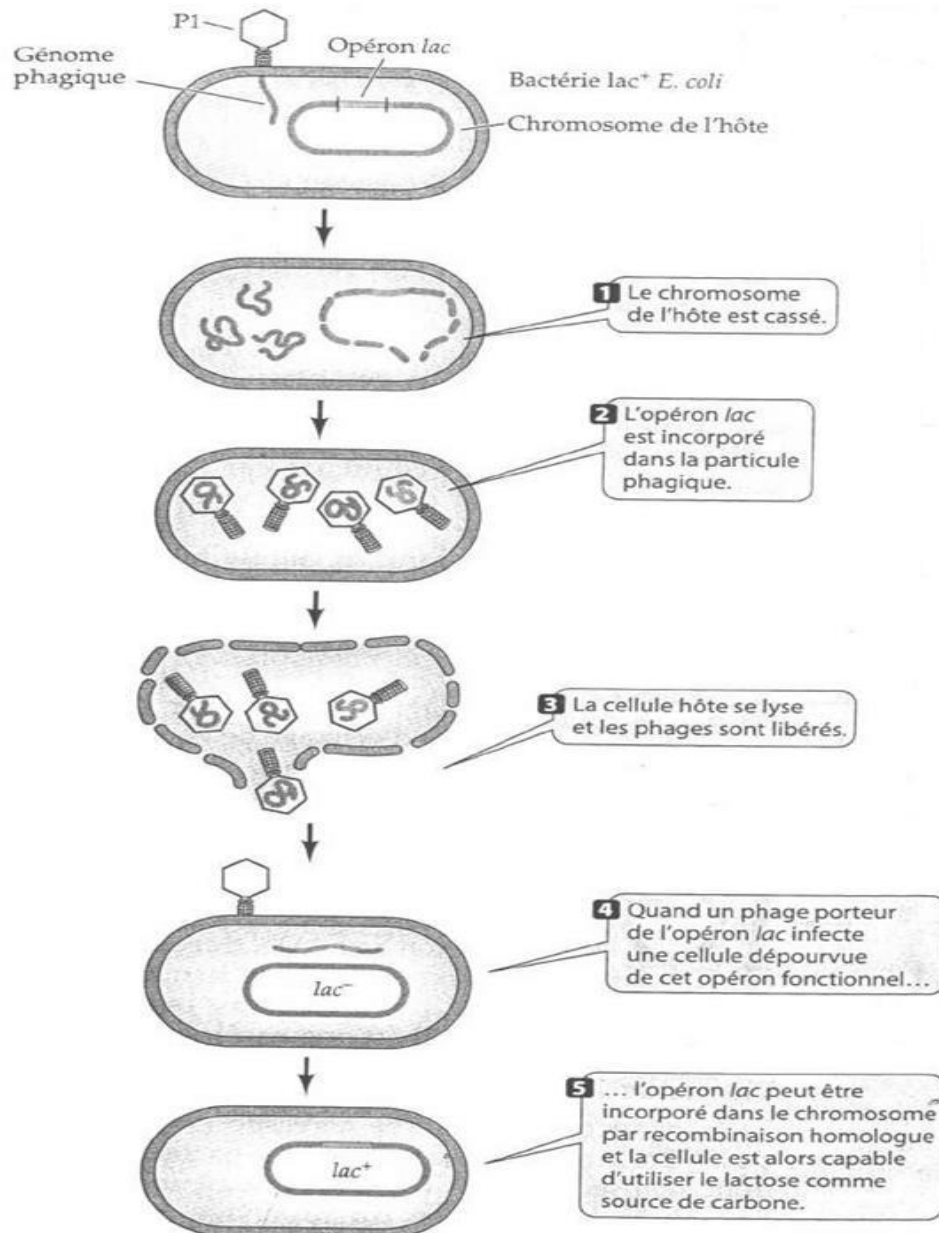


Figure 07. Generalized transduction.

Generalized transduction performed by bacteriophage P1

Specialized transduction

Specialized transduction requires the incorporation of viral DNA into the bacterial chromosome and is therefore only carried out by lysogenic viruses. It occurs after the formation of the prophage, with the integration of viral DNA at a specific site on the host cell's chromosome.

The prophage therefore replicates for several generations with the bacterial chromosome, of which it is an integral part. Occasionally, it can excise itself and initiate a lytic cycle, involving the replication of viral DNA, packaging, and lysis of the host cell. This excision can sometimes be imprecise and take along a part of the flanking chromosomal DNA (Figure 08).

This excised DNA is then included in the viral DNA; it replicates with it and is packaged in each viral particle produced. After cell lysis, the released virulent bacteriophages will infect new host cells, leading to viral replication and a new lytic cycle.

However, as with the initial bacteriophage, the transduced virus can begin a lysogenic cycle involving chromosomal integration of the viral DNA. If the host cell has sequences homologous to the bacterial DNA sequences transduced by the bacteriophage, reciprocal recombination events are likely to occur, leading to the stable incorporation of these genes into the bacterial chromosome independently of the establishment of lysogeny.

Specialized transduction allows the transfer by phages of genes located near the integration site of the lysogenic prophage. Although this process

can only involve these proximal genes, it is extremely efficient, because once the erroneous excision has occurred, the bacterial genes then become an integral part of the viral DNA and are therefore very efficiently transferred.

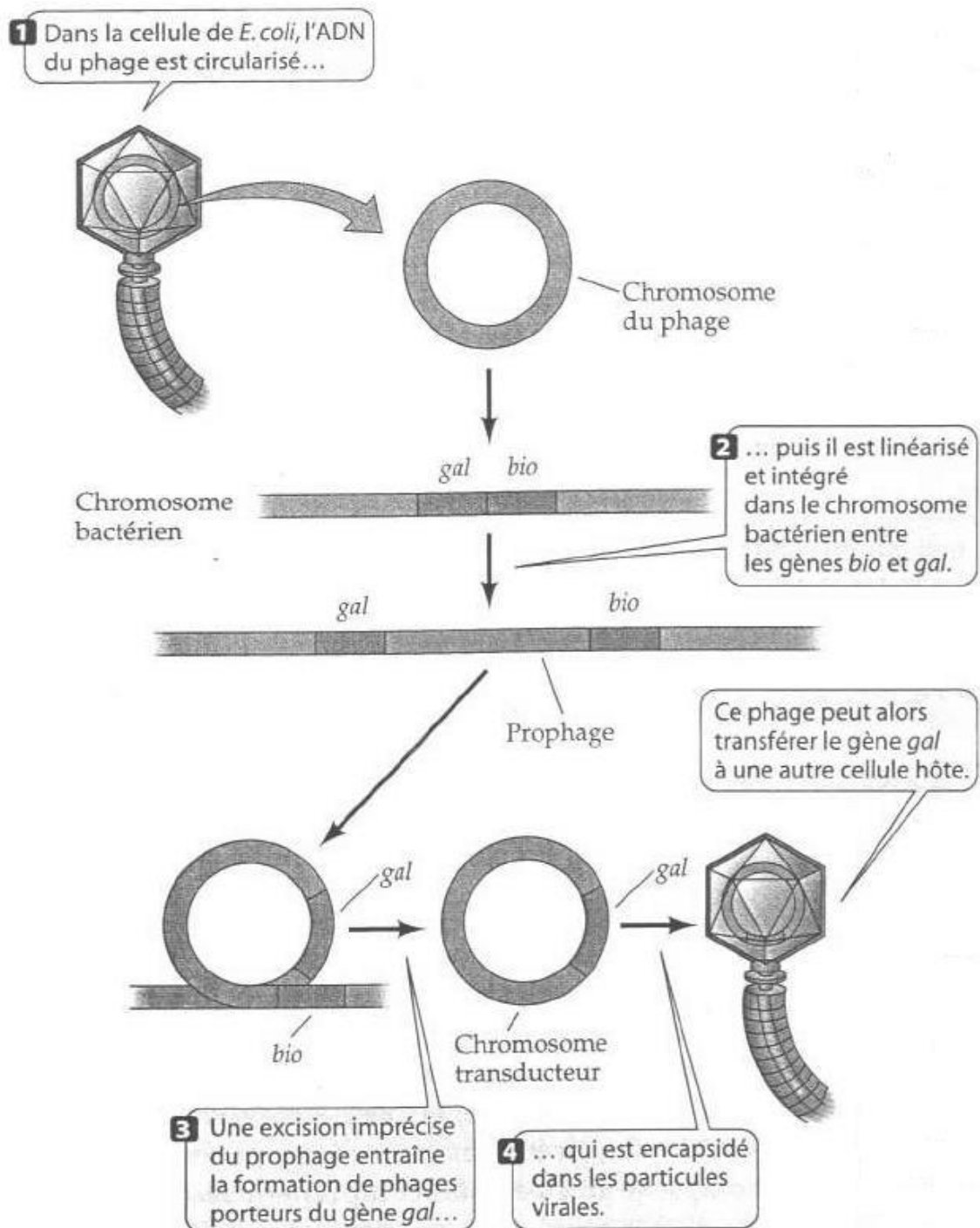


Figure 08. Specialized transduction

Specialized transduction performed by bacteriophage lambda

MOBILE GENETIC ELEMENTS AND TRANSPOSONS

This course has so far dealt with genetic exchanges of DNA fragments transferred from one cell to another by being integrated into phage or plasmid units. Occasionally, a gene can be moved from one location to another on the same chromosome, or between the chromosome and a plasmid contained in the same cell. This process is called transposition.

These genetic movements by transposition involve recombination events between the transposed DNA and its integration site. The particular mechanism involved does not require the presence of homologous sequences as in general recombination (non-homologous recombination).

Site-specific recombination requires the presence of specific DNA sequences specifying the transposition site, and that of a specific enzymatic machinery that catalyzes the transposition event.

Insertion sequence

The insertion sequence or IS is the simplest form of a mobile gene. A number of insertion sequences have been characterized in bacteria, ranging in size from 700 to 5000 bp. All these IS elements have a common characteristic: they contain short inverted repeated sequences of 16 to 41 bp at their ends. The IS also all code for at least one enzyme, called transposase, which specifically catalyzes the site-specific recombination carried out during transposition.

The transposition of an IS element into a new site can lead to the appearance of mutations if it breaks a sequence coding for a polypeptide.

The presence of this large fragment of DNA in an operon (a group of genes transcribed from a single promoter) can lead to polar mutations, that is, prevent the expression of all genes located downstream of the integration site. This phenomenon is explained by the inability of RNA polymerase to traverse an IS element, probably due to the existence of transcription termination signals located in these elements.

Transposons

Transposons are more complex forms of mobile genetic elements, which are characterized by the presence of other genes, in addition to the genes necessary for transposition. Most genes commonly found in transposons are those coding for proteins involved in antibiotic resistance mechanisms. However, other genes such as those coding for toxins have also been found in transposons.

Classification of transposons

The simplest form of transposon is the composite transposon, which is a mobile genetic element in which two IS are found on either side of a gene.

Transposition then involves the translocation of a copy of the entire element (the two IS and the gene) to a new site, using the transposase encoded by one of the two IS.

Composite transposons can serve as a source of transposition of IS sequences because the latter, if they contain the gene for a functional transposase, can transpose independently of the composite transposon. Two examples of these transposons are presented in the Figure. Some composite transposons, like the Tn5 transposon represented in this figure, contain genes coding for multi-drug resistance.

A distinct class of transposons is characterized by the absence of IS elements at their ends, for example the Tn3 transposon (Figure 09). This transposon is a segment of DNA bounded at its ends by short inverted repeats of 38 bp. Inside this transposon, genes code for antibiotic resistance (β -lactamase in Tn3), and two genes are involved in transposition: one coding for transposase and the other for resolvase, which is an endonuclease.

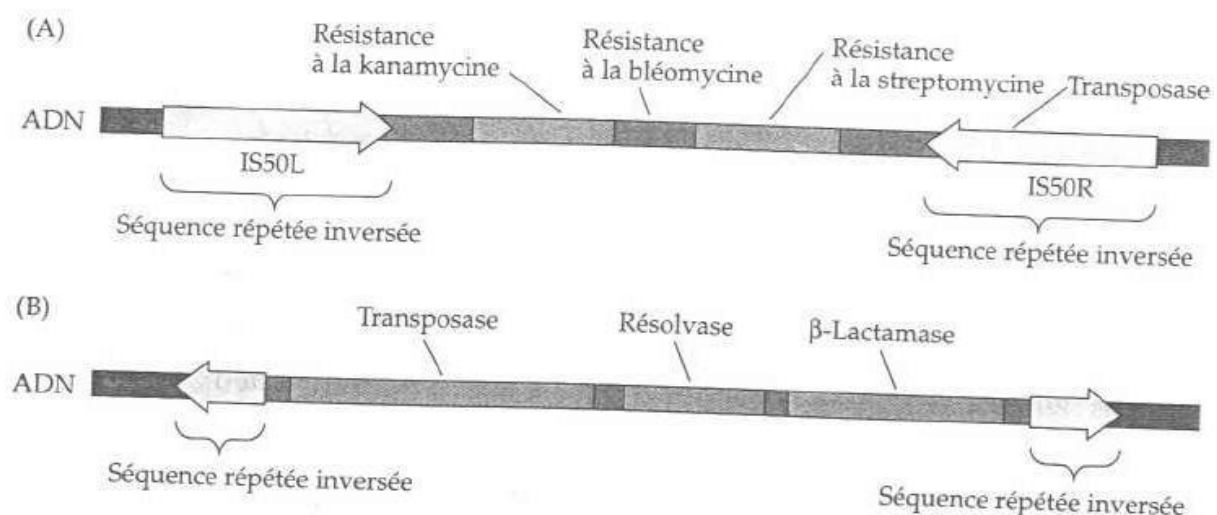


Figure 09. Composite transposons.

Structures of bacterial transposable elements. A composite transposon contains genes coding for proteins involved in antibiotic resistance flanked by two insertion sequences. (A) The Tn5 transposon is represented here with its inverted repeated sequences. (B) Transposon Tn3.