TRANSPOSONS

A transposable element (TE, transposon, or jumping gene) is a DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genetic identity and genome size. Transposition often results in duplication of the same genetic material. Barbara McClintock's discovery of them earned her a Nobel Prize in 1983.

Transposable elements make up a large fraction of the genome and are responsible for much of the mass of DNA in a eukaryotic cell. Although TEs are selfish genetic elements, many are important in genome function and evolution. Transposons are also very useful to researchers as a means to alter DNA inside a living organism.

There are at least two classes of TEs: Class I TEs or retrotransposons generally function via reverse transcription, while Class II TEs or DNA transposons encode the protein transposase, which they require for insertion and excision, and some of these TEs also encode other proteins.

Barbara McClintock discovered the first TEs in maize (*Zea mays*) at the Cold Spring Harbor Laboratory in New York. McClintock was experimenting with maize plants that had broken chromosomes.

EUKARYOTIC TRANSPOSABLE ELEMENTS

Transposons have been discovered in eukaryotic organisms also, e.g., controlling elements in maize, Tam1 elements in *Antirrhinum majus*, Ty elements in Yeast and FB elements in Drosophila. These elements can be divided into two main classes.

1. This class includes the transposable elements that are similar to those found in bacteria. These elements contain inverted repeats at their ends and generate short direct repeats of the target DNA at the sites of their insertion. These elements are always located in the host genome and cannot survive outside the genome. Controlling elements in maize and P elements in Drosophila belong to this class of transposable elements.

2. Retrotransposons (Retroposons): Retrotransposons are DNA elements formed by the reverse transcription of retroviruses. The class of retroviruses and other sequences are transposed via RNA. Transposition of retroposons occur through RNA intermediates.

In the winter of 1944–1945, McClintock planted corn kernels that were self-pollinated, meaning that the silk (style) of the flower received pollen from its own anther. These

kernels came from a long line of plants that had been self-pollinated, causing broken arms on the end of their ninth chromosomes. As the maize plants began to grow, McClintock noted unusual color patterns on the leaves. For example, one leaf had two albino patches of almost identical size, located side by side on the leaf. McClintock hypothesized that during cell division certain cells lost genetic material, while others gained what they had lost. However, when comparing the chromosomes of the current generation of plants with the parent generation, she found certain parts of the chromosome had switched position. This refuted the popular genetic theory of the time that genes were fixed in their position on a chromosome. McClintock found that genes could not only move, but they could also be turned on or off due to certain environmental conditions or during different stages of cell development.

McClintock also showed that gene mutations could be reversed. She presented her report on her findings in 1951, and published an article on her discoveries in *Genetics* in November 1953 entitled "Induction of Instability at Selected Loci in Maize".

Her work was largely dismissed and ignored until the late 1960s–1970s when, after TEs were found in bacteria, it was rediscovered. She was awarded a Nobel Prize in Physiology or Medicine in 1983 for her discovery of TEs, more than thirty years after her initial research.

Approximately 90% of the maize genome is made up of TEs, as is 44% of the human genome.

PROKARYOTIC TRANSPOSABLE ELEMENTS

The following points highlight the two main types of transposable elements found in prokaryotes. The types are: 1. Insertion Sequences 2. Transposon.

Type # 1. ***Insertion Sequences (IS):***

IS elements are the simplest transposable elements and are normal constituents of bacterial chromosomes and plasmids. The IS elements range in size from 768 bp to more than 5,000 bp and are found in most cells. All such elements end with perfect or nearly perfect terminal inverted repeats (IR's) of 9 to 41 bp.

Thus, essentially the same sequence is found at each end of an IS, but in opposite orientations. The IS elements encode an enzyme transposase that is essential for their own transposition. This enzyme recognises those sequences that initiate transposition.

Integration of IS elements along the chromosome may cause mutation by disrupting either the coding sequence of a gene or a gene's regulatory sequence. In addition, the presence of IS elements can cause deletion or inversion type of mutations in the adjacent DNA. Finally, deletion and inversion events may also occur as a result of crossing over between duplicated IS elements in the genome.

Example:
In *E. coli*, a number of IS elements are known, e.g., IS1, IS2, IS1OR etc. Each genome may contain up to 30 copies of IS elements and each has a characteristic length and nucleotide sequence. For example, IS1 has 768 bps and is present in 4 to 19 copies in *E. coli* chromosome. The inverted repeats of IS1 consist of 23 bp with not quite identical sequences.

Mechanism of transposition:
The IS elements integrate at a target site with which the element has no sequence homology. At the time of insertion, first a staggered cut is made at the target site and the IS element is then inserted, becoming joined to the single-stranded ends.

DNA polymerase and ligase then fill in the gaps, making an integrated IS element with two direct repeats of the target site sequence flanking the IS element. Here 'direct' means that the two sequences are repeated in the same orientation. These small direct repeats are called target site duplications and their size varies from 4 to 13 bp.

Type # 2. ***Transposon (Tn):***
The transposon is more complex mobile genetic element than the IS element. The Tn contains gene coding for transposase as well as other proteins.

In prokaryotes two types of transposons are found:

(a) Composite transposons and

(b) Non-composite transposons.

(a) **Composite transposons**:
These Tn elements may be 1000 bp long and have a complex structure with a central region containing genes that confer resistance to antibiotics. They are flanked by IS elements of same type on both sides which are called ISL for the left one and ISR for the right one.

The ISs themselves have terminal inverted repeats in addition to terminal inverted repeats of the composite transposon. So the ISL and ISR may be in the same or inverted orientation, relative to each other.

Example:
Tn10: Here the IS elements help in the transposition of the composite transposons. The transposase is supplied by one or both IS elements and recognise the inverted repeats of the IS elements at the two ends of the transposon to initiate transposition. Like IS elements, they produce target site duplication after transposition, which is 9 bp long in case of Tn10. Transposition of Tn10 is rare, occurring once in 10^7 cell generations.

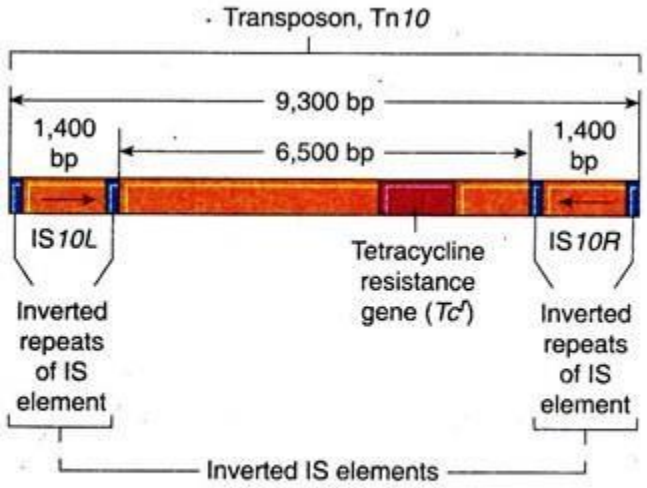

Fig. 9.36 : Structure of Tn10. The central region carries gene/genes and flanked by inverted IS elements at each end

(b) **Non-composite transposons:**
Unlike the composite transposon, non-composite transposons do not contain IS elements at their ends, but has the repeated sequences at their ends that are required for transposition.

Example:
Tn3: It has 38 bp inverted terminal repeats and contains three genes in its central region. One of the genes is bla encoding β-lactamase which breaks down ampicillin and, therefore, makes the cells with Tn3 resistant to ampicillin.

The other two genes are tnpA and tnpB encoding the enzymes transposase and resolvase, respectively. Transposase catalyses insertion of Tn into new sites, and resolvase helps in the recombinational events associated with transposition. Like composite transposons they also cause target site duplication while they move. For example, Tn3 produces a 5 bp site duplication.

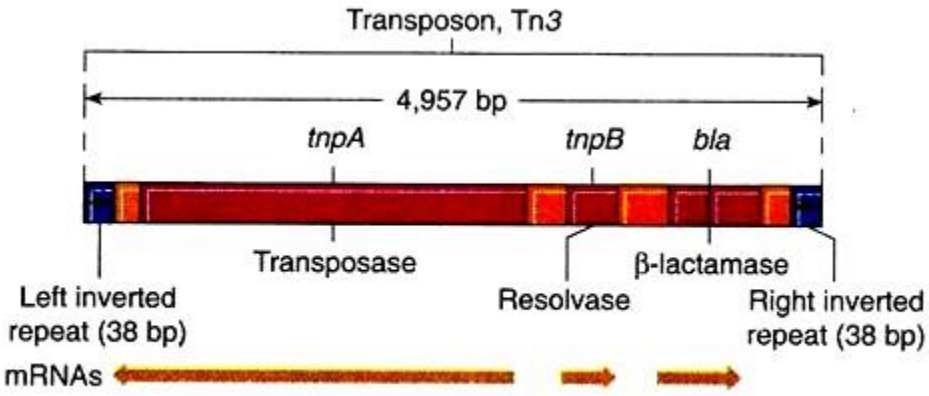

Fig. 9.37 : Structure of non-composite transposon Tn3

Mechanism of transposition:

Several models have been proposed regarding the transposition of transposons.

Two most popular models are discussed here:

(i) **Replicative transposition:**

According to this model, transposition of transposon occurs from one genome to another. For example, from a plasmid to a bacterial chromosome or vice versa. In this case, the donor DNA with the Tn elements fuses with the recipient DNA. During this, the Tn element becomes duplicated with one copy located at each junction between donor and recipient DNA.

This fused product is called a co-integrate. Then, the co-integrate is resolved into two products, each with one copy of the transposable element. Since the Tn element becomes duplicated, this process is called replicative transposition.It involves two enzymes:

(a) Transposase acts on the end of original Tn element and

(b) Resolvase acts on the duplicated copies.

Tn3 and other non-composite transposons show this type of transposition.

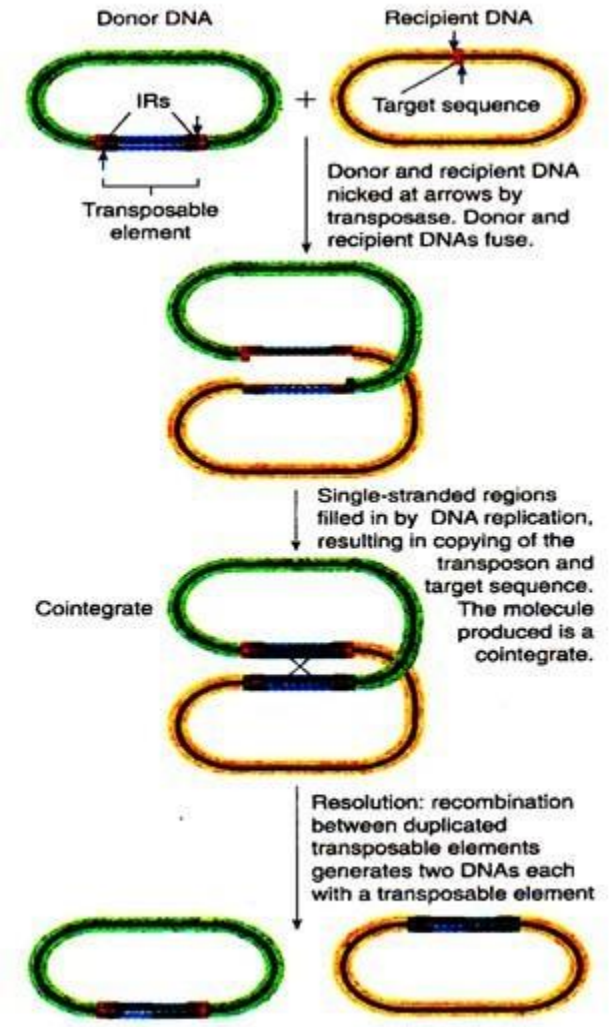

Fig. 9.38 : Transposition of a Transposable element by replicative transposition

(ii) **Non-replicative transposition:**

It allows the transposon to move physically from one location to another on the same or different DNA, without any replication of the element. This mechanism is called conservative transposition because here every nucleotide bond is conserved.

The Tn element is lost from the original position leaving a break at the donor DNA which is lethal unless it is repaired. Tn10 transposon transposes by conservative transposition.

Insertion of a transposon into the reading frame of a gene may cause loss of function of that gene.

USE OF TRANSPOSONS

1. Mutagenesis: Since it is clear that transposons transpose to new sites at certain frequencies, one might expect that you "mutagenize" a strain by taking a strain carrying the transposon (in a site considered "uninteresting" in terms of the system being studied) and looking for cases where the Tn has moved and generated an "interesting" mutation. Such a situation is achieved if the Tn is introduced into a recipient cell on a non-replicating vector. The vector should also not be able to easily integrate itself into a replicon, as would be the case for a specialized phage. In this case, the only way for a "stably" drug-resistant cell to exist is for the Tn to become associated with a replicon by transposition.

2. Selection for a mutant phenotype: Most mutations leading to the loss of gene function do not have a selectable phenotype. This means that they cannot easily (i.e. selectably) be moved from one strain to another using the gene transfer. A mutation caused by a transposon therefore has a distinct advantage, since it has two phenotypes: the loss of function of the affected gene and the drug-resistance of the transposon. The latter is selectable, but brings the former along.

3. Cleanliness: As a mode of mutagenesis, transposons are relatively clean; that is, in contrast to other forms of mutagenesis they do not cause a large number of alterations in the genome.

4. Organism specificity: Because most MGEs borrow some host machinery during transposition, they tend to be limited to hosts with "compatible" machinery. Most MGEs found in Gram-negative bacteria therefore tend to be specific for that group and fail to function in Gram- positives.

5. Genetically altered transposons: An increasing number of genetically altered transposons are being produced with useful and amusing properties: (1) elements with drug resistance of one transposon but with a transposase and the ends of another, thus yielding, for example, elements with a Tn5 randomness

and a variety of drug-resistant properties. (2) Elements that transpose more or less frequently than the originally isolated version due to differential expression of the transposase. (3) Elements that transpose only once and then are stable. These latter types are generated by removing the transposase gene from the element but supplying the gene product in "*trans*" by placing that gene elsewhere in the donor cell. (4) Elements that carry a gene or operon that is unexpressed due to the lack of a promoter, but expressible if the element is inserted (in the appropriate orientation) into an actively transcribed region. Such elements have been devised so that they do not disrupt transcription entering one end of the element but allow such transcription of the promoterless region they carry.

6. Physically detectable homology: When a transposon is used to generate a mutant, the mutated region can be physically isolated using probes for the transposon sequence. The mutated region can then be used in turn as a probe for the wild-type sequence.