e-content for Program: M.Sc. Botany (4th semester)
Course: MBOTEC1
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Topic: Transposons: Structure and types of transposons (Prokaryotic and Eukaryotic); Mechanism of transposition (replicative and non-replicative); Retroposons; Application of transposon

1st Part (Transposon: Structure and types; Mechanism of transposition)

Genetic studies of Maize by Barbara McClintock (1940s) yielded results that greatly upset the classical genetic picture of gene residing only at fixed loci on the chromosome. She suggested the existence of genetic element of main genome that can move from one location to another. These findings were ignored for many years but are now clear that such mobile element is wide spread in nature. Barbara Mc Clintock got Nobel Prize in 1983.

Many names have been applied to these elements like controlling agent, cassettes, jumping gens, mobile genes etc. The term **“transposon”** was coined by Hedges and Jacob in 1974. These are also known as “Transposable genetic elements” (most appropriate name).

Definition: “Short segment of genetic elements that can move or transpose from one position on the chromosome to another position on the same chromosome or on different chromosome”. The process is called “transposition”.

Although transposons were first detected in eukaryotes (Maize), the molecular nature was first understood in bacteria and phages.

Types:

[I] DNA mediated transposon

1. Bacterial insertion sequences (IS)
2. Bacterial transposons
3. Eukaryotic transposons

[II] RNA mediated transposons

1. Viral Retroposons
2. Non viral Retroposons

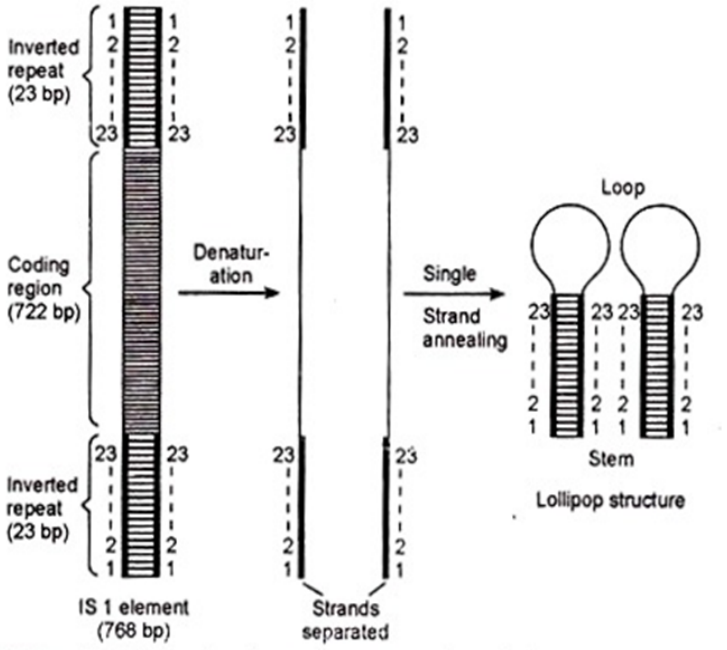
[I] DNA mediated transposon:

[1] Bacterial Insertion sequences or IS elements:

-First IS element was discovered in bacteria (*E.coli*) in 1967.
-These are short segment of DNA (150-1500bp) with inverted repeats of 15-40 base pairs at the ends.
-On denaturation and self-annealing they generate “Lollipop” figure.
-ISs found in both chromosomal and plasmid DNA as well as in certain bacteriophages. Eg. IS1, IS2, IS3 etc.

Some Prokaryotic ISs

IS	Number of copies	Number of base pairs	Inverted repeats (IR)
IS1	5-8 on genome	768	23
IS2	5 on genome and 1 on plasmid	1327	41
IS3	5 on genome and 2 on plasmid	1400	38
IS4	1-2 on genome	1400	18
IS5	10-11 on genome	1534	16
IS101	On plasmid pSC101	201	37



. Constitution of IS1. Denaturation causes separation of the two strands. On single strand annealing, the inverted repeats present at the two ends of the same strand pair, while the coding region forms a loop, giving rise to the "lollipop" or "keyhole" structure.

[2] Bacterial Transposons:

-Larger than IS
-These are actually composite transposon.
-Carry some other genes (protein coding genes) in addition to those needed for transposition.
-Extra genes include drug resistance genes.
-Carry two inverted repeat (IR) sequences. The IR sequences together with their contained genes have been collectively called a transposon (Tn).

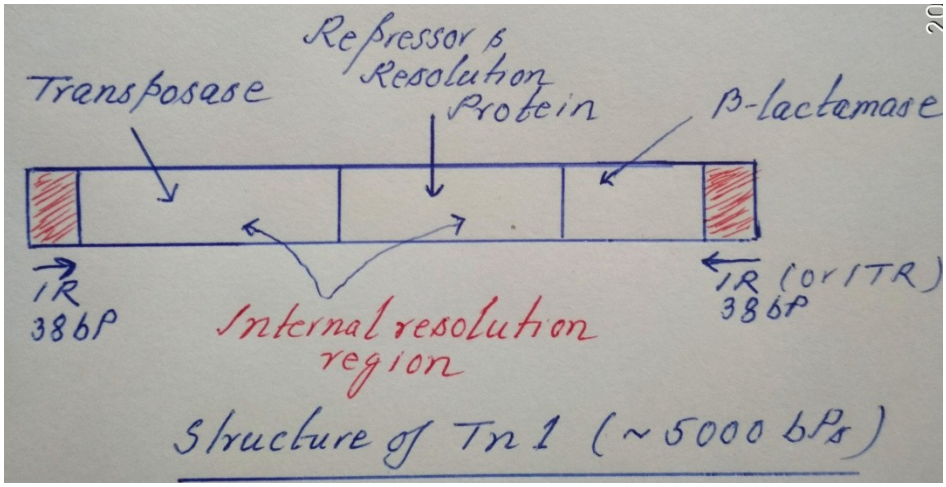
Some Prokaryotic Tns

IS	Markers	Number of base pairs	Inverted repeats (IR)
Tn1	Ampicilin resistance	4957	38
Tn5	Kanamycin resistance	5400	1500
Tn9	Chloramphenicol resistance	2638	18/23
Tn10	Tetracycline resistance	9300	1400

Tn1 family of transposons consists of quite large elements (5000bps). Each transposon carries 3 genes.

1. Gene encoding beta-lactamase enzyme (confers Ampicilin resistance)
2. Gene encoding Transposase enzyme- required for transposition
3. Repressor gene-Regulate the transposase gene

In addition to above genes, an Internal Resolution site is also necessary for resolution of Tn1 conintegrates.



Mechanism of transposition:

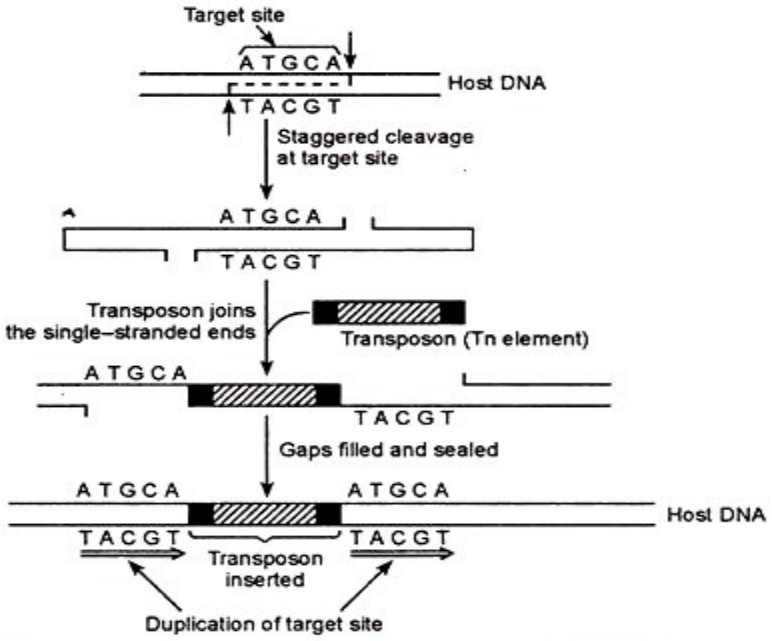
Two methods- Non-replicative and Replicative

[A] Non-replicative: Also known as **Cut-and-Paste Transposons.** eg. Tn10

In this case is excised from one location in the chromosome and become reinserted at a second location. The copy number remains one. The element is lost from the original site.

Steps:

(i) Trasposase makes blunt ends cut in donor DNA and staggered cut in target.
(ii) Trasposase ligate excised Tn into the staggered sites in the target DNA.
(iii) ssDNA is filled by cellular DNA polymerase. Ligase join 3’ and 5’ ends of DNA.



General mechanism of transition of a *Tn* element. The target site is duplicated as direct repeat.

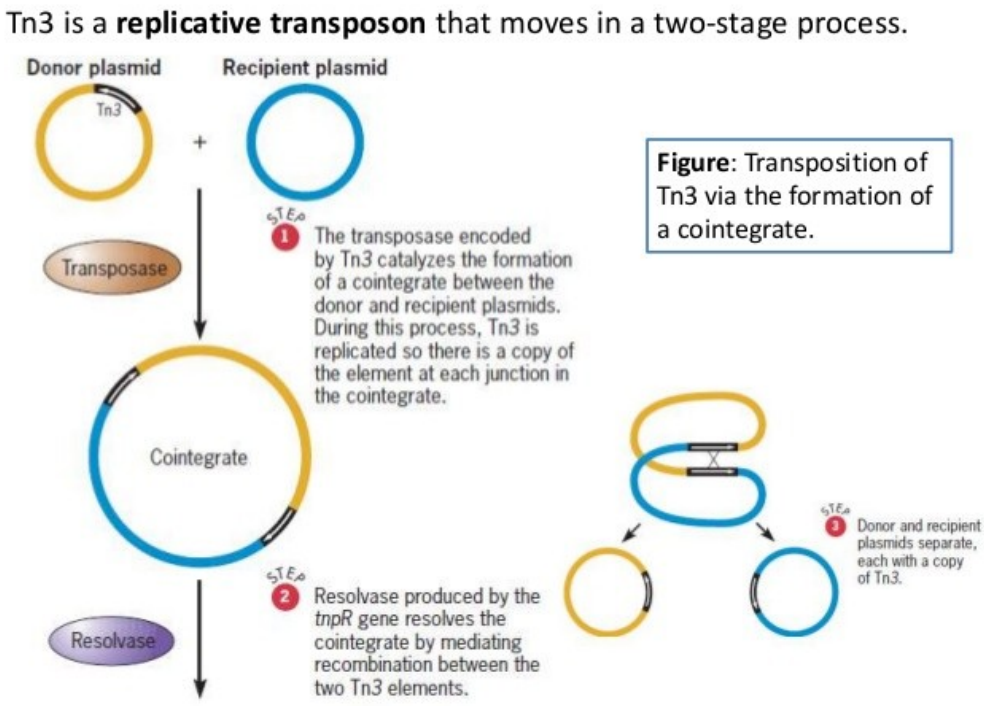
The process result in the duplication of target site sequence on each side of inserted Tn elements. The number of base pairs duplicated in the target is a characteristic of each element. In bacteria 5bp and 9bp repeats are most common.

[B] Replicative transposition: In this case a new copy of the transposon is generated during the transposition event. So one copy appears at the new site and one copy remains at the old site. Eg. Transposition in two circular genetic elements.

-Cointegrate is formed as an intermediate which consist of two complete copies of transposons in the DNA.

-The cointegrate intermediate is converted into separate products by site-specific recombination in which specialized recombinase promote required deletion reaction. Internal Resolution site (IRS) participates in this process.

- In this type of transpositions the number of copies of the transposon increases. Two endonucleases are involved in transposition: the enzyme transposase acts on the ends of the original transposon while another enzyme resolvase acts on the duplicated copy.



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[3] Eukaryotic transposon:

(i) Transposons in Maize: Ac-Ds system

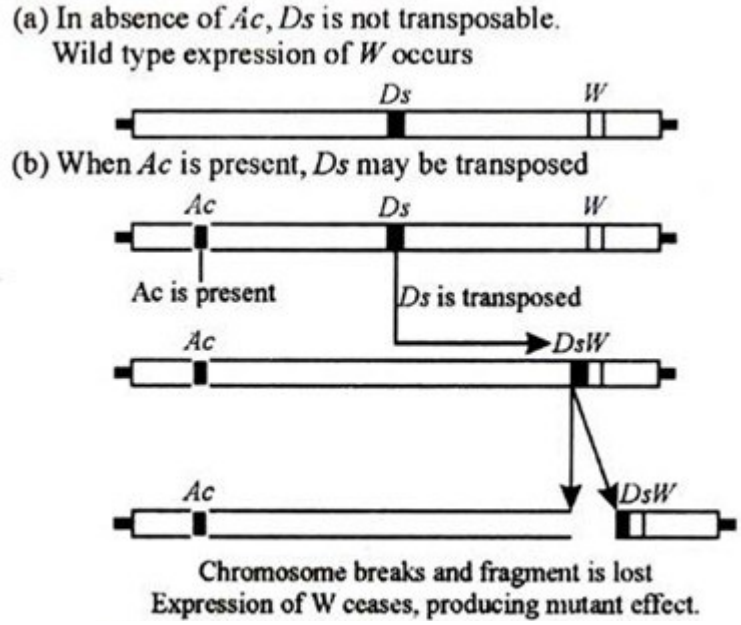
-This system of transposable elements in maize was analysed and given by Barbara Mc. Clintock.

- The transposition involving this Ac-Ds system produces altered kernel phenotypes.

-Here Ac stands for Activator and Ds for Dissociation. Barbara found that Ds and Ac genes were sometimes mobile and moved to different chromosomal locations thus resulting in different kernel phenotypes.

-Ds element is activated by Ac and on activation it serves as the site provider for breakage in chromosome.

-Ac can move autonomously while Ds can move only in the presence of Ac.



Effect of transposition involving *Ac-Ds* system in Maize

(ii) Copia like elements:

-Reported by Hogness *et al*. (1975) in *Drosophila.*

-Name refers to copious amount of RNA transcribed from this sequence.

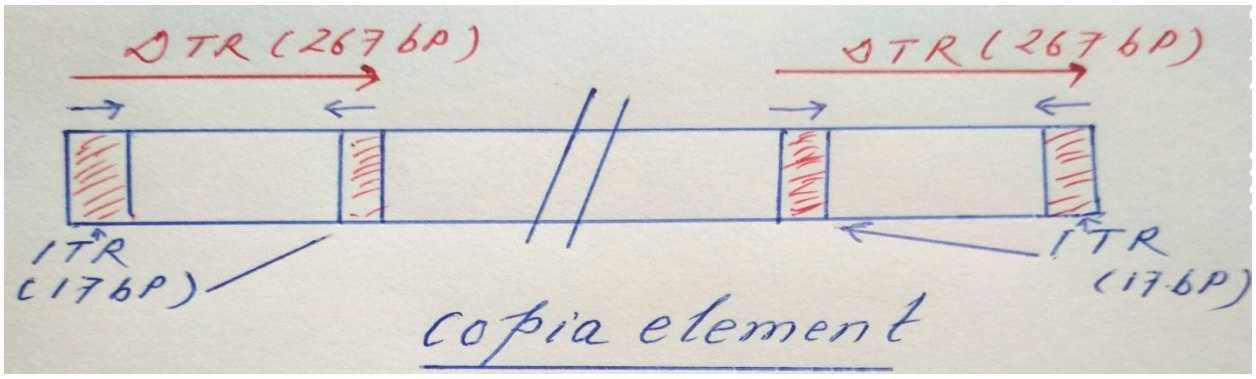
-7 families of copia elements observed.

-Each copia gene consists of about 5000 bps to 8500bps.

-Members of each family appear at 10-100 position in the *Drosophila* genome.

Each member carries a long direct terminal repeat (DTR) sequence of **267bps** at each end.

-Within each repeat is a short inverted terminal repeat (ITR) of **17bps.**



-In addition to copia about 30 related but not identical sequence, called copia like also identified.

-Copia and related elements range in number from 21-60/genome and account for about 3% of *Drosophila* DNA.

-As copia produce lots of RNA, it is believed that RNA molecules may be an intermediate in transposition of these elements with the use of reverse transcriptase (a type of retrotransposon).

(iii) Ty elements in yeast:

-Two major classes of Ty-Ty1 and Ty917 observed.

-Ty1: 35 copies present in each genome, 6.3kb long carrying 330bps long termini **(DR, direct repeats, also called as δ).** [Unlike prokaryotic transposon that carry IR]. However, like prokaryotes 5bps of target DNA are repeated on either side of inserted Ty element.

-Ty917: 6 copies/genome, contain about 100 copies of independent δ elements called **solo δs.**