e-content for Program: M.Sc. Botany (4th semester)
Course code: MBOTEC1
Name of course: Cytogenetics and Crop Improvement
Name of teacher: Prof. Birendra Prasad
Department of Botany, PU
Email:bprasad.pu@gmail.com
Mob.No.9431457533

Topic: Transposons: Structure and types of transposons (Prokaryotic and Eukaryotic); Mechanism of transposition (replicative and non-replicative); Retroposons; Application of transposon

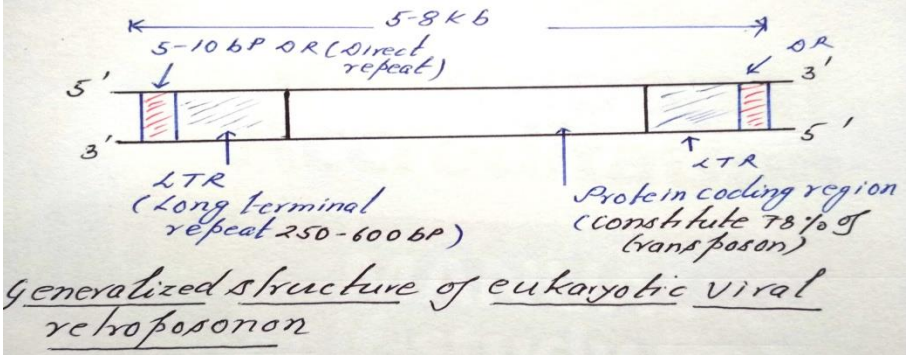
2nd Part (Retroposons; Application of transposon)

[II] RNA mediated transposition:

-All eukaryotes from yeast to human contain retrotransposons.
-Mobile DNA element transpose through an RNA intermediate utilizing a reverse transcriptase (RT).
-These elements are divided into two categories-Viral and Non-viral retrotransposons.
(a) Viral retrotransposons:
-Abundant in yeast (eg.Ty) and in *Drosophila* (eg. Copia).
-In mammals non-viral retroposons are the most common type of mobile elements. Still viral retroposons are estimated to account for about 4% of human DNA.

Generalized Structure of Viral retrotransposons:

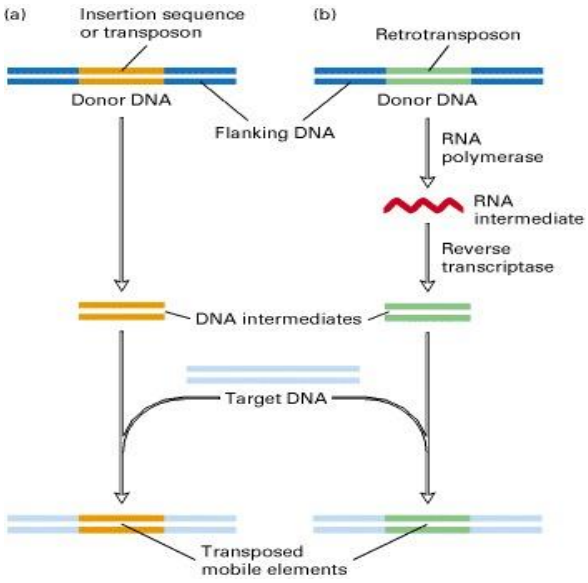
-Central protein coding region flanked by 2 LTR which are element specific.
-Like other transpososn, retrotransposons have short target site direct repeat (DR) at their 3' and 5' ends.
-These elements encode reverse transcriptase and integrase which assist in transposition by converting the RNA intermediate into DNA and inserting the DNA into the target site in a manner similar to retrovirus (Ty and Copia elements also inserted by same mechanism.



generalized structure of eukaryotic viral retroposonon

Basic difference of mechanism transposition and retrotransposition:

The basic difference of transposon and retrotransposons: can be explained by following diagram:



i. Viral retrotransposon synthesizes RNA intermediate with the help of RNA polymerase enzyme
ii. Reverse transcriptase synthesizes cDNA intermediates.
iii. It integrate in target sequence by the same mechanism by which retrovirus integrate in host genome.

[2] Non-viral retrotransposons-

-Lack LTRs

-Mostly belong to 2 classes of moderately repeated DNA sequences found in mammalian genome.

- LINES (Long Interspersed Elements- Size about 6-7kb, Found in human.
- SINES (Short interspersed Elements, Size about 300bp.

LINES and SINES have accumulated over evolutionary time by repeated copying of a sequence and insertion of the copies at new position.

LINES-

-Most common LINE is L1 LINE

-Approximately 6,00,000 copies of L1 occurs in human genome accounting for about 15% of the total human DNA.

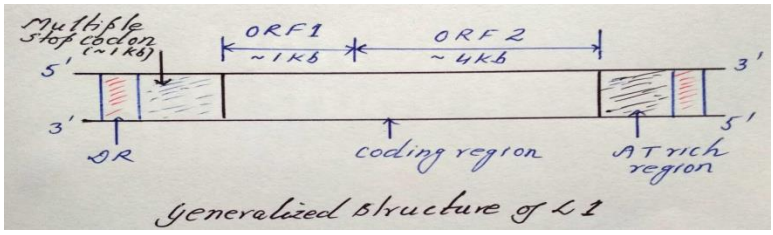
-L1 flanked by short direct repeats (SDR). These are generated from the target site sequence during insertion.

-The sequence contains 2 long open reading frames (ORFs).

-ORF1- size 1kb, encode a RNA binding protein.

-ORF2-size 4kb, encode protein similar in sequence to RNAase of retrovirus and viral retroposons.

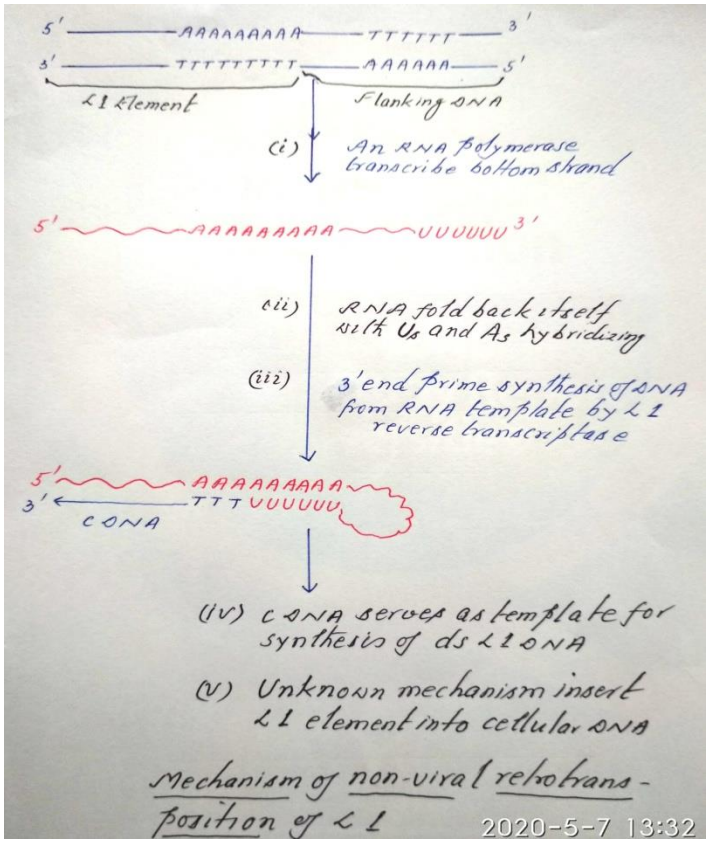
-AT rich region at the right end is thought to function in retrotransposition.



Proposed Mechanism of non-viral retrotansposition of L1 elements:

-It has been suggested that transposon transposed to new sites through an RNA intermediate.
-In vitro studies indicated that AT rich region at the right end generates a stretch of A-residues in their RNA transcript.
-Based on these properties of L1, the model of L1 retrotransposition has been proposed.
-Steps are:

i. An RNA polymerase transcribes the bottom strand.
ii. RNA folds back on itself with Us and As hybridizing.
iii. 3' end primes synthesis of DNA from RNA template by L1 RTase.
iv. cDNA serves as template for synthesis of ds L1 DNA.
v. Unknown mechanism inserts L1 element into cellular DNA.



SINES and Alu sequences:

-SINES are the second major class of moderately repeated DNA in mammals.

-Many repetitive sequences in human DNA contains a recognition site for the restriction enzyme *Alu,* therefore called *Alu* repetitive sequence.

- *Alu* sequences are collectively called SINES (Short interspersed Element).

-These retroposons are not similar to retroviruses.

- *Alu* sequence is about 300bps and present at about 1 million sites in human genome amounting for about 10% of the total genomic DNA.

-The *Alu* sequence closely resembles that of the 7SL RNA (a small cellular RNA in signal recognition and help in secretion of newly formed polypeptides through membrane of endoplasmic reticulum).

- Like all other mobile elements, *Alu* sequence are usually flanked by direct repeats (DR) and also contains AT-rich region at one end similar to L1.

-So transposition thought to be similar to L1 elements possibly by RTase and other proteins expressed from functional L1 elements.

Application of transposons:

-According to one school, these elements have no function. Rather they a type of genetic parasite that can spread within host genome and may cause serious adverse effects (Selfish DNA).

-Second group (more appropriate) argues that transposition is a key mechanism in creating genetic changes that leads to biological evolution of modern-day organisms. Many spontaneous mutations in *Drosophila* result from insertion of mobile DNA element into or near a transcription unit. As it also occurs in human it may play great role in human also. Transposons contributed to the evolution of the immune system

-**Transposons as Genetic Tools:** Terminal-inverted repeat (TIR) DNA transposable elements (TEs) are exceptionally useful genetic tools for insertion mutagenesis and isolation of the mutated gene ('transposon tagging'). Today TEs are also increasingly used as highly efficient vectors for gene transfer in mammalian cells. It is possible to use transposons as bi-component systems, in which virtually any DNA sequence of interest can be placed between the transposon TIRs and mobilized by transposes enzyme in the form of an expression plasmid. This feature makes transposons natural and easily controllable DNA delivery vehicles that can be used as tools for versatile applications, ranging from somatic and germline transgenesis to functional genomics and gene therapy