TRANSPOSONS

'Jumping genes'

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Transposons are elements that can hop or transpose; from one place in DNA to another.

Overview and general properties

- First discovered in corn by Barbara McClintock in early 1950s, 20 years later in bateria.
- The term "transposon" was coined by Hedges and Jacob in 1974.
- Transposons exists in all organisms on earth.
- Transposons play an important role in evolution.
- Half of the human DNA may be transpsosons-Human Genome project.
- Transposition: The process by which transposons move is called transposition and the enzyme that promote, transposases.
- Transposon can enter other genera of bacteria.
- Frequency of transposition once in every 10^3 cell division to once in every 10^8 cell division.
- The process by which a transposon moves is called transposition.

Why transposons are known as jumping genes?

- Transposons encodes its own transposases, hence can hop each time it moves- ability to hop conferred hence known as **'Jumping genes'**.

Nomenclature

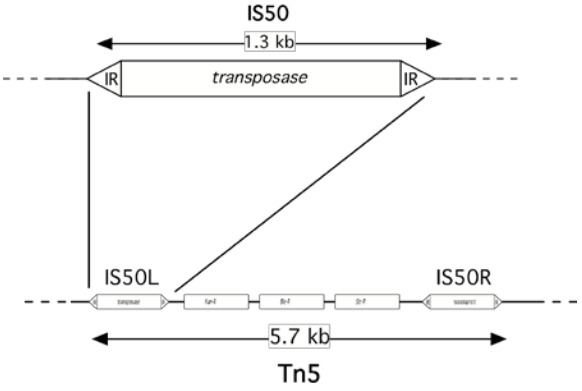
Designated with a prefix of Tn and assigned a sequential number e.g. Tn1, Tn2, Tn3, etc. The allocation of numbers and database administration was carried out by the late **Dr. Esther Lederberg from Stanford University Medical School, CA, USA**. Lists for the registry of Tn number allocations were subsequently published (Lederberg, 1981, 1987) taking the continuous system up to Tn4685. However, Tn numbers up to and above Tn5500 were allocated but a list of these has not been published.

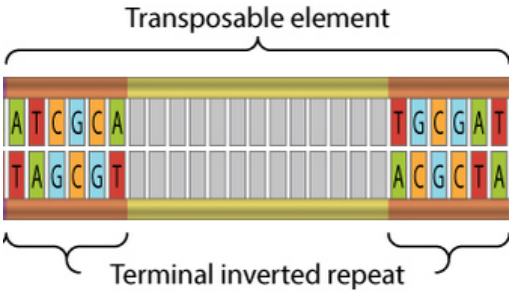
The allocation of Tn numbers stopped with the retirement of Dr. Lederberg and gradually a variety of rules were adopted for naming newly discovered transposons. Tn number, written in italics to conform with previous guidelines, e.g. Tn3 or Tn5397. A registry for the assignment of Tn numbers has been set up on the UCL website (http://www.ucl.ac.uk/eastman/tn/) where researchers can request as many Tn numbers as needed and log details (e.g. description, accession numbers and references) of newly discovered, fully sequenced and/or functional transposons in the registry

Additionally, and due to their prolific use in the literature, the terms ICE and IME for integrative conjugative element and integrative mobilizable element are interchangeable with the terms CTn for conjugative transposon and MTn for mobilizable transposon, respectively. For example, if an element was designated Tn5999 and subsequently shown to be conjugative it can be subsequently named CTn5999 or ICE5999. The same is true for elements found to be mobilisable, a MTn or IME prefix can be substituted.

Structure of a Transposon

Transposons are stretches of DNA that have repeated DNA segments at either end.
A transposon consists of a central sequence that has transpose gene and additional genes. This is flanked on both sides by short repeated DNA segments.
The repeated segments may be direct repeats or inverted repeats. These terminal repeats helps in identifying transposons.

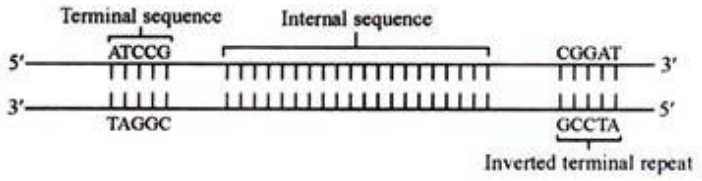




Types of Bacterial Transposons

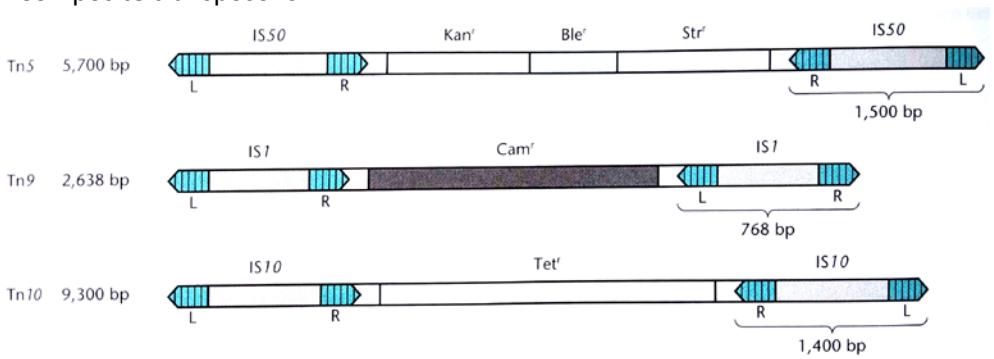
1. Insertion sequence elements

- Smallest bacterial transposons usually about 750-2500 bp long and encode little more than transposase enzyme.
- Does not carry selectable genes.
- Can insert at different sites in the bacterial chromosomes. only inactivate the gene in which it is inserted.
- Species specificity.
- Important in assembly of plasmids and information of Hfr strains.
- IS-elements contain ITRs (Inverted Terminal Repeats). The ITRs present at the ends of IS-elements are an important feature which enables their mobility. The ITRs present in the IS-elements of E.coli usually range between 18-40 bp. Originally 4 different IS elements were found in *Escherichia coli* K12 i.e., IS1, IS2,IS3 and IS4.
- The term **'Inverted Terminal Repeat'** (ITR) implies that the sequence at 5 end of one strand is identical to the sequence at 5′ end of the other strand but they run in inverse opposite direction.



An insertional sequence (IS element) with inverted terminal repeats (ITRS)

2. Composite transposons



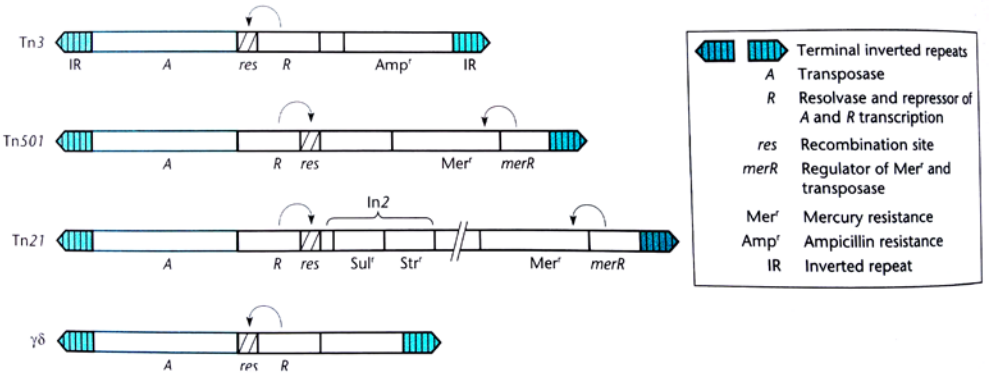
- Composite transposon is made up of two IS elements, one present at each end of a DNA sequence which contains genes whose functions are not related to the transposition process. These transposons have been found to have inverted repeats at the ends.

- The length of these inverted repeats ranges from a few nucleotides to about 1500 bp.

- The IS elements of same type, bracketing other genes to form larger transposon called composite transposon. e.g., Tn5, Tn9 and Tn10
- Tn5 contains genes for kanamycin(Kan^r), Bleomycin (Ble^r) and streptomycin (Str^r)resistance bracketed by copies of is elements IS50.
- Tn9 contains genes for chloramphenicol (Cam^r)resistance bracketed by copies of IS elements IS1.
- Tn10 two copies of IS10 flank a gene for tetracycline resistance (Tet^r).
- Bracketing is elements are in same orientation in case of t9, whereas T5 & T10 have orientation of is elements in opposite direction.

3. Non-composite transposons

- Bracketed by short IRs, but resistance gene is part of the minimum transposable unit.



Mechanism of transposition

The major categories of TEs are distinguished by their transposition mechanisms:

1. Cut-and-Paste (non-replicative) Transposons - found in prokaryotes and eukaryotes.
    - Insertion Sequence (IS) Elements
    - Composite Transposons (example, Tn5)
2. Replicative Transposons - prokaryotes only (example, Tn3)
3. Retrotransposons - eukaryotes only

RNA intermediate in transposition/Revrese Transcriptase some are closely related to retroviruses

Transposases:

The enzymes that promote transposition are a type of recombinase called transposase.
Use distinct catalytic mechanisms for break/rejoining of DNA. e.g., some transposases cut/transfer/paste original DNA, while others copy DNA into the target site.

Five families have been classified.

1. DDE transposases
2. Tyrosine (Y) transposases
3. Serine (S) transposases
4. Rolling-circle (RC), or Y2 transposases
5. Reverse transcriptases/endonucleases (RT/En)

1. DDE transposases

These transposases carry a triad of conserved amino acids: aspartate (D), aspartate (D) and glutamate (E), which are required for the coordination of a metal ion required for catalysis, although the DDE chemistry can be integrated into the transposition cycle in differing ways.
These employ a **cut-and-paste mechanism** of the original transposon.
This family includes the maize Ac transposon, as well as the Drosophila P element, bacteriophage Mu, Tn5 and Tn10, Mariner, IS10, and IS50.

2. Tyrosine (Y) transposases

These also use a **cut-and-paste mechanism** of transposition, but employ a site-specific tyrosine residue. The transposon is excised from its original site (which is repaired); the transposon then forms a closed circle of DNA, which is integrated into a new site by a reversal of the original excision step. These transposons are usually found only in bacteria, and include Kangaroo, Tn916, and DIRS1.

3. Serine (S) transposases

These transposases use a **cut-and-paste (cut-out/paste-in)** mechanism of transposition involving a circular DNA intermediate, which is similar to that of tyrosine transposases, only they employ a site-specific serine residue. These transposons are usually found only in bacteria, and include Tn5397 and IS607.

4. Rolling-circle (RC), or Y2 transposases

These employ either a **copy-in** mechanism, where they copy a single strand directly into the target site by DNA replication, so that the old (template) and new (copied) transposons both have one newly synthesized strand. These transposons usually employ host DNA replication enzymes. Examples include IS91 and helitrons.

5. Reverse transcriptases/endonucleaseses (RT/En)

Retrotransposons can vary in their mechanism of transposition. Some use the RT/En method, employing an endonuclease to nick the target site DNA, the nick serving as a primer for reverse transcription of an RNA copy by the reverse transcriptase enzyme. Examples include LINE-1 and TP-retrotransposons

Assays of transposition

1. Use of Suicide Vectors

- Any DNA, including plasmids or phage DNA, that cannot replicate (not a replicon) in a particular host can be used as suicide vector.
- Suicide vectors can enter cells in which they cannot replicate - essentially kills themselves.
- To assay transposition with suicide vector , we can use one to introduce a transposon carrying an antibiotic resistance gene into appropriate host.
- Introducing suicidal vectors

Plasmids- can be introduced into the cell by conjugation.

Phage- cell could be infected.

- Methods should be very efficient- Since transposition is a rare event.
- Once in the cell- Suicide vector remain unreplicated and eventually lost as the cell population grows.
- Transposons can survivive moving to and confer antibiotic resistance another DNA moleculae – capable of autonomous replication. i.e., replicating plasmid / DNA.
- Cells under study are plated on antibiotic containing agar and incubated, the appearance of colonies as a result of multiplication of antibiotic resistant bacteria, is evidence for transposition. These cells have been mutagenized by the transposon, since the transposon has moved into a cellular DNA molecule either chromosome or plasmid-causing insertion mutation.
- Similarly phage suicide vectors or plasmid suicide vectors can also be used for assaying transposition.
- a Insertion elements creates mutations when they hop into a gene. It is laborious to distinguish insertion mutations from the myriad mutations
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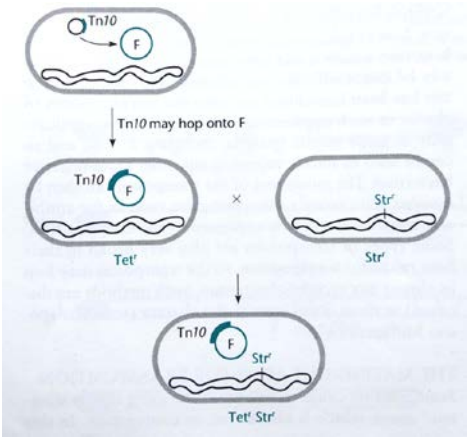
2. 'Mating out' Assay



Regulation and Control of Transposition

Transposition is potentially deleterious to the host as well as the transposon, whose replication and propagation depend on the survival of their host. Thus, the development of ways to decrease the impact of transposition on host fitness is beneficial for both host and transposon. Strategies for transposon control :

Overproduction Inhibition (OPI)

The transposase itself can act as a transposition inhibitor, as when it exceeds a threshold concentration, transposon activity is decreased.

Vertical Inactivation

It has been suggested that this is the result of selective pressure to reduce damage to the host genome . In addition, **inactive elements could produce inactive transposases** that would impede the transposition of active elements, by OPI or by competition with the active transposases.

Other Mechanisms: One way used by the host to silence a Tc1/*mariner* element is **DNA methylation**, thereby **preventing its transcription**, or using post-transcriptional **silencing mechanisms such as RNA interference** .

Uses of Transposons:

1. **Contributing to adaptation of bacteria to the environment**
   Integrons integrates antibiotic resistance genes from large storage areas into transposons that can than transpose into other DNA.
   Conjugate elements and integrating elements can move these transposons frombacterium to bacterium and even into other generaof bacteria.
   Allows bacterial genome to remain small but still have access to many types of genes that increase their ability to adapt to different environments.
   One way this impacts humans directly is in the acquisition of antibiotic resistance by bacteria.
2. **Transposon mutagenesis**
   a. **Gene mapping:** Gene marked with transposon is easy to map using arbitrary PCR or ligation mediated PCR.
      Transposon insertion usually inactivate a gene - lethal event in haploid bacterium, if gene is essential for growth. Generally used to mutate genes that are non-essential /essential under certain conditions.
   b. **Gene Cloning:** Genes marked with transposons are relatively easy to clone by selecting for selectable genes carried on the transposons.
   c. **Random gene fusion:** Ability of some transposons to make random gene fusion. Fusing a gene to a reporter gene whose product is easy to monitor (green fluorescence protein) is easy to monitor, can make regulation of the gene much easier.
      Trasnposons have been engineered to make either transcriptional or translational fusions.

- *Sleeping Beauty* (SB) transposon system has been developed as the leading non-viral vector for gene therapy. This vector combines the advantages of viruses and naked DNA.

Summary

- Conjugative elements-plasmids and integrating conjugative elements can move transposon from bacterium to bacterium and even into other genera of bacteria.
- Presumably, allows bacteria genomes to remain small, but still have access to many types of genes that increase their **ABILITY TO ADAPT TO DIFFERENT ENVIRONMENTS**.
- Acquisition of antibiotic resistance by bacteria.
- Transposon can contribute to widespread antibiotic resistance.
- Inducing mutations.
- Resulting in ultimate evolution.

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