WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 18, 382-407. Review Article ISSN 2277– 7105

JUMPING GENE (TRANSPOSONS)

***Abhishek Dubey, Vikas Gupta, Rahul Chaurasiya, Gaurav Dubey and Sambodhan Dhawane Mandar Chavan**

India.

Article Received on
23 August 2018,

Revised on 13 Sept. 2018,
Accepted on 03 October 2018

DOI: 10.20959/wjpr201818-13429

***Corresponding Author**
Abhishek Dubey
India.

ABSTRACT

The phenomenon of jumping genes was initially discovered by Nobel Laureate, Barbara McClintock, in her work on maize chromosome in fifties. The Jumping genes transpose from one position to another in horizontal fashion within the same chromosome or even to other chromosomes. In this paper, it is to present how this genetic transposition, after transforming into a computational method, can enhance the evolutionary multiobjective optimization. The fundamental concept, design of operations, performance ju The characteristics of bacterial mobile genetic elements are presented in relation to the mobilization of resistance genes and to the occurrence of complex assemblies of resistance transposons on plasmids. Their significance as an important factor in bacterial evolution is discussed. stification and applications of the Jumping Gene evolutionary approach will be outlined. Transposons are well-known architects of genetic change but their role in insecticide resistance has, until recently, only been speculated upon. Transposon insertion, or transposon-mediated transposition, could alter either metabolic enzymes capable of degrading pesticides or could change the functionality of insecticide targets. The recent work of Aminetzach and co-workers suggests an exciting alternative, that transposon insertion can cause resistance by altering gene product function. This hypothesis is discussed in the light of other examples in which transposons have been implicated in insecticide resistance.

KEYWORDS: Genetic Algorithm Multiobjective Optimization Nobel Laureate Multiobjective Problem Maize Chromosome.

INTRODUCTION

Mobile DNA sequences can ‘jump’ or transpose from one location to another in a host genome, and can have profound biological effects on an organism. Many of these elements,

found in diverse organisms from bacteria to humans and including retroviruses such as HIV, transpose using a similar mechanism. Our understanding of this process comes largely from extensive studies on bacteriophage Mu, for which a defined in vitro system is available. Mu DNA transposition is mediated by a series of higher-order nucleoprotein complexes or transpososomes (for reviews see[1],[2]). The assembly of this transposition machinery is an intricately choreographed process, requiring multiple proteins, multiple DNA sites and a complex circuit of cooperative protein–protein and protein–DNA interactions on a supercoiled DNA substrate. The process also requires divalent metal ions, bending of the DNA and the intertwining of domains from separate monomers of the transposase (Mu A) to construct functional active sites. The active sites then promote chemical reactions at the opposite ends of the Mu DNA (in trans) from where Mu A is bound.

The complexity of the transposition process is now reaching mind-boggling proportions, and a recent flurry of papers has contributed much to our understanding of the reaction. It is becoming increasingly clear that the assembly process ensures that all the working parts and substrates are firmly bolted in place before the machine will run. This tight regulatory safeguard ensures that DNA transposition occurs in the right place at the right time, and avoids damage to the host genome by preventing partial reactions that cannot be completed.

Machine components

In vitro, the higher-order Mu transpososomes are constructed from various parts.[1],[2],[3]

The Mu A transposase, a 663 amino-acid multi-domain protein (Figure 1), promotes the essential DNA-strand cleavage and joining reactions at the two ends of the Mu transposon (the ends are termed left, or L, and right, or R). Each end encompasses three ‘end-type’ transposase binding sites (L1, L2, L3 and R1, R2, R3, numbered from the extreme ends of Mu inwards). The Mu enhancer, located roughly 950 base pairs from the left end, is also specifically bound by transposase, albeit through a different domain than that used to bind the ends. A second Mu-encoded protein, Mu B, greatly stimulates the strand-cleavage and transfer activities of the Mu A transposase and plays an important part in target-site capture as well as immunity (which keeps the phage DNA from transposing into itself). The host, Escherichia coli, also contributes components to the transposition machinery: both HU and IHF are DNA-flexing/bending proteins that serve as architectural elements in the assembly of Mu transposition intermediates.

Figure 1: Domain structure of the 663 amino-acid Mu A transposase protein. Domains I, II and III are depicted in red, blue and green, respectively, with sub-domains indicated. Numbers above the boxes refer to amino-acid positions at the beginning of each functional region. Asterisks denote positions of DDE motif residues (see[18]). The 26 amino-acid peptide from domain IIIα (residues 575–600) displays both non-specific DNA binding and nuclease activities.[11] Non-specific DNA binding activities have also been attributed to domain Iγ (residues 178–243) and domain II (residues 491–560), on the basis of partial proteolysis (see[1]) and crystallization[19] studies, respectively.

Construction of the transpososome

Successful gene jumping requires that both transposon ends be cut from the initial substrate and then joined to a new target site in a coordinated fashion. In the case of Mu, this is accomplished by tightly coupling chemical catalysis with proper synapsis of the Mu ends in a multi-step assembly process (Figure 2). The Mu A protein normally exists as a chemically inert monomer that does not recognize the DNA-cleavage sites at the Mu ends. Construction of the transpososome serves to assemble the catalytic components needed for DNA transposition and to direct the machinery to the scissile bonds at the 3′ ends of Mu.

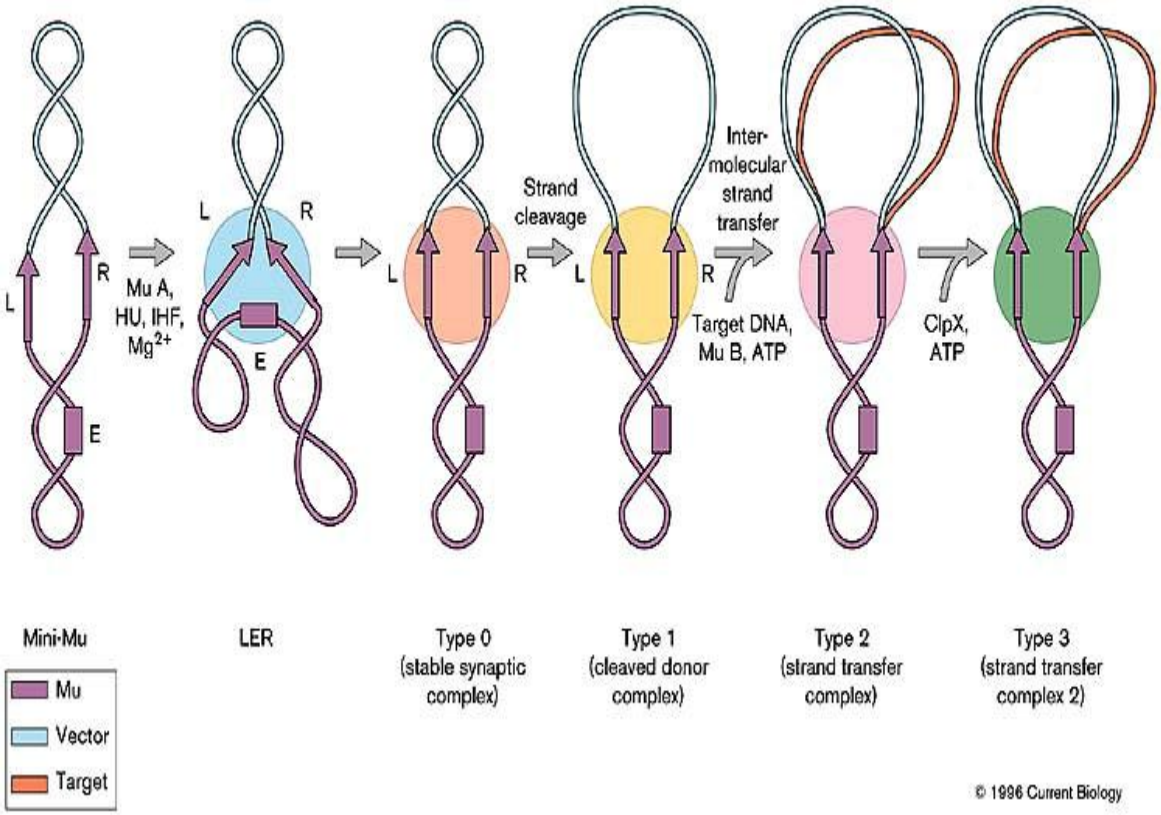


Figure 2: Protein–DNA complexes involved in Mu DNA transposition. The standard substrate for the in vitro reaction is a supercoiled mini-Mu plasmid containing the left and right ends of Mu along with the trans positional enhancer. The earliest characterized synaptic complex is the LER[5] in which the two Mu ends (L and R) and the enhancer (E) are brought together by a complex circuit of interactions involving the transposase Mu A and aided by the host HU and IHF proteins. The LER is converted to the Type 0 complex (or SSC, stable synaptic complex), in which the enhancer has been released and the two Mu ends are engaged within the active sites of the complex. DNA cleavage results in the formation of the Type 1 complex (or CDC, cleaved donor complex), in which a nick has been introduced at each end of the Mu DNA, exposing 3′ OH groups and resulting in relaxation of the vector domain. In the presence of Mu B, strand transfer of these 3′ ends into a random site on a target DNA molecule generates a Type 2 complex (or STC, strand transfer complex). Subsequently, the action of the ClpX chaperone induces a conformational change in the Type 2 transpososome and weakens the interactions which hold it together.[16] The product of this transformation is the Type 3 or STC2 transpososome, which then functions in the assembly of proteins required for DNA replication. For further details see.[1],[2]

- Recent work has provided some insight into how this process is affected. The assembly cofactors, HU and IHF, introduce precisely positioned DNA bends at the Mu left end (between two of its end-type transposase-binding sites[4]) and at the enhancer, respectively (Figure 2). These architectural cues promote a complex circuit of contacts between Mu A monomers bound to the left end, the enhancer (E) and the right end, forming a transient three-site synaptic intermediate called the LER.[5] This pre-transpososome complex, in which the DNA-cleavage sites have not yet been engaged by the protein, is converted into the more stable Type 0 complex (or stable synaptic complex), in which the chemically active transposase tetramer has engaged the terminal base pairs and is poised for action. Although we have illustrated assembly as a single defined pathway, variations certainly exist. For example, the Mu B protein can join the party at any of several locations, and pathways for enhancer-independent assembly can be found under appropriate conditions.[6],[7]

The oligomeric state of its protein components is not the only important aspect of the transpososome structure. The transposase bends each of its end-type binding sites on the DNA by 60–90°, and this could play a part in end–end and/or end–enhancer interactions. In addition, structural perturbations of the DNA conformation at or near the cleavage sites have been demonstrated in both the Type 0 and Type 1 complexes, and these may contribute to the lability of the phosphodiester bond hydrolysed at the junction between Mu and host DNA (see[1],[8],[9]).

Interlocking parts build active sites

Recently, the spotlight has centred on relationships between transposase monomers in the Mu transpososome. The experiments have used selected mutant derivatives of Mu A, which are inactive in the in vitro transposition reaction. Surprisingly, certain pairwise combinations of the mutant proteins generated active transpososomes. The results have created a picture of an intimate protein–DNA complex in which the sharing of domains between transposase monomers builds composite active sites in the tetramer.

Disabling the transposase metal-ion-binding pocket (the DDE motif; Figure 1) in domain II by mutation results in loss of DNA-strand cleavage and transfer activity. Similarly, deletion of domain IIIα gives inactive transposase. However, a mixture of these two mutant Mu A proteins cleaves the DNA at the Mu ends to form the Type 1 transpososome. The domain-sharing model that has been proposed to explain these results[10] suggests that there are

contributions from both mutants in order to promote the strand cleavage reaction — the domain II DDE comes from the domain IIIα mutant and domain IIIα comes from the DDE mutant. The contribution of domain IIIα of the transposase to the DNA-cleavage reaction has been investigated further, and putative catalytic residues have been localized to a 26 amino-acid region[11] (Figure 1). This region is thought to activate the cleavage sites for catalysis, possibly through the stress induced by DNA bending.

The latest developments take us a step further, by fitting DNA into the scenario described above. A simplified in vitro assay was used to identify the end-type transposase-binding sites occupied by specific monomers in the tetramer (those contributing either the domain II DDE or domain IIIα for catalysis of strand cleavage or strand transfer). The normally supercoiled substrate was replaced by short linear DNA fragments carrying only the R1 and R2 end-type transposase-binding sites.[8]

Under modified reaction conditions, the transposase tetramer were assembled on two right ends and it successfully catalysed cleavage and strand transfer. Transposase mutants were localized to specific end-type sites either by preincubation before mixing[12] or by cross-linking to DNA.[13] Both studies.[12],[13]

Show that, for the strand-transfer step, the monomers contributing domain II (DDE) are bound at the R1 sites, whereas those providing domain IIIα are at the R2 sites (Figure 3). A tentative model for the strand-cleavage step[12] reverses the arrangement for strand transfer, consistent with the proposal of reciprocal sharing of domains II and IIIα.[12]

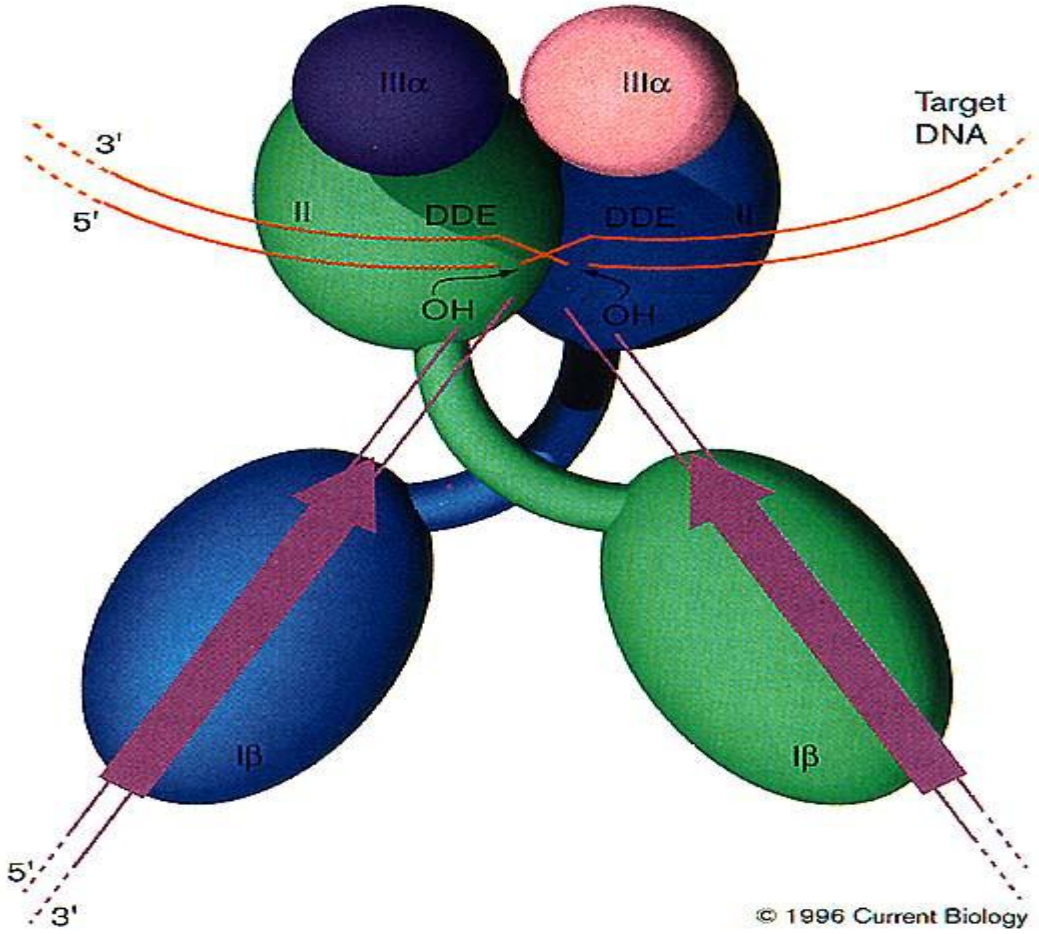


Figure 3: Model of the Mu transposase subunits that promote the strand-transfer reaction. This view is a close-up of the events at the end of the Mu DNA during formation of the Type 2 complex (see Figure 2). Different colours denote the four Mu A monomers in the core tetramer. Note the interlocking of domains from separate monomers to build an active site, and the participation of domain II in Trans or across the synapse from where domain Iβ of the same monomer is bound. The two IIIα domains are from the R2-bound subunits. For simplicity, vector DNA is not shown. (Modified from[13], with permission).

- **The means to an end**

Having identified the end-type binding sites occupied by specific monomers in the transposase tetramer, there remained the question of whether DNA-strand cleavage and transfer are catalysed by monomers acting on the Mu end to which they are bound (in cis), or on the other end (in Trans). Two groups tackled this question, again using the simplified in vitro system described above. The data reveal that domain II (DDE) of transposase operates intrans for both strand-cleavage[14] and strand-transfer (Figure 3).[13],[14]

Thus, the theme of intimacy in the transpososome core deepens with the revelation that transposase monomers not only share protein domains to build active sites, but also reach across the complex to mediate the chemical steps of transposition at the Mu end bound by their partner in the tetramer. It is still unclear whether domain IIIα mediates catalysis in cis or intrans. Nonetheless, the recently published papers.[12],[13],[14]

Contribute an impressive amount of detailed information, and reveal a structural basis for the requirement to form the transpososome before chemical reactions can occurs. The characteristics of bacterial mobile genetic elements are presented in relation to the mobilization of resistance genes and to the occurrence of complex assemblies of resistance transposons on plasmids. Their significance as an important factor in bacterial evolution is discussed.

Classification

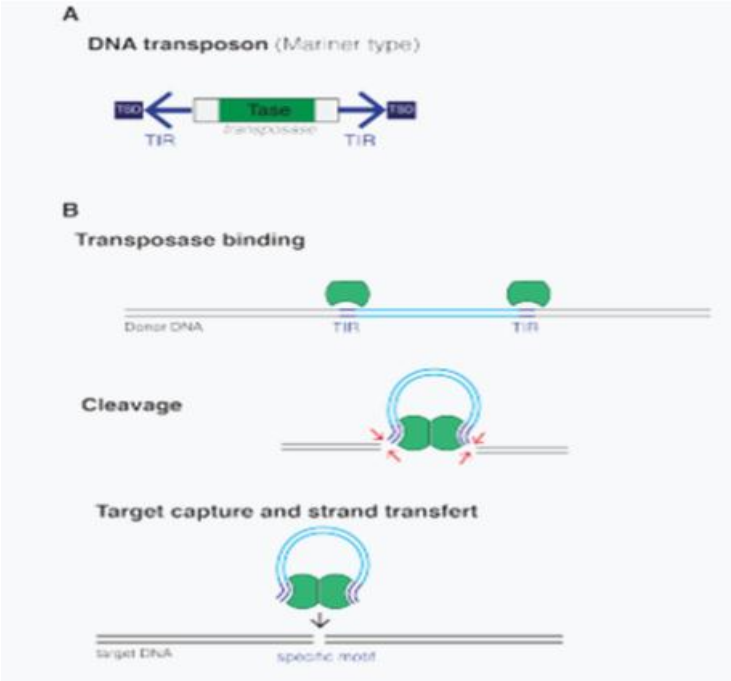
Class I (retrotransposons)[edit]

Class I TEs are copied in two stages: first, they are transcribed from DNA to RNA, and the RNA produced is then reverse transcribed to DNA. This copied DNA is then inserted back into the genome at a new position. The reverse transcription step is catalysed by a reverse transcriptase, which is often encoded by the TE itself. The characteristics of retrotransposons are similar to retroviruses, such as HIV.

Retrotransposons are commonly grouped into three main orders:

- Retrotransposons, with long terminal repeats (LTRs), which encode reverse transcriptase, similar to retroviruses.
- Retroposons, Long interspersed nuclear elements (LINEs, LINE-1s, or L1s), which encode reverse transcriptase but lack LTRs, and are transcribed by RNA polymerase II.
- Short interspersed nuclear elements (SINEs) do not encode reverse transcriptase and are transcribed by RNA polymerase III.

[Note: Retroviruses can also be considered TEs. For example, after conversion of retroviral RNA into DNA inside a host cell, the newly produced retroviral DNA is integrated into the genome of the host cell. These integrated DNAs are termed proviruses. The provirus is a specialized form of eukaryotic retrotransposon, which can produce RNA intermediates that may leave the host cell and infect other cells. The transposition cycle of retroviruses has similarities to that of prokaryotic TEs, suggesting a distant relationship between the two].



A. Structure of DNA transposons (Mariner type). Two inverted tandem repeats (TIR) flank the transposase gene. Two short tandem site duplications (TSD) are present on both sides of the insert. B. Mechanism of transposition: Two transposases recognize and bind to TIR sequences, join together and promote DNA double-strand cleavage. The DNA-transposase complex then inserts its DNA cargo at specific DNA motifs elsewhere in the genome, creating short TSDs upon integration.[15]

Class II (DNA transposons)[edit]

The cut-and-paste transposition mechanism of class II TEs does not involve an RNA intermediate. The transpositions are catalysed by several transposase enzymes. Some transposases non-specifically bind to any target site in DNA, whereas others bind to specific target sequences. The transposase makes a staggered cut at the target site producing sticky ends, cuts out the DNA transposon and ligates it into the target site. A DNA polymerase fills in the resulting gaps from the sticky ends and DNA ligase closes the sugar-phosphate backbone. This results in target site duplication and the insertion sites of DNA transposons may be identified by short direct repeats (a staggered cut in the target DNA filled by DNA polymerase) followed by inverted repeats (which are important for the TE excision by transposase).

Cut-and-paste TEs may be duplicated if their transposition takes place during S phase of the cell cycle, when a donor site has already been replicated but a target site has not yet been

replicated.[citation needed] Such duplications at the target site can result in gene duplication, which plays an important role in genomic evolution.[16]:284

Not all DNA transposons transpose through the cut-and-paste mechanism. In some cases, a replicative transposition is observed in which a transposon replicates itself to a new target site (e.g. helitron).

Class II TEs comprise less than 2% of the human genome, making the rest Class I.[17]

Autonomous and non-autonomous [edit]

Transposition can be classified as either "autonomous" or "non-autonomous" in both Class I and Class II TEs. Autonomous TEs can move by themselves, whereas non-autonomous TEs require the presence of another TE to move. This is often because dependent TEs lack transposase (for Class II) or reverse transcriptase (for Class I).

Activator element (Ac) is an example of an autonomous TE, and dissociation elements (Ds) is an example of a non-autonomous TE. Without Ac, Ds is not able to transpose.

Types of Transposons

Transposition occurs by two methods

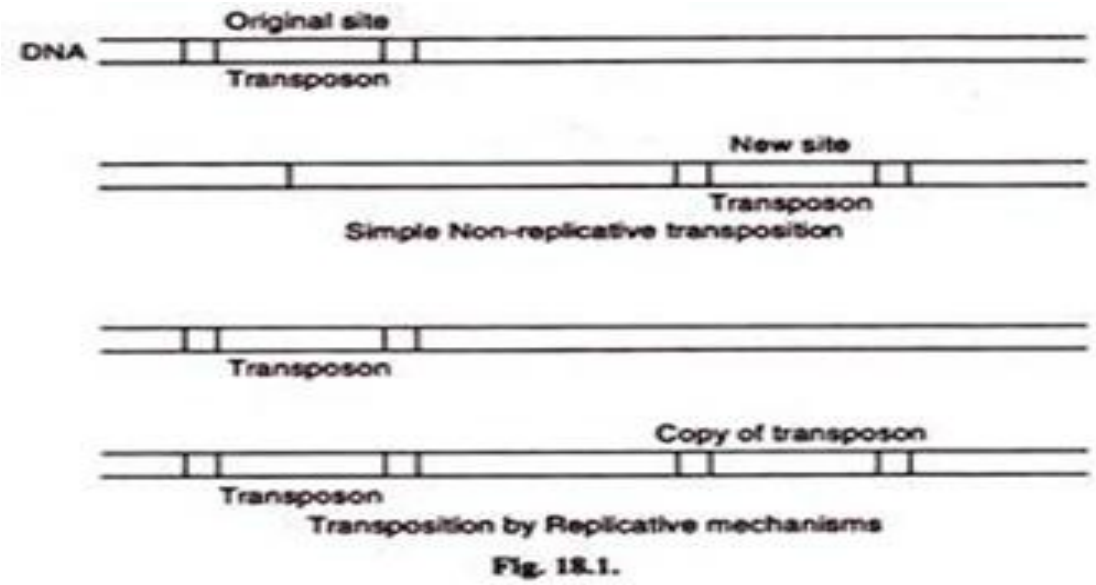


Fig. 18.1.

1. Simple non-replicative transposition

It involves excision of the transposon from its original location to the new DNA site. This is also known cut-and-paste transposition.

2. DNA transposition by replicative mechanism

Transposable segments generate a new copy by replication. The first copy remains at the original site and second copy moves to a new site anywhere within the genome.

The movement to the target site requires breaking up of the chromosome at the new site and inserting the transposon between the two ends generated. The enzymes required for breaking and re-joining the chromosome are present in the transposon itself.

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 transposes 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. This terminal repeats help in identifying transposons.

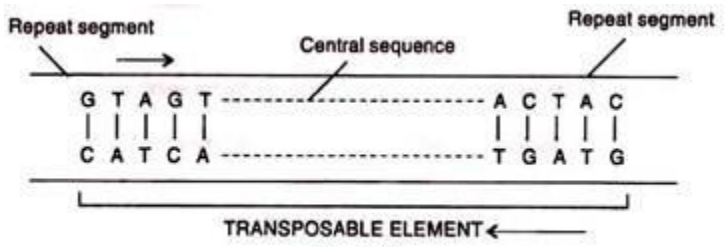


Fig. 18.2.

The number of repeated nucleotides is uneven 5 or 7 or 9 nucleotides are due to its method of insertion at the target site.

Target Sequence

The site where a transposon is inserted is called target site or recipient site. Before the transposon is moved into the target site, the target sequence is duplicated.

The two copies formed move apart. The transposon is inserted in between the two copies of the target sequences.

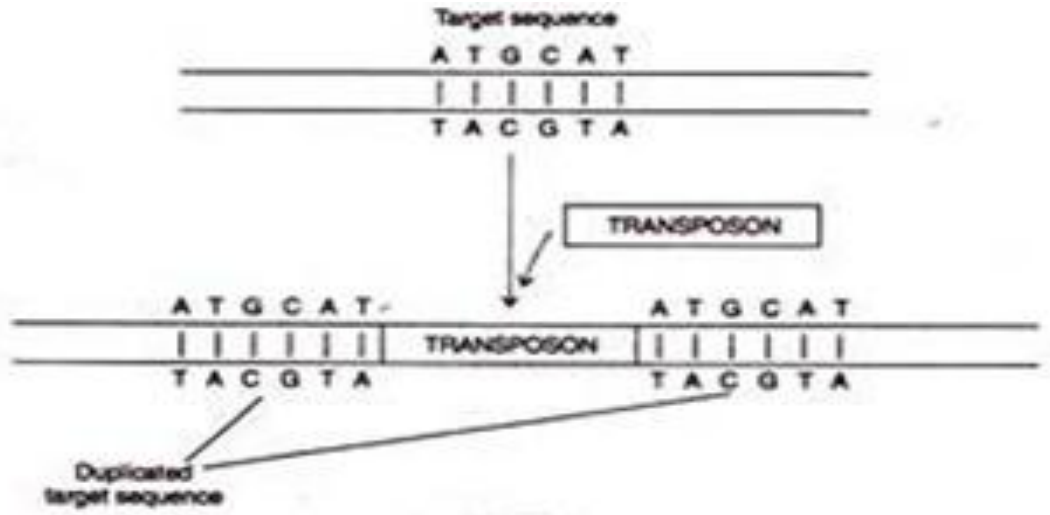


Fig. 18.3.

Mechanism of Transposition

The enzyme transposase present in the transposon itself makes nicks or cuts in each strand of the target sequence. The target sequence is duplicated and two copies move away to make way for the transposon in the centre. The transposon then fixes itself into the two free ends generated. The nicks are sealed by ligase and two strands become continuous.

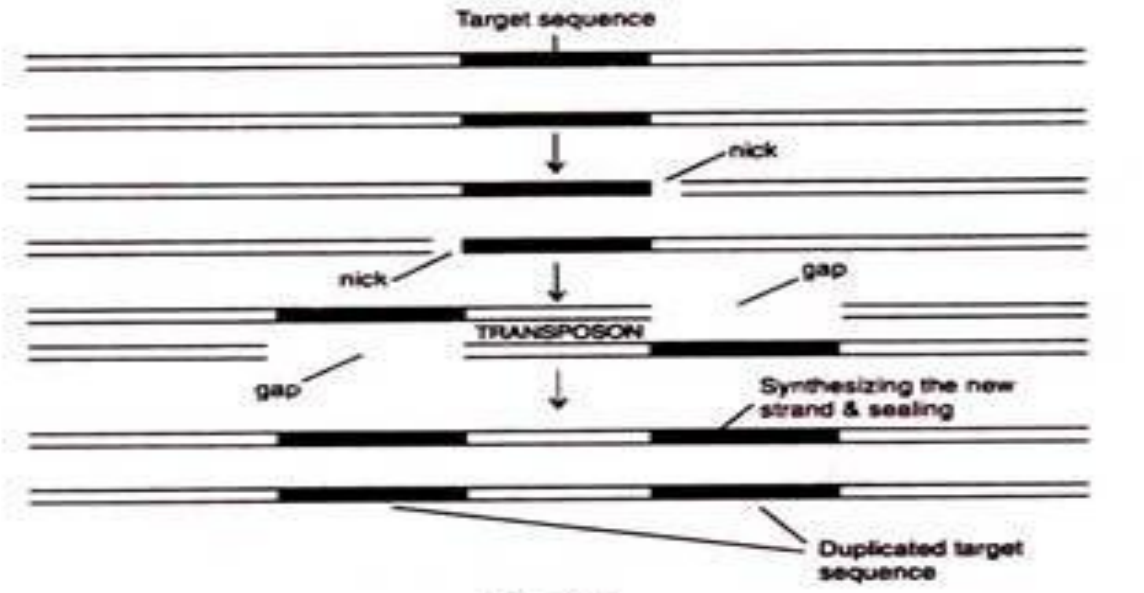


Fig. 18.4.

Functions of Transposons

Mutation Caused by Transposons

Transposons are inserted within genes affecting their function, thus cause disruption of their functions. When they are inserted within the regulatory sequence of genes, they cause change

in their expression. They are most common source of mutation. Transposons may insert stop codons thus producing truncated proteins. In drosophila, majority of spontaneous mutations are caused by transposons jumping into a gene. The mutant white-eyed drosophila is produced by a transposable element inserted into the gene, which normally produces red pigment.

In human beings, transposons cause many genetic diseases. Transposons lead to the development of functional immune system in vertebrates.

In bacteria, the transposable elements are present on extra chromosomal DNA called plasmid. Transposable elements on plasmids carry genes for proteins that nullify the effects of antibacterial drugs and toxins.

Formation of New Genes

How do cells make new genes? Often it is done by exon shuffling by which the functional units of two existing genes recombine generating a new gene. Exon duplication and divergence also play their role in formation of new genes.

Pseudogenes

Transposons introduce great genome flexibility. Sometimes duplicated genes and other transposons do not succeed in making a functional gene and therefore they become.

Transposons or Jumping Genes

Pseudogenes or dead by products of evolution. The pseudogenes are frequent in mammalian genome and they are unable to produce mRNA for translation.

Causes

Mobile DNA can jump to another location on the same chromosome or a different chromosome each time a cell divides. If this happens in sperm or egg cells, it will be passed on to the next generation. The current estimate of such events occurring ranges from 1 in 20 to 1 in 1,000 births.

These jumps can disrupt normal gene function and result in spontaneous emergence of heritable diseases, such as blood disorders, neurodegeneration, and age-related macular degeneration.

Other cell populations also seem particularly prone to mobile DNA rearrangements. Several epithelial cancers, such as those lining the gastrointestinal tract, are known to harbor mobile genetic elements at diverse locations.

Whether these events are at the root of the cancer or a side effect is not currently known, and the human genome is much more complex than previously thought. While jumping genes are just one part of the puzzle, scientists are beginning to appreciate the genetic contribution that microbes make to human diversity and disease. The team believes that studying insect species will help find more evidence of cross-species transfer. They also aim to study other jumping genes and explore the possibility of aquatic vectors, such as sea worms and nematodes. TEs are mutagens and their movements are often the causes of genetic disease. They can damage the genome of their host cell in different ways:[29]

- a transposon or a retrotransposon that inserts itself into a functional gene will most likely disable that gene;
- after a DNA transposon leaves a gene, the resulting gap will probably not be repaired correctly;
- multiple copies of the same sequence, such as Alu sequences, can hinder precise chromosomal pairing during mitosis and meiosis, resulting in unequal crossovers, one of the main reasons for chromosome duplication.

Diseases often caused by TEs include haemophilia A and B, severe combined immunodeficiency, porphyria, predisposition to cancer, and Duchenne muscular dystrophy.[30][31] LINE1 (L1) TEs that land on the human Factor VIII have been shown to cause haemophilia[32] and insertion of L1 into the APC gene causes colon cancer, confirming that TEs play an important role in disease development.[33] Transposable element dysregulation can cause neuronal death in Alzheimer's disease and similar tauopathies.[34]

Additionally, many TEs contain promoters which drive transcription of their own transposase. These promoters can cause aberrant expression of linked genes, causing disease or mutant phenotypes.

Mode of Transmission

- The most common transposable element in humans is the Alu sequence. It is approximately 300 bases long and can be found between 300,000 and one million times

in the human genome. Alu alone is estimated to make up 15–17% of the human genome.[22]

- Mariner-like elements are another prominent class of transposons found in multiple species, including humans. The Mariner transposon was first discovered by Jacobson and Hartl in Drosophila.[23] This Class II transposable element is known for its uncanny ability to be transmitted horizontally in many species.[24][25] There are an estimated 14,000 copies of Mariner in the human genome comprising 2.6 million base pairs.[26] The first mariner-element transposons outside of animals were found in Trichomonas vaginalis.[27] These characteristics of the Mariner transposon inspired the science fiction novel The Mariner Project by Bob Marr.
- Mu phage transposition is the best-known example of replicative transposition.
- Yeast (Saccharomyces cerevisiae) genomes contain five distinct retrotransposon families: Ty1, Ty2, Ty3, Ty4 and Ty5.[28]
- A helitron is a TE found in eukaryotes that is thought to replicate by a rolling-circle mechanism.
- In human embryos, two types of transposons combined to form noncoding RNA that catalyses the development of stem cells. During the early stages of a foetus's growth, the embryo's inner cell mass expands as these stem cells enumerate. The increase of this type of cells is crucial, since stem cells later change form and gives rise to all the cells in the body.
- In peppered moths, a transposon in a gene called cortex caused the moths' wings to turn completely black. This change in coloration helped moths to blend in with ash and soot-covered areas during the Industrial Revolution.

Prevention

Transposons, also called transposable elements, are ancient viruses that have become a permanent part of our genes. Around half of the human genome is made of transposons, many are damaged, but some can become active. Active transposons can be harmful because they move about the genome. When transposons move they can damage genes, leading to genetic illnesses and playing a part in some cancers.

Chemical markers in DNA called methylations can keep transposons inactive. Cells often use methylations to inactivate pieces of DNA, whether they are genes or transposons. Yet, in each new generation most methylations are temporarily erased and renewed by a process

called epigenetic reprogramming. This means that, during sperm and egg production there is a short time when methylations do not control transposon activity, leaving them free to damage genes and shuffle DNA.

The new findings show that transposons become active when cells erase DNA methylation and they are shut down by the endosiRNA system. Just like active genes, active transposons produce messages in the form of RNA molecules, which have many similarities to DNA. The study reveals that cells can detect these transposon RNA messages and use them to create specific endogenous small interfering RNAs (endosiRNAs). The endosiRNAs then act like a trap allowing a protein called Argonaute2 (Ago2) to seek and destroy transposon messages before they cause any harm.

Speaking about the research lead author on the paper, Dr Rebecca Berrens, said: "Epigenetic reprogramming plays a vital role in wiping the genome clean at the start of development, but it leaves our genes vulnerable. Understanding the arms race between our genes and transposon activity has been a long-running question in molecular biology. This is the first evidence that endosiRNAs moderate transposon activity during DNA demethylation. EndosiRNAs provide a first line of defence against transposons during epigenetic reprogramming.".

The effects of active transposons vary, often they have no effect, only occasionally will they alter an important gene. Yet, transposons can affect almost any gene, potentially leading to different kinds of genetic disease. Studying the control of transposons, adds to our understanding of the many ways that they can impact on human health.

Transposons sit within genes and are read in the opposite direction to the surrounding gene. It is this arrangement that allows cells to identify RNA messages from transposons. RNA messages read from the same piece of DNA in opposite directions are complementary, meaning they can join to form a structure called double-stranded RNA (dsRNA), which initiates the creation of endosiRNAs.

Senior scientist on the paper, Professor Wolf Reik, Head of the Epigenetics Laboratory at the Babraham Institute, said: "Transposons make up a large part of our genome and keeping them under control is vital for survival. If left unchecked their ability to move around the genome could cause extensive genetic damage. Understanding transposons helps us to make sense of

what happens when they become active and whether there is anything we can do to prevent it."

Much of this research was carried out using embryonic stem cells grown in the lab, which had been genetically modified to lack DNA methylations. Natural epigenetic reprogramming happens in primordial germ cells, the cells that make sperm and eggs, but these are harder to study. The researchers used primordial germ cells to verify the key results from their study of stem cells.

Altogether, these results show that distinct factors initiate, establish, and maintain MuDRsilencing. Muk initiates silencing by targeting mudra with its hairpin RNAs, leading to the destruction of mudra transcripts and methylation of the transposon's terminal inverted repeats. NAP1 is required to establish heritable silencing, likely by changing chromatin into a transcription-unfriendly state. Mop1/RDR2 then maintains silencing by using RNA processing to mediate continued DNA methylation.

Given the damage that transposons can cause by inserting themselves into essential genes, it's not surprising that organisms have evolved enduring mechanisms to keep jumping genes in their place. This study contributes a valuable framework for identifying the factors that regulate the enigmatic epigenetic processes that defend the genome against invasive elements—and helps explain how these changes can persist and be transmitted to the next generation.

Diagnosis

In early 1978, Ned Boyer, a senior faculty in McKusick's Division of Medical Genetics, sent Alan Scott, a postdoc, to Alec Jeffreys's lab in England to learn Southern blotting. After Alan returned, Phillips suggested and I agreed that he learn Southern blotting from Alan. Y.W. Kan had recently found a HpaI polymorphic site 3′ of the beta-globin gene, and demonstrated its usefulness in prenatal diagnosis of sickle cell anemia.[6] Soon thereafter, Jeffrey found two polymorphic HindIII sites in the gamma-globin genes.[7] Phillips used Southern blotting to find extended linkage disequilibrium involving the Beta S mutation and the HpaI and HindIII sites.[8] Out of eight possibilities for the three sites, there were only four varieties of Beta S-bearing chromosomes. The 60% with the HpaI site lacked both HindIII sites, whereas the 40% without the HpaI site usually contained one HindIII site. This setup allowed an increase in precise prenatal detection of sickle cell anaemia by linkage analysis from 60% to

85%.[8] This paper was crucial to the thinking that went into our later work characterizing mutations in beta-thalassemia. In 1979, we hired Corinne Boehm to run our molecular diagnosis program, which consisted only of prenatal diagnosis of sickle cell anaemia by linkage analysis.

In the genome of every human being, there are about 80-100 active LINE-1s that are capable of mobilizing by a copy and paste mechanism to a new location in the genome.

Upon jumping to a new site, the LINE-1 might alter the function of nearby genes, or knock out a gene by jumping into its protein coding region.

We now know of over 100 examples of patients that acquired a disease because a LINE-1 jumped into one of their genes.

LINE-1s have also played roles in evolution, for example by likely providing the reverse transcriptase sequences for enzymes called telomerases that stabilize the ends of chromosomes.

These are very, very old sequences, probably older than 500 million years as they are found in certain yeast.

So over evolutionary time they have jumped in the genome and then those jumped copies have become inactivated by mutation so now we end up with over 500,000 copies of LINE-1s, mostly dead, that make up almost 20% of the human genome.

On top of those copies, the proteins of LINE-1 are also responsible for mobilizing other human jumping genes, so overall LINE-1 is responsible for over 1/3 of our genome.

The inactive copies have often been co-opted to provide sequences that affect expression of nearby genes. They can provide new splice sites within genes, all kinds of functions can be newly acquired by inactive LINE-1 sequences.

In addition, two inactive LINE-1s of very similar sequences that are say 5 million base pairs apart in the genome can be mispaired and produce a deletion or a duplication of the sequences between those LINE-1 sequences.

You look for a LINE-1 sequence that is 6 kb in length because most of the inactive sequences have lost their front ends during jumping. Active elements need their front ends in order to be expressed.

Then you make sure that the sequences that encode the two LINE-1 proteins are still intact and that they have other tell tales of human specific sequences. If these criteria are met, there is an excellent chance that the element is active as determined GW researcher receives grant to examine link between liver fluke infection and bile duct cancer.

- For nursing home patients, breast cancer surgery may do more harm than good.
- Genetics study uncovers key factors for fracture risk in osteoporosis.

We and others have found that there are somatic new insertions of jumping genes occurring in certain types of cancers, particularly those of epithelial (lining) cell origin, such as cancers of the GI tract.

We now believe that most of these LINE-1 insertions occur in normal cells of the GI tract with specific insertions in different proportions of cells, i.e., some insertions may be in 1 cell in 1 million and others may be in 1 cell in every.[10]

Depending upon which normal cell becomes cancerous, the insertions present in that normal cell become clonal in the cancer. Other insertions may occur during cancer development.

A LINE-1 insertion might knock out the function of one copy of a gene that has a tumour suppressor function. However, another hit would be required in the second copy of the gene.

It would be unlikely to cause tumour progression without that second hit. So it would merely cause susceptibility to the cancer, but this is like any other mutation. For metastasis, the insertion could knock out a gene whose function is important to slow or prevent metastasis.

This is the 64,000 dollar question. I suspect that most insertions are merely passengers and not involved in cancer development. However, we do know of at least two instances where LINE-1 insertions are extremely likely to be causative of cancer development.

Thus, the big question is, Are LINE-1 insertions involved in 1 in 1000 cases of GI cancer or are they important in 1in 50 cases? If the latter, then they are quite important in etiology.

LINE-1 insertions are stuck in the genome where they land. That is why we have so many in our genome.

If one could obtain a small piece of the cancer at surgery and find the LINE-1 insertions in the cancer that appear clonal, one could screen the patient periodically for the insertion in the blood. Upon finding the insertion in blood DNA, one could determine that the tumour had re-appeared.

We want to determine whether we can find insertions in tumours in the blood later. Can we determine that these insertions can be used as biomarkers for the tumour?.

We are also wondering whether these insertions can be used as therapeutic markers for the tumour if they are we could then attempt to kill all the tumour cells while killing 1 in 100,000 of the normal cells.

Also, we want to determine whether any of these insertions are important for the tumour phenotype. In tissue culture of cancer cells, we hope to remove the insertion and determine whether the phenotype revert back toward the normal state.

Treatment

There are also hints that an endogenous retrovirus that became embedded in the DNA of a primate ancestor might help boost the production of the digestive enzyme amylase, which helps to break down starch, in our saliva.[8,9]

To peer deeper into the effects of transposable elements in humans, geneticist Nels Elde and his colleagues at the University of Utah in Salt Lake City used CRISPR–Cas9 gene editing to target an endogenous retrovirus called MER41, thought to come from a virus that integrated into the genome perhaps as far back as 60 million years ago. The scientists removed the MER41 element from human cells cultured in a dish. In humans, MER41 appears near genes involved in responding to interferon, a signalling molecule that helps our immune response against pathogens. Notably, as compared with normal cells, cells engineered to lack MER41 were more susceptible to infection by the vaccinia virus, used to inoculate people against smallpox. The findings, reported last year, suggest that MER41 has a crucial role in triggering cells to launch an immune response against pathogens through the interferon pathway.[10]

The gene that they detected multiple copies of in squirrel monkeys is called charged multi vesicular body protein 3, or CHMP3. Each squirrel monkey seems to have three variants of the gene. By comparison, humans have only the one, original variant of CHMP3. The gene is thought to exist in multiple versions in the squirrel monkey genome thanks to transposable elements. At some point around 35 million years ago, in an ancestor of the squirrel monkey, LINE-1 retrotransposons are thought to have hopped out of the genome inside the cell nucleus and entered the cytoplasm of the cell. After associating with CHMP3 RNA in the cytoplasm, the transposable elements brought the code for CHMP3 back into the nucleus and reintegrated it into the genome. When the extra versions of CHMP3 were copied into the genome, they were not copied perfectly by the cellular machinery, and thus changes were introduced into the sequences. Upon a first look at the data, these imperfections seemed to render them non-functional 'pseudogenes'. But as Elde's team delved into the mystery of why squirrel monkeys had so many copies of CHMP3, an intriguing story emerged.

Hiding in plain sight: Squirrel monkeys carry extra copies of the CHMP3 gene. Credit: Ariadne Van Zandbergen Alamy.

The discovery of pseudogenes is not wholly uncommon. There are more than 500,000 LINE-1 retrotransposons in the human genome[11], and these elements have scavenged and reinserted the codes for other proteins inside the cell as well. Unlike with the endogenous retroviral elements in the genome, which can be clearly traced back to ancient viruses, the origin of LINE-1 retrotransposons is murky. However, both types of transposable elements contain the code for an enzyme called reverse transcriptase, which theoretically enables them to reinsert genetic code into the genome in the cell nucleus. This enzyme is precisely what allowed LINE-1 activity to copy CHMP3 back into the genome of the squirrel-monkey ancestor.

Elde couldn't stop thinking about the mystery of why squirrel monkeys had multiple variants of CHMP3. He knew that in humans, the functional variant of the CHMP3 gene makes a protein that HIV uses to bud off of the cell membrane and travel to and infect other cells of the body. A decade ago, a team of scientists used an engineered vector to prompt human cells in a dish to produce a truncated, inoperative version of the CHMP3 protein and showed that the truncated protein prevented HIV from budding off the cells.[12] There was hope that this insight would yield a new way of treating HIV infection and so prevent AIDS. Unfortunately, the protein also has a role in allowing other important molecular signals to facilitate the

formation of packages that bud off of the cell membrane. As such, the broken CHMP3 protein that the scientists had coaxed the cells to produce soon caused the cells to die.

Given that viruses such as HIV use a budding pathway that relies on normal CHMP3 protein, Elde wondered whether the extra, altered CHMP3 copies that squirrel monkeys carry confers some protection against viruses at the cellular level. He coordinated with researchers around the globe, who sent squirrel-monkey blood from primate centres as far-reaching as Bastrop, Texas, to French Guiana. When Elde's team analysed the blood, they found that the squirrel monkeys actually produced one of the altered versions of CHMP3 they carry. This finding indicated that in this species, one of the CHMP3 copies was a functional pseudogene, making it more appropriately known as a 'retro gene'. In a further experiment, Elde's group used a genetic tool to coax human kidney cells in a dish to produce this retro gene version of CHMP3. They then allowed HIV to enter the cells, and found that the virus was dramatically less able to exit the cells, thereby stopping it in its tracks. By contrast, in cells that were not engineered to produce the retro gene, HIV was able to leave the cells, which means it could theoretically infect many more.

In a separate portion of the experiment Elde's group demonstrated that whereas human cells tweaked to make the toxic, truncated version of CHMP3 (the kind originally engineered a decade ago) die, cells coaxed to make the squirrel-monkey retro gene version of CHMP3 can survive. And by conducting a further comparison with the truncated version, Elde found that the retro gene—what he calls retroCHMP3—in these small primates had somehow acquired mutations that resulted in a CHMP3 protein containing twenty amino acid changes. It's some combination of these twenty points of difference in the protein made by the retro gene that he thinks makes it nontoxic to the cell itself but still able to sabotage HIV's efforts to bud off of cells. Elde presented the findings, which he plans to publish, in February at the Keystone Symposia on Viral Immunity in New Mexico Meyer stresses that these insights elevate the already eminent discoveries by McClintock. “I would hope she would be extremely gratified and vindicated,” he says. “She recognized a type of sort of factor of genomic dynamism that no one else had seen before. And I am firmly convinced that it's going to only become more and more and more central to our understanding of how genomics works.”

Craig's team has worked on many families of DNA transposons. "Part of what we've done is to show the fundamental chemical similarity between transposons of the same family," Craig

says. "It turns out that they all work the same way, but we didn't know that when we started out."

Craig and other scientists are interested in understanding how transposons pick their sites, because transposons can be used in gene therapy. It's possible to take a DNA transposon from an insect or a fish, for example, and put it in a mammalian cell, then watch it work. Now Craig is studying just how the host fits into the process—whether cells somehow inhibit or activate DNA transposons and which host proteins get involved.

The ways in which transposons are wreaking havoc in cancer cells can also happen during meiosis, a type of cell division that occurs in developing sperm and eggs. As in cancer cells, transposons can cause recombination. And transposons can even move genes around, if the transposition process accidentally copies some neighbouring DNA.

Understanding more about how retrotransposons work. He'd like to take tumour cells in culture, edit out the insertions that seem significant and see whether the cancer changes its behaviour. With another group, he's trying to catch retro transposition events in live stem cells by periodically analysing them as they grow and divide.

Conclusions and future directions

The longstanding idea that neuronal genomes contain stable, unchanging DNA is now being challenged by mounting evidence that somatic retro transposition occurs in neurons. This review has highlighted the evidence that retrotransposons, McClintock's “jumping genes,” must now be considered as a viable source of genetic variability in the brain and in health and disease of the CNS. The advances in genomic technologies have enabled a deeper understanding of the role of retrotransposons in neuronal function and evolution. This is indeed an exciting time for what was once dismissed as genomic “parasites” or “selfish” entities of the genome.

The sheer abundance of retrotransposons in eukaryotic genomes begs us to reconsider their role in biology and in neuroscience in particular. Altered retrotransposon expression or function appears to be associated with stress, alcohol, neurodegeneration, and aging. However, we need to better define the functional consequences of these retrotransposons, which could lead to novel treatment strategies for a multitude of psychiatric and neurological disorders.

Finally, the evolutionary significance of retrotransposons, especially in the evolution of brain function, is paramount in understanding why nature has produced such a biological mechanism. Cross-species analysis of the function of retrotransposons in the brain will undoubtedly provide clues to their origin and their potential as drivers of genomic innovation.

REFERENCES

1. https://www.ncbi.nlm.nih.gov/pubmed/6314499.
2. https://link.springer.com/content/pdf/10.1007%2F978-3-642-28664-3_1.
3. https://europepmc.org/abstract/med/16369944.
4. https://www.cell.com/current-biology/fulltext/S0960-9822(02)00603-6#back-BIB1.
5. Madigan M, Martinko J, eds. (2006). Brock Biology of Microorganisms (11th ed.). Prentice Hall. ISBN 0-13-144329-1.
6. Jump up^ Kazazian HH Jr; Moran JV (May 1998). "The impact of L1 retrotransposons on the human genome". Nature Genetics., 19(1): 19–24. Doi:10.1038/ng0598-19. PMID 9590283.
7. http://www.biologydiscussion.com/gene/transposons-or-jumping-genes-types-structure-mechanism-and-functions/16499.
8. https://www.medicalnewstoday.com/articles/318978.
9. https://economictimes.indiatimes.com/.../jumping-gene...is...cause.../64918768.cms.
10. Belancio VP, Hedges DJ, Deininger P (March 2008). "Mammalian non-LTR retrotransposons: for better or worse, in sickness and in health". Genome Res., 18(3): 343–58. Doi:10.1101/gr.5558208. PMID 18256243.
11. Jump up^ Kazazian H.H.; Goodier J.L. (2002). "LINE drive: retro transposition and genome instability". Cell., 110(3): 277–80. Doi:10.1016/S0092-8674(02)00868-1. PMID 12176313.
12. Kapitonov V.V., Pavlicek, A., Jurka, J. (2006). "Anthology of Human Repetitive DNA". Encyclopedia of Molecular Cell Biology and Molecular Medicine. Doi:10.1002/3527600906.mcb.200300166.
13. Jump up^ Kazazian, HH Jr; et al. (1988). "Haemophilia a resulting from de novo insertion of L1 sequences represents a novel mechanism for mutation in man". Nature., 332(6160): 164–6. Bibcode:1988Natur.332.164K. Doi:10.1038/332164a0. PMID 2831458.
14. Jump up^ Miki, Y.; et al. (Feb 1992). "Disruption of the APC gene by a retro transposal insertion of L1 sequence in colon cancer". Cancer Research, 52(3): 643–5. PMID 1310068.

15. Jump up^ Sun, Wenyan; Samimi, Hanie; Gamez, Maria; Zare, Habil; Frost, Bess (23 July 2018). "Pathogenic tau-induced piRNA depletion promotes neuronal death through transposable element dysregulation in neurodegenerative tauopathies". Nature Neuroscience, 21(8): 1038–1048. Doi:10.1038/s41593-018-0194-1. PMC 6095477. PMID 30038280.
16. https://www.sciencedaily.com/releases/2017/11/171102121435.
17. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1563490.
18. Kazazian HH, Moran JV (May 1998). "The impact of L1 retrotransposons on the human genome". Nat. Genet., 19(1): 19–24. Doi:10.1038/ng0598-19. PMID 9590283.
19. Jump up^ Jacobson JW, Medhora MM, Hartl DL (November 1986). "Molecular structure of a somatically unstable transposable element in Drosophila". Proc. Natl. Acad. Sci. U.S.A., 83(22): 8684–8. Bibcode:1986PNAS...83.8684J. Doi:10.1073/pnas.83.22.8684. PMC 386995. PMID 3022302.
20. Jump up^ Lohe AR, Moriyama EN, Lidholm DA, Hartl DL (January 1995). "Horizontal transmission, vertical inactivation, and stochastic loss of mariner-like transposable elements". Mol. Biol. Evol., 12(1): 62–72. Doi:10.1093/oxfordjournals.molbev.a040191. PMID 7877497.
21. Jump up^ Lampe DJ, Witherspoon DJ, Soto-Adames FN, Robertson HM (April 2003). "Recent horizontal transfer of mellifera subfamily mariner transposons into insect lineages representing four different orders shows that selection acts only during horizontal transfer". Mol. Biol. Evol., 20(4): 554–62. Doi: 10.1093/molbev/msg069. PMID 12654937.
22. Jump up^ Mandal PK, Kazazian HH (October 2008). "SnapShot: Vertebrate transposons". Cell., 135(1): 92–192.e1. Doi:10.1016/j.cell.2008.09.028. PMID 18854165.
23. Jump up^ Carlton JM, Hirt RP, Silva JC, et al. (January 2007). "Draft genome sequence of the sexually transmitted pathogenTrichomonas vaginalis". Science, 315(5809): 207–12. Bibcode:2007Sci...315.207C. Doi:10.1126/science.1132894. PMC 2080659. PMID 17218520.
24. Jump up^ Kim JM, Vanguri S, Boeke JD, Gabriel A, Voytas DF (May 1998). "Transposable elements and genome organization: a comprehensive survey of retrotransposons revealed by the complete Saccharomyces cerevisiae genome sequence". Genome Res., 8(5): 464–78. Doi:10.1101/gr.8.5.464. PMID 9582191.

25. https://www.news-medical.net/news/20151006/Jumping-genes-a-marker-for-early-cancer-diagnosis-An-interview-with-Dr-Kazazian.aspx.
26. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2667999.
27. https://www.hopkinsmedicine.org/research/advancements-in-research/fundamentals/in-depth/jumping-genes-and-cancer.
28. https://www.scientificamerican.com/article/medicines-movable-feast-what-jumping-genes-can-teach-us-about-treating-disease.
29. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3818539.