Transposable dna elements

Most genes occupy fixed sites on the chromosomes. However, researchers have found that some genes (DNA sequences) can actually change position in the chromosomes. These mobile DNA sequences are calledtransposable elementsor simplytransposons. Typically they are quite small ranging from 500 to 10, 000 nucleotide-pairs, but some are larger. Transposable elements are ubiquitous specific DNA sequences of the genome found in both prokaryotes and eukaryotes. At the molecular level, these elements exhibit considerable variation in structure and function. Transposable elements were discovered by Barbara McClintock in maize in 1950.

Transposable elements are generally of two types. One type encodes proteins which induces the transposable element to move directly to a new position or replicate the transposable element to produce a new element that integrates elsewhere in the genome. These types of elements are found in both prokaryotes and eukaryotes. The second type of transposable element is related to retrovirus. Such elements encode a reverse transcriptase for making copies of DNA from their RNA transcripts, which subsequently integrate at new sites in the genome. Such elements are found only in eukaryotes.

In eukaryotes, transposable elements can move to a new position within the same chromosome or to a different chromosome. In prokaryotes, they can move to a new position on the single chromosome present. Transposable elements can inserts into new chromosomal locations without having a homologous sequence; and therefore the process of insertion is different from homologous recombination. Such recombination is callednon-homologous recombination.

Transposable elements can induce various genetic changes. By inserting themselves into the coding region of a gene, they can alter the reading frame or introduce stop codons. If inserted into the regulatory region of a gene, they can disrupt expression of the gene. They can also create double-stranded breaks, inversions and translocations in the chromosomes. Transposable elements have contributed to the evolution of the genomes through induction of chromosomal rearrangements. Usually the frequency of transposition varies with different elements, but it is very low, as higher frequency may lead to lethality.

Insertion sequences (IS) or IS elements is the simplest transposable element found in bacterial chromosomes and plasmids. IS elements were first identified in *E. coli* , from the observations that some mutations affecting the expression of genes, that control the metabolism of the sugar glucose, did not have properties typical of a point mutations or deletions. It was later found that such changes were induced due to insertion of an approximately 800 base-pair DNA segment in to a gene. This particular segment of DNA is called insertion sequence 1, or IS1 (Fig. 6. 19).

E. coli contains several IS elements (e. g. IS1, IS2 etc), each may present up to 30 copies per genome. Each one of them has a characteristic length (ranging from 768 bp to > 5000 bp) and unique nucleotide sequence. All IS elements end with identical or near identical inverted repeats (IR’s) of 9 to 41 bp, but in opposite orientations. For example, the inverted repeats of IS1 consist of 23 bp, but the sequences are not completely identical.

An IS elements can integrate at random locations along the chromosome often inducing mutations, by disrupting either the coding or regulatory sequence of a gene. Promoters present within the IS elements may also effect by altering the expression of nearby genes. IS elements can also induce deletions and inversions in the adjacent DNA thereby causing mutations.

An enzyme calledtransposase, encoded by the IS element, is responsible for transposition of the IS elements. Presences of inverted repeats (IR’s) are essential as transposase recognizes these sequences to initiate transpositions. Insertion of IS element takes place at a target site with which the IS element has no sequence homology (Fig. 6. 20). First, a staggered cut is made in the target site and the IS element is then inserted, and joined to the single stranded ends. Then DNA polymerase and DNA ligase fills-in the gaps. The IS element is thus integrated into the chromosome with two direct repeats (oriented in the same direction) of the target-site sequences flanking the IS element. The direct repeats are called target-site duplications.

The second type of transposons found in prokaryotes is calledTn transposonswhich are similar to IS elements but it contains additional genes. Transposons are of two types; composite transposons and noncomposite transposons. Composite transposons are comparatively complex in nature, having functional genes (e. g. resistance against antibiotics), flanked on both sides by IS elements (Fig. 6. 21). The IS elements are called ISL (left) and ISR (right) and basically of same type, however they may be in the same or inverted orientation depending on the transposon. As the IS’s elements have terminal inverted repeats, the composite transposons also have terminal

inverted repeats. Tn10 is an example of composite transposon. The enzyme transposase, produced by one or both IS elements, recognizes the inverted repeats of the IS elements and process of transposition is similar as described for IS element. Composite transpositions produce target site duplications in the new location after transposition.

Noncomposite transposons also contain functional genes like antibiotic resistance, but ARE elements are not present in the terminal ends (example Tn3). They have repeated sequences at genes present in the central region. The enzyme transposase catalyzes the insertion of a transposon into new sites, and the second enzyme, resolvase is involved in the recombinational events associated with transposition. Noncomposite transposons also cause target-specific duplications when moved to a new location.

In *E. coli* transfer of genetic material between two mating types (F^+ and F^-) is the result of the function of the fertility factor F. The F^+ strains have the F factor within the cell whereas F^- cells does not. F factor is an episome, an extrachromosomal genetic element, which is capable of self-replication and integration into bacterial chromosome. When F^+ strains and F^- were co-cultivated, the F factor can move from F^+ strains to F^- strain and thereby convert the F^- cells to F^+ strain (seeChapter 4). F factor contains four IS elements, namely one copy IS2, two copies IS3, and one copy of an insertion sequence element called gamma-delta. The *E. coli* chromosome also has copies of these four insertion sequences at various locations. Thus, integration of the F factor into the main chromosome of the bacteria can be achieved by pairing of the homologous sequences and subsequently

exchange of genetic material. Depending upon how the F factor has paired with its homologous segments in the chromosome, integration of the F factor may have different orientations.

While studying black Mexican sweet corn in 1930s, Rhodes observed that when allele *a* of A1 locus is present in homozygous condition, it cannot synthesize purple anthocyanine pigments, and the aleurone layer of the seed remains colourless. However, the presence of Dt (dotted) gene on another chromosome can influence the phenotype of the aleurone layer. Plants of genotype *a/a Dt/Dt* or *a/a Dt/dt* produce seeds with dots of purple colour as if allele *a* got mutated to the dominant wild type allele A1. Moreover, the number of doses of the *Dt* allele affected the number of dots – one dose produced on the average 7. 2 dots per seed, two doses produced 22. 2, and three doses produced 121. 9. Rhodes called *Dt* asmutator gene, a gene which can increase the spontaneous mutation frequency of another gene.

McClintock carried a series of experiments with maize during 1940s and 1950s that led her to put forward a hypothesis on existence of what she called ‘ controlling elements’, which can move freely in the genome and can modify or suppress gene activity. Later the controlling elements she studied were shown to be transposable elements. Recent work has showed that Rhode’s mutator gene *Dt* is also a transposable element.

Presence of transposable elements has been shown in many eukaryotes, which include yeast, fruit fly, maize and human. Structurally and functionally the transposable elements found in prokaryotes and eukaryotes are very similar. Eukaryotic transposable elements have genes that encode enzymes

required for transposition, and they can integrate into chromosomes at a number of sites. Effects of such insertions are similar as described for prokaryotic organisms.

The yeast carries a transposable element called *Ty* in its haploid genome, which is about 5. 9 kb long and bounded by 340 bp long DNA segment called the delta sequence at each end. Each delta sequence is oriented in the same direction and is called long terminal repeats (LTRs). Sometimes LTRs becomes detached from a *Ty* element, producing solo delta. Each delta contains a promoter and sequences recognized by transposing enzymes. The *Ty* element encodes a single mRNA of 5, 700 nucleotides, which begins at the promoter in the delta at the 5’ end of the element (Fig. 6. 23). The mRNA transcript contains two open reading frames (ORFs), designated *Ty* A and *Ty* B. They encode two different proteins required for transposition. On average there exist 35 copies of Ty element in each yeast cells, although the number may vary in different strains.

The genetic organization of the *Ty* elements is similar to that of eukaryotic retrovirus. This single stranded RNA virus synthesizes DNA from their RNA after infecting the cell. The DNA then inserts itself into a site in the genome, where it can be transcribed to produce progeny viral RNA genomes and mRNAs for viral proteins. This inserted material has same overall structure as in yeast *Ty* element. *Ty* elements transpose by making RNA copies of the integrated DNA sequence and then creating a new *Ty* element by reserve transcription. The new *Ty* elements would then integrate at new sites in the chromosome. *Ty* elements have two genes, *A* and *B* , which are analogous to the *gag* and *pol* genes of retrovirus. The two genes produces virus like

particles inside yeast cells, but it is not known whether these particles are infectious. Because of their overall similarity to the retroviruses, *Ty* element is also calledretrotransposonsand the process is calledretrotransposition.

Several families of transposons have been identified in plants. Each family has two forms of transposons – autonomous elements and non-autonomous elements. Autonomous elements can transpose themselves, whereas non-autonomous elements cannot transpose themselves, as they lack the genes for transposition. Therefore, non-autonomous elements require the presence of an autonomous element to carry out transposition. Usually, within the family, the non-autonomous element is derived from autonomous element, with loss of function of one or more genes required for transposition.

When an autonomous element is inserted into a functional gene, the resultant mutant gene is unstable, because the element can excise and transpose to another location. On the other hand, when the non-autonomous element is inserted into a gene, the mutant is stable, because the element is unable to transpose by itself. However, if the autonomous element of its family is present in the genome, it may provide the necessary enzymes required for transposition, and the non-autonomous element also will be able to transpose, making the mutant gene unstable.

While working with maize, Barbara McClintock observed that purple coloured kernel is produced by the wild–type gene *C* , and colourless by the mutant *c* . Sometimes rather than being either of a solid purple colour or colourless, kernels with spots of purple pigment on the otherwise white (colourless) background are produced. After careful genetical studies, she concluded that

the spotted phenotype was due to what she called ‘ controlling elements’, which is actually transposon. During kernel development, the mutant gene *c* reverts to the wild-type *C* , leading to development of spotted purple pigment. The earlier the reversion occurs, the larger is the purple spot. McClintock found that the *c* (colourless) mutation resulted from a ‘ mobile controlling element”, called *Ds* (for dissociation) being inserted into the *C* gene. It is now known that this insertion take place by the transposition of a nonautonomous transposon. A second mobile controlling element called *Ac* (for activator) is required for transposition of *Ds* into the gene. *Ac* can also excise *Ds* out of the *c* gene, producing a wild-type revertant *C* . *Ac* has now been identified as autonomous transposable element.

Molecular basis of *Ac-Ds* system was understood only in 1983. The autonomous *Ac* element is 4, 563 base pair long, with a single gene encoding the enzyme transposase and short terminal inverted repeats. Upon insertion into the genome, an 8 base pair direct duplication of the target site is generated. *Ds* elements have all the same terminal inverted repeats (IRs) as *Ac* elements, but are heterogeneous in length and sequence. The variation in length and sequence occur as most *Ds* elements are generated from *Ac* element by deletion of segments or by more complex sequence rearrangements. Because of these variations, *Ds* elements remain transposition-defective and cannot exert transposition activity themselves.

Insertion of *Ac* element takes place through conservative transposition mechanism (cut-and-pest) during chromosome replication (Fig. 6. 22). When a chromosome with one *Ac* element (present at a site called donor site) replicates, two copies of *Ac* will be produced, one on each daughter

chromatids. Depending upon which chromatid is involved, there are two possible results of *Ac* transposition, one involving the replicated and other unreplicated chromosome site. If we consider transposition to a replicated chromosome site, and if one of two *Ac* elements transposes to an already replicated site, an empty donor site is left on one chromatid, and *Ac* element remains in the homologous donor site on the other chromatid (Fig. 6. 22). The inserted *Ac* element is inserted into the same chromosome. Thus there is no net increase in the number of *Ac* elements in the chromosome. On the contrary, if the *Ac* element is transposed to a nonreplicated chromosomal site, it will create an empty donor site, but since it was inserted into an unreplicated site it will be duplicated after replication. Thus there will be increase in the number of *Ac* elements in the chromosome. Transposition of *Ds* elements occurs in similar fashion but requires the enzyme transposase encoded by *Ac* elements.