Review

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Plant Transposable Elements and Functional Genomics

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Abstract

Transposon tagging is the direct way of gene identification and cloning in living organisms. In plants, well characterized transposable elements are available from *Zea mays* and *Antirrhinum majus*. These have been used as simple insertion mutagens to clone genes in both native and several heterologous plant species. Transposon mediated techniques are also increasingly being used to study the pattern and regulation of gene expression in plants. Recently, transposons have been used in ingenious ways to bring about deletion and inversion of chromosomal segments. The transposon-based reverse genetics and its potentials in assigning biological functions to the known DNA sequences makes it useful in functional genomics.

This review traces the developments in the use of plant transposons, from a simple technique for insertional mutagenesis to a powerful tool for gene discovery and study of gene function.

The past decade has seen many path-breaking developments in the field of plant genetic engineering. Most notably perhaps, is the perfection of techniques to genetically transform plants. Plant transformation has become routine for many species including important food crops and holds great promise for crop improvements in the future. To realize this promise in good measure, there is now, an urgent need to identify and isolate relevant genes and regulatory elements which can be manipulated to increase yields, reduce losses and improve quality.

Several methods have been in use for the identification and isolation of plant genes. Methods like subtractive hybridization, functional complementation and genomic methods like map-based cloning have been in use for many years. The use of mutants, either spontaneous or induced, to identify genetic regions controlling specific traits is also not new. These methods, however, have several limitations; low efficiency, limited applicability and sometimes technical complexity, to cite a few.

A relatively recent development is the use of characterized DNA as insertional mutagens to tag and isolate genetic regions. This method overcomes many of the limitations of the earlier techniques and opens up many more exciting possibilities.

In plants there are two types DNA insertional mutagens used for cloning genes, they are the T-DNA of *Agrobacterium tumefaciens* and transposable elements (transposons). Both have been widely used for gene tagging in different plant species [1-3]. T-DNA tagging is limited to those species which can be transformed by *Agrobacterium* mediated methods. On the other hand, some of the better characterized transposable elements, particularly from maize are known to transpose in heterologous host species tested so far, like petunia [4], Arabidopsis [2, 5, 6], tomato [3], tobacco [7] and flax [8] and have been successfully used to tag genes from these plants. Further, several recent developments in the design of synthetic transposons and strategies to use them for gene isolation has made this a powerful gene discovery tool.

This review is intended to discuss the use of transposons as a gene identification and isolation tool, as well as its use in studying gene function in plants.

1. Plant transposable elements exists as families.

More than half a century ago, Barbara McClintock discovered transposable elements in maize [9]. Similar genetic elements have been subsequently discovered and characterized in bacteria, yeast, animals and other plants. In plants, the transposons are organized into families. Each family has an autonomous element and a series of dependent elements. The autonomous element is a complete transposon; all the functions required for transposition, namely the transposase protein and the cis-acting sequence motifs are encoded within it. The dependent elements are defective transposons, usually internal deletion derivatives of the autonomous element with only the cis-acting sequences intact. The ORF encoding the transposase is partially or completely deleted. The dependent elements are therefore, active only in the presence of an autonomous element, which provides the transposase function in trans.

The maize *Ac-Ds* (Activator-Dissociation) and

En-I (Enhancer-Inhibitor) are among the well characterized, typical plant transposable element families. The autonomous element *Ac*, described first by Barbara McClintock is 4565 bp long [10, 11] and contains a 11 bp terminal inverted repeat (TIR). A 3.5 kb mRNA is transcribed from a single ORF in *Ac* which is translated into an 807 amino acid long transposase protein [12]. The cis-determinants of transposition in *Ac* are represented by about 200 bp at each end which includes the TIR. The dependent element of the *Ac* family is the Dissociation element (*Ds*) [9]; the 11 bp TIR is conserved in all the *Ds* elements. Some *Ds* elements are internal deletion derivatives of the autonomous element *Ac*. The *Ds* element in the genome is active only if the genome has an autonomous *Ac* element.

Compared to the *Ac*, the autonomous Enhancer or Suppressor-mutator element (*En/Spm*) of maize has a complex organization; it is 8287 bp long [13, 14]. The *En* encodes two functional proteins, named as *TNPA* and *TNPD* [15, 16] and both of them are required for transposition. The cis-determinants of *En/Spm* is represented by about 200 bp at the 5′ end and 300 bp on the 3′ end of the element which includes a 13 bp TIR [reviewed in 17].

The maize transposons *Ac* and *En/Spm* have short TIRs while another member in this species, Mutator (*Mu*) [18], has long TIRs, approximately 200 to 500 bp. Unlike *Ac* and *En/Spm*, *Mu* element is not well characterized at the molecular level. Another species where the transposable elements have been extensively studied is *Antirrhinum majus*. *Tam*1 is an autonomous element in this species and has strikingly similar structural organization of *En/Spm* [19]. Another element from this species, *Tam*3, on the other hand, is more like *Ac* [20].

Several other transposable element systems and transposable element like sequences have been identified by different researchers in other species like *Arabidopsis* [21, 22] and rice [23]. Apart from these transposable elements which transpose as a DNA molecule, the plant genomes also contain retrotransposons [24] which transpose through an RNA intermediate. Retrotransposons can insert into the genome, but cannot excise and therefore, they generate permanent disruption of the gene.

2. Transposable elements are mutagenic

Transposons, by their very capacity to excise from a location in the genome and get inserted into a new genomic location, have tremendous potential to induce mutations by disrupting gene function. In fact, the very discovery of transposons was based on the instability of certain traits. The disrupted gene can be cloned using the inserted transposon as a 'molecular tag' (transposon tagging). A generalized strategy for transposon tagging and gene isolation is outlined in **fig. 1**.

Several genes have been cloned from maize and *Antirrhinum* using the native transposons as molecular tags. For *e.g.*, genes from maize such as *A*1, *Bz*1, *Bz*2, all involved in anthocyanin biosynthesis [25-27], hcf-106, involved in chloroplast development [28], the nuclear restores genes *rf*2, *rf*1 and *rf*8 [29-31] and from *Antirrhinum majus*, *deficins*, a regulatory gene [32] and *pallida*, a locus encoding an enzyme required for anthocyanin biosynthesis [33].

Mutations induced by the insertion of the transposon are prone to reversion if autonomous elements are present in the system. Mutations induced by a dependent element can however, be stabilized if the autonomous element is removed from the system through genetic recombination and segregation.

Transposition is generally a random event and therefore mutations can be expected to be induced at random in the genome. Nevertheless, there are subtle differences in the behavior of different transposon families and these considerations have to be factored into strategies for transposon mediated mutagenesis.

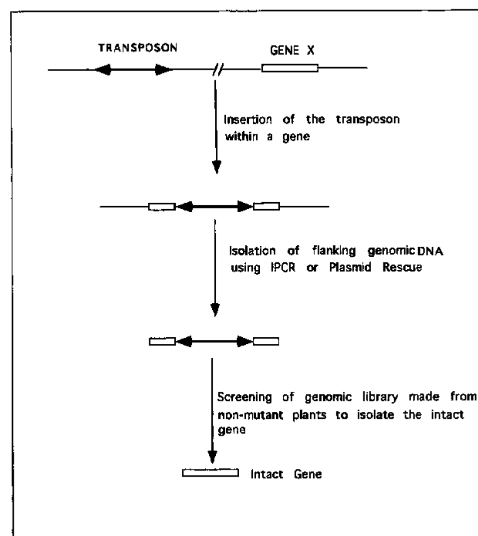

Fig. 1 Transposon tagging and gene isolation. The insersion of the transposon into Gene X results in a mutant phenotype. The genomic region flanking the inserted transposon, which is the target gene, can now be isolated by Inverse Polymerase Chain Reaction (IPCR) with primers based on the transposon sequence or by rescuing the transposon carrying the genomic region as a plasmid in an *E. coli* host if the transposon is engineered to have the appropriate functions. The flanking regions can then serve as a probe to screen the total genomic or cDNA library from a wild type plant to isolate the intact gene.

For *e.g.*, the maize *Ac-Ds* system has a tendency to transpose to closely linked sites [34] and if random mutagenesis is desired, a selection system against closely linked transpositions has to be built into the gene isolation strategy.

The frequency of transposition and consequently the mutagenic potential of the transposon is influenced by several factors of which certain environmental stimuli and the genetic background of the host seem to be important [35].

3. Gene tagging in heterologous systems

The mutagenic potential of transposons was initially exploited only in maize and snapdragon, where active, well-characterized endogenous [36] transposons were present. The discovery that the maize transposon can function efficiently in other plant species [1-8, 37, 38] has led to the development of transposon tagging systems in several species, even some in which no transposon has yet been detected. The methods and considerations in using transposons for mutagenesis has been reviewed by Walbot [39].

Transposon tagging in a species using the native transposons may get complicated by the presence of multiple copies of the element in the system. Further, the mutations tend to be unstable due to the presence of autonomous elements in the system which will reactivate the transposon. The transposon may jump out of the mutated site before the region is isolated. It is known that all excision events do not lead to reversion to the wild phenotype; often the excision is imprecise. These problems are to a large extent avoided if the transposon is used in a heterologous species.

Transposon tagging using autonomous elements can lead to instability of the mutants and problems in isolation of the relevant genetic region. A two-component system has been developed to overcome this problem (**fig. 2**). This system consists of two transgenic lines, one with an immobilized autonomous element (*Ac or En*) to provide the transposase enzyme and the other with a synthetic dependent element (*Ds* or *dSpm*) which can be activated by the transposase in trans. Transposition is initiated by crossing these two transgenic lines. The lines carrying stable insertions could be recovered in the segregating generations. By linking appropriate selectable markers with the immobilized autonomous element and the synthetic dependent element constructs, the stable transposants can be selected. The transposase source is often the autonomous element which has been made immobile by modifying the cis-determinants of transposition, usually by deleting one of the termini. The minimum requirement for the synthetic dependent element is the intact cis-determinants, but often they are engineered to carry several other features. A gene conferring an antibiotic resistance (selectable marker) is often engineered into the dependent element. This feature helps in tracking the presence of the dependent transposon in the progenies. It should be noted that all the transposons that excise do not reintegrate and get lost or genetic segregating can give rise to progenies devoid of the dependent element.

An excision marker gene to select the lines where the transposon has excised from the original site is an useful feature, particularly when the natural transposition frequencies are low. The excision marker also helps in reducing the number of progenies to be screened for new mutations. For the speedy recovery of the tagged region the features for plasmid rescue function can be engineered into the dependent element. These functions include resistance to an antibiotic like ampicillin and plasmid origin of replication. For transposon families like the *Ac-Ds*, which have a tendency to jump to closely linked sites, it is important to have markers flanking the synthetic *Ds* element. These markers get inactivated if the transposon jumps into them, thus helping in selecting transposants with more randomly distributed *Ds*-insertions. In a heterologous system it is important to have appropriate promoters driving the transposase and the marker genes for greater efficiency. The frequency of transposition could be increased by using a strong promoter to drive the transposase gene [1, 40-42].

The *Ac-Ds* system has been successfully used in

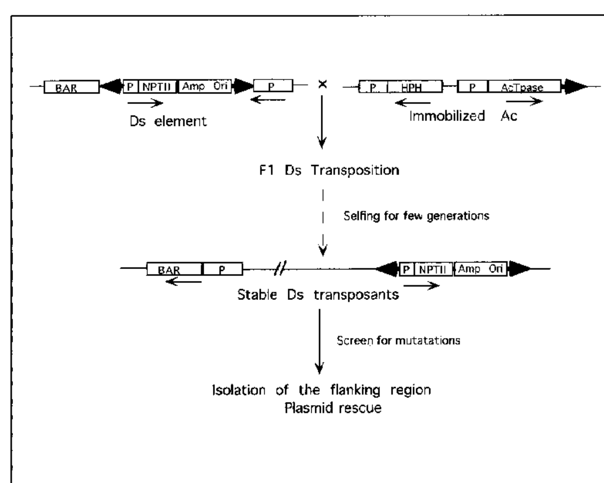

Fig. 2 A two-component system for gene tagging. P, appropriate plant promoter; *BAR*, *HPH*, *NPT II*, selectable markers conferring resistance to ammonium glufocinate, hygromycin and kanamycin respectively; Amp, bacterial selection marker conferring resistance to ampicillin ; Ori, origin of plasmid DNA replication; Ac Tpase, transposase gene.

more plant species compared to the En-I system. The *Ac- Ds* system was used to clone a leaf morphology gene [2] and an albino gene from *Arabidopsis* [5], flower colour gene from petunia [4], gene conferring resistance to *Cladosporium fulvum Cf*-9 and the dwarf gene from tomato [3, 43]. The first gene cloned using the *En-I* element system in a heterologous host is the male sterility gene from *Arabidopsis* [1].

4. Transposon traps are more potent

Conventional transposon tagging using the two-component system has a major limitation. It only helps in the identification of genes which when mutated, produce an obvious mutant phenotype. Whereas, most eukaryotic genes do not have an obvious phenotype on disruption and therefore are not amenable to conventional transposon tagging approaches. It has been estimated that in yeast, *Saccharomyces cerevisiae*, 60 to 70 percent of the genes do not have a mutant phenotype [44-46].

This is partly due to genetic redundancy. Also, a good proportion of the genes are regulated; spatially, temporally or in response to environmental stimuli. To extend the application of the transposon tagging technique to such genes, a modified approach was developed first in *Drosophila* [47] and then in mouse embryonic stem cells [48]. The modification was essentially the inclusion of a reporter gene in the synthetic transposon. The expression of the reporter gene is dependent on the endogenous transcription signals. Such a transposon helps in the "trapping" of even those genetic regions which do not have a mutant phenotype, as the expression of the reporter gene is indicative of the transposon sitting in an active genetic region. Moreover, the expression pattern of the reporter gene reflects the activity and regulation of the genetic region which has been tagged.

The transposon constructs used for gene trapping comes in two versions, the enhancer trap and the gene trap (**fig. 3**). The enhancer trap has a minimal promoter, which drives reporter gene expression if activated by a chromosomal enhancer region. Thus, enhancer traps are very useful in identifying and isolating cis-acting regulatory sequences and genes activated by them. The gene trap on the other hand, has absolutely no promoter sequence to drive the reporter gene expression. The reporter is therefore expressed only if the transposon gets inserted into a transcribed region in the genome, in the proper frame and orientation. However, a splice acceptor sequence is included immediately 5′ of the reporter gene. This ensures that the reporter is part of the translated mRNA and is not spliced out if the transposon inserts into an intron region of the target locus.

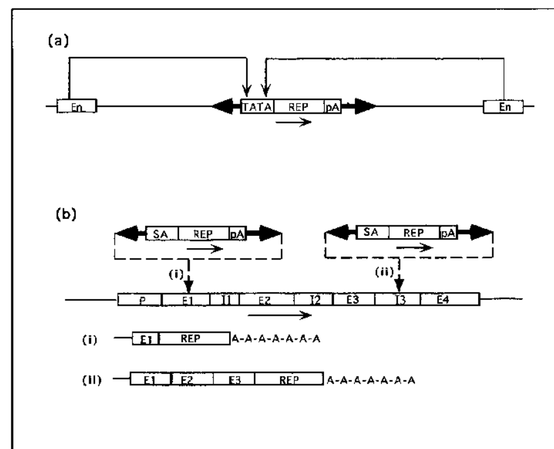

Fig. 3 Transposon trap constructs. (a) Enhancer trap. (b) Gene trap. TATA, minimal promoter; REP, reporter gene (*e.g. GUS*); pA, polyadenylation signal sequence; En, chromosomal enhancer region; E1, E2, E3 and E4, exon sequences; I1, I2, I3, intron sequences. (i) insersion of the gene trap into an exon and the resultant mRNA having truncated E1. (ii) insertion of the gene trap into an intron and the resultant mRNA.

In plants, the enhancer and gene traps were first successfully used in *Arabidopsis* [49]. The traps were based on the maize *Ac-Ds* transposon family and the reporter gene was the bacterial *uidA* or *GUS* (β-glucuronidase). The expression of *GUS* can be visualized by simple histochemical staining methods [50]. Also, the *GUS* gene can tolerate large amino-terminal fusions [51]. These qualities make *GUS* a valuable reporter for inclusion in transposon trap constructs.

We are developing transposon trap lines in rice to clone genes of agronomic importance [52, **Fig. 4**]. The potential of transposon traps as a powerful tool for studying gene function and for discovery of new genes is being increasingly recognized.

5. Transposons versus T-DNA

Both transposons and T-DNA sequences insert into plant chromosomes by illegitimate recombination and hence they should be able to mutate any gene in the genome. In the case of small genomes like Arabidopsis, with a haploid genome size of about 80 Mb [53], saturation-T-DNA tagging is feasible by generating large number of independent insertions through independent genetic transformations. This may not be feasible in other plants where the labor involved in generating sufficient number of insertions makes it impractical. Reports indicate that in a large proportion of the T-DNA inserted mutants, the T-DNA does

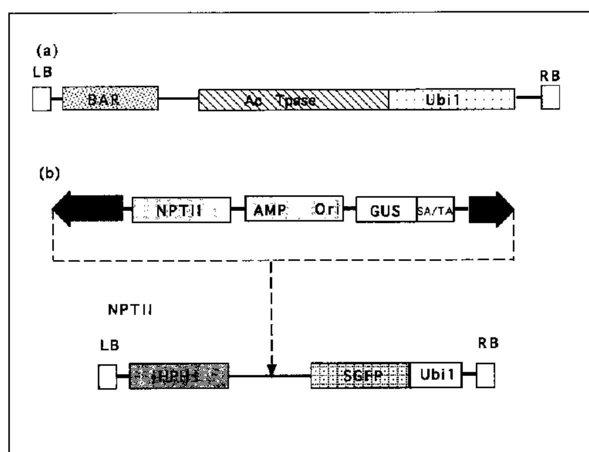

Fig. 4 Transposon trap constructs for isolating genetic regions from rice. (a) Transposase source. An immobilized *Ac* transposase gene (Ac Tpase) is driven by the maize ubiquitin promoter (*Ubi1*) for high expression in rice tissues. The selectable marker is *BAR* which confers resistance to ammonium glufocinate. (b) Enhancer/Gene trap. The reporter gene *GUS* is flanked upstream by a minimal promoter (TATA) in the enhancer trap and the splice acceptor sequence (SA) in the gene trap. The trap constructs also have a plant selectable marker (*NPT II*), a bacterial selectable marker (AMP) and a bacterial plasmid origin of replication sequence (Ori) within the *Ds* termini. This synthetic *Ds* element is inserted into a T-DNA between an *HPH* gene (hygromycin resistance) and the *SGFP* marker gene (synthetic green flurescent protein). Both the transposase and the trap constructs have been cloned in a binary vector for *Agrobacterium* mediated transformation of rice. For more details about these constructs, see reference [52].

not co-segregate with the mutant phenotype [54-56]. Such mutations may have resulted from the insertion of the vector backbone sequence [57] into the gene instead of the T-DNA alone. In the case of transposon tagging the mutated gene can be reverted back by crossing the mutant with the transposase expressing plant to check whether the mutation was due to the insertion of the transposon, however, such a check is not possible with T-DNA tagging.

6. Chromosomal rearrangements using transposable elements

A combination of DNA transposition and site-specific recombination can be used to generate inversions and deletions of specific chromosomal regions [58]. Recently this development was demonstrated in *Arabidopsis* [59] using the combination of *Ac-Ds* system with the site specific recombinase system *Cre-lox* from the bacteriophage P1 [60]. The *Cre-lox* recombinase system comprises the *Cre* recombinase protein which catalyses the recombination between two *lox* sites. The *lox* site is a palindromic sequence of 34 bp with an asymmetric core of 8 bp. The deletion or the inversion is dependent on the orientation of the *lox* site. The *Ds* element can be engineered to carry a *lox* sequence within it and the other *lox* sequence engineered into the flanking sequence of the T-DNA. The transposition of the *Ds* element leads to the separation of the *Ds-lox* by a portion of the chromosomal region. The transposition event would now bring the *lox* sequences in the direct or indirect orientation. While crossing these plants with plants carrying the *Cre* recombinase gene, the intervening chromosomal region will be deleted, if the *lox* sites are in direct orientation or flipped around if the *lox* sites are in the inverted orientation. This localized chromosomal rearrangement or deletion will help in understanding the significance of the organization of different chromosomal segments in the genome. This methodology should be applicable to plants species with reasonably small genomes.

7. Reverse genetics

Conventional methods of gene isolation usually start with a mutable trait and then work backwards to identify the associated genetic sequence. These days, with the development of automated, high throughput techniques to sequence large regions of the genome, the sequence comes first and then their assigned function and the traits they control. Such efforts have come to be known as reverse genetics.

The expressed sequence tag (EST), cDNA and genome sequencing efforts are on now in plants such as Arabidopsis and rice. This will result in the identification of many novel genes by sequence alone. Already a large number of gene sequences are known from several species, but their biological functions are unknown. The biological function of such gene sequences can be elucidated by studying the phenotypic effect of the gene disruption using transposon based-reverse genetics. The principle behind this approach is to generate a large population of transposon insertion mutants. It is ideal to have the probability of insertions in any given gene. The genomic DNA from all the individuals of the population is then isolated and screened using PCR for insertions within the gene of interest. The PCR amplification is carried out using one set of the primer complementary to the transposon sequence and the other complementary to the gene sequence. The insertion of the transposon inside the gene can established by the presence and size of the PCR product. In order to facilitate handling of a large number of

samples, the DNA can be pooled according to a three dimensional matrix for screening by PCR. This technique has been successful in the mutagenized population of maize containing high copy number of *Mu* insertions [61].

8. Activation tagging

Another exciting development is activation tagging. Here the synthetic dependent element is designed to carry a constitutive promoter that reads out of the transposable element terminus. The insertion of such an element will lead to the production of mutants by gene disruption if it inserts within the gene or activation of normally silent genetic regions if it inserts in the upstream region. Insertion at the downstream end in the reverse orientation could sometimes lead to gene silencing by the anti-sense expression. These possibilities make activation tagging a very potent tool to study gene function.

9. Conclusion

To improve the efficiency of transposon mediated methods of gene discovery in plants, efforts are now on to saturate the maps of each species with the trap element. Practically, this means the generations of many independent transgenic trap lines with the trap element integrated into different map locations. Such trap lines where the map positions of the trap is known will be an extremely valuable resource for gene cloning, especially with the maize *Ac-Ds*-based systems which have a tendency to transpose to closely linked sites. If the approximate map location of the target locus is known, then the trap line, where the trap is in a close map location will greatly increase the chance of getting insertions into the target locus.

Large number of genomic regions could be isolated, sequenced and their physiological significance studied with inserted transposons. The transposon based techniques thus, will make a significant contribution in this dawning age of genomics and reverse genetics.

Acknowledgments

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