

# Movers and shakers: maize transposons as tools for analyzing other plant genomes

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Transposons have been successfully exploited as insertional mutagens for the efficient identification and isolation of genes (transposon tagging) in many organisms. Plants are no exception. The maize Activator and Suppressor-mutator transposons function when transferred into heterologous plant species, and many different gene tagging systems have been developed. These systems have recently been used to clone novel and important genes, including disease resistance loci from *Nicotiana tabacum*, tomato and flax.

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## Introduction

Transposons are ubiquitous genetic elements that have the property of mobility. An autonomous transposon encodes its own transposase protein, and always retains the potential to transpose. A non-autonomous transposon does not encode a transposase, but may be induced to transpose by the transposase expressed by another transposon or gene. The study of insertional mutagenesis caused by transposons ('tagging') has occurred concurrently with the appearance of physical maps and the positional cloning of genes in a number of different plant genomes. Although nuclear gene replacement by homologous recombination in plants has been reported [1], it has not yet been widely applied. Thus transposons have been viewed as potentially important genetic resources. This assumption has now proven true, as the past two years have seen a number of successes, in both specific and non-specific mutagenesis experiments. Furthermore, the transposons have been used as tools to reconstruct genomes, and as molecular probes for gene location and expression.

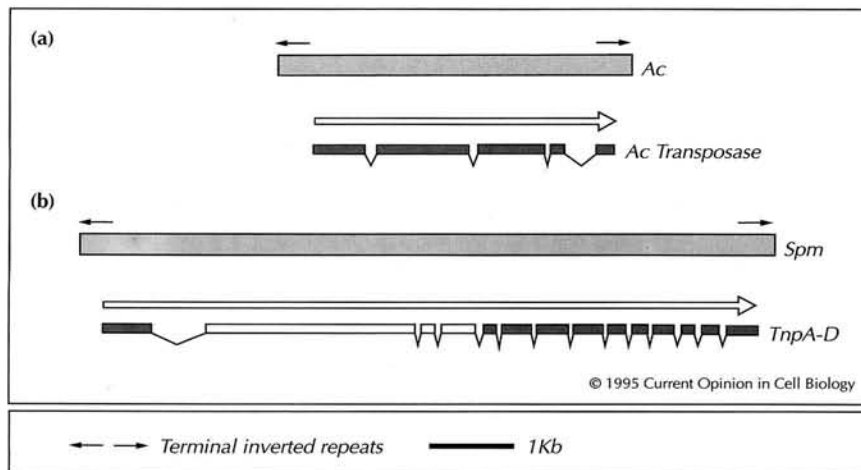
The utilization of the maize transposons Activator/Dissociation (Ac/Ds) and Suppressor-mutator and its derivatives (Spm/dSpm) as mutagens in other plant species has drawn the attention of a number of laboratories for the past several years. The inception of these studies came, of course, with the cloning of the transposons themselves (Ac [2]; Spm [3]), and with the demonstrations that these genetic elements retain the ability to transpose in the transgenic plants [4,5]. These efforts also showed that the transposons could remain intact after transposition, and that engineered transposons could be made from the autonomous starting material.

The autonomous element Ac is a member of a family of transposons that includes Tam3 from *Antirrhinum* (snapdragon) and hobo from *Drosophila*. These elements have been so classified solely on the basis of the similarities between their respective predicted transposase sequences, termini, and their common property of generating an 8 bp direct duplication upon insertion [6]. Ac transposition is associated with DNA synthesis, in that Ac frequently excises conservatively after its replication into proximal, unreplicated DNA [7]; transposition can thereby result in an increase in copy number. Ac encodes one protein, the enzyme that catalyzes its transposition (the transposase), which binds to multiple, subterminal 6 bp repeats present in both termini (Fig. 1a; [8]). Ds is a related non-autonomous element that lacks an intact transposase gene. An Ac cloned from maize was found to transpose in *Nicotiana tabacum* [4], as well as in other flowering plants including *Arabidopsis*, rice, flax, and tomato [9–12]. An unusual, conserved property of Ac and Ds is their tendency to transpose to sites that are genetically linked (i.e. physically close) to their point of origin in these species [13–15].

The autonomous maize transposon Spm (also called Enhancer, En) can also transpose in flowering plants, including *Arabidopsis* and *Nicotiana tabacum* [15,16]. Studies on this complex element in the latter species have shown that Spm encodes four proteins (TnpA–TnpD) that are translated from alternatively spliced mRNAs (Fig. 1b; [17]). TnpA is a DNA-binding protein whose 12 bp recognition sites are reiterated in the termini of the element [18]. Expression of the TnpA and TnpD products is necessary and sufficient for the induced transposition of non-autonomous derivatives, called dSpm [5,17]. These transposons create 3 bp direct repeats on

## Abbreviations

Ac—Activator; **Chl<sup>R</sup>**—chlorsulfuron resistant; **Ds**—Dissociation; **Gn<sup>R</sup>**—gentamicin resistant; **GUS**— $\beta$ -glucuronidase; **Hyg<sup>R</sup>**—hygromycin resistant; **Kan<sup>R</sup>**—kanamycin resistant; **Nam<sup>S</sup>**—naphthalene acetamide sensitive; **PCR**—polymerase chain reaction; **Ppt<sup>R</sup>**—phosphonothricin resistant; **Spm**—Suppressor-mutator; **Str<sup>R</sup>**—streptomycin resistant; **TMV**—tobacco mosaic virus.



**Fig. 1.** Schematic diagram of the Ac (a) and Spm (b) transposons showing the exons and introns of the transposase transcripts. The constitutively expressed exons are shaded. The alternative exons of Spm (2, 3 and 4) are shown in white. Differential splicing of these exons gives rise to the TnpA, B, C, and D gene products.

insertion [19]; their excision footprints could therefore create silent mutations [5]. Other elements related by sequence to Spm include Tam1 of *Antirrhinum* and Tgm of *Glycine max* [20].

The anticipated behavior of these transposons in transgenic plants has been strongly conditioned by our understanding of the elements in the native maize genome. Some pertinent properties include the relative stability of a given insertion over the generations (germinal transmission), a detectable degree of somatic excision, and, in the case of Ac/Ds, the tendency of the transposon to transpose to nearby sites [21]. These characteristics have, for the most part, been conserved. There have been a number of variations on these themes, however, particularly as the elements were evaluated in different species, where they display differing behaviors. We propose that one cause of these disparities is differing expression of the transposase genes in host plants, and that work can be directed towards the perfect reconstruction of the elements for desired purposes by fusing characterized promoters to transposase coding regions. However, researchers have achieved complete success tagging genes in precisely directed selection schemes using unmodified transposons (see below).

### Transposon tagging strategies

Two different approaches have been taken to gene tagging using these transposons, using either one or two elements (Fig. 2). In the one-element strategy, a single autonomous transposon launches from a T-DNA, using antibiotic resistance as the selection for excision (Fig. 2a). In the two-element approach, a T-DNA is constructed bearing a non-autonomous transposon containing selectable or screenable marker genes inserted into an excision marker (Fig. 2b, Table 1). Plants bearing these T-DNAs are crossed to plants bearing a second T-DNA expressing transposase function (yielding an F1 hybrid). A double-selection for excision and for the marker within the transposon can yield F2 plants with transposed elements. These plants may be homozygous

mutants or they may be heterozygous for a mutagenic insertion. The two-element approach has a particular advantage with respect to Ac/Ds, as the transposed Ds is likely to be unlinked to the transposase, facilitating the independent segregation of the two T-DNAs and the stabilization of the Ds insertion. The gene disrupted by the insertional event is 'tagged' by the presence of the transposon, which can be detected by techniques such as Southern hybridization or the polymerase chain reaction (PCR).

### One-element systems

In the experiments described below, unmodified autonomous elements were introduced into a variety of plants, and one conclusion has been that the activity of Ac or Spm can vary widely between host species. For example, Spm excised somatically at a low frequency in transgenic *Nicotiana tabacum* plants, as evinced by the appearance of occasional, small  $\beta$ -glucuronidase (GUS)<sup>+</sup> sectors due to restoration of the function of a GUS chromogenic reporter gene upon Spm excision in the cells of a plantlet's leaves [22]. However, more frequent germinal transmission of transposed Spm was detected in *Arabidopsis* [16]. A comparison of the expression of the TnpA and TnpD mRNAs in these two species allowed the straightforward proposal that the increased somatic and germinal excision of Spm in *Arabidopsis* was due to a near-optimal ratio of the two transcripts (100:1), closely resembling that found in maize.

In contrast, the autonomous Ac element appeared to be an inefficient mutagen in *Arabidopsis* and lettuce by virtue of its low rate of germinal excision [9,23]. Tomato, *Nicotiana tabacum*, and flax were capable of supporting higher rates, however, as well as displaying occasional instances of dramatic amplification of copy number [13,24,25]. These latter species have been the subjects of valuable mutant screens using one- or two-element strategies.

One species, *Petunia hybrida*, which supported Ac germinal excision from a T-DNA at a modest frequency [26], hosted the first example of insertional mutagenesis by a

**Table 1.** The selectable and screenable components of some recently described two-element systems. The name of the gene is followed by its conferred phenotype in parentheses.

Ds or dSpm marker	Excision marker	Transposase marker	Reference
–	–	Nos-HPT (Hyg <sup>R</sup> )	[33 •]
35S-HPT (Hyg <sup>R</sup> )	35S-SPT (Str <sup>R</sup> )	p2'-GUS	[51]*
19S-aph4 (Hyg <sup>R</sup> ) + GUS†	35S-ALS (Chl <sup>R</sup> )	35S-tms2 (Nam <sup>S</sup> )	[40]
Nos-HPT (Hyg <sup>R</sup> )	35S-SPT (Str <sup>R</sup> )	p2'-GUS	[35•]
ALS (Chl <sup>R</sup> )§	p1'-NPT (Kan <sup>R</sup> )	p2'-GUS	[50]‡
35S-GUS	35S-HPT (Hyg <sup>R</sup> )	Nos-NPT (Kan <sup>R</sup> )	[52]#
–	p2'-GUS	Nos-NPT (Kan <sup>R</sup> )	[16]
Nos-HPT (Hyg <sup>R</sup> )	35S-BAR (Ppt <sup>R</sup> ) or p1'-GUS	p2'-GUS	[44]**
Nos-BAR (Ppt <sup>R</sup> )†† or Nos-SPEC (Str <sup>R</sup> )††	Nos-SPEC (Str <sup>R</sup> ) or 35S-BAR (Ppt <sup>R</sup> )	p2'-iaaH (Nam <sup>S</sup> ) or p2'-GUS	[46]
35S-BAR (Ppt <sup>R</sup> ) + AAC§§	35S-surA,B (Chl <sup>R</sup> )	35S-R-Lc	[41•]

\*Ac transposase source was a stable 3' deletion of Ac, with a 5' leader deletion.  
†Promoterless GUS, for detection of adjacent genes after Ds transposition.  
‡The *Arabidopsis* *csr-1* gene. §Ac transposase fused to 35S promoter, *rbcS*, and chalcone synthase promoters. #Ac transposase source was an autonomous Ac.  
\*\*Ac transposase source was a stable 3' deletion of Ac. ††These Ds-borne genes were irreversibly inactivated on crossing to lines expressing Ac transposase.  
§§Promoterless AAC confers Gn<sup>R</sup> when fused to the 35S promoter upon *lox-lox* recombination.

maize transposon in a heterologous plant [27•]. Several independent transformants bearing transposed Ac were selfed and a floral mutation was found segregating within a family. Southern hybridization analysis showed that this mutation co-segregated with an Ac-hybridizing band, and hybridizations using the genomic region flanking the Ac insertion as a probe indicated that all the mutants (and only the mutants) were homozygous for the insertion. Wild-type, revertant progeny of mutants concomitantly regained a wild-type restriction fragment, indicative of excision of the transposon. The mutation caused by Ac was found to be allelic to a pre-existing one, *ph6*. It is a fitting coincidence that the first mutation demonstrably caused by Ac insertion in another species should display this superb variegation (due to somatic excision), given the wondrous deductions of McClintock based on her observations of analogous, mutable phenotypes (kernel color) in maize [28,29].

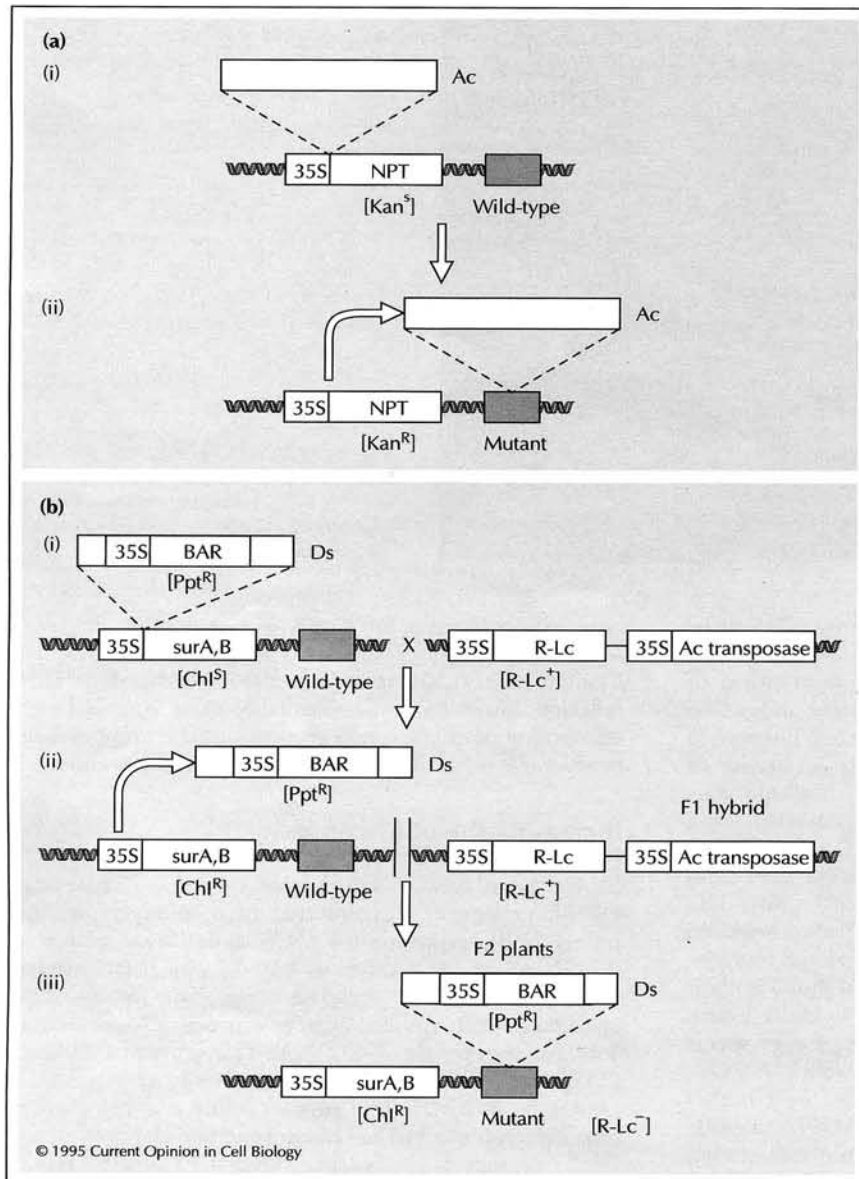
Tobacco was used as a host in the first example of heterologous transposon tagging as a consequence of a specific, large-scale screen [30•]. The target was the *N* gene, which confers resistance to tobacco mosaic virus (TMV). A conditional selection system was devised in which plants bearing inactivated *N* (*n*) would survive TMV application, on the basis of the observation that *N*-bearing plants completely resist local infection but succumb to

massive, overall TMV infection. Sixty four thousand *Nn* plantlets from three *NNAc* × *nn* crosses were thus selected, yielding 36 putative *nn* loss-of-function mutants (generated by the action of the transposon in the *Nn* plantlets). Of these, one displayed the variegated, diseased and resistant phenotype expected of an unstable allele generated by Ac insertion. The identity of the *N* gene was confirmed by co-segregation of Ac-hybridizing restriction fragments and the *n* phenotype in this mutant, and by the complementation of *nn* plants with the cloned *N* sequence. The flax *L6* gene, which confers resistance to the fungal pathogen *Melampsora lini*, was also isolated using a similar Ac tagging scheme (GJ Lawrence, EJ Finnegan, MA Ayliffe, JG Ellis, unpublished data).

## Two-element systems

Most recent efforts have been applied to the study of two-element systems, particularly where the unmodified elements transposed at less than a desired frequency. These approaches take the general form of transcriptional fusions of 'strong' or regulable promoters to transposase genes, in combination with a transposon bearing any of a multitude of marker genes, enhancers, rare sites, recombinogenic sites, or indicator genes that may be activated by adjacent, *cis*-acting positive elements (such as promoters). Table 1 shows some of the combinations that have been used, indicating the wealth of available marker genes in plant systems. Some of these selections, such as resistance to chlorsulfuron (Chl<sup>R</sup>), phosphonothricin (Ppt<sup>R</sup>) or kanamycin (Kan<sup>R</sup>) can be applied to plants growing in soil, reducing labor. One of the screenable markers (35S-R-Lc [31]) is a dominant, visible marker in *Arabidopsis*, conferring a deeply pigmented, hirsute phenotype (Fig. 3) [32]. Counter-selections to eliminate plants bearing transposase T-DNAs (and stabilize the germinally transmitted Ds insertion) have also been used successfully (*tms2* or *iaaH*; [32]). The 35S promoter of cauliflower mosaic virus was fused to transposase in these systems unless otherwise noted.

A two-element system within one T-DNA, was used to mobilize a dSpm (also called Inhibitor, I) in one of the first demonstrations of insertional mutagenesis of an *Arabidopsis* gene by an introduced transposon [33•]. This particular vector bore both the defective Spm element and a fusion between the 35S promoter and Spm [22]. A family of plants each bearing five to ten transposed dSpm elements was analyzed, and a male sterility mutation (*ms2*) was discovered in the family. The co-segregation of dSpm and the *ms2* mutation was established by Southern analysis using the genomic region flanking dSpm as probe. Some mutant plants contained large sectors of fertile, wild-type tissue, as in the previously described tagging of *Ph6* by Ac. That these were reversion events was confirmed by sequence analysis of the empty dSpm excision sites, which were shown to contain typical, differing Spm footprints. It is notable that six new *ms2* alleles were found in this collection



**Fig. 2.** One- and two-element approaches to transposon tagging. The conferred phenotypes are indicated in square brackets. **(a)** An example of the one-element approach using the autonomous transposon Ac. (i) The Ac T-DNA contains Ac inserted into the 35S-NPT antibiotic resistance gene; the presence of the transposon inactivates the gene, conferring the Kan<sup>S</sup> phenotype. (ii) Ac excision from this gene confers Kan<sup>R</sup>; the Kan<sup>R</sup> selection enriches for those plants containing Ac insertions. **(b)** An example of the two-element approach using the non-autonomous transposon Ds [42]. (i) The Ds T-DNA construct contains Ds bearing 35S-BAR (Ppt<sup>R</sup>) inserted into the 35S-surA,B gene. The 35S-Ac transposase T-DNA construct, which provides the transposase necessary for the Ds element to transpose, also contains the visual marker gene 35S-R-Lc (R-Lc<sup>+</sup>). (ii) Plants bearing Ds and 35S-Ac transposase T-DNAs are crossed to create the F1 hybrid (R-Lc<sup>+</sup> and Ppt<sup>R</sup>); Ds excision from the 35S-surA,B gene confers Chl<sup>R</sup>. (iii) F2 plants selected for the Chl<sup>R</sup>, Ppt<sup>R</sup>, R-Lc<sup>+</sup> phenotype contain stable Ds insertions (the Ac transposase construct having been lost).

of revertants where dSpm had excised from within the coding region, leaving differing footprints.

Two-element systems with Ds have been used successfully to insertionally mutagenize the *Arabidopsis* genome in a non-specific way [34,35,36]. These reports also address the important issue of the ratio of tagged and untagged mutants in mutagenized populations. Untagged mutations that are not linked to the introduced T-DNA may arise, by unknown mechanisms, during T-DNA transformation, or by Ds insertion and subsequent excision. In one study [34], a double-selection for hygromycin and streptomycin resistance (Hyg<sup>R</sup> Str<sup>R</sup>; Table 1) was used to isolate 487 lines bearing an estimated 238 independent transposed Ds elements. The progeny of plants bearing transposed Ds were examined in the absence of selection, and five recessive mutations were

observed. Three of the five mutations were linked to a transposed Ds (Hyg<sup>R</sup>), and one mutation, *drl1* (deformed roots and leaves) was chosen for further study. This pleiotropic mutation was somatically unstable in 28 of 36 plants containing both the single transposed Ds and the Ac transposase T-DNA (GUS<sup>+</sup> plants), but was stable in the absence of transposase (GUS<sup>-</sup> plants). The sequence analysis of the insertion and the empty excision sites from these revertants confirmed that Ds insertion had caused the *drl1* lesion.

Another report [35] also described the use of a Hyg<sup>R</sup> Str<sup>R</sup> selection (Table 1), where 1678 independent F2 families were so selected, yielding 758 F2 families containing at least one Hyg<sup>R</sup> Str<sup>R</sup> individual. Three hundred and forty nine of the 758 families also contained at least one Hyg<sup>R</sup> Str<sup>R</sup> plant lacking the 35S-Ac





**Fig. 3.** *Arabidopsis* plants with and without an *Ac* transposase T-DNA bearing a 35S-R-Lc transgene [41•]. This fusion confers a deeply pigmented, hirsute phenotype.

T-DNA (GUS<sup>-</sup>), and 201 F3 families were screened for visible mutations. Seven mutations were observed, and four were examined for evidence of co-segregation of Ds and phenotype. Three of the four were judged to be possible tagged mutations, by virtue of linkage to the Ds-borne marker or, further, somatic instability of the mutation in plants bearing 35S-Ac. An albino mutation, *alb3*, that met both criteria was proved to be a Ds-induced lesion by a combination of Southern analysis and sequence analysis of empty excision sites from plants that were germinal revertants of *alb3* [35•]. The authors also discussed the possible discrepancy between the mutagenicity of random T-DNA insertional mutagenesis [36] and the frequency of visible mutations in their screen, which appears to be four- to fivefold lower. Some explanations included the differing target specificities of the two mutagens (T-DNA versus *Ac*/Ds), higher copy number of inserted T-DNAs, or a higher incidence of untagged mutations in T-DNA mutagenized populations. The sample sizes in these studies do not allow conclusive comparisons, but it is certain that this question will continue to draw attention.

Two-element systems are generally more suited to experimental manipulation than one-element systems. An exposition of this was a study of the effects of the Spm gene products (TnpA–TnpD) on the reversible inactivation of the ability of Spm to excise from *Nicotiana tabacum* chromosomes [37•]. This inactivation correlates with methylation of cytosine residues around the transcriptional start site of the element. Expression of TnpA, and not of TnpB, C, or D, increased the activity of Spm, and an accompanying decrease in methylation of the pertinent cytosine residues was detected by restriction enzyme cleavage. What is notable is that the TnpA binding sites are near the assayed restriction sites, but do not overlap with them; however, whether the promotion of Spm activity by TnpA is direct or indirect could not be ascertained. This, and related questions

concerning the role of TnpA as a transcription factor, were addressed by experiments using the Spm promoter fused to the luciferase reporter gene [38•]. This work revealed TnpA to be a remarkable protein, effective as transposase, transcriptional repressor of the unmethylated promoter, and activator of the methylated promoter.

### Increasing the utility of Ds

Ds has been used as a bearer of specialized sequences, extending its role beyond that of a mutagen. In one report [39•], promoterless GUS genes were placed at the 5' end of Ds (similar to Mu-*lac* constructs used in *Escherichia coli* [40]), creating a detector for *dis*-acting promoter elements in adjacent genes in *Arabidopsis*. The *Ac* transposase T-DNA in this scheme contained a 35S-tms2 fusion, conferring sensitivity to naphthalene acetamide (NAMS). The authors found a strong correlation between the NAMS<sup>S</sup> phenotype and the presence of GUS<sup>+</sup> sectors in Ds-bearing (Hyg<sup>R</sup>) F1 and F2 plants, indicating frequent Ds transposition to genic regions.

Another two-element scheme incorporated *lox* sites from the bacteriophage P1 Cre-*lox* site-specific recombination system into both Ds and the Ds T-DNA [41•]. Thus Ds transposition distributes *lox* sites about the *Arabidopsis* genome, where they become substrates for Cre recombinase introduced via a cross to Cre-expressing plants. Selection for *lox-lox* recombination was achieved by placing the *lox* site in Ds next to a promoterless AAC reporter gene. Thus, *lox-lox* recombination conferred gentamicin resistance by fusing the AAC sequence in Ds to a 35S promoter in the Ds T-DNA. The *Ac* transposase and Cre recombinase T-DNAs in this work were marked with a 35S-R-Lc transgene, which is an obvious, visible marker (Fig. 3). We describe the efficient creation of inversions of 5.6 and 16.5 centiMorgans (cM; 1 cM is roughly 200 kb in *Arabidopsis*), one of which was germinally transmitted. This system will tend to generate

inversions and deletions, given the tendency of Ds to transpose to linked sites in *Arabidopsis* [15]. This property is unusual amongst transposons, and it has been displayed by Ac and Ds in all transgenic settings examined so far [13–15].

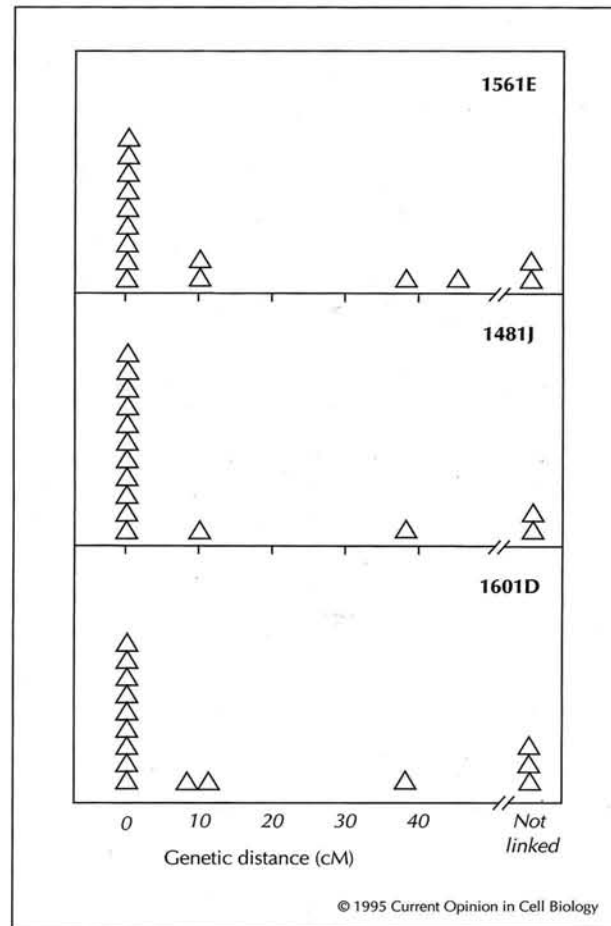
### Targeted tagging

Researchers have begun to avail themselves of a resource that maize geneticists have used successfully for some time, that of 'targeted tagging'. In this approach one chooses an Ac or Ds element genetically linked to a given mapped locus in order to mutagenize it. The first example of the use of this strategy in transgenic plants was the isolation of the tomato fungal resistance gene *Cf-9*, using a Ds T-DNA situated 3 cM distant [42]. *Cf-9* plants specifically resist *Cladosporium* carrying the avirulence gene *Avr9*, which encodes a small peptide that is somehow identified by the plant, perhaps by the *Cf-9* gene product. The authors devised a lethal selection based on the observation that *Cf-9/+* plants, but not *+/+* plants, die as seedlings when bearing an *Avr9* transgene. Thus, *Cf-9/Cf-9* plants bearing a terminally deleted, stable Ac (which produces transposase but does not itself transpose) [43] and the Ds T-DNA were crossed to plants homozygous for the *Avr9* transgene. One hundred and eighteen survivors were isolated (from 160 000 selected) of which 37 were considered to bear Ds insertions into *Cf-9* by virtue of phenotypes that could be destabilized by the stable Ac. The power of the strategy was shown by the resultant map of the *Cf-9* gene, containing 28 independent Ds insertions, scattered over its 3 kb length [42].

Some groups have anticipated the importance of targeted tagging by mapping transposon-bearing T-DNAs in plant genomes. For example, two collections of Ac or Ds T-DNAs mapped in the tomato genome have been described [44,45]. These collections also include large numbers of transposed elements. An exemplary distribution of Ds elements transposing from three different mapped T-DNAs in tomato is shown in Figure 4 [46], where the majority of transposed elements are linked to their T-DNA.

### Transposition from an episomal vector

The experiments described below [47,48] introduced Ds and Ac transposase transcriptional fusions simultaneously and directly into cultured, regenerable plant cells. Work of this type is particularly important for those plant species that cannot be efficiently transformed with *Agrobacterium*, such as rice. Although previous reports had shown excisions of transposons from episomes, no complete transpositions had been characterized. Selections were applied for the Ds-borne markers, and the regenerated tissues were examined for signs of Ds transposition from the episomal vector into the plant genome. The vector bearing Ds in the first report [47] contained



**Fig. 4.** The distances between germinally transmitted, transposed Ds elements and their T-DNAs in tomato [46]. Each map represents a different T-DNA, and shows the genetic distance between the stable T-DNA and a number of independent, transposed Ds elements. Recombinant progeny were identified using element-borne markers and PCR analysis.

two open reading frames encoding replication factors from the miscanthus streak virus and the viral replication origin. Rice calli were regenerated after electroporation using selection for Hyg<sup>R</sup>. Of the 16 calli examined, 5 were shown by Southern analysis to contain single Ds insertions, and not to contain vector sequences. DNA from these 5 calli also contained Ds sequence flanked by the 8 bp direct repeats that should be generated on *de novo* insertion, isolated by inverse PCR. In a second report [48], 32 independent transgenic *Nicotiana plumbaginifolia* plants were regenerated using the Kan<sup>R</sup> selection conferred by Ds. Nineteen of 32 plants contained Ds sequence alone, and one or more copies of Ds, according to physical analysis and genetic analysis of selfed progeny. One plant examined in detail by inverse PCR cloning contained Ds sequence flanked by the predicted 8 bp repeats. This latter report used a plasmid presumed to be non-replicating to bear Ds; however, the literature has still not yielded the definitive



answer on the necessity of replication for Ac/Ds excision, as no data was furnished to prove that this vector had, in fact, not replicated *in vivo*.

## Conclusions

These recent experiments show the fundamental efficacy and flexibility of transposon systems in plants. Precise screens will be designed and have hopes of success, particularly in those situations where an Ac or Ds element can be located genetically linked to the target gene. General screens may be implemented as well, perhaps using transposons bearing sequences such as promoterless GUS for gene detection, or in lines of plants with specific reporter genes. We believe that transposition is not yet optimally efficient in plants such as *Arabidopsis*, however. The solution may lie in the control of transposase transcription by fusion of the transposase gene to developmentally regulated promoters. In this way, multiple insertions may be isolated from a single plant when the transposase is expressed around gametogenesis. Efforts have been directed to this end [49–51], but no ideal solutions have been provided to date.

The integration of marked elements into plant genomes, and then into their genetic maps, will aid the construction of those maps and the manipulation of the plants themselves as genetic material, as the genes within the transposons are easy to score. We may also expect to see the use of transposons as physical delimiters of the genome for long range mapping, as many workers report the inclusion of cleavable, rare sites in their vectors. We reasonably expect to find transposons occupying the same lofty position in transgenic plants that they do in other experimentally evolved organisms.

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- of special interest
- of outstanding interest

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