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# The splicing of transposable elements and its role in intron evolution

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## Abstract

Recent studies have demonstrated that transposable elements in maize and *Drosophila* are spliced from pre-mRNA. These transposable element introns represent the first examples of recent addition of introns into nuclear genes. The eight reported examples of transposable element splicing include members of the maize *Ac/Ds* and *Spm/dSpm* and the *Drosophila P* and *412* element families. The details of the splicing of these transposable elements and their relevance to models of intron origin are discussed.

## Introduction

The origin of introns is a question that has long fascinated molecular biologists and has led to the formulation of two opposing theories (Doolittle, 1987). The introns-early hypothesis proposes that introns were present in primordial genes as linkers of short functional coding regions, and were subsequently lost in prokaryotes but retained in eukaryotes. In contrast, the introns-late hypothesis assumes that the primordial genes were intronless, and that intervening sequences were later inserted into eukaryotic genes (Cavalier-Smith, 1985; Rogers, 1990). Recently, it has been suggested that introns were incorporated into genes at various times during evolution (Cavalier-Smith, 1991).

The lack of evidence for a mechanism of intron addition has weakened arguments supporting models of a late origin for introns. The introns-late theory presumes that insertion of mobile elements is the likely mechanism for intron gain (Cavalier-Smith, 1985). However, the absence of donor and acceptor splice sites precisely at the termini of known transposable elements has been cited as evidence against transposons as the progenitors of introns (Sharp, 1985).

Studies of transposable element-induced mutations have demonstrated that mobile elements can also function as introns (Wessler, 1989). These elements, which include members of four element families from maize and *Drosophila*, are spliced out from pre-mRNA despite the lack of precise terminal splice sites. Instead, splice sites within the element and in flanking host sequences are utilized. Thus, the splicing of element sequences is not perfect - part of the element persists in the transcripts and some host sequences may be deleted. This imperfect splicing may reflect the dual nature of these sequences as both mobile elements and introns. In this review, we will summarize specific examples of transposable element splicing from pre-mRNA and their implications on intron origin and evolution.

## Splicing of transposable elements

The cellular RNA processing machinery interacts with transposable element insertions in several ways (Weil & Wessler, 1990); the particular interaction depends greatly on the location of the element within a gene. Insertions in introns can modify RNA processing patterns by altering host gene splice site choice and creating alternative processing pathways (Mount, Green & Rubin 1988; Varagona, Purugganan & Wessler, in preparation). In contrast, insertions in exons can function as new introns and be spliced from pre-mRNA. Our dis-

cussion will deal exclusively with the latter class of insertions, since they illustrate a means by which transposable elemens might evolve into nuclear introns. The reader is referred to several excellent reviews for a more thorough treatment of element structure and biology in maize (Gierl *et al.*, 1988; Fedoroff, 1989) and *Drosophila* (in Berg & Howe, 1989).

## Maize *Ac/Ds* splicing

The maize *Activator/Dissociation* (*Ac/Ds*) transposable element family consists of the autonomous 4.6 kb *Ac* element and several non-autonomous *Ds* elements. The *Ac* element is comprised of a single transcription unit that encodes the transposase required for its own transposition as well as for the transposition of non-autonomous *Ds* elements in the genome. Genes containing *Ds* insertions have a stable mutant phenotype if *Ac* is not also present in the genome.

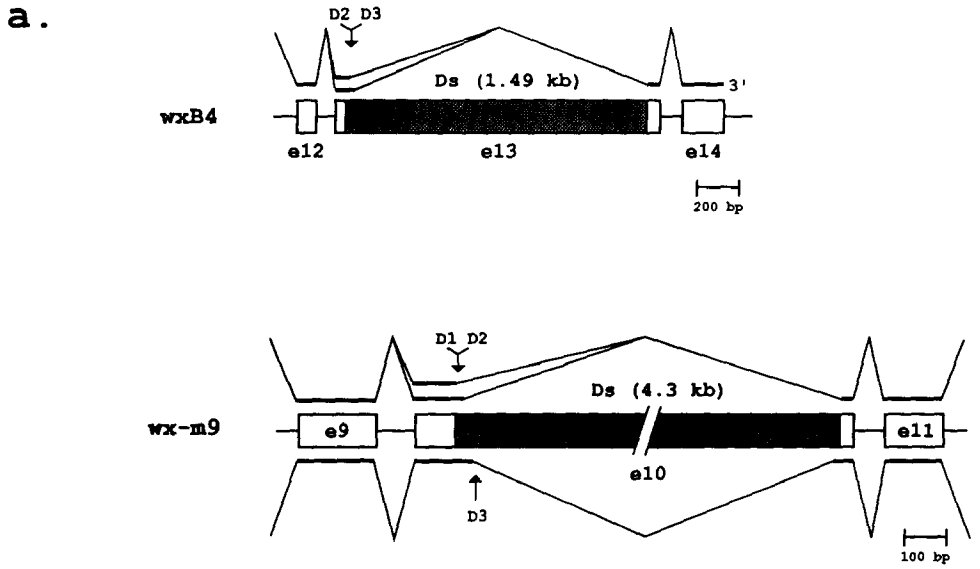
The first suggestion that transposable elements could behave as introns came from observations that *Ds* alleles have residual gene expression (Wessler, Baran & Varagona, 1987; Dennis *et al.*, 1988). Specifically, *Ds* alleles of the *adh* and *wx* loci with intermediate expression have been identified. Whereas *adh* encodes alcohol dehydrogenase, the *wx* gene encodes a starch granule-bound ADP glucose glucosyl-transferase involved in amylose biosynthesis in endosperm and pollen tissues. The *Ds*-containing alleles *wx-m9* and *adh1-Fm335* display stable intermediate phenotypes and encode wild type-sized transcripts when *Ac* is not present (and *Ds* cannot transpose). Both genes have been cloned and, surprisingly, each allele contains a *Ds* insertion within exon sequences. In addition to these leaky alleles, two null alleles of *wx*, *wxB4* and *wx-m1* (Wessler, 1991b), have also been found to encode wild-type-sized *wx* transcripts despite the presence of *Ds* insertions in *wx* exons.

Characterization of mutant transcripts from these strains reveals that inserted *Ds* elements are spliced from the pre-mRNA (Wessler, 1988; Wessler, 1989). The processing of the *Ds* elements in all four of these alleles proceeds in a very similar manner despite the fact that the structure of each *Ds* element is different. The *wx-m9* allele contains a 4.37 kb *Ds* insertion in *wx* exon 10 (Fedoroff, Wessler & Shure, 1983). This *Ds* element is a derivative of *Ac* and differs by a 194-bp deletion within exon 3 of the *Ac* transcription unit. In the absence of *Ac*, *wx-m9* encodes a *wt*-sized *Wx* protein and *Wx* mRNA and has about 10% of wild-type *Wx* enzymatic activity, even though there is a large *Ds* insertion within a translated exon.

The *adh1-Fm335* allele has a 405-bp *Ds* element in the untranslated leader of *adh1* (Dennis *et al.*, 1988). In the absence of *Ac*, *adh1-Fm335* has about 10% of wild-type *Adh* enzymatic activity and encodes a wild-type-sized *Adh* transcript. A similar 409-bp *Ds* element is also found as an insertion in exon 9 of *wx-m1*. This allele has no *Wx* enzymatic activity in the absence of *Ac*; nevertheless, it encodes wild-type-sized *Wx* mRNA (Wessler, 1991a). The small *Ds* element in both *adh1-Fm335* and *wx-m1* are referred to as *Ds1* elements, whereas elements with more extensive sequence homology to *Ac* are simply called *Ds*. Although *Ds1* can transpose in response to *Ac* in the genome, this class of element is only homologous with the 11-bp inverted repeat *Ac* termini and a short sequence of approximately 20 bp adjacent to one terminus. The remainder of the *Ds1* sequence is almost 80% A and T residues.

Like *wx-m1*, *wxB4* encodes wild-type-sized *Wx* mRNAs but has no *Wx* enzymatic activity in the absence of *Ac*. The 1.49 kb *Ds* element in *wxB4* is in exon 13 and is comprised of approximately 300 bp and 250 bp of the 3′ and 5′ ends, respectively, of the *Ac* termini (Varagona & Wessler, 1990). The approximately 1 kb between the termini are unrelated to *Ac* sequences.

The wild-type-sized transcripts encoded by these mutant *wx* and *adh* alleles result from the splicing of most of the *Ds* sequences from pre-mRNA. The new introns created by the insertion of two of these *Ds* elements are shown in Figure 1a. The donor and acceptor splice sites within the *Ds* and *Ds1* elements are shown in Figure 1b. Each *Ds* insertion is oriented such that, when transcribed, the 20-bp region adjacent to the inverted repeat and conserved among the different *Ds* elements contains several consensus splice donor sites that are utilized *in vivo*. These *Ds* donor sites may be ligated to acceptor sites that are located (i) near the 3′ end of the *Ds* insertion (*wx-m1*, *wx-m9*), (ii) within the 8-bp direct repeat of target sequences generated upon element insertion (*wxB4*, *wx-m1*, *adh1-Fm335*), or




b.

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                 D1          D2  D3
Ds:   TAGGGATGAAA ACGGTCGGTAACGGTCGGTAAAATACCT —⌐¬— GTACGGGATTTTCCCATCCTAC TTTCATCCCTA
                 D1          D2          D3                  A1  A2
Ds1:  TAGGGATGAAA ACGGTCGGAAACGGTATTTATTCGGTAT —⌐¬— CCTAACAGCTCAGAATTATCAC TTTCATCCCTA
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*Fig. 1.* The splicing of *Ds* elements from pre-mRNA. (a) Splicing in *wxB4* and *wx-m9*. The unshaded boxes marked e11, e12, and so on represents exons, and the fine lines connecting them are the introns in the wild-type gene. The shaded region indicates the position of the *Ds* insertion in the exon. The pre-mRNA is drawn above and, for *wx-m9*, below these boxes. Heavy lines above and below the genomic sequence are exons in the mutant alleles; they are connected by diagonal lines that represent the introns. A diagonal line rises from a donor site and descends to an acceptor site; sites may be within the transposable element or the host gene; - (b) Comparison of terminal splice sites within *Ds* and *Ds1* elements. Donor and acceptor sites are designated by solid and open arrowheads, respectively. The arrows underneath the sequence designate terminal inverted repeats for these elements.

(iii) downstream of the insertion site (*wx-m9*). In all cases *Ds* splicing does not produce a normal transcript, since both donor and acceptor sites are not located within the direct repeats generated upon insertion. Although the processed transcripts differ from wild-type, the positions of the multiple donor and acceptor sites within *Ds* allow alternatively spliced transcripts with different reading frames to be generated. The ability to be alternatively spliced may increase the likelihood of retaining some gene function despite element insertions.

## Maize *Spm/dSpm* splicing

The maize *Suppressor-mutator* (*Spm*) transposable element family consists of the 8.3 kb autonomous *Spm* element and several smaller derivatives called *defective Spm* (*dSpm*) elements. *Spm/dSpm* family members have 13-bp terminal inverted repeats and generate 3-bp target sequence duplications upon insertion (Fedoroff, 1989). The element encodes at least two functions; a (i) mutator (*m*) function required for element mobility, and a (ii) suppressor

(*sp*) function that acts to suppress residual gene expression from hypomorphic *Spm/dSpm* alleles.

The *dSpm* element has been shown to be spliced from alleles of the bronze (*bz*) and anthocyaninless (*a2*) genes. The *bz* locus is one of several genes responsible for the production of the purple anthocyanin pigment in the maize kernel (Klein & Nelson, 1984). Null alleles are bronze in color due to the lack of UDP-glucose:flavonol glucosyltransferase (UFGT) activity. In the absence of *Spm* elsewhere in the genome, kernels containing the *bz-m13* allele are purple in color and encode wild-type-sized *Bz* mRNA and 5%-10% UFGT activity despite a 2.2 kb *dSpm* insertion in the second *bz* exon. The low level UFGT activity in *bz-m13* is sufficient to condition a non-mutant purple phenotype.

*Bz* expression in *bz-m13* results from the splicing of the inserted *dSpm* from the *bz* gene (Kim *et al.*, 1987; Raboy *et al.*, 1989). Unlike the splicing of *Ds* from pre-mRNA, the *dSpm* insertion in *bz-m13* utilizes a wild-type *bz* donor site and one of two cryptic acceptors in *dSpm* (see Fig. 2a). The transcript produced by ligation of *bz* D to AS1 within the 13-bp dSpm inverted repeat encodes a functional Bz protein, even though this splice removes 33 bp of *bz* exon 2. Splicing of *bz* D to AS2 results in a non-functional protein.

In the presence of *Spm*, *bz-m13*-containing kernels are bronze-colored with purple spots. This phenotype illustrates the action of the two components of *Spm* - its suppressor function suppresses residual *Bz* expression (hence the bronze, not purple, background) while its mutator function mediates element excision and sectorial restoration of *Bz* expression. The residual gene expression resulting from the splicing of *dSpm* in *bz-m13* is suppressed by the *Spm*-encoded *tnpA* gene. It has been hypothesized that binding of the TNPA product to subterminal repeats in *dSpm* prevents transcriptional readthrough in this allele (Gierl, 1990).

Unstable alleles can give rise to new unstable alleles with heritable differences in the frequency and timing of element excision (see Table 1). In several instances, analysis of these change-in-state (CS) derivatives has revealed that the derivatives have sustained deletions of element sequences. It is believed that these deletions are mediated by the *Spm* transposase.

The *bz-m13CS9* allele is a change-in-state deriv-

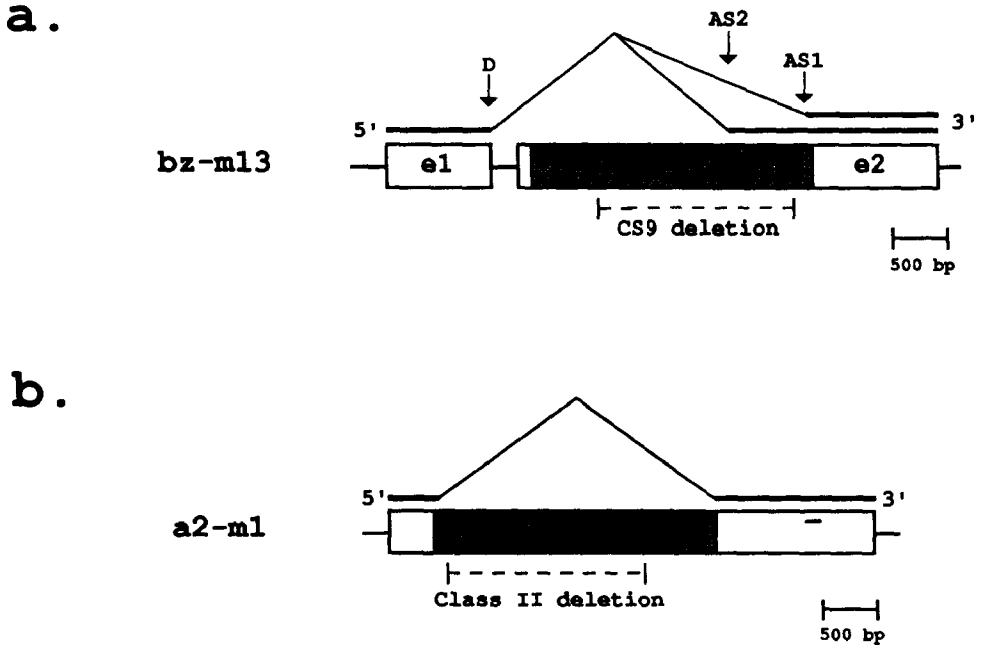


*Fig.* 2. Splicing of *dSpm* elements from pre-mRNA. The examples shown are (a) *bz-m13*, and (b) *a2-m1*. The dashed line under each *dSpm* element indicates the extent of the deletion in the change-of-state alleles *bz-m12CS9* and *a2-m1 (Class II)* in (a) and (b), respectively.

*Table 1.* Alleles with transposable element introns.

| Allele | Element | Size | Location | Comments |
|---|---|---|---|---|
| *wx-m1* | *Ds1* | 409 bp | exon 9 | *Ds1* splicing yields 5 messages; no *Wx* activity |
| *wx-m9* | *Ds* | 4.37 kb | exon 10 | deletion derivative of *Ac*; two protein isoforms detected; 10% of wild type *Wx* activity |
| *wxB4* | *Ds* | 1.49 kb | exon 13 | no *Wx* activity |
| *adh1-Fm335* | *Ds1* | 405 bp | exon 1 | 10% wild-type levels of *adh1* activity |
| *bz-m13* | *dSpm* | 2.2 kb | exon 2 | *Bz* wild-type donor splices to two internal *dSpm* acceptor sites; 5%-10% wild-type levels of UFGT activity; *Spm*-suppressible |
| *bz-m13CS9* | *dSpm* | 1.3 kb | exon 2 | internal *dSpm* deletion in *bz-m13* removes proximal A2 acceptor site; 69% wild-type levels of UFGT activity; reduced *Spm*-dependent excision rate |
| *a2-m1* | *dSpm* | 2.2 kb | -[a] | *dSpm* spliced using internal sites in element; low-level kernel pigmentation; *Spm*-suppressible |
| *a2-m1 (Class II)* | *dSpm* | 1.3 kb | -[a] | deletion derivative of *a2-m1*; wild-type A2 phenotype; *Spm*-dependent excision abolished |
| $v^{k}$ | *412* | 7.5 kb | exon 1 | *412* spliced from pre-mRNA using LTR sites; leaky *vermilion* expression; *Su(s)*-suppressible |
| $v^{+37}$ | *412-B104* | 11.1 kb | exon 1 | 3.6 kb *B104/roo* insertion in *412* 5′ end contains two donor sites; splicing of insertion more efficient |
| $y^{76d28}$ | *P* | 1.1 kb | exon 1 | element spliced using internal *P* and cryptic *y* splice sites; leaky *yellow* expression; *Su(s)*-suppressible |
| $y^{13\text{-}11}$ | *P* | 0.4 kb | exon 1 | deletion derivative of $y^{76d28}$; wild-type phenotype |

[a] the wild-type *a2* gene has no introns

ative of *bz-m13* that differs from its progenitor in two ways: i) in the absence of *Spm*, *CS9* has 69% UFGT activity compared to the 5%-10% for *bz-m13*; and ii) in the presence of *Spm*, *CS9* germinal excision frequencies drop to 1%-2%, compared to 50%-70% for *bz-m13*. A 1340-bp deletion in the *dSpm* element is responsible for both alterations in phenotype (Raboy *et al.*, 1989). First, the deletion removes the unproductive AS2 splice site (see Fig. 2a) resulting in elevated use of AS1 and increased UFGT activity. Second, the deletion also removes a portion of the *dSpm* termini that is necessary for the efficient transposition of the element. Thus, the 902-bp *dSpm* cannot excise as frequently as its 2.2 kb progenitor.

The *bz-m13* allele and its *CS9* derivative illustrate one way in which a transposable element may evolve into a better intron. A single deletion in the *CS9* element removes both the AS2 splice site and part of the subterminal repeat required for efficient transposition. Thus, the 902-bp element-intron permits a higher level of UFGT activity and it transposes at a lower frequency.

The *a2-m1* and *a2-m1* (*Class II*) alleles provide another example of how *dSpm* elements may evolve into introns (Menssen *et al.*, 1990). Like the *bz* gene, *a2* encodes an enzyme in the anthocyanin pathway that conditions purple pigment in the kernel. The *a2-m1* allele contains a 2.2 kb *dSpm* insertion in the intronless *a2* transcription unit. Despite this insertion, *a2-m1* conditions a low level of kernel pigmentation in the absence of *Spm*. In the presence of *Spm*, *a2-m1* expression is suppressed resulting in colorless kernels with frequent sectors of wild-type pigmentation due to element excision.

Gene expression in the absence of *Spm* results from the splicing of all but 21 nt of the 2.2 kb *dSpm* element from *a2* pre-mRNA (see Fig. 2b). The splice acceptor site utilized in *a2-m1* is identical to AS1 of the *bz-m13 dSpm* insertion. The donor site for *a2-m1*, however, is located within the element near the terminus.

The *a2-m1* (*Class II*) derivative differs from the progenitor *a2-m1* allele in two ways: i) in the absence of *Spm*, *a2-m1* (*Class II*) kernels are almost fully pigmented, whereas *a2-m1* kernels are pale; and, ii) in the presence of *Spm*, *a2-m1* (*Class II*) kernels are colorless with no revertant sectors,

while *a2-m1* kernels are heavily spotted on a colorless background. Like *bz-m13CS9*, a 900-bp deletion in *a2-m1* (*Class II*) is responsible for both phenotypic changes: the increase in *A2* gene expression and the decreased transposition.

### *Drosophila P* and *412* element splicing

The *P* element family in *Drosophila* are a class of transposable elements that are mobilized in the germline in P-M hybrid crosses. The 2.9 kb wild-type *P* element contains a single transcription unit which encodes an 87 Kd transposase that catalyzes its own transposition as well as movement of defective *P* elements elsewhere in the *Drosophila* genome (Spradling & Rubin, 1982).

Splicing of *P* element insertions has been reported in the *yellow* alleles $y^{76d28}$ and $y^{13\text{-}11}$ (Geyer *et al.*, 1991; Geyer *et al.*, 1988). The *yellow* gene is responsible for the brownish-black cuticular pigmentation in larval and adult flies, and null alleles of this gene condition yellow flies (Chia *et al.*, 1986). The allele $y^{76d28}$, which contains a 1.1 kb *P* insertion in the untranslated leader region of exon 1, conditions an intermediate tan cuticular color. In the late pupal stage of flies carrying $y^{76d28}$, two transcripts, 1.9 kb and 3.0 kb in size, accumulate at low levels. The sizes correspond to a wild-type *yellow* transcript (1.9 kb) and a compositie *yellow-P* element insertion transcript (3.0 kb). Analysis of the 1.9 kb transcripts reveals that most *P* sequences are removed when a GT in the *P* element inverted repeat is alternatively spliced to any of three cryptic acceptor sites in *y* exon 1 (see Fig. 3a). Two of the three acceptor sites are in the untranslated leader sequence, while the third site is downstream of the start of translation. The splicing of *P* in $y^{76d28}$ is reminiscent of *Ds* splicing in *wx-m9* and *adh1-Fm335*; both maize and *Drosophila* elements are spliced using donor sites near the element's 5′ termini and cryptic acceptor sites in the host genes' exon. The 5′ donor sites in *P*, like the *Ds* elements, are located in *cis*-sequences required for *P* transposition.

Phenotypic revertants that affect *P* element splic-

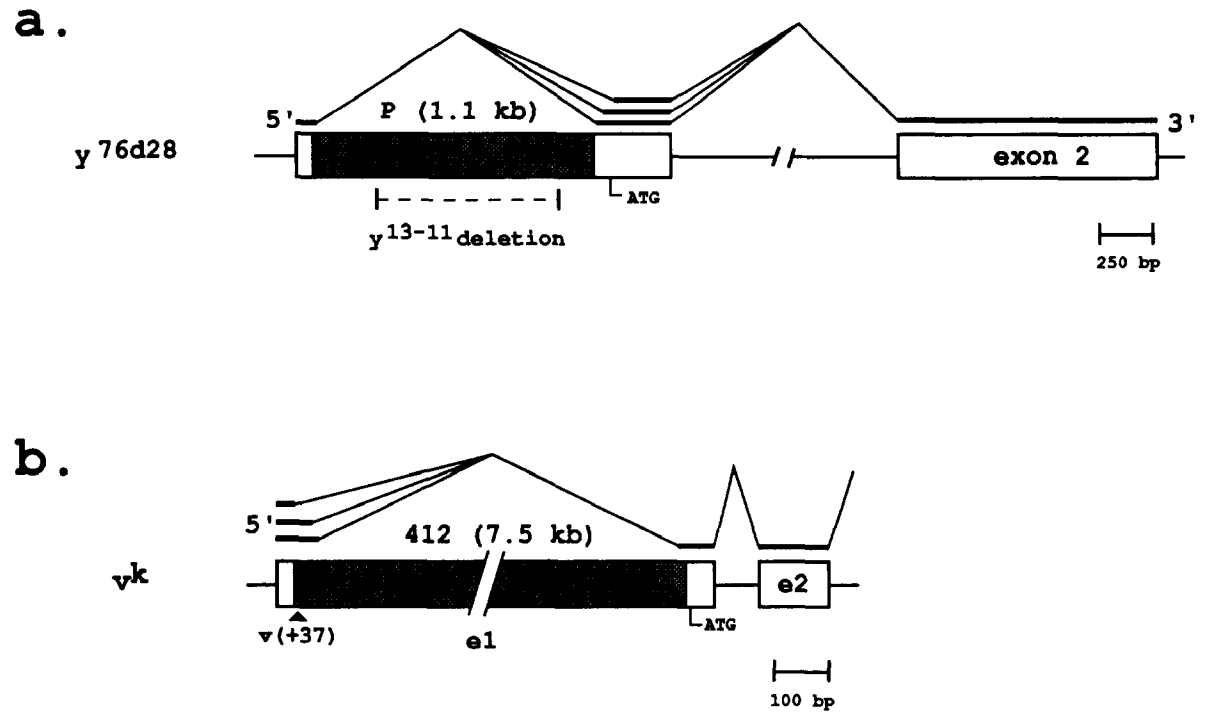


*Fig. 3.* The splicing of Drosophila elements from pre-mRNA. (a) *p* splicing in $y^{76d28}$. The start of translation is indicated as ATG. The dashed line under the *P* element indicates the extent of the deletion in the revertant allele $y^{13\text{-}11}$. A splicing event using only cryptic sites in the yellow gene is not shown; - (b) *412* element splicing in $v^{k}$. The solid arrow under the *412* element indicates the position of the *B104/roo* insertion in the revertant allele $v^{+37}$. A splicing event to a chimeric donor site between *412* and *vermilion* sequences is not shown.

ing have been isolated and found to fall into two classes: i) intragenic revertants that contain sequence rearrangements, including deletions, within the element; and, ii) intergenic suppressor mutations which act in *trans* to suppress the mutant phenotype.

Several examples of the first class of $y^{76d28}$ derivatives have been isolated and found to contain intra-element deletions (Geyer *et al.*, 1988); these element deletions are believed to be the result of *P* element activity. The derivative $y^{13\text{-}11}$ has a 700-bp deletion in the inserted *P* element and conditions nearly wild-type levels of 1.9 kb spliced *yellow* transcripts and cuticular pigmentation. The $y^{13\text{-}11}$ allele is similar to the derivatives of the maize *bz-m13* and *a2-m1* alleles in two important ways: in both cases (i) intra-element deletions increase gene expression by improving splicing efficiency, and (ii) the deletions are mediated by transposase activity.

Intergenic suppressors of the $y^{76d28}$ mutation have also been isolated. One of the loci identified in this way is *suppressor-of-sable [Su(s)]*, which also affects insertion mutations at the *vermilion* and *singed* loci. Flies that are homozygous for both *su(s)* and $y^{76d28}$ have increased cuticular pigmentation and both spliced and unspliced *yellow* transcripts when compared to $y^{76d28}$ in a *Su(s)* background (Geyer *et al.*, 1991). The wild-type *Su(s)* product contains RNA-binding domains (Voelker *et al.*, 1991) and is believed to participate in the degradation of unprocessed pre-mRNA (Geyer *et al.*, 1991). To explain *su(s)* suppression of element-induced mutations, it has been suggested that pre-mRNA containing transposon sequences are normally degraded by the *Su(s)* product before element sequences can be spliced. According to this model, $y^{76d28}$-encoded pre-mRNA would be more stable in a *su(s)* background, resulting in higher levels of spliced transcripts and cuticular pigmentation.

Splicing of a *Drosophila* retrotransposon has also been reported (Fridell, Pret & Searles, 1990). The hypomorphic *vermilion* allele $v^k$ contains a 7.5 kb *412* element in the untranslated leader region of exon 1. The *vermilion* gene encodes tryptophan oxygenase and is responsible for brown pigment synthesis in the eye. Despite the presence of a 7.5 kb element in exon 1, $v^k$ conditions an intermediate phenotype and encodes wild type-sized *vermilion* mRNAs. Analysis of $v^k$ cDNAs demonstrates that the *412* insertion is spliced from pre-mRNA using splice sites within the element's long terminal repeats (LTRs). Three donor sites clustered at the 5′ termini of *412* are spliced to an acceptor site just upstream of the element's 3′ end (see Fig. 3b). An additional chimeric donor site containing both *vermilion* and *412* sequences is also used (not shown in figure). Splicing of *412* shares some features with *dSpm* splicing in *a2-m1* and *Ds1* splicing from *wx-m1*, where both donor and acceptor sites are also within element sequences.

Both intragenic revertants and second-site suppressors of $v^k$ expression have been isolated. The intragenic revertant $v^{+37}$ contains a 3.6 kb *B104/roo* element insertion at the 5′ end of the *412* insertion (see Fig. 3b) (Pret & Searles, 1991). Surprisingly, this 11.1 kb *412-B104/roo* insertion appears to be more efficiently spliced than the *412* element alone, possibly because the *B104/roo* element provides two donor splice sites that are a better match with the consensus sequence for *Drosophila* donor sites.

Mutations in *Su(s)* also suppress the $v^k$ mutant phenotype (Fridell, Pret & Searles, 1990). Whereas only trace levels of wild type-sized *vermilion* mRNA are detected when $v^k$ is in a *Su(s)* background, approximately 10%-20% of $v^k$ transcripts are of wild type size in a *su(s)* background. Loss of the *Su(s)* product may stabilize $v^k$ pre-mRNA just as it may stabilize the $y^{76d28}$ transcripts and lead to the suppression of the phenotypes of both alleles.

## Evolutionary implications of transposable element splicing

The splicing of transposable elements from pre-mRNA strengthens the introns-late hypothesis by providing a plausible mechanism for intron insertion. It was originally believed that transposable elements had to have splice sites at their termini in order to function as introns (Cavalier-Smith, 1985). None of the examples presented here resemble these idealized intron-elements; in all cases, imprecise splicing of element sequences alters transcripts and leads to modifications in 5′ leader sequences (*adh1-Fm335*, $y^{76d28}$, $v^k$) or in protein structure (*wx-m9, wx-m1, wxB4, bz-m13, a2-m1*).

Given that these transposable element introns are less than perfect, is it reasonable to suggest that

they play a role in the origin of introns? We believe that the answer is yes, in part because of accumulating evidence that not all introns are of ancient origin and that the position of some introns are not strictly conserved between related species (Rogers, 1985, 1990; Shah *et al.*, 1983; Fornwald *et al.*, 1982; Cavalier-Smith, 1991; Finnegan, 1989). However, the relatively few examples of introns of this type lead us to conclude that transposable elements were not the original introns and that most introns did not arise from transposable elements. Rather, transposable elements probably evolved to take advantage of existing splicing mechanisms because the ability to be spliced provides a selective advantage to these elements and their host (Hickey, 1982).

There are two reasons to believe that transposable elements are evolving into introns. First, examination of maize and *Drosophila* elements indicate that 5′ and 3′ splice sites are as close as possible to the elements' termini. The 5′ splice sites in *Ds*, *P* and *412* are the first GT residues encountered at one terminus, whereas the 3′ splice sites in *Ds1*, *dSpm* and *412* are the last AG residues at the other terminus. Previous studies have suggested that *cis*-requirements for transposition in *Ds*, *dSpm* and *P* also reside within or adjacent to the terminal inverted repeats (Hehl & Baker, 1989; Mullins, Rio & Rubin, 1989). Thus, the position of splice sites within these elements may reflect an evolutionary compromise between the ability to transpose and the ability to be spliced. Second, transposable elements have the ability to evolve more rapidly than non-element sequences, facilitating their conversion into introns. Aborted transposition events have been hypothesized to mediate deletions in maize and *Drosophila* elements, creating change in state derivatives such as *bz-m13CS9* and *a2-m1 (Class II)*. These derivatives are more intron-like than their progenitor in two ways: (i) they permit higher levels of gene expression, and (ii) the elements transpose at a reduced frequency.

Mobile introns have also been reported in fungal mitochondria. The spread of these self-splicing introns are facilitated either by (i) reversible self-splicing reactions (Cech, 1990), or (ii) intron-encoded endonucleases (Lambowitz, 1989). Group I and II introns are perfect introns in that they are precisely removed from pre-mRNA. However, they are poor transposable elements because they have very restricted target sites. In contrast, transposable elements such as *Ds* and *P* are poor introns because they are imprecisely removed from pre-mRNA, but are superb transposable elements with few constraints as to where they can insert. These contrasting strengths and weaknesses may reflect the original function of each class of element: group II introns may be introns evolving into transposable elements whereas some maize and *Drosophila* element insertions may be transposable elements evolving into introns.

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## References

Berg, D. & M. Howe (eds.), 1989. Mobile DNA. Washington, DC: Am. Soc. Microbiol.

Cavalier-Smith, T., 1985. Selfish DNA and the origin of introns. Nature 315: 283-284.

Cavalier-Smith, T., 1991. Intron phlogeny: a new hypothesis. Trends in Genetics 7: 145-148.

Cech, T., 1990. Self-splicing in group I introns. Ann. Rev. Biochem. 59: 543-568.

Chia, W., G. Howes, M. Martin, Y. Meng, K. Moses & S. Tsubota, 1986. Molecular analysis of the yellow locus of Drosophila. EMBO J. 5: 3597-3605.

Dennis, E., M. Sachs, W. Gerlach, L. Beach & W. Peacock, 1988. The Ds1 transposable element acts as an intron in the mutant allele Adh1-Fm335 and is spliced from the message. Nucleic Acids Res. 16: 3315-3328.

Doolittle, W., 1987. The origin and function of intervening sequences in DNA: a review. Am. Nat. 130: 915-928.

Fedoroff, N., 1989. Maize transposable elements, pp.375-411 in Mobile DNA, edited by D. Berg and M. Howe, Washington, DC, Am. Soc. Microbiol.

Fedoroff, N., S. R. Wessler & M. Shure, 1983. Isolation of the transposable maize controlling elements Ac and Ds. Cell 35: 235-242.

Finnegan, D., 1989. Eukaryotic transposable elements and genome evolution. Trends in Genetics 5: 103-107.

Fornwald, J., G. Kuncio, I. Peng & C. Ordahl, 1982. The complete nucleotide sequence of the chick α-actin gene and its evolutionary relationship to the actin gene family. Nucleic Acids Res. 10: 3861-3876.

Fridell, R., A.-M. Pret & L. Searles, 1990. A retrotransposon 412 insertion within an exon of the Drosophila melanogaster vermilion gene is spliced from the precursor RNA. Genes & Dev. 4: 559-566.

Geyer, P., K. Richardson, V. Corces & M. Green, 1988. Genetic instability in Drosophila melanogaster: P-element mutagenesis by gene conversion. Proc. Natl. Acad. Sci. USA 85: 6455-6459.

Geyer, P., A. Chien & V. Corces & M. Green, 1991. Mutations in the su(s) gene affect RNA processing in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 88: 7116-7120.

Gierl, A., 1990. How maize transposable elements escape negative selection. Trends in Genetics 6: 155-158.

Gierl, A., H. Cuypers, S. Lutticke, A. Pereira, Z. Schwarz-Sommer, *et al.* 1988. Structure and funtion of the En/Spm transposable element of Zea mays, pp. 155-120 in Plant Transposable Elements, edited by O. Nelson Jr., New York, Plenum.

Goodall, G. & W. Filipowicz, 1991. Different effects of intron nucleotide composition and secondary structure on pre-mRNA splicing in monocot and dicot plants. EMBO J. 10: 2635-2644.

Hehl & Baker, 1989. Induced transposition of Ds by a stable Ac in crosses of transgenic tobacco plants. Mol. Gen. Genet. 217: 53-57.

Hickey, D., 1982. Selfish DNA: a sexually-transmitted nuclear parasite. Genetics 101: 519-531.

Kim, H., J. Schiefelbein, V. Raboy, D. Furtek & O. E. Nelson, 1987. RNA splicing permits expression of a maize gene with a defective Suppressor-mutator transposable element insertion in an exon. Proc. Natl. Acad. Sci. USA 84: 5863-5867.

Klein, A. & O. E. Nelson, Jr. 1984. Characterization of an Spm-controlled bronze-mutable allele in maize. Genetics 106: 769-779.

Lambowitz, A., 1989. Infectious introns. Cell 56: 323-326.

Menssen, A., W. M. Hohmann, P. Schnable, P. A. Peterson, H. Saedler & A. Gierl. The En/Spm transposable element of Zea mays contains splice sites at the termini generating a novel intron from a dSpm element in the A2 gene. EMBO J. 9: 3051-3057.

Mount, S., M. Green & G. Rubin, 1988. Partial revertants of the transposable element-associated suppressible allele white-apricot in Drosophila melanogaster: structures and responsiveness to genetic modifiers. Genetics 118: 221-234.

Mullins, M., D. Rio & G. Rubin, 1989. Cis-acting DNA sequence requirements for P-element transposition. Genes & Dev. 3: 729-738.

Pret, A. & L. Searles, 1991. Splicing of retrotransposon insertions from transcripts of the Drosophila melanogaster vermilion gene in a revertant. Genetics 129: 1137-1145.

Raboy, V., H. Kim, J. Schiefelbein & O. E. Nelson Jr., 1989. Deletions in a dSpm insert in a maize bronze-1 allele alter RNA processing and gene expression. Genetics 122: 695-703.

Rogers, J., 1985. Exon shuffling and intron insertion in serine protease genes. Nature 315: 458-459.

Rogers, J., 1990. The role of introns in evolution. FEBS Letters 268: 339-343.

Searles, L., R. Ruth, A. Pret, R. Fridell & A. Ali, 1990. Structure and transcription of the Drosophila melanogaster vermilion gene and several mutant alleles. Mol. Cell. Biol. 10: 1423-1431.

Shah, D., R. Hightower & R. Meagher, 1983. Genes encoding actin in higher plants: Intron positions are highly conserved but the coding sequences are not. J. Mol. Appl. Gen. 2: 111-126.

Sharp, P., 1985. On the origin of RNA splicing and introns - a review. Cell. 42: 397-400.

Spradling, A. & G. Rubin, 1982. Transposition of cloned P elements into Drosophila germline chromosomes. Science 218: 342-347.

Varagona, M. & S. R. Wessler, 1990. Implications for the cis-requirements for Ds transposition based on the sequence of the wx-B4 Ds element. Mol. Gen. Genet. 220: 414-418.

Voelker, R., W. Gibson, J. Graves, J. Sterling & M. Eisenberg, 1991. The Drosophila suppressor-of-sable gene encodes a polypeptide with regions similar to those of RNA-binding proteins. Mol. Cell. Biol. 11: 894-905.

Walbot, V., 1985. On the life strategies of plants and animals. Trends in Genetics 1: 165-169.

Weil, C. & S. Wessler, 1990. The effects of plant transposable element insertion on transcription initiation and RNA processing. Ann. Rev. Plant Phys. Plant Mol. Biol. 41: 527-552.

Wessler, S., 1988. Phenotypic diversity mediated by the maize transposable elements Ac and Spm. Science 242: 399-405.

Wessler, S., 1989. The splicing of maize transposable elements from pre-mRNA - a minireview. Gene 82: 127-133.

Wessler, S., 1991a. Alternative splicing of a Ds element from exon sequences may account for two forms of Wx protein encoded by the wx-m9 allele. Maydica 36: 317-322.

Wessler, S., 1991b. The maize transposable Ds1 element is alternatively spliced from exon sequences. Mol. Cell. Biol. 11: 6192-6196.

Wessler, S., G. Baran & M. Varagona, 1987. The maize transposable element Ds is spliced from RNA. Science 237: 916-918.

Wessler, S., G. Baran, M. Varagona & S. Dellaporta, 1986. Excision of Ds produces waxy proteins with a range on enzymatic activities. EMBO J. 5: 2427-2432.