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The Maize Transposable Element *Ds* Is Spliced from RNA

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In some instances, insertion of maize transposable elements into exons does not result in the total loss of enzymatic activity. In other instances, messenger RNAs of wild-type size are encoded by genes known to contain the maize transposable element *Dissociation* (*Ds*) in exons. To understand how *Ds* is processed from RNA, a study was made of transcripts encoded by two alleles of the maize *waxy* (*wx*) gene containing *Ds* insertions in exon sequences. The analysis was carried out in strains where the *Ds* element could not excise from the *wx* gene. Despite insertions of 4.3- and 1.5- *Ds* elements, the predominant transcripts encoded by these two genes were wild type in size. For both alleles, DNA sequencing of complementary DNAs revealed that the *Ds* elements had been spliced in a similar manner. Splicing was accomplished by the utilization of multiple 5′ donor splice sites in the *Ds* termini and a 3′ acceptor site within the *wx* gene adjacent to the *Ds* element. The net effect in both cases was the removal of most of the *Ds* element from the messenger RNA.

THE EXPRESSION OF GENES CONtaining *Ds* (*Dissociation*) insertions can be analyzed either in the presence or absence of the *Ac* (*Activator*) element. Recent analyses of these elements have focused on gene expression in the presence of *Ac*, that is, when the *Ds* element can transpose. These studies have revealed how the excision of *Ds* can alter gene expression by adding nucleotides to a gene at the site of excision (*1–5*). In the present study, we analyzed the influence of *Ds* insertion on RNA transcripts in the absence of *Ac*; that is, when *Ds* cannot excise from the gene in which it is inserted. This study was prompted by two findings: (i) *Wx* (*waxy*) protein of wild-type size is produced in a strain containing a *wx* allele with a 4.3-kb *Ds* element in one of the exons (*6*), and (ii) two alleles of the *adh1* locus produce wild-type sized *Adh1* messenger RNAs (mRNAs) despite *Ds* insertions in exons (*7, 8*). To determine the sequences involved in the removal of *Ds* sequences from the primary transcripts, we characterized the RNAs produced by two alleles of the *wx* locus that have *Ds* insertions in two distinct exons.

The *wx* gene encodes a starch granule–bound enzyme responsible for the synthesis of amylose in endosperm tissue. The gene has been cloned (*9*) and sequenced (*10*) and the position and effect of several *Ac* and *Ds* insertions have been described (*4, 11, 12*). The positions of two *Ds* elements within the *wx* gene are displayed in Fig. 1B. The cloning (*11*) and sequencing (*13*) of the *Ds* insertion in the *wx-m9* allele has also been described. Despite insertion of a 4.3-kb *Ds* element, endosperms harboring the *wx-m9* allele have significant amylose and produce reduced amounts of a wild-type sized *Wx* protein (*6*). In contrast to *wx-m9*, endosperms harboring the *wxB4* allele contain no amylose and have no *Wx* enzymatic activity (*14*).

Steady-state transcripts produced by these two *wx* alleles were analyzed by Northern blots (Fig. 2). Surprisingly, we found that in both cases the predominant *Wx* transcript is approximately 2.4 kb, the same size as the wild-type transcript. Rehybridization of the *wx-m9* blot with a *Ds*-specific probe indicated that the 2.4-kb transcript had no detectable homology with *Ds* but the minor transcripts of 6.7 kb and 1.8 kb were homologous with both *Ds* and *Wx* sequences (Fig. 2A). These data led us to conclude that the 6.7-kb transcript is a pre-mRNA that includes *Wx* mRNA (2.4 kb) and the entire *Ds* element (4.3 kb) while the 2.4-kb transcript arises following the processing of *Ds* from pre-mRNA. The 1.8-kb transcript is not homologous with *Wx* sequences downstream from the 3′ site of *Ds* insertion and may represent a transcript that terminates within the *Ds* element. The precise nature of this small transcript has not been investigated further.

The relative abundance of the 2.4-kb transcripts in the *wx-m9* and *wxB4* strains suggested that the *Ds* elements were efficiently spliced from *Wx* mRNA. To determine how these processed transcripts arose, we used samples of the RNA analyzed by Northern blots (Fig. 2) as templates for complementary DNA (cDNA) synthesis. Double-stranded cDNAs were inserted into λgt10 and plaques screened with *Wx* specific probes. Recombinant phage DNAs that hybridized with *Wx* probes were subcloned and the DNA near the site of *Ds* insertion was sequenced (Fig. 1, A and D).

In addition to cDNA, genomic DNA containing the *wxB4 Ds* insertion was also cloned and DNAs containing the *Ds* terminal and adjacent *Wx* sequences were subcloned and sequenced (Fig. 1C). We found that the *wxB4 Ds* insertion is 1.5 kb and the termini have 316 and 257 bp, respectively, homologous with the *Ac* termini. The central 900 bp of this *Ds* element is not homologous with *Ac* (*15*). Pohlman *et al.* (*13*) previously determined that the 4369-bp *Ds* element in the *wx-m9* allele was derived from the 4563-bp *Ac* element by a 194-bp internal deletion.

The sequences compared in Fig. 3A reveal splicing sites (*16*) that would result in removal of most of the *Ds* element during RNA processing. Of the eight cDNAs analyzed, three had the sequence of cDNA I while five were like cDNA II (see the legend to Fig. 1D). The two cDNAs differ by the choice of 5′ donor sites located only 4 bp apart. The splice acceptor site for both cDNAs is the same and is part of the 8-bp direct repeat of *Wx* sequences that is generated upon *Ds* insertion and flanks the element (Fig. 3). DNA sequencing also revealed that both cDNAs I and II utilize the same poly(A) addition site and that this site is one of three utilized in nonmutant *Wx* strains (*10*).

The cDNA I differs from wild-type *Wx* cDNA by the addition of 23 bp: 24 bp added from the 5′ *Ds* terminus and 1 bp deleted from *Wx* sequences on the 3′ end of the intron. The 8-bp direct repeat is also deleted but it was added to the *Wx* sequences upon insertion of *Ds* and one copy

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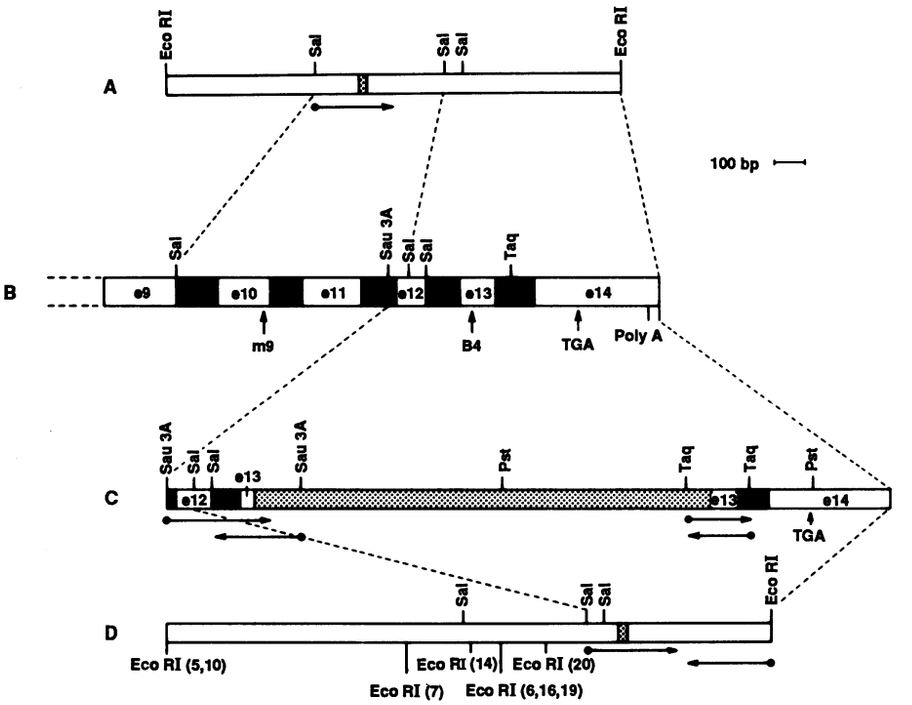

Fig. 1. Subclones and sequencing strategies for *wxB4* genomic DNA and cDNAs from *wx-m9* and *wxB4* (*20*). (**A**) *wx-m9* cDNA. (**B**) Detail of exons 9 through 14 in the *Wx* gene including relevant restriction sites and the sites of the *Ds* insertions in *wx-m9* and *wxB4*. The position of introns, exons, and poly(A) addition were determined by Klosgen *et al.* (*10*). Cloning and characterization of the *wx-m9* allele has also been described previously (*11*). (**C**) *wxB4* genomic DNA containing the *Ds* insertion and adjacent *Wx* sequences. To clone the 1.5-kb *Ds* element, genomic DNA was isolated from leaf tissue (*11*) of a *wxB4* homozygote and restricted with Bgl II, and an 8-kb fragment was cloned into the Bam HI site of EMBL3 (*21*). (**D**) *wxB4* cDNAs. For (A) to (D) darkly shaded areas are introns, unshaded areas are coding DNA, and lightly shaded areas are *Ds* sequences. Double-stranded cDNA was prepared from 2 μg of endosperm poly(A)$^+$ RNA from strains harboring either the *wx-m9* or *wxB4* allele according to the protocol of Gubler and Hoffman (*22*). Double-stranded cDNAs were ligated with Eco RI linkers, cloned into λgt10 (*23*), packaged in vitro (*24*), plated on the permissive host LE392, and screened with ^{32}P-labeled *Wx* fragments. DNA from phage containing *Wx* sequences was subcloned into M13mp18 or M13mp19 (*25*) and sequenced by the dideoxy chain termination protocol (*26*). The heavy arrows indicate the regions and strands sequenced. For *wx-m9*, ten cDNAs were recovered from 10^6 primary recombinants screened; only the one shown in (A) was long enough to include the mutated region. For *wxB4*, eight cDNAs with the different sized Eco RI inserts displayed in (D) were recovered from 200,000 primary recombinants screened. Complementary DNAs designated 5, 10, and 14 had 24 bp of *Ds* sequences and cDNAs 6, 7, 16, 19, and 20 had 28 bp of *Ds* sequences.

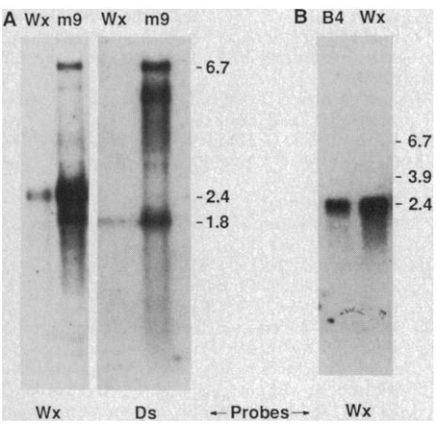

Fig. 2. Northern blot analysis of *Wx* RNAs encoded by the (**A**) *wx-m9* or (**B**) *wxB4* allele and compared with nonmutant (*Wx*) transcripts. Polyadenylated RNA was isolated from endosperm tissue harvested 18 to 21 days after pollination (*9*), fractionated on formaldehyde-containing agarose gels (*27*), transferred to nitrocellulose, and probed with ^{32}P-labeled fragments of the *Wx* gene or the *Ds* element (the 1.6-kb internal Hind III fragment shown in Fig. 3B). The amount of poly(A)$^+$ RNA loaded was (A) *m9*, 5 μg; *Wx*, 0.5 μg; and (B) *B4*, 3 μg; *Wx*, 0.5 μg. The molecular weight markers are in kilobases. The apparent band of 1.8 kb displayed in the *Wx* RNA lane probed with *Ds* is in fact a streak on the autoradiograph.

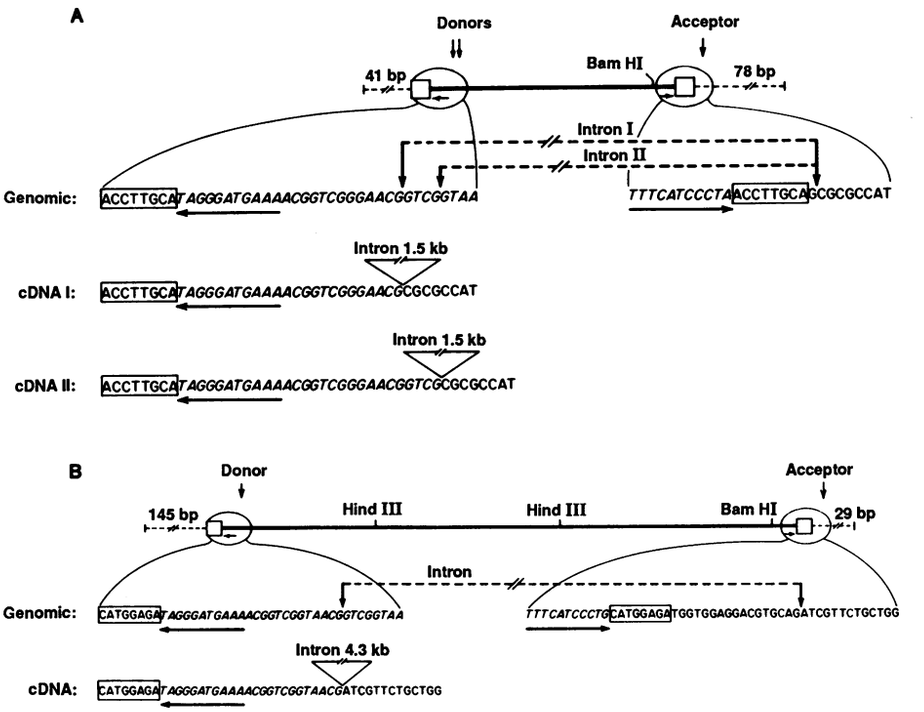

Fig. 3. The DNA sequences of genomic and cDNA subclones from *wx-m9* and *wxB4* reveal how the *Ds* elements are spliced from *Wx* RNA. (**A**) The multiple donor and single acceptor sequences in *wxB4*. The position of the 1.5-kb *Ds* element within exon 13 is shown. The regions within the ellipses have been expanded to show the genomic sequence at the *Wx*/*Ds* junctions. *Ds* sequences are italicized. The heavy arrows indicate the 11-bp inverted repeat at the *Ds* termini. The boxed region and sequences within are the 8-bp direct repeat of *Wx* DNA generated upon *Ds* insertion. The position of the new intron, as determined by comparing the genomic and cDNA sequences, is shown. (**B**) The donor and acceptor sequences in *wx-m9*. The heavy line at the top represents the 4.3-kb *Ds* element and the position of the element within exon 10. The arrows and boxes represent the repeats described for (A).

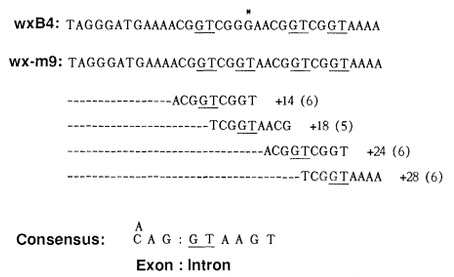

Fig. 4. Homologies with the donor splice consensus sequence in the *Ds* termini. The numbers in parentheses indicate the extent of homology with the consensus sequence. The +24-bp and +28-bp sites are used in vivo; donors at +14 bp and +18 bp are predicted. The GT residues that would be the 5′ end of each intron are underlined throughout. The asterisk indicates a difference in the sequence of these two *Ds* termini. For *wxB4*, this base change eliminates the +18-bp donor because the GT dinucleotide is absolutely required for splicing. This T to G transversion also makes the +14-bp donor in *wxB4* a poorer fit (a 5-bp rather than a 6-bp match). This may explain why our analysis of eight cDNAs from *wxB4* did not include any using the +14-bp donor.

of the direct repeat is still present in both cDNAs. The cDNA II maintains the correct reading frame with the addition of 27 bp to the coding region; 28 bp added from the *Ds* terminus and 1 bp deleted from *Wx* sequences. If translated, this mRNA would encode a protein with an additional nine amino acids.

In contrast to the *wxB4* cDNAs, only one cDNA that was sufficiently long to contain the mutated region was recovered from the *wx-m9* library (Fig. 3B). The sequence of this cDNA reveals that, like *wxB4* cDNA I, the donor splice sequence at position +24 is utilized in vivo. Also, like both *wxB4* cDNAs, the acceptor site is in *Wx* sequences. This cDNA differs from wild-type *Wx* cDNA by the addition of 7 bp: 24 bp added from the 5′ end of the *Ds* element and 17 bp deleted from *Wx* sequences (not including the direct repeat, one copy of which is still present in the cDNA sequence).

Our data may explain how a reduced amount of a wild-type sized *Wx* protein is produced in endosperms harboring the *wx-m9* allele. It is unlikely that the sequenced *wx-m9* cDNA with its additional 7 bp represents a transcript that encodes this protein. It is possible that the correct reading frame may be maintained by utilizing a different donor site at the 5′ end of the *Ds* element. The DNA sequence of the *Ds* terminus contains other potential splice donor sites (Fig. 4) in addition to those shown in Fig. 3 at +24 bp and +28 bp. In fact, for the *wx-m9 Ds*, there are four regions within the *Ds* terminus with homology to the donor splice consensus sequence derived from a comparison of plant genes (*17*). If the putative +14 donor (Fig. 4) is coupled with the acceptor found in vivo at −17 bp (Fig. 3B), the reading frame would be maintained. The sequencing of additional *wx-m9* cDNAs should reveal if this, in fact, occurs.

We have demonstrated that two *Ds* elements inserted into different exons of the *wx* gene are spliced in a similar manner. As mentioned above, two *Ds* insertions into exons of the *adh1* gene are apparently spliced (*7, 8*). At this time we do not know if the donor sites we have found within *Ds* are also utilized by these alleles.

It is unclear what purpose is served by the splicing of *Ds*. Possibly a splicing mechanism has evolved to allow some gene function despite insertion into exons. In support of this hypothesis is our finding that splicing can generate multiple reading frames and that most of the *Ds* is removed from the mature transcript. However, most *Ds* alleles are like *wxB4*, that is, they have a null phenotype in the absence of *Ac*. Our data suggest that gene function is not restored in *wxB4* because splicing does not generate a normal transcript; part of the *Ds* terminus persists in all cDNAs examined. However, in support of the notion that splicing has evolved to permit gene expression, it could be argued that all *Ds* alleles were selected for their mutant phenotypes; *Ds* alleles might never be detected if splicing restored gene function. Thus, the *Ds* alleles available for analysis may represent a biased sample and may not reflect the behavior of the 25 to 100 copies of "silent" *Ds* sequences estimated to be in all maize genomes (*1, 11, 18*).

Recently, Kim *et al.* (*19*) determined that splicing of a 902-bp *dSpm* element from the *bz-m13CS9* allele permits 50% of nonmutant enzymatic activity. The splicing of *dSpm* from the *bronze* gene is very different from that described here for *Ds* (*19*). Despite this difference, the finding that two distinct maize transposable elements are spliced from RNA supports the idea that splicing has evolved to permit gene expression.

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