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Excision of *Ds* produces *waxy* proteins with a range of enzymatic activities

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The *waxy* (*wx*) locus of maize encodes an enzyme responsible for the synthesis of amylose in endosperm tissue. The phenotype of the *Dissociation* (*Ds*) insertion mutant *wx-m1* is characterized by endosperm sectors that contain different levels of amylose. We have cloned the *Wx* gene from this allele and from two germinal derivatives, *S5* and *S9*, that produce intermediate levels of amylose. The *Ds* insertion in *wx-m1* is in exon sequences, is 409 bp in length and represents an example of a class of *Ds* elements that are not deletion derivatives of the *Activator* (*Ac*) controlling element. The two germinal derivatives, *S5* and *S9*, lack the *Ds* element but contain an additional 9 and 6 bp, respectively, at the site of *Ds* insertion. The level of *Wx* mRNA and *Wx* protein in *S5* and *S9* is essentially the same as in normal endosperm tissue but Wx enzymatic activity is reduced. Thus, the lesions in *S5* and *S9* lead to the addition of amino acids in the *Wx* protein, resulting in *Wx* enzymes with altered specific activities. This work supports the notion that the maize transposable elements may serve a function in natural populations to generate genetic diversity, in this case, proteins with new enzymatic properties.

Key words: dissociation (Ds)/DNA sequences/excision/enzymatic activity/*wx-m1*

Introduction

There are several unstable alleles of the *waxy* (*wx*) locus of maize caused by insertion of either the *Activator* (*Ac*) or *Dissociation* (*Ds*) controlling element. For each allele the element is inserted in a different position within the *Wx* transcription unit (Fedoroff *et al.*, 1983; Behrens *et al.*, 1984; Varagona and Wessler, unpublished data) and, in each case, the mutant allele has a unique, unstable phenotype (McClintock, 1948, 1952, 1963, 1964; Nelson, 1968). It is our goal to understand the molecular basis of these different phenotypes.

The *wx* locus encodes a starch granule-bound glucosyl transferase responsible for the synthesis of amylose in the endosperm of the developing kernel. Insertion of either *Ac* or *Ds* into the locus results in the partial or total loss of *Wx* function. Excision in somatic tissue generates a sectored endosperm that displays clones of cells with and without amylose. The eight *Ac*/*Ds* alleles of *waxy* differ in the size, amylose content and/or frequency of these endosperm sectors. Excision in germinal tissue can result in the production of new *wx* alleles. The frequency with which these new alleles are produced and the amount of *Wx* activity they possess is characteristic of each of the eight *Ac*/*Ds* alleles.

Molecular analysis of new alleles generated following excision of *Ac* or *Ds* from the *wx* (Pohlmann *et al.*, 1984), *Adh1* (Peacock *et al.*, 1984) and *sh* loci (Weck *et al.*, 1984) indicate that virtually all have lost the *Ac* or *Ds* element but retain some of the 8 bp host sequence duplication generated upon insertion. Thus, the phenotypes of the *Ac*/*Ds* alleles of *waxy* may depend upon whether the element has inserted into intron or exon sequences. Furthermore, if inserted into exon sequences, the position within the protein coding region should have differing effects on protein structure and function. These molecular studies are consistent with prior genetic studies that indicated that the position of an element within a locus influenced the phenotype (Peterson, 1976). In addition, previous biochemical examination of the protein products of new alleles derived from the excision of controlling elements found altered *bronze* (Dooner and Nelson, 1976) and *waxy* (Echt and Schwartz, 1981; Shure *et al.*, 1983) protein products.

Recently Schwarz-Sommer *et al.* (1985) have suggested that the insertions and subsequent excisions of plant transposable elements may be responsible for protein evolution. They analyzed several *Wx* revertants produced following *Enhancer* (*En*) induced excision of a 2.1 kb element from the *wx-m8* allele. Although phenotypically wild type, the *Wx* genes in many of these strains encoded an additional amino acid. These alterations in the *Wx* protein did not apparently diminish *Wx* expression.

In this report we demonstrate that excision of *Ds* from the *wx* locus can have a profound effect on gene expression. Excision of *Ds* from the locus in strains harboring the *wx-m1* allele produces new alleles (germinal derivatives) that encode *Wx* proteins with altered enzymatic activities. We have cloned the locus from strains harboring the *wx-m1* allele and from two germinal derivatives that have intermediate levels of amylose in their endosperms. DNA sequence analysis demonstrates that the *Ds* element is inserted into exon sequences and that the two derivatives have additional amino acids within the coding region. Evidence is presented suggesting that the reduced amount of amylose in these alleles is not caused by reduced amounts of *Wx* mRNA or *Wx* protein but rather by alterations in the enzymatic properties of the *Wx* enzyme.

In addition, DNA sequence analysis of the *Ds* insertion in the *wx-m1* allele is also presented. This element represents only the second example of a *Ds1*-like element, that is, it is homologous with an unusual *Ds* element found, thus far, only in the *Adh1-Fm335* allele (Sutton *et al.*, 1984). The *Ds* element is very short (409 bp), is AT rich, and is not a deletion derivative of the *Ac* element.

Results

Phenotype of the wx-m1 allele

The *wx-m1* allele was the first *Ds* insertion at the *wx* locus isolated by McClintock (1948). Its phenotype and that of one germinal derivative can be seen in Figure 1 where kernels have been filed to reveal the underlying endosperm then stained with an I/KI solution to indicate the presence and relative amounts of amylose. The endosperm starch of strains harboring a normal *Wx* gene

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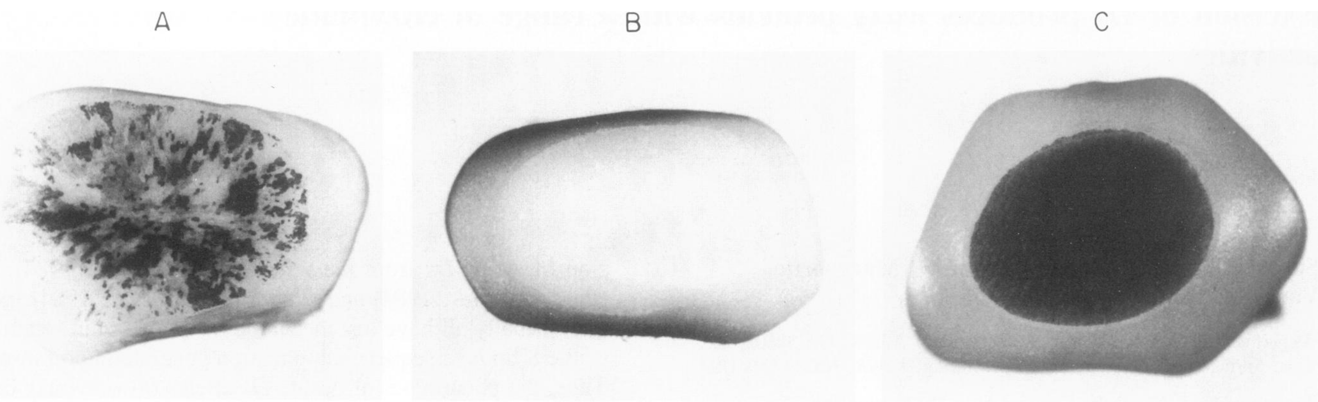

Fig. 1. Endosperm phenotypes of kernels with various *wx* alleles. Endosperms stained with I/KI from kernels with the following genotypes: Kernel A: *wx-ml/wx-ml/wx-ml* (+*Ac*); Kernel B: *wx-ml/wxlml/wx-ml* no *Ac*; Kernel C: *wxS9/wxS9/wxS9*. Kernels with the *wx-ml* mutation in the absence of an active *Ac* element show undetectable levels of amylose judged by I/KI staining (Kernel B). When *Ac* is present, *Ds* at the *wx-ml* allele is somatically unstable. Excision of *Ds* from *wx-ml* results in clonal endosperm sectors exhibiting various levels of I/KI staining (Kernel A). If *Ds* excision is premeiotic, germinal *wx-ml* derivatives can be recovered that exhibit stable but sometimes altered levels of amylose (Kernel C).

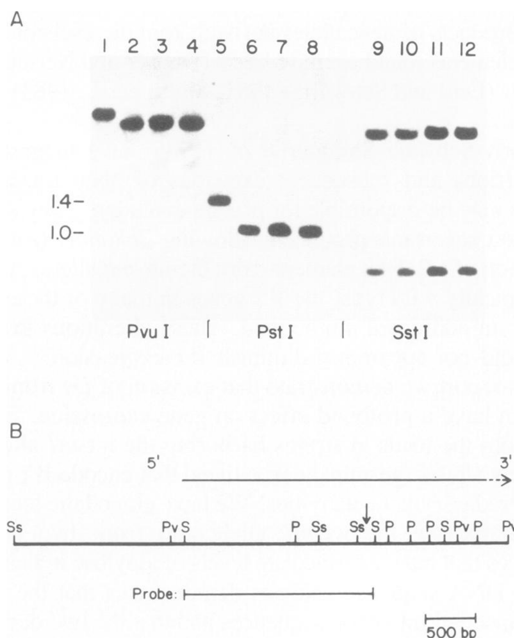

Fig. 2. (A) Southern blot of *wx-ml*, *S9*, *S5* and *Wx* DNA probed with a *Wx* specific probe. DNA (10 μg) was restricted with the enzyme shown for each panel, electrophoresed through 1% agarose and transferred to nitrocellulose. Lanes 1, 5 and 9, *wx-ml* (−*Ac*); lanes 2, 6 and 10, *S9*; lanes 3, 7 and 11, *S5*; lanes 4, 8 and 12, *Wx*. The size of fragments were determined by comparison with lambda and pBR322 restriction fragments that were transferred and probed simultaneously. **(B)** Schematic map of a region of the *Wx* gene containing the entire transcription unit (as delimited by the horizontal arrow). The position of the *Sal*I probe used for the Southern blot is noted. The vertical arrow indicates the position of the *Ds* insertion in the wx-ml allele.

contains approximately 22−28% amylose and 72−78% amylopectin (Whistler and Weatherwax, 1948) and stains black in color. In contrast, strains harboring the *wx-ml* allele, in the absence of the autonomous controlling element *Ac* contain no amylose and display no stain (Figure 1B). When *Ac* is present in the genome with the *wx-ml* allele the *Ds* element can excise from the locus, restoring *Wx* expression and resulting in darkly staining endosperm sectors (Figure 1A). One characteristic of the unstable *wx-ml* phenotype is that these sectors have varying amounts of amylose, as indicated by the intensity of staining. This suggests that somatic excision of *Ds* can result in a range of *Wx* gene expression.

In the presence of *Ac*, stable germinal derivatives can also be isolated from this allele. McClintock (1951) found that the amount of amylose in the endosperm of these new *wx* alleles can range from 0 to 28% of the total endosperm starch. We have chosen to focus on two derivatives isolated by Curme and Sprague (Curme, 1955) that no longer respond to *Ac* in the genome and have 5 and 9% of the total endosperm starch as amylose. They are designated *S5* and *S9* respectively. These levels represent approximately 21% (*S5*) and 36% (*S9*) of non-mutant amylose content. The endosperm of the *S9* allele displays a paler stain with I/KI (Figure 1C) indicating reduced amounts of amylose relative to non-mutant.

Localization of the Ds insertion in wx-ml

To determine the size and position of the *Ds* insertion in the *wx-ml* allele, a Southern blot analysis was performed (Southern, 1975). Genomic DNA isolated from the *wx-ml* allele, a normal *wx* allele, and the *S5* and *S9* derivatives were digested, electrophoresed, blotted and hybridized with a labelled probe from the *Wx* transcription unit. The results of such an analysis are shown in Figure 2 and can be summarized as follows. The *wx-ml* allele has a fragment that is about 400 bp larger than *S5*, *S9* and non-mutant *Wx* DNA, when digested with *Pvu*I, *Pst*I (Figure 2A) or *Sal*I (data not shown) and hybridized with the *Sal*I probe shown in Figure 2B. Since the insertion is in the 1.0 kb *Pst*I fragment homologous with the *Sal*I probe but not within either the 4.0 kb or 0.4 kb *Sst*I fragments (Figure 2A), it must be inserted in the 200 bp *Sst*I−*Sal*I fragment shown in Figure 2B.

These data indicate not only the position of the *Ds* insertion but its approximate size (400 bp), and the fact that, at this level of resolution (50 bp), the *S5* and *S9* alleles are indistinguishable from a non-mutant *wx* allele.

Cloning and sequencing of the Ds insertion

The *wx-ml* allele was cloned on a 14.5 kb *Eco*RI fragment using bacteriophage EMBL 4 as described in the Methods section. The *Sst*I−*Sal*I fragment containing the *Ds* insertion was subcloned into M13mp18 and M13mp19 and sequenced by the dideoxy chain termination method (Sanger *et al.*, 1977). The sequence of the *Ds* insertion is shown in Figure 3. Unlike virtually all *Ds* elements analyzed to date (reviewed in Doring and Starlinger, 1984), this element is not a deletion derivative of the *Ac* ele-

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wx-m1       TAGGGATGAAAACTGACGGAAA-CGGTATTTATTCGGTAATCAGTTTTTTGGTCGTTTTTCTTTGATTACGAATAAATAG
Adh1-Fm335               G T      T        T                  A                 G

            GATATATAATGCAGTATACAAATTTATATTCTTGTTTATAACATTGAGCTTGTAAAGATTCATAAAAGTTAATCCTCAAA
                  G   AT CA          G           T      CC       T               -  A

            TTCATCATATATTTTCTCAAA-GATT-GATATAAAACTTCGGTATGGCATTCGAAAACAAATGTCGGTAATATTTTCAAC
                  C              TA A A        -           -     G   T    -        T

            TTTTTTTGCTGTGAAGTGAGCAAATAATACATAAAACAATTTATGTAATATTTTATTCTTATTTGTAATAAATG--CTTG
            C    A  T   --  G                       G            -    A     C  C   -  TG

            AGAACATAACACAAGATCACTATCAAATTTTATACATATCTATTTTAAAATAATT-AATTTGTCCTAACAGCTCAGATTA
             T       G A       A              T                 -  A     A         T  G

            TCACTTTCATCCCTA
            C
```

Fig. 3. DNA sequence of the *Ds* insertion in the *wx-m1* allele and comparison with the *Ds* element in *Adh1-Fm335* (Sutton *et al.*, 1984). Nucleotide differences are noted in the *Adh1-Fm335* sequence; dashes represent deletions. The 11-bp inverted repeat is underlined.

```
wx-m1  ←148bp— CTC ACC GGC ATC ACC ——Ds (409bp)—— GC ATC ACC GGC ATC GTC —72bp→

Wx             CTC ACC GGC ATC ACC GGC ATC GTC
                L   T   G   I   T   G   I   V

wxS5           CTC ACC GGC ATC ACG GCC ATC ACC GGC ATC GTC
                L   T   G   I   T   A   I   T   G   I   V

wxS9           CTC ACC GGC ATC ACC ATC ACC GGC ATC GTC
                L   T   G   I   T   I   T   G   I   V
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Fig. 4. DNA sequence analysis of *wx-m1* and its germinal derivatives. The amino acids deduced from the correct reading frame (Klosgen *et al.*, 1986) are shown for each allele. Boldface type indicates the fate of the 8-bp target sequence in the *Wx* allele following *Ds* insertion (*wx-m1*) and subsequent excisions (*S5*, *S9*). A 9-bp direct repeat (indicated by arrows) was found to pre-exist at the site of *Ds* insertion. The position of the sequence shown for the *Wx* allele, with respect to the 5′ and 3′ borders of exon 9 is indicated.

ment. It is very short (409 bp), AT rich and has termini containing 11 bp inverted repeats. These inverted repeats are homologous with 10 of 11 bp of the inverted repeats of the *Ac* controlling element. In addition, 11 of 15 bp adjacent to the left inverted repeat are homologous with *Ac* sequences located in the same position. However, the rest of the element shares no homology with *Ac*. Rather, this element is 87% homologous with the *Ds1* element described by Sutton *et al.* (1984). A comparison of the *wx-m1 Ds* element and the *Ds1* sequence is also displayed in Figure 3.

Cloning and sequencing of the insertion site in the Wx gene and the excision sites in the S5 and S9 alleles

To determine the target site of *Ds* insertion, the *Sst*I – *Sal*I fragment (Figure 2B) was subcloned from plasmid pWx5 (Shure *et al.*, 1983) which contains the entire *Wx* transcription unit. The target site as determined following sequencing of this fragment is displayed in Figure 4. The *Ds* element of *wx-m1* has inserted assymetrically into a 9 bp direct repeat already present in the *Wx* gene. This repeat is located within the ninth exon of the *Wx* transcription unit (Klosgen *et al.*, 1986). The precise location of insertion within this exon is shown in Figure 4. Like all other *Ds* insertion events examined to date, an 8 bp direct repeat of host sequences was generated upon insertion and flanks the *Ds* element (Doring and Starlinger, 1984; Figure 4, boldface type).

Southern blot analysis revealed that most or all of the *Ds* element has excised from the *Wx* gene in the *S5* and *S9* alleles (Figure 2A). To determine precisely their molecular lesions, the *S5* and *S9* alleles were cloned on 14-kb *Eco*RI fragments as described in the Methods section. In each case the 200-bp *Sst*I – *Sal*I fragment was subcloned into M13mp18 and sequenced (Yanisch-Perron *et al.*, 1985). As compared with the *Wx* target sequence, *S5* has an additional 9 bp and *S9* has an additional 6 bp (Figure 4). Most of this extra DNA remains from the 8-bp direct repeat generated upon insertion of the *Ds* element. Since this DNA is in exon coding sequences (Klosgen *et al.*, 1986), the extra bases in *S5* and *S9* encode three and two additional amino acids, respectively, in the *Wx* protein. Figure 4 also shows the identity of these amino acids based upon the determination of the correct reading frame by Klosgen *et al.* (1986).

Strains harboring the S5 and S9 alleles have normal levels of Wx protein and Wx RNA but have reduced Wx enzymatic activity

Wx mRNA, protein and enzymatic activity were quantified in strains *S5* and *S9* to determine at what level *Wx* gene expression is affected. Northern analysis of endosperm poly $(A)^+$ RNA indicates that the size and approximate amount of *Wx* mRNA encoded by *S5* and *S9* are the same as that encoded by a normal *Wx* gene (Figure 5A). To quantify more rigorously the steady-state levels of *Wx* RNA, dot blots were employed using total RNA isolated from endosperm tissue. The results of this analysis, displayed in Figure 5B, indicate that strains harboring either the *Wx*, *S5* or *S9* allele have the same amount of *Wx* RNA. Thus, the steady-state level of *Wx* mRNA is apparently unaffected in the *S5* and *S9* derivatives.

Starch granule-bound proteins were examined by SDS – polyacrylamide gel electrophoresis to determine the relative amount of *Wx* protein in *S5* and *S9* versus non-mutant *Wx* strains. The *Wx* protein comprises almost 80% of the granule bound protein at 18 – 21 days after pollination (Echt and Schwartz, 1981; Shure *et al.*, 1983). From Figure 5C it can be seen that, as with RNA levels, the amount of protein in *S5* and *S9* that comigrates with the *Wx* protein is at least as great if not greater than that observed in the *Wx* sample. This result was repeated several times with different granule preparations and less than 10% variation was observed. However, the absolute level of *Wx* protein present in

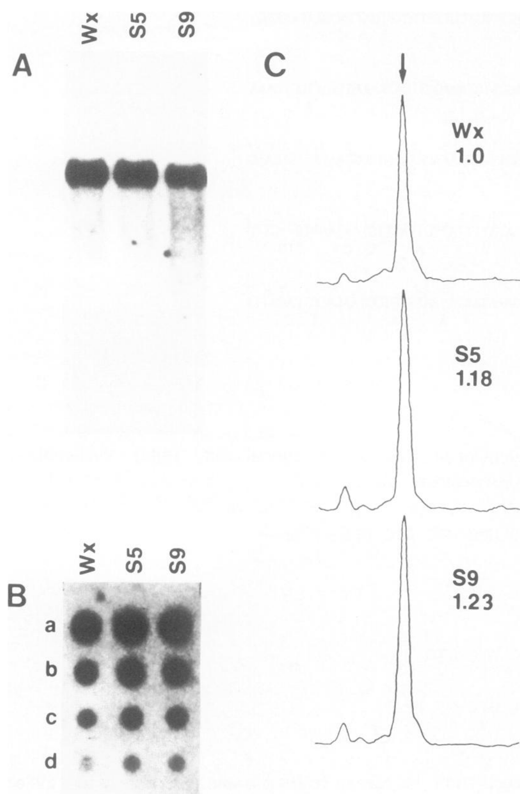

Fig. 5. Analysis of *Wx* RNA and protein levels in *Wx*, *S5* and *S9* alleles. (A) Northern blot analysis of poly(A)$^+$ RNA (0.2 μg each) from the strains noted. Procedures are described in the Materials and methods section. Autoradiography was for 48 h with an intensifying screen. (B) Dot blot analysis of total RNA. Several fourfold dilutions of each RNA sample were applied; (a) 2.0 μg; (b) 0.5 μg; (c) 0.125 μg; (d) 0.031 μg. Autoradiography was for 16 h with an intensifying screen. (C) Analysis of starch granule-bound proteins by SDS–polyacrylamide gel electrophoresis. Proteins were extracted from 5 mg of purified starch granules. Aliquots containing the extracted proteins, equivalent to 0.75 mg of granules were electrophoresed, stained and scanned as described in the Materials and methods. The position of the *Wx* protein is indicated by the arrow. The numbers to the right of each tracing represent the relative amount of protein in each sample normalized to the *Wx* protein level.

Table I. *Wx* enzymatic activity

Wx allele[a]	UDP-glucosyl transferase activity[b]	Normalized activity[c]	% of non-mutant activity
Wx	6.9	6.9	100
S9	4.5	3.7	53
S5	2.6	2.2	32

[a]Each allele was present in three doses in endosperm tissue.
[b]nmoles glucose incorporated/mg starch granules/30 min.
[c]Normalized to the amount of *Wx* protein in the granule preparations, from Figure 5C. *S9* and *S5* activity divided by 1.23 and 1.18 respectively.

non-mutant starch granules is dependent on the genetic background. Over two-fold variation was found when four genetic backgrounds were surveyed. The *Wx* sample shown in Figure 5C represents the highest level observed.

The same granule preparations used for protein determination were assayed for *Wx* enzymatic activity. The results of this analysis are shown in Table I. We find that the specific activity of the starch granule-bound UDP-glucosyl transferase is reduced to 53% of non-mutant for the *S9* allele and 32% of non-mutant for the *S5* allele.

Discussion

Recent molecular studies have demonstrated that the phenotypic variation exhibited by *Ds* alleles can result from a variety of insertion and excision events. Of primary importance is whether insertion is into introns or exons. This is due to the fact that excision of *Ds* is usually imprecise and results in the addition of nucleotides to the transcription unit. Excision from exon sequences can produce a complex array of phenotypes ranging from null (Peacock *et al.*, 1984) to intermediate (Sutton *et al.*, 1984) to normal expression (Pohlmann *et al.*, 1984). In this report we have determined a molecular basis for the gradient of *Wx* expression produced by a single *Ds* allele, the *wx-m1* allele.

We find that the *wx-m1* phenotype results from *Ac* mediated excision of a 409 bp *Ds* element inserted in exon sequences near the middle of the *Wx* gene. Examination of two germinal derivatives, *S5* and *S9*, that have different but intermediate levels of amylose in their endosperm tissue, reveals an additional 9 and 6 bp, respectively, in the *Wx* coding region at the site of *Ds* excision. Mutant and normal gene expression was compared by quantifying *Wx* protein, mRNA and enzymatic activity. The relative amount of *Wx* protein and mRNA is indistinguishable in these strains. However, *Wx* enzymatic activity is reduced in *S5* and *S9*. We conclude that the additional nucleotides in the *Wx* transcription unit have altered *Wx* enzymatic activity resulting in the lower amylose content.

A direct assay of *Wx* enzymatic activity in these strains indicates that three doses of *S5* and *S9* have 32% and 53%, respectively, of non-mutant activity. The fact that this value is not as low as might be expected from the amylose content (approximately 21% of non-mutant for *S5* and 38% of non-mutant for *S9*) is not surprising. We and others have found that although the *in vitro* activity of this starch granule bound glucosyl transferase is proportional to the number of *Wx* alleles in the triploid endosperm (Tsai, 1974), the *in vivo* amylose content is not (McClintock, 1951). It was found that endosperms with one dose of *Wx* (*Wx/wx/wx*) had 18% of their starch as amylose while endosperms with two and three doses had 20% and 22% respectively (Sprague *et al.*, 1943). Apparently *in vivo*, the amount of *Wx* enzymatic activity is not a limiting factor in amylose biosynthesis.

A comparison of the *S5* and *S9* sequences with the sequence of the *Wx* gene determined by Klosgen *et al.* (1986) indicates that the additional amino acids in *S5* are alanine, isoleucine and threonine while the additional amino acids in *S9* are isoleucine and threonine (Figure 4). All represent amino acids with neutral charges. Consistent with these changes is our finding that the *S5* and *S9 Wx* proteins co-migrate with non-mutant *Wx* protein on two-dimensional polyacrylamide gels (Baran and Wessler, unpublished).

S5 and *S9* represent just two of many germinal derivatives that can be isolated from the *wx-m1* allele. As can be seen in Figure 1A, excision of *Ds* in the presence of *Ac* produces somatic sectors with a range of amylose content. If these excision events are premeiotic, stable germinal derivatives that vary in their endosperm amylose content (like *S5* and *S9*) can be isolated. McClintock isolated new alleles of *wx-m1* that had from 0 to 28% amylose in the endosperm (McClintock, 1951). These alleles are no longer available but we are currently isolating new derivatives from strains harboring the *wx-m1* allele and an *Ac* element. Char-

acterization of these alleles will reveal whether each allele is distinguished by the addition of a unique set of amino acids or whether more complex changes are also involved. Of particular interest are those alleles that apparently produce more amylose than non-mutant *Wx* strains. In these strains, *Ds* excision may have engineered a *Wx* protein that is more active than the non-mutant protein.

S5 has nine additional nucleotides at the site of *Ds* excision. Thus far, all *Ds* excision events analyzed at the molecular level have an additional eight or fewer nucleotides at the site of excision; eight being the number of the host target duplication generated upon insertion. In fact, to generalize even further, for the plant elements *Ac*, *Spm*, *Tam1* and *Tam2* and *Ds*, the nucleotides remaining following excision of the element have been found to be equal to or less than the length of the host duplication for all germinal derivatives analyzed (Saedler and Nevers, 1985).

The *wx-m1* insertion is only the second example of a mutation caused by what can now be referred to as a class of *Ds* elements that are not deletion derivatives of the *Ac* element. These so-called *Ds1*-like elements are named after an *Ac* responsive insertion in the *Adh1* locus in the allele *Adh1-Fm335* (Sutton *et al.*, 1984). In addition to the *Ds1* elements and the *Ds* elements that are deletion derivatives of *Ac*, there is a third class of *Ds* elements that have 550 bp and 300 bp of *Ac* termini sequences flanking short repeats of *Ac* sequences and, in addition, they contain a 200-bp GC-rich sequence that is not derived from *Ac* (Merckelbach and Starlinger, 1986). This latter class of *Ds* elements, called *Ds2* elements, is found in the *Adh1-2F11* allele and has recently been found in a *bz2-m* allele (Theres and Starlinger, 1986).

With the cloning and characterization of these three classes of *Ds* elements, a comparison can be made between the structure of the element and the genetic behavior of each class. All three classes are capable of transposing at a high frequency in the presence of a standard *Ac* element. However, *Ds* elements display a varied response to *Ac* elements that appear to produce a transposase with reduced activity. Genetic studies indicate that the *Ds1* like element in *wx-m1* has a very different frequency of transposition in the presence of *Ac2* or *Ac-w* elements when compared with the frequency of transposition displayed by *bz2-m* (*Ds2*) or *wx-m9* (an *Ac* deletion derivative) (Rhoades and Dempsey, 1983; Schwartz, 1985). These data may indicate that sequences other than those common to all three elements (the short inverted repeats and a 15-bp sequence adjacent to one of the repeats) may interact with an *Ac* gene product(s).

In this regard it is interesting to note that the nucleotides of *Ds1* that are not homologous with *Ac* are AT rich (about 75% for both elements) and cannot encode more than 37 amino acids. In addition, it has been reported that the maize genome has 30 – 50 copies of *Ds1* like sequences and that these sequences can also be found at about the same copy number in a distant relative of *Zea mays*, *Tripsacum dactyloides* (Peacock *et al.*, 1984). It would be interesting, though premature to speculate that one reason these non-coding sequences have been conserved is because of the requirements of an *Ac* gene product(s).

Materials and methods

Maize strains

The *wx-m1* mutation was originally isolated by McClintock (1948) and obtained for this study from Drew Schwartz. The *S5* and *S9* alleles were isolated from the *wx-m1* allele by Curme and Sprague (1955) and obtained from Oliver Nelson. The strains examined in this study were all homozygous for the designated allele and maintained as such by outcrossing to *Wx* lines and subsequent self-pollination. *Wx* RNA was isolated from the inbred line HY (from B.Bear) and *Wx* protein was isolated from the inbred lines HY, W23 (from G.Neuffer), Ga 221 and Ga 219 (from A.Flemming).

Genomic DNA and RNA preparation and filter hybridization

DNA was purified from 2 – 4-week-old plantlets as described previously (Shure *et al.*, 1983). Restricted DNA was electrophoresed through 1% agarose, blotted (Southern, 1975) and hybridized under conditions described by Fedoroff *et al.* (1983a). Total and poly $(A)^+$ RNA was purified from the dissected endosperms of kernels harvested 18 – 21 days after pollination (Shure *et al.*, 1983). For Northern blot analysis, poly$(A)^+$ RNA samples were denatured at 65°C for 5 min in 2.2 M formaldehyde, 50% (v/v) deionized formamide, 5 mM $NaPO_4$, pH 7.0 and electrophoresed through 1% agarose containing 2.2 M formaldehyde, 0.2 M 3-[N-morpholino]propanesulfonic acid (MOPS), 50 mM sodium acetate and transferred to nitrocellulose as described by Thomas (1977). For dot blots, total RNA was denatured as for Northerns and immediately diluted with 9 vol. of 11 × SSC, 9% formaldehyde, 5 mM $NaPO_4$. Subsequent dilutions were in 10 × SSC, 6% formaldehyde, 3.3 mM $NaPO_4$ (all chemicals supplied by Sigma). The samples were applied to nitrocellulose (equilibrated with 10 × SSC) using a 96-well manifold (Hybrid-Dot, BRL). Hybridization conditions were as for DNA blots. For Northerns, Southerns and dot blots autoradiography was for 24 h with an intensifying screen.

Genomic library construction and screening

Two μg of *Eco*RI-digested DNA and 5 μg of lambda EMBL 4 *Eco*RI arms were coligated at 4°C for 24 h in a 15-μl reaction volume containing 50 units of T4 DNA ligase (New England Biolabs). Three μl of the ligation mixture was packaged *in vitro* as described previously (Hohn, 1979). Phage were plated on *E. coli* LE392 (Enquist *et al.*, 1977) and transferred to nitrocellulose filters according to Maniatis *et al.* (1982). The filters were prehybridized for 2 h at 65°C in 6 × SCP (1 × SCP = 100 mM NaCl, 30 mM Na_2HPO_4, and 1 mM EDTA, pH 6.5), 2% sarcosine and 500 μg/ml heparin (Type II Sigma). Hybridization was performed for 12 h at 65°C in the above buffer containing denatured salmon sperm DNA (final concentrtion 100 μg/ml) and denatured nick-translated probe. The filters were washed as described above. Positive recombinant clones were purified and a recombinant phage clone containing a 14-kb *Eco*RI fragment was confirmed by genomic blot analysis to contain the *wx-m1* region with the *Ds* insertion element. Genomic libraries were constructed from DNA from plants homozygous for the *S5* and *S9* alleles and recombinant phage analyzed as described above.

Restriction mapping, plasmid subcloning and sequence analysis

The position of the *Ds* element in the *wx-m1* allele was determined by the Southern blot analysis described in the Results section. The *Sal*I fragments (Figure 2B) containing this region were purified from agarose gels by electroelution (Drezten *et al.*, 1981) and sublconed into the *Sal*I site of pUC18. *Sal*I inserts were further subcloned as *Sal*I – *Sst*I fragments into the polylinker sites of M13mp18 and M13mp19 (Yanisch-Perron *et al.*, 1985) for dideoxynucleotide sequence analysis. The *S5* and *S9* insertion sites were sequenced directly following digestion of phage DNA with *Sst*I – *Sal*I, shotgun cloning into M13mp18 and screening of desired clones with the *Sal*I *Wx* fragment (Figure 2B) by methods described above. Sequencing of the *Sst*I – *Sal*I inserts were performed by universal primer extension using [^{35}S]adenosine triphosphate (New England Nuclear, Biggin *et al.*, 1983). Buffer gradient gels and autoradiography was performed as described by Biggin *et al.* (1983).

Preparation of Wx protein

Starch granules were prepared from frozen endosperms dissected from kernels harvested 18 – 21 days after pollination (Shure *et al.*, 1983). Five mg of lyophilized starch granules suspended in 0.5 ml KOH (pH 12) were incubated at 37°C for 30 min and then washed three times with 50 mM Tricine (pH 8.0). The starch granule pellets were dispersed in 100 μl 9 M urea, 5% Triton X-100 and 100 μl of 2 × SDS electrophoresis extraction buffer. The mixture was sonicated for 5 – 10 s, incubated at 37°C for 15 min, and the starch pelleted in a microfuge for 10 min. Aliquots of the supernatant were electrophoresed through a 10 – 18% polyacrylamide gradient gel, stained with Coomassie Blue, destained and scanned at 550 nm in a Beckman DU-7 spectrophotometer.

Measurement of Wx enzymatic activity

UDP-glucosyl transferase activity was assayed as described by Nelson *et al.* (1978). For the activities listed in Table I, four samples containing between 1 and 10 mg of starch granules were assayed in duplicate for each strain. Linear regression analysis was performed to obtain the best line to fit the data. All lines had greater than 98% correlation.

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