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Analysis of *sh-m6233*, a mutation induced by the transposable element *Ds* in the sucrose synthase gene of *Zea mays*

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The unstable allele *sh-m6233* caused by insertion of the transposable element *Ds* into the sucrose synthase gene of maize, was cloned. The mutation is caused by the insertion of an ~4 kb DNA segment, consisting of two identical *Ds* elements of ~2000 bp length, of which one is inserted into the center of the other in inverted orientation. This structure is, at the level of restriction mapping and partial DNA sequencing, identical to the double *Ds* element found in a larger insert in the mutant allele *sh-m5933*. 8 bp of host DNA are duplicated upon insertion. In a revertant, a 6-bp duplication is retained.
Key words: maize/transposable elements/sucrose synthase

Introduction

The controlling element system consisting of the two elements *Activator* (*Ac*) and *Dissociation* (*Ds*) in *Zea mays* was discovered and studied by McClintock (1951, 1965). Of these two elements, *Ac* is able to excise and to transpose itself. *Ds* is transposed or excised only if *Ac* is present in the same cell. In the absence of *Ac, Ds* is completely stable at the site of insertion.

The *Shrunken* (*Sh*) locus on chromosome 9 encodes the endosperm enzyme sucrose synthase (Chourey and Nelson, 1976). McClintock isolated a series of *shrunken* mutations caused by *Ds*. These mutations originated in two maize lines, each of which carried *Ds* in the vicinity of *Sh* (McClintock, 1952, 1953). Some of these mutations revert phenotypically to wild-type in the presence of *Ac*. Five of the mutable alleles are available. Four of them have been studied at the DNA level. Surprisingly, these mutations have quite different structures.

The *sh-m5933* allele carries a 30-kb insertion in an intron of the *Sh* gene. Part of the insertion and adjacent DNA is duplicated in the mutant. The 30-kb insertion is removed upon reversion to the non-mutant *Sh* phenotype, but the duplication is retained (Courage-Tebbe *et al.*, 1983). *sh bz-m4* is a double mutant, of which only the bronze allele is unstable. In this mutant, the *Shrunken* gene is deleted (Burr and Burr, 1981; Chaleff *et al.*, 1981; Döring *et al.*, 1981).

The *sh-m6258* and *sh-m6795* alleles were reported to be ~20 kb insertions into another intron. Reversion of *sh-m6795* to a phenotypic wild-type does not remove the insertion, but the restriction pattern of the inserted DNA is altered (Burr and Burr, 1982). Other interpretations of these data have been offered (Fedoroff *et al.*, 1983a).

Thus, *Ds* gave rise to different types of mutations and reversions. We therefore decided to investigate *sh-m6233*, the fifth available mutant of this series. Here we report that this mutation is caused by the insertion of an ~4-kb segment of DNA that is identical to a double *Ds* element located in the *sh-m5933* allele at the junction between the 30-kb insert and the 3′ part of the sucrose synthase gene (Courage-Tebbe *et al.*, 1983; Döring *et al.*, 1984a).

Results

DNA blotting experiments

Blotting experiments were done to find DNA rearrangements associated with the *sh-m6233* mutation. With the enzymes *Bst*EII, *Pst*I, *Sst*I as well as, after double digestion, with *Bgl*II and *Bst*EII or with *Sst*I and *Pst*I, fragments were detected that were ~4 kb larger than the corresponding fragments cleaved from the progenitor *Sh* DNA. This indicated the presence of a 4-kb insertion within the *Sh* locus in the *sh-m6233* mutant. Other enzymes cleaved within the insert. In these cases, two fragments were detected, one of which hybridized to probes derived from one side of the insertion site, and the other one of which hybridized to a probe homologous only to DNA on the other side of the insertion site. Such results were obtained with *Bam*HI, *Bgl*I, *Sph*I, *Pvu*II and *Hind*II. We will refer to the *Sh* DNA located on either side of the insertion as the 5′ and the 3′ part of the gene, depending on whether this DNA encodes the 5′ or the 3′ segment of the mRNA of the *Sh* gene (Döring *et al.*, 1983). If two fragments were found, the sum of their lengths was always smaller than the corresponding wild-type fragment with a 4-kb insertion. This indicated that all enzymes used cleave at least twice within the insert. The cleavage sites determined were always those located next to the junction with *Sh* DNA on the side from which the probe was derived. Combining these data, the map shown in Figure 1 was constructed.

Genomic cloning

In an initial attempt to clone DNA from the *sh-m6233* mutant, we obtained an insert spanning the junction between the

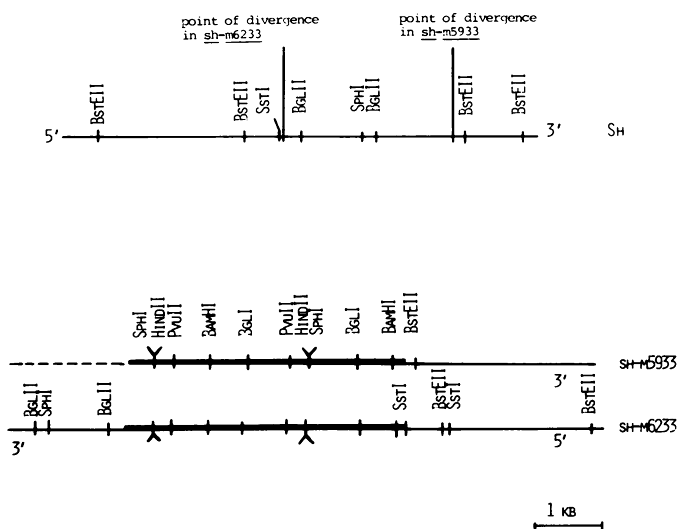

Fig. 1. The restriction maps of the *Sh* allele and of the *sh-m5933* and *sh-m6233* alleles.

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a

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WT
 ooooooooo o                                        oooooooo  oo
ACCGTGTCCC CTGATGAGCT CAAGCGCTCG CCTTAGCCGC GTCCTTGTCC CCCGCCATTT

sh-m6233
CAAGCGCTCG CCTTAGCCGC GTCCTTGTCC CTAGGGATGA AA..TTTCAT CCCTACTTGT CCCCCGCC

R 6233
CAAGCGCTCG CCTTAGCCGC GTCCTTGTCC TTGTCCCCCG CC
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b

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              5'                               3'
sh-m5933      ....TTTCATCCCTACCACTTCGGCTAGCC....

sh-m6233      ....TTTCATCCCTACCACTTCGGCTAGCC....
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Fig. 2. (**a**) The sequences at the insertion site of the double *Ds* element in the *sh-m6233* allele, its progenitor, and its revertant *Sh-m6233 r1*. The inverted repeats at the termini of *Ds* are underlined by arrows. The sequence duplication caused by the insertion is boxed. A sequence duplication existing prior to insertion is indicated by dots above the sequence. (**b**) The sequence of *sh-m5933* and *sh-m6233* are compared at the insertion site of the central *Ds* element in the double *Ds*. Symbols are as in **a**.

3′ end of the sucrose synthase gene and the inserted *Ds* element. This junction was sequenced from the *Hin*dIII site as shown in Figure 2. The *Ds* element terminates in the 11-bp sequence TTTCATCCCTA characteristic for the termini of *Ds* elements (Sachs *et al.*, 1983; Döring *et al.*, 1984a). The adjacent sequence is found in sucrose synthase DNA and is located in the first intron of the gene (W. Werr, W.-B. Frommer, and P. Starlinger, in preparation). Restriction analysis of this clone shows that it contains ~17.5 kb of maize DNA including the 3′ terminus of the *Shrunken* gene up to the junction and of 2.5 kb of *Ds* DNA. Adjacent to this fragment, the clone contains a DNA segment that hybridizes neither to sucrose synthase DNA nor to *Ds* DNA. Presumably it is an unrelated DNA fragment from the partial *Sau*3A digest. The *Ds* part of the cloned fragment contains a characteristic *Bam*HI site. The DNA sequence located near this *Bam*HI site was determined and is also shown in Figure 2.

The other junction of the *Ds* insert with sucrose synthase DNA was determined from a cloned ~14-kb *Eco*RI/*Bgl*II fragment containing the complete *Ds* element in the sucrose synthase gene. The sequence was determined from the left *Bam*HI site and is shown in Figure 2.

The 8 bp adjacent to the *Ds* element at both junctions are repeated. These 8 bp are present only once in the corresponding wild-type sequence (W. Werr, W.-B. Frommer, P. Starlinger, in preparation).

The former insertion site in the *sh-m6233-r1* revertant was sequenced from the *Sst*I site. This sequence is also given in Figure 2. The *Ds* is not present and 6 bp of the 8-bp duplication flanking the *Ds* element in the mutant are retained in the revertant.

Discussion

The *sh-m6233* allele has an insertion within the sucrose synthase gene. The insertion is located within the first intron of the gene (W. Werr, W.-B. Frommer and P. Starlinger, in preparation). The insertion site in another unstable *shrunken* allele, *sh-m5933*, is located ~2.5 kb closer to the 3′ end of the gene (Courage-Tebbe *et al.*, 1983). By restriction analysis, the insertion in the *sh-m6233* allele is ~4 kb long. It is indistinguishable from the complex *Ds* element found as part of a 30-kb insertion in the *sh-m5933* allele. In the *sh-m5933* allele, the 4-kb *Ds* element is at the junction of the 30-kb insertion and the 3′ region of the *Sh* gene. DNA sequencing showed it to be composed of two *Ds* elements, of which one is inserted into the center of the other one in inverted orientation (Döring *et al.*, 1984a).

The similarity between the double *Ds* elements in *sh-m5933* and *sh-m6233* is further supported by partial DNA sequencing of the latter. The termini of the 4-kb insertion in *sh-m6233* consist of the inverted 11-bp repeats TAGGGATGAAA characteristic of all *Ds* elements sequenced so far (Sutton *et al.*, 1984; Döring *et al.*, 1984a). The *Ds* element inserted in the alcohol dehydrogenase gene in the *2F11* mutant (Döring *et al.*, 1984b) also terminates in the same 11-bp inverted repeats (A. Merckelbach, personal communication). The internal structure of the double *Ds* in *sh-m6233* is also identical to that of the *Ds* element in the *sh-m5933* allele. The point where the centrally located *Ds* element is inserted into the recipient *Ds* element is the same in both *Ds* elements, as can be seen from the DNA sequence in Figure 2.

There is an 8-bp duplication at the site of insertion of the double *Ds* element in the *sh-m6233* allele. This, too, is characteristic of all the *Ds* elements mentioned above. A 10-bp sequence (one mismatch) is repeated at a distance of 31 bp from the insertion site in the *sh-m6233* allele. This duplication is present prior to the insertion (Figure 2). A nearby duplication was found at the insertion site of the *Ds* elements in the *sh-m5933* allele (Döring *et al.*, 1984a) and in the *Adh1-Fm335* allele (Sutton *et al.*, 1984). This might indicate an integration specificity of *Ds* elements.

In other respects, however, the two mutants differ. While the *sh-m6233* mutation carries an insertion of the double *Ds* element by itself, the double *Ds* element in the *sh-m5933* allele is part of a larger insertion. The orientation of these two double *Ds* structures with respect to the sucrose synthase gene is also inverted in the two mutants. We conclude that the double *Ds* element is itself transposable and can give rise to different insertion mutations.

Upon reversion, the 8-bp duplication generated when the *Ds* element inserted is neither retained nor eliminated. A duplication of 6 bp is present at the original insertion site. The 6-bp duplication probably represents part of the original 8-bp duplication and arose by an imperfect excision of the *Ds* element.

Retention of the duplication created by *Ds* insertion, but in a mutated form was first observed by Sachs *et al.* (1983) in a series of revertants of the *Fm335* allele of the *Adh1* gene. The same structure was found in a revertant of the *wx-m9* allele (Pohlman *et al.*, 1984). The phenomenon is not confined to *Ds*, as it was also found in a revertant of a *Tam1*-caused mutation of the *nivea* gene encoding chalcone synthase in *Antirrhinum majus* (Bonas *et al.*, 1984).

A mechanism allowing the decrease in size of the duplication from 8 to 6 bp will allow the phenotypic reversion of insertions within exons, avoiding frame-shifts but adding two amino acids to the protein. This may be an interesting mechanism for protein evolution. Altered proteins were observed after excision of *Ac* or *Ds* from the *Bz* and *Wx* genes (Dooner and Nelson, 1979; Echt and Schwartz, 1981; Shure *et al.*, 1983). In the *sh-m6233* allele, as in the case of the other mutations in the sucrose synthase caused by *Ds* insertion, the *Ds* element was located in an intron, and even the retention of the 8-bp duplication would probably not have been detrimental to the expression of sucrose synthase in the

revertants. *Ds* insertions can occur in exons, however, as in the case of *adhl 2F11* (Döring *et al.*, 1984b; Merckelbach, unpublished observations).

The presence of the double *Ds* element in two independent *shrunken* mutations raises the question of its origin. The *sh-m6233* allele is derived from strain *Ds4864A*, while the *sh-m5933* allele originated in strain *Ds5245* (McClintock, 1952). In both strains, *Ds* is located very near the *Sh* locus, in the interval between the *C* and *Sh* loci. Both of these lines are derived from a common progenitor carrying *Ds* in the standard position between the *Wx* locus and the centromere, where *Ds* was first mapped by McClintock (1947, 1949). In the cultures designated *Ds4864A* and *Ds5245*, the *Ds* element had been removed from the standard position and McClintock inferred that it had transposed to a new location in the vicinity of *Sh*.

The similarity in the structure of the double *Ds* elements in the *sh-m5933* and *sh-m6233* alleles suggests that the double *Ds* element did not arise during the last transposition event, but already existed in the strain that gave rise to the *Ds4864A* and *Ds5245*. We must consider this argument with some caution, however, as it is now known that *Ds* elements are present in many copies within the maize genome (Geiser *et al.*, 1982; Fedoroff *et al.*, 1983b; Sachs *et al.*, 1983). Caution is also indicated in view of the results of studies on two *Ds* insertions in the alcohol dehydrogenase gene which were both derived from the same progenitor strain carrying only one Ds element by genetic criteria. This *Ds* element was located on the same chromosome in the *Bz2* gene. The two *Ds* insertions in the alcohol dehydrogenase gene have quite different DNA structures, however, and may thus be derived from different *Ds* elements of which only one or neither might have originated from the *Bz2* gene (Sachs *et al.*, 1983; Döring *et al.*, 1984b).

Ds elements located in the standard position proximal to the *Wx* gene on chromosome 9, as well as in the mutable sucrose synthase alleles cause chromosome breakage. McClintock designated these elements *Dissociation (Ds)*, because *Ds*-induced breaks were discovered before *Ds*-induced mutations of particular genes or the phenomenon of transposition was known. Later, McClintock discovered that the element *Ac* is required to cause both chromosome breaks at *Ds* and *Ds* transpositions.

McClintock (1965) suggested that *Ds* is derived from *Ac* by mutation. For *Ds* elements in the *Shrunken* and *Waxy* genes it was shown by DNA analysis that they are internal deletions of *Ac* (Fedoroff *et al.*, 1983a, 1983b; Döring *et al.*, 1984a). We therefore expect that *Ac* causes the same biological effects as *Ds* does in the presence of *Ac*. This is not true in the case of chromosome breaks, however. The *Ac wx-m9* mutant does not cause chromosome breaks (McClintock, 1963), and *Ac*-dependent chromosome breakage is observed rarely or never in other situations (McClintock, personal communication). Chromosome breaks are not associated with all *Ds* elements either. McClintock distinguished a state I of *Ds*, causing frequent chromosome breaks, from state II causing chromosome breaks rarely (McClintock, 1949).

The double *Ds* element differs from *Ac* in three features. (i) In the double *Ds* element, the terminal inverted repeats are perfect. In the *Ac* element sequenced so far, the outermost nucleotides of the inverted repeats are not complementary (Döring *et al.*, 1984a; Sutton *et al.*, 1984; Pohlman *et al.*, 1984; J. Yoder, unpublished experiments). It is not easily seen, how this difference could be responsible for breaks. (ii) A 2-kb inversion could pair in sister strand exchanges giving rise to U-shaped dicentrics and acentrics upon recombination. Such events were observed in yeast as a consequence of the insertion of the 2 μ plasmid (which carries a pair of 0.6-kb inverted sequences) into chromosomes (Falco *et al.*, 1982). The involvement of *Ac* in the production of *Ds*-induced breaks is not easily explained by this hypothesis. (iii) Two pairs of the 11-bp inverted repeats are present in double *Ds*. Thus, two of these sequences each form direct repeats (and are thus inverted relative to the normal situation). Recognition of the direct, instead of the 11-bp inverted repeats could cause an *Ac*-dependent break. This possibility appears most plausible to us.

It will be interesting to see whether chromosome breaks are produced in situations where a *Ds* element is known not to be a double *Ds*. Mutants *wx-m6, wx-m9* and the *Ds* insertions in the alcohol dehydrogenase *Fm335* and *2F11* are cases in point. Due to the fact that many more *Ds* copies are present in the cells than are known from genetic effects, it will be important in these cases, however, to show that breaks are produced at the site of the *Ds* in question and not at some other site on the same chromosome.

Sh-m6233 r1 still shows chormosome breakage. Is the *Ds* of *sh-m6233* transposed to a nearby position? If it were true, this revertant would differ from revertants of *sh-m5933*. In these cases, breaks of chromosome 9 are due to the persistence of a large duplication containing *Ds*. The 30-kb insert, however, was not detected in the DNA of the revertants when blotting experiments were carried out with unique sequences isolated from the insert (M. Motto, unpublished experiments).

In summary, *sh-m6233* differs from the other mutations caused by *Ds* insertion in the sucrose synthase gene as well as from the *Ds* insertions in the alcohol dehydrogenase and *Waxy* genes (Burr and Burr, 1982; Fedoroff *et al.*, 1983a; Sachs *et al.*, 1983; Courage-Tebbe *et al.*, 1983). *Ds* is thus capable of giving rise to a variety of different mutations.

Materials and methods

Seeds of *C sh-m6233* were obtained from J. Mottinger. Seeds carrying *Ds* in standard position and *Sh* (McClintock, 1951) were used as a source of the progenitor *Sh* allele. Plants were made homozygous by selfing. Homozygosity for *Ds* was verified by crosses to a tester strain homozygous for both *c* and *Ac*. The F1 was scored for kernel color variegation caused by chromosome breaks at *Ds* and consequent losses of *C* on the acentric fragment produced by the break. The *Sh-m6233 r1* revertant was selected as a single non-shrunken kernel from an ear that was heterozygous for the *sh-m6233* allele and an *sh* deletion mutation (Mottinger, 1970; McCormick *et al.*. 1982). The non-shrunken phenotype proved heritable and the chromosome carrying the revertant allele showed chromosome breakage, as judged by the loss of dominant markers, in the presence of an *Ac* element.

Preparation of DNA and DNA blotting experiments

These experiments were done as previously described (Döring *et al.*, 1981; Geiser *et al.*, 1982).

The restriction endonucleases

These enzymes were obtained from Boehringer Mannheim; BRL, Neu-Isenburg; and Biolabs, Dreieich. They were used as recommended by the suppliers.

Cloning of sh-m6233 from genomic DNA

In the first experiment, a partial *Sau*3A digest was cloned in lambda 1059 (Karn *et al.*, 1980). In the second experiment, *sh-m6233* DNA was digested to completion with *Eco*RI and *Bgl*II. Botting to a sucrose synthase DNA probe showed the mutated gene to be located on a 14-kb fragment. To clone this fragment with two restriction ends, two aliquots of EMBL 4 DNA (Lehrach, Frischauf, Ponstka and Murray, personal communication) were digested with *Eco*RI and *Bam*HI, respectively. The preparations were mixed with each other and with the digested maize DNA, ligated and processed further as previously described (Geiser *et al.*, 1982).

Sucrose synthase DNA from the revertant Sh-m6233-r1

This was cloned as a 16.5-kb *Bam*HI fragment in *Bam*HI-digested EMBL 4 DNA.

DNA sequencing

This was done by the chemical degradation method (Maxam and Gilbert, 1977).

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