Genomic clones of a wild-type allele and a transposable element-induced mutant allele of the sucrose synthase gene of *Zea mays* L.

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In an attempt to isolate the transposable genetic element *Ds* from *Zea mays* L., we cloned DNA fragments hybridizing to a cDNA clone derived from the sucrose synthase gene in a λ vector (λ::*Zm Sh*). The fragments cloned from wild-type and from the *Ds*-induced mutant *sh-m5933* (λ::*Zm sh-m5933*) share a segment 6 kb long while a contiguous segment of 15 kb of λ::*Zm sh-m5933* (mutant-derived DNA) does not hybridize to the DNA segment cloned from the wild-type. Restriction maps are given, and the junction point between the two DNA segments in the mutant clone was determined. Hybridization of DNA fragments, present in the wild-type DNA of λ::*Zm Sh*, but not in the mutant clone, λ::*Zm sh-m5933*, to genomic DNA of *sh-m5933* showed that no part of this DNA is deleted. It cannot be said whether the DNA found in the mutant, but not in the wild-type clone, has been brought there by *Ds* insertion or by another *Ds*-dependent DNA rearrangement. The mutant-derived DNA was hybridized to genomic DNA of various maize lines digested by several restriction endonucleases. Approximately 40 bands were detected. The mutant-derived DNA contains two pairs of inverted repeats several hundred nucleotide pairs long, one of which is located at the junction to wild-type-derived DNA.
Key words: endosperm/maize/shrunken gene/sucrose synthase/transposable element *Ds*

Introduction

The transposable element *Ds* (dissociator) which undergoes transposition under the *trans*-acting influence of another transposable element *Ac* (activator) is one of the most thoroughly investigated transposable genetic elements in maize (McClintock, 1951, 1956, 1965). It can integrate into genes or into their vicinity, thereby causing null mutations as well as less severe decreases in gene expression. Several *shrunken (sh)* mutants on the short arm of chromosome 9 have been isolated and shown to be caused by *Ds* which maps near the gene (McClintock, 1952, 1953). The *Shrunken* gene encodes endosperm sucrose synthase (Chourey and Nelson, 1976). No enzyme encoded by this gene is detected in mutants *sh-m5933*, *sh-m6233*, and *sh bz-m4*. The former two mutants frequently revert phenotypically to the wild-type in the presence of *Ac*, while the double mutant *sh bz-m4*, which lacks both sucrose synthase and the product of gene *Bronze*, UDPG-flavonoid glucose transferase (Dooner and Nelson, 1977), reverts to expression of the transferase gene, but not with respect to the expression of sucrose synthase.

As the unstable *shrunken* mutants are caused by the presence of *Ds* in the *Shrunken* gene or in its vicinity, they might provide a suitable material for the isolation of *Ds* DNA. Cloning of sucrose synthase DNA from the wild-type and one of the mutants might reveal DNA present in the latter but not in the former. This mutant-derived DNA might be *Ds* DNA.

cDNA clones obtained from sucrose synthase mRNA that can serve as hybridization probes for the identification of λ clones containing genomic DNA were described (Geiser *et al.*, 1980; Burr and Burr, 1981a; Chaleff *et al.*, 1981). Restriction maps of the DNA surrounding the region complementary to the cDNA were constructed by Southern blotting (Burr and Burr, 1981b; Chaleff *et al.*, 1981; Döring *et al.*, 1981). The restriction maps obtained from wild-type and several mutants coincide on one side of the map, but differ from each other on the other side of the map. They thus support the notion that the mutation is caused by a DNA rearrangement rather than by a point mutation. DNA obtained from the double mutant *sh bz-m4* failed to hybridize to the cDNA clone, supporting the hypothesis that the mutation is a deletion of the *Shrunken* gene extending from the site of insertion of *Ds* beyond gene *Shrunken* to gene *Bronze*. The hypothesis that mutants *sh-m5933* and *sh-m6233* are also caused by deletions adjacent to *Ds*, rather than by transposition of this element into gene *Sh*, was discussed by Döring *et al.* (1981).

We report here the cloning of a 16.3-kb *Bam*HI fragment from *Sh* maize and a 21-kb *Bcl*I fragment from mutant *sh-m5933* in the λ vector *1059* (Karn *et al.*, 1980).

## Results

Genomic DNA clones in λ*1059*

We showed previously (Geiser *et al.*, 1980; Döring *et al.*, 1981) that the *Sh* maize DNA hybridizing to the sucrose synthase cDNA clone pKS500 is located on a *Bam*HI fragment. We cloned this fragment from genomic DNA after enrichment of the appropriate size fraction by salt gradient centrifugation. λ*1059* (Karn *et al.*, 1980) was used as a vector. The maize DNA insert was subcloned in pBR322, plasmid DNA was prepared and a restriction map constructed which is shown in Figure 1a. Its size was determined as 16.3 kb.

For reasons still unknown, *Bam*HI digestion of DNA prepared from mutant *sh-m5933* does not yield bands clearly visible upon blotting analysis. A hybridizing band of ~21 kb was detected, however, after digestion with *Bcl*I. As *Bam*HI and *Bcl*I produce identical sticky ends, we could clone this *Bcl*I fragment in λ*1059*.

As expected, the *Bcl*I maize DNA insert could not be excised from the *Bam*HI sites of λ*1059* DNA. Comparison of the wild-type and mutant restriction maps (Figure 1a, b) showed that the segment of 6 kb located at the right of the insert of *sh-m5933* DNA corresponds to a 6-kb segment in the DNA cloned from *Sh*. This segment extends from the right-most *Bcl*I site to the point of divergence between wild-type and mutant DNA as deduced from the restriction maps of genomic DNA. The 15-kb segment located at the left side of the

16,3 kb BamHI fragment of λ::Zm Sh

A

point of divergence
in sh-m5933

21 kb BclI fragment of λ::Zm sh-m5933

B

point of divergence
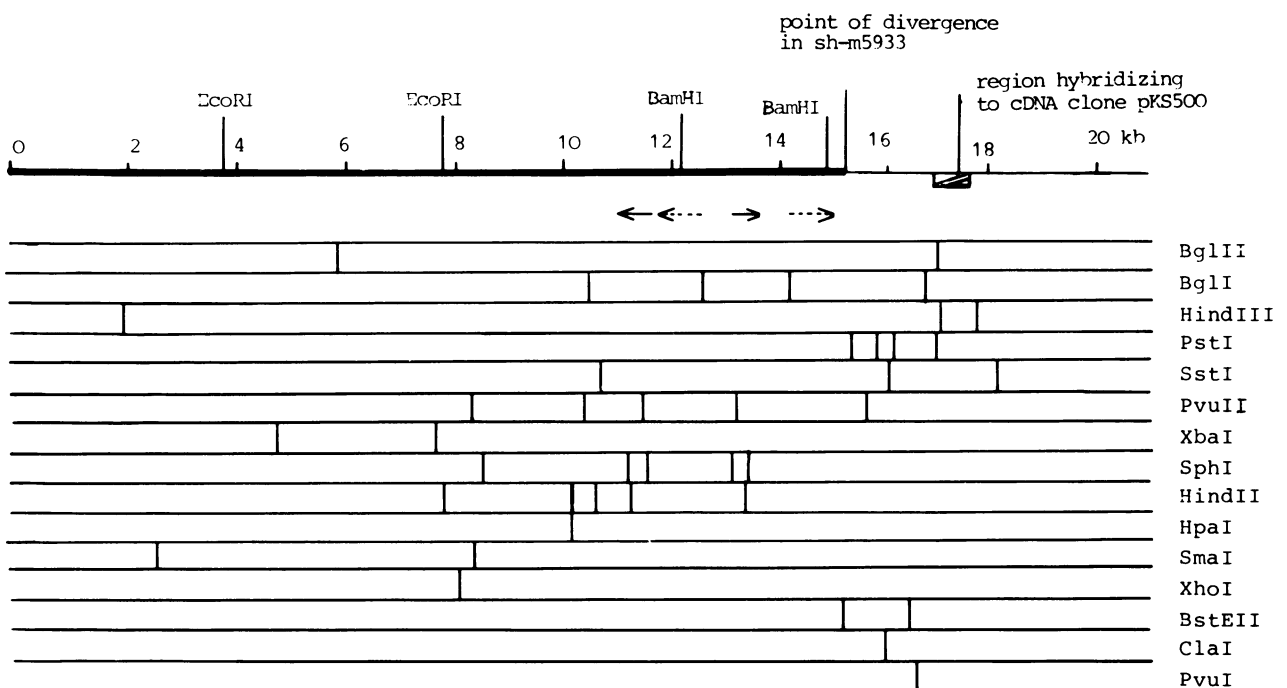in sh-m5933
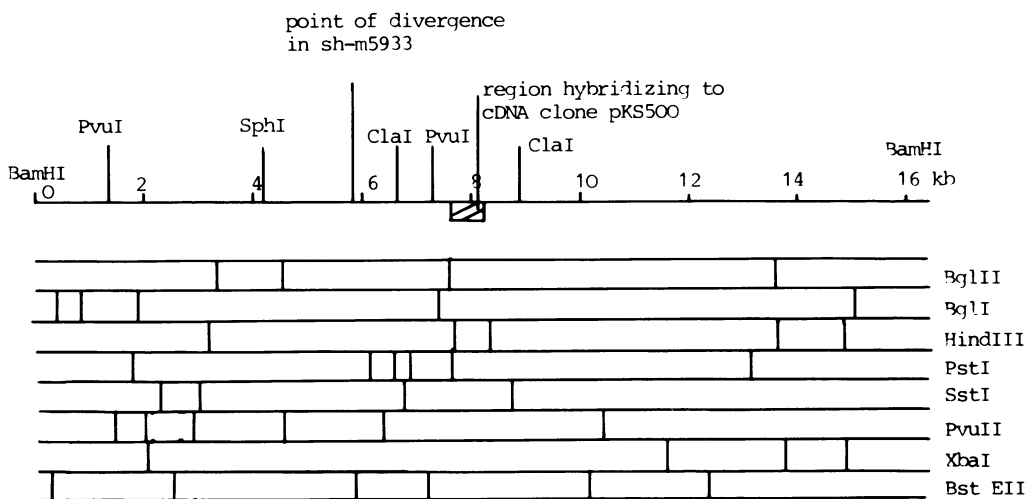
Fig. 1. Restriction maps of cloned DNA segments obtained from *Sh* (**A**) and *sh-m5933* (**B**). The maps were constructed using single and double digests separated on agarose gels. Fragments smaller than 200 bp may have escaped detection. (**A**) a 0.25-kb *Bgl*II fragment and a 0.3-kb *Pvu*II, 0.2-kb *Xba*I fragment were detected but not placed on the map. On the right part of the map, five unmapped *Sst*I sites are present. (**B**) This map is incomplete with regard to sites to the left of the *Eco*RI site at coordinate 7.8 and to the right of the *Hind*III site at coordinate 17.2. The direction of transcription (5' to 3') is from left to right (Döring *et al.*, 1982).

sh-m5933 insert does not hybridize to the wild-type clone. The junction between common DNA and mutant-derived DNA is located on a 2.25-kb *Hind*III/*Bam*HI fragment, adjacent to which are located a 2.7-kb *Bam*HI fragment, a 4.4-kb *Bam*HI/*Eco*RI fragment, and a 4.0-kb *Eco*RI fragment (Figure 1b). These four fragments were subcloned in pBR322, plasmid DNA was prepared and used for the construction of a more detailed restriction map.

The two maps obtained from wild-type and mutant DNA cloned in λ*1059* coincide with the maps obtained by Southern blotting of genomic DNA at those sites that could be determined by the latter method (Döring *et al.*, 1981), with the exception of the *Bam*HI sites that cannot be detected in genomic DNA (see above). The genomic maps differ from the cDNA clone by the presence of a *Hind*III site ~200 bp to the right of the *Bgl*II site in the former but not in the latter.

A B C D

2200 —

1200 —
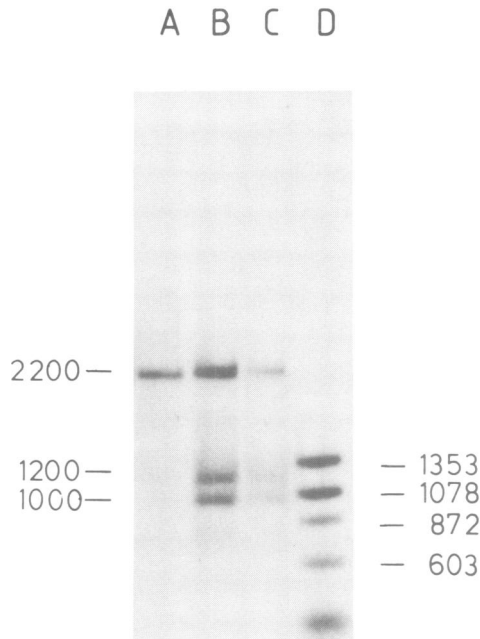1000 —

— 1353
— 1078
— 872
— 603

Fig. 2. Estimation of the divergence point between the DNAs cloned from wild-type and mutant *sh-m5933* by digestion of a DNA heteroduplex with endonuclease S1. (**A**) a 2.2-kb *BglII/ClaI* (~50 μg) fragment from wild-type labeled at the *ClaI* 5' end treated as in **lane B** in the absence of mutant DNA fragment. (**B**) a 2.2-kb *BglII/ClaI* fragment and 1.2-kb *ClaI/BamHI* fragment from mutant *sh-m5933* mixed at a ratio of 1:3, denatured, renatured and incubated for 30 min at 25°C with 10 U endonuclease S1. (**C**) Same as lane B, but 1 h S1 digestion. (**D**) *HaeIII*-digested φX174 DNA.

1 2 3   4 5   6 7

Bgl II   Bgl II   Cla I   Bgl II

Fig. 3. Blot hybridization of *BglII/EcoRI*-digested *Sh* and *sh-m5933* DNA. Lanes 1, 5, and 7: *Sh* DNA. Lanes 2, 3, 4, and 6: *sh-m5933* DNA. Lanes 1, 2, and 3 probed with the 1.1-kb *BglII* fragment (see Figure 1a). Lane 2: *sh-m5933* DNA obtained from N. Fedoroff. Lanes 4 and 5 probed with the 2.4-kb *BglII/ClaI* fragment (left end of 3.3-kb *BglII* fragment). Lanes 6 and 7 probed with the 0.9-kb *ClaI/BglII* fragment (right end of 3.3-kb *BglII* fragment). The insert shows the same experiment as lane 4, but the DNA was electrophoresed for 24 h in order to clearly resolve the ~9.5-kb band into two bands.

This *Hind*III site is located in an intron of 162 bp, as determined by DNA sequence analysis (W.B. Frommer and W. Werr, unpublished data).

To demonstrate identity between the two clones in the region extending from the right-most *Bgl*II site in maize DNA up to the junction with λ DNA (where only a few restriction sites are located on the maps given in Figure 1), we first showed that the 6.0-kb *Bgl*II fragment prepared from wild-type DNA does hybridize to undigested λ *Zm::sh-m5933* DNA. Secondly, we digested the above *Bgl*II fragment and a 5-kb *Bgl*II fragment from cloned *sh-m5933* DNA extending into the right λ arm with *Hpa*II, labeled them at their termini with ^{32}P and separated them on two adjacent lanes on a polyacrylamide gel. We observed six and four fragments, respectively, of which three migrated identically. According to the restriction maps, the junction point is located between the *Bam*HI site and the left-most *Bst*EII site on the 2.25-kb *Bam*HI/*Hind*III fragment. In the wild-type DNA, this *Bst*EII site is located on a 2.2-kb *Cla*I/*Bgl*II fragment. To localize the junction point more accurately, we prepared heteroduplex molecules between one strand of this fragment, labeled with ^{32}P, and a 1.2-kb *Cla*I/*Bam*HI fragment derived from the mutant. The latter was used in 3-fold excess. The heteroduplex DNA was digested with endonuclease S1, separated on agarose by electrophoresis, and autoradiographed. In Figure 2 two new bands are seen: 1.0 and 1.2 kb. We interpret the 1.0-kb fragment as extending from the *Cla*I site to the junction point, while the 1.2-kb band corresponds to the length of the mutant fragment and can be explained as a side product of the reaction. This places the junction point ~200 bp to the right of the *Bam*HI site.
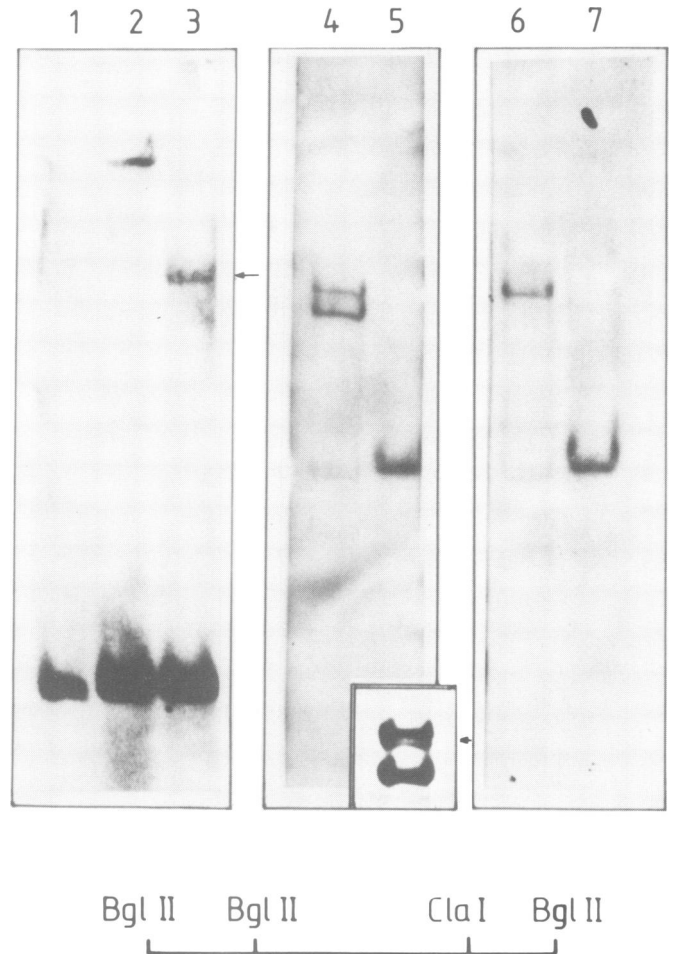
The structure of the sucrose synthase gene in sh-m5933

The DNA segment located on the 3' side of the junction in the wild-type is also present in genomic *sh-m5933* DNA. This is seen in blotting experiments using, as a probe, a 0.9-kb *Cla*I/*Bgl*II fragment located entirely on the 3' side, and either *Bgl*II or *Bgl*II/*Eco*RI-digested genomic DNA. An ~11-kb band and a ~9.5-kb fragment are detected, respectively. These bands migrate identically with the *Bgl*II and *Bgl*II/*Eco*RI junction fragments obtained from the cloned *sh-m5933* DNA, when they were separated side by side on the same gel (data not shown).

When the 1.1-kb *Bgl*II wild-type fragment located on the 5' side of the junction is used, a fragment of the same size is detected in genomic *sh-m5933* DNA. When the 2.4-kb *Bgl*II/*Cla*I fragment spanning the junction is used, the fragments observed with the 3' probe after *Bgl*II and *Bgl*II/*Eco*RI digestion are seen. In addition, an ~11-kb *Bgl*II and an 8.2-kb *Bgl*II/*Eco*RI fragment, respectively, are detected which do not hybridize to the 3' probe. These fragments probably span the 5' junction of the DNA rearrange-
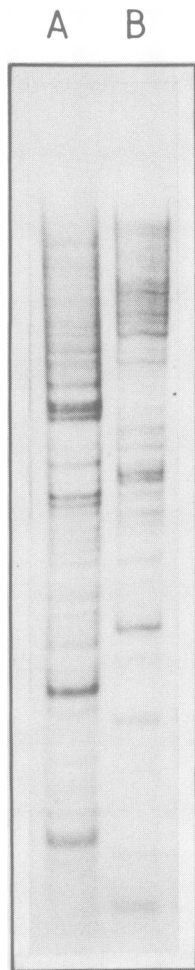
Fig. 4. Hybridization of subfragments of the genomic DNA cloned from *sh-m5933* to genomic DNA digested with *Bam*HI (A) and *Eco*RI (B). The maize DNA preparation was obtained from an unfertilized ear of a plant from a cross of *W22* with a *bz2-m* mutant obtained from D. Schwartz, Bloomington, USA. Similar patterns were observed with other maize DNAs (see Figure 5). In many experiments, however, the resolution of the bands was less satisfactory after autoradiography and subsequent photography. We thank K. Theres for this blot.

Fig. 5. DNA by different maize lines, digested with *Bam*HI, separated by electrophoresis on an agarose gel and hybridized to nick-translated pBR322 DNA carrying, as an insert, the 2.7-kb *Bam*HI segment of λ::*Zm sh-m5933* DNA. **A**: *sh-m6233*, **B**: *sh-m5933*, **C**: *sh-m6795*, (another mutable *shrunken* allele obtained from B. McClintock), **D**: *bz-m4*, **E**: *Sh*, **F**: *sh* (a stable *shrunken* allele obtained from B. McClintock).

ment present in *sh-m5933*.

Qualitatively, these experiments show unambiguously that the DNA located on the 5′ side of the junction in the wild-type is not deleted in the mutant. Our attempts to construct a restriction map of the DNA carrying the 5′ junction or to estimate the size of the DNA rearrangement have not been successful yet. This is mainly due to the reproducible appearance of additional bands (Figure 3, arrows) hybridizing both to the 5′ probe and to the junction-spanning probe. These bands are observed in three different DNA samples prepared from different plants in two years. N. Fedoroff (personal communication) did not detect the additional DNA fragment (arrow) with the 5′ probe in *sh-m5933* DNA, and we confirmed this with a DNA sample kindly given to us (Figure 3, lane 2).

The mutant-derived DNA cloned from sh-m5933 contains two pairs of inverted repeats

We found that a region of at least 300 bp bounded by two *Sph*I sites, and containing one *Hind*II site and one *Pvu*II site is located both on the 4.4-kb *Eco*RI/*Bam*HI fragment and on the 2.7-kb *Bam*HI fragment. The subfragments obtained by
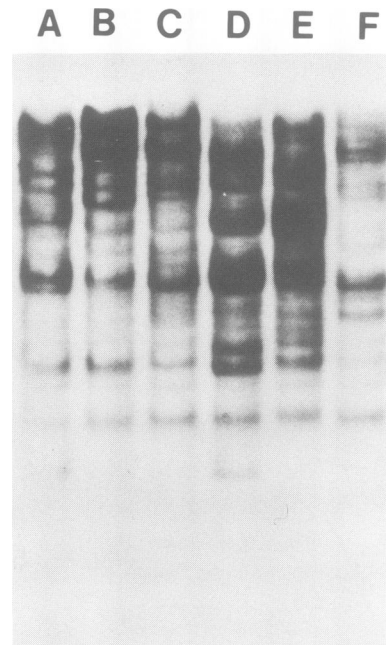
appropriate double digests from both regions are of identical size, and blotting analysis shows hybridization between these two fragments in the regions bounded by the two *Sph*I sites (data not shown). The two fragments are inverted with respect to one another. Another pair of inverted repeats includes the *Bam*HI sites separated by 2.7 kb on cloned *sh-m5933* DNA. This was first seen by hybridization experiments. The 4.4-kb *Eco*RI/*Bam*HI fragment hybridizes to the 2.25-kb *Bam*HI/*Hind*III fragment. The right and left halves of this *Bam*HI fragment hybridize to each other. The presence of inverted repeats was confirmed by DNA sequence determinations around the *Bam*HI sites. The right member of this pair of repeats terminates at the junction point to wild-type DNA (unpublished results). The positions of the two pairs of inverted repeats are shown in Figure 1b.

Several bands of wild-type and mutant genomic DNA hybridize to the putative Ds DNA cloned from sh-m5933

The DNA present in the clone from *sh-m5933* DNA but not in the wild-type clone is possibly *Ds* DNA. We were interested to see whether this DNA is unique, or present in more than one copy in the genomic DNA. The 2.7-kb *Bam*HI fragment internal to the putative *Ds* DNA, was labeled with ^{32}P. The probe was hybridized to genomic DNA. The autoradiogram shows ~40 bands (Figure 4). Comparison of DNA samples from different mutants show similarities as well as differences (Figure 5).

Discussion

The two DNA fragments cloned in λ*1059* show a restriction pattern in agreement with the respective genomic DNAs in most of the sites that could be determined in the latter. This excludes major cloning artifacts.

A discrepancy is seen after *Bam*HI digestion of *sh-m5933*

DNA. The two *Bam*HI sites found in the cloned DNA predict a *Bam*HI fragment, including the DNA hybridizing to the cDNA clone pKS500, of ~ 11 kb; this is not observed. As the DNA of *sh-m5933* cloned in λ*1059* does not extend far enough to the right, we cannot investigate as yet the reason for this discrepancy. Either the right *Bam*HI site seen in the wild-type is missing from *sh-m5933* DNA due to restriction polymorphism, or else the *Bam*HI sites detected in the cloned DNA cannot be cleaved in genomic DNA. The former possibility is not unlikely. Even the two wild-type lines with the genotypes *C-I Sh Bz Wx Ds* and *C Sh Bz Wx Ds*, differ in the location of one *Eco*RI site, as was seen in the blotting experiments reported by Burr and Burr (1981a) and by us (Döring *et al.*, 1981).

The hybridization experiments clearly show that the DNA cloned from *sh-m5933* is divided into two segments of ~ 6 kb and 15 kb, respectively. The 6-kb segment is homologous to a segment of the same length contained in the DNA cloned from the wild-type, and extends from the right *Bcl*I site to the junction point. The identity of the restriction maps of these segments shows that the two DNA fragments are derived from the same DNA sequence not yet altered by restriction polymorphism. This supports the assumption that the cloned DNAs are derived from the DNA region containing the endosperm sucrose synthase gene.

The 15-kb segment located to the left of the junction point does not share any homology with the DNA cloned from the wild-type. The junction point was mapped by S1 digestion and is located ~ 200 bp to the right of the *Bam*HI, at the end of the *Bam*HI/*Hind*III fragment in *sh-m5933* DNA. We had previously assumed that the mutation causing the shrunken phenotype in *sh-m5933* would turn out to be a deletion (Döring *et al.*, 1981) and that the endpoint of this deletion would be located outside of the coding region. Our hybridization experiments rule out this possibility at least for *sh-m5933*. In the absence of DNA clones containing the left junction as well as the complete DNA between the junctions, the structure of the DNA rearrangement in *sh-m5933* cannot be elucidated definitely. A few conclusions can be derived, however.

(1) In the genomic DNA of *sh-m5933*, no deletion was detected. As in *sh bz-m4* a considerable segment of DNA including the 1.1-kb *Bgl*II fragment and the 3.3-kb *Bgl*II fragment is deleted (data not shown), the two mutants must have originated by a different mechanism.

(2) We have not been able to decide whether the appearance of more than one band hybridizing to the 5′ probe or more than two bands hybridizing to the junction-spanning probe of the junction is due to non-random partial cleavage of the DNA or to the duplication of parts of it. Because of this uncertainty, and because of differences between our restriction maps and those reported by Burr and Burr (1982), we cannot, at present, support their conclusion that *sh-m5933* is caused by the insertion of a DNA segment of 22 kb. Our restriction data agree (apart from the anomolous band) with those obtained by N.Fedoroff (personal communication).

Hybridization of the 2.25-kb junction fragments to genomic DNA revealed ~ 20 − 40 bands (data not shown). This indicates that the cloned DNA is neither unique DNA nor repetitive DNA placed near the *Shrunken* gene by a rearrangement. We are aware that at present we cannot be sure whether the DNA placed next to the sucrose synthase gene in the cloned *sh-m5933* DNA is (part of) *Ds*. If this DNA is *Ds* DNA, its hybridization to several bands indicates

the presence of several copies of *Ds*. A similar number of bands is detected, when the internal 2.7-kb *Bam*HI fragment is used as a probe and the genomic DNA is digested by the same enzyme. This indicates heterogeneity of different copies of *Ds*. Heterogeneous transposable elements have been described in yeast (Cameron *et al.*, 1979, Roeder *et al.*, 1980) and in *Drosophila* (Potter, 1982; Rubin *et al.*, 1982).

Materials and methods

Radioactive compounds

The radioactive compounds used were purchased from Amersham-Buchler, Braunschweig.

Enzymes

The enzymes used for cloning, e.g. restriction endonucleases and T4 DNA ligase were purchased from Biolabs, Dreieich, Boehringer, Mannheim, and BRL, Neu-Isenburg. They were used as recommended by the suppliers. S1 was from Sigma, München.

Microorganisms

The vector phage λ*1059* was propagated and used for cloning as described by Karn *et al.* (1980). It was kindly provided by J. Karn. The vector plasmid pBR322 (Bolivar *et al.*, 1977) and its derivative pKS500, carrying an insert of endosperm sucrose synthase cDNA from maize (Geiser *et al.*, 1980) were propagated on *Escherichia coli* K12 strains, usually HB101. λ*1059*, *E. coli* Q358 and Q364 were as described (Karn *et al.*, 1980) and were obtained from J.Karn. *E. coli* lysogens BHB2690 and BHB2908 were obtained from B. Hohn (Hohn, 1979).

Microbiological methods

The methods used were as described by Miller (1972).

Growth of maize and preparation of genomic DNA

The methods used were decribed previously (Döring *et al.*, 1981). Mutant *sh-m5933* was originally obtained from B. McClintock as a heterozygote *C-I Ds sh-m5933 Bz Wx / C sh bz wx*. A copy of *Ac* originally present at the tip of chromosome 9 was lost or inactivated, as the kernels show no variegation due to breaks at *Ds*, leading to loss of *C-I* and therefore to the appearance of coloured regions on a colourless background.

We selfed plants derived from these kernels and chose kernels homozygous for *C-I*, as shown by the lack of segregation of seed colour in F₂. This test does not exclude heterozygosity *sh-m5933/sh*, due to recombination in the interval *C-I - Sh.*, We have not verified homozygosity genetically for *sh-m5933* for all of the plants used for DNA preparation, due to the lack of appropriate tester strains (which did not grow in all years in our climate). In those cases, we verified homozygosity biochemically. Digestion of the DNA with *Eco*RI and hybridization of Southern blots to the cDNA clone (Geiser *et al.*, 1980) gave only one band of the expected size instead of two bands characteristic of *sh-m5933* and *sh* DNA from a heterozygote.

Plasmid DNA

Plasmid DNA was prepared in small amounts by the method of Birnboim and Doly (1979), and in larger quantities by the method of Clewell and Helinski (1969).

DNA fractionation

This was performed on a 5 − 20% linear NaCl gradient in 10 mM Tris pH 8.0, 5 mM EDTA in an SW40 Ti rotor at 35 000 r.p.m. for 4 h at 4°C. Fractions were collected, ethanol precipitated, and aliquots were subjected to blotting analysis with the cDNA clone of sucrose synthase mRNA as a probe, in order to select fractions enriched for the fragment to be cloned.

Cloning in λ1059

λ*1059* was cloned as described by Karn *et al.* (1980). The strains described by the authors were used.

In vitro packaging of λ DNA

The packaging was carried out as described by Enquist and Sternberg (1979), with minor modifications (Blattner, personal communication).

Nick-translation

This procedure followed the protocol of Rigby *et al.* (1977).

Plaque hybridization

This was performed according to Benton and Davis (1977).

Blotting analysis used the method of Southern (1975) with the modification of Botchan *et al.* (1976) and Wahl *et al.* (1979), but 5 x SSC without formamide was used at 68°C.

Estimation of the divergence point between wild-type and mutant 5933

The 7.1-kb *Cla*I fragment from wild-type was labeled with T4 polynucleo-tide kinase (Boehringer), cleaved with *Bgl*II, and the 2.2-kb *Cla*I/*Bgl*II fragment isolated on a low melting 1% agarose gel (Seaplaque, Marine Colloids). This fragment was mixed with the 1.2-kb *Bam*HI/*Cla*I fragment from mutant *sh-m5933*, isolated from the 2.25-kb subclone, in a ratio of ~3:1, denatured (5 min, 80°C) and renatured (temperature gradient 55 – 35°C over 4 h) in 70% formamide 0.3 M NaCl. 0.006 M EDTA 0.015 M Tris/HCl pH 8.5. The digestion with S1 endonuclease was carried out as described by Berk and Sharp (1978). The products were electrophoresed on a 1% agarose gel and autoradiographed after drying.

Recombinant DNA

All recombinant DNA was used in a P2 facility according to the rules laid down by the Zentralkommission für die Biologische Sicherheit.

Acknowledgements

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