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MOVABLE GENETIC ELEMENTS

DETECTION OF CHANGES IN MAIZE DNA AT THE *Shrunken* LOCUS DUE TO THE INTERVENTION OF *Ds* ELEMENTS

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This report describes our initial attempts at the molecular characterization of a maize controlling element. We have prepared a cDNA probe and used it to detect changes at a locus where Ds elements are found. Evidence of their presence are indicated by changes in the restriction patterns, but there is as yet no information on the physical nature of the controlling elements nor on the kinds of rearrangements they cause. It should be remembered that Barbara McClintock provided extensive evidence for the physical presence of controlling elements in the 1951 Cold Spring Harbor Symposium.

The endosperm, which constitutes the major portion of the maize kernel, is primarily made up of starch and storage protein. Genetic lesions which interfere with these major biosynthetic pathways have readily observable morphological effects. One of the mutants affecting the accumulation of starch has a collapsed kernel phenotype called shrunken. (In maize genetic nomenclature Sh denotes a wild-type allele, while sh denotes recessive mutant alleles.) The locus is genetically defined by a recessive allele discovered on an Indian reservation in Nebraska (Hutchison, 1921). Schwartz (1960) found that the mutant seed lacked a major soluble protein. Induced electrophoretic variants of the protein were described by Chourey and Schwartz (1971). Finally, Chourey and Nelson (1976) showed that this protein was the enzyme sucrose synthetase. The enzyme carries out the physiological reaction: sucrose + UDP $\longrightarrow$ UDP glucose + fructose. Sucrose entering the kernel from the leaves is broken down to a nucleotide diphosphate glucosyl donor which is indirectly used in starch synthesis. When this reaction is blocked, starch synthesis is inhibited and this results in the shrunken phenotype. Controlling elements have been described as being associated with the locus and, as mentioned, the locus makes an abundant gene product. It therefore seemed to provide a highly accessible system by which a cloned probe might be prepared that eventually could be used to describe maize controlling elements.

McClintock (1952) identified two transpositions of Ds from its original position between Wx and the centromere on the short arm of chromosome 9 to a subsequent position just distal to Sh (Fig. 1). From these she isolated many mutant shrunken alleles as well as other events adjacent to the new location of Ds (McClintock 1952, 1953). In the presence of the regulatory element Ac, Ds is located by its ability to break chromosomes and by the subsequent dicentric

formation leading to the breakage-fusion-bridge cycle (McClintock, 1951). If the chromosome carrying Ds is heterozygous with a homologue carrying recessive markers, initial breaks can be observed by the loss of distal markers. Subsequent fusion of sticky-ended chromatids leads to random breaks of the dicentric chromosome and loss of proximal markers which appear as subsectors within the first. The three shrunken mutables which McClintock propagated have Ds at the locus. All have normal transmission and viability. In the presence of Ac dicentric formation occurs although one of the alleles does occasionally give whole kernel Sh revertants. When Ac is removed by segregation, the mutable alleles behave as stable recessive markers and are thus suitable material for biochemical analysis.

Sucrose synthetase, because of its abundance, is easily purified to homogeneity. The protein has a molecular weight of 89,000. Antibodies have been prepared to it and their specificity has been demonstrated by immunoelectrophoresis against total endosperm proteins. The messenger RNA can be enriched on DMSO-sucrose density gradients and it appears to have a molecular length of approximately 2.5 kb. Double-stranded cDNA was prepared to size-fractionated poly(A)+ RNA. After blunt end ligation with synthetic Eco RI linkers the duplex was inserted into the chloramphenicol resistance gene of the plasmid pBR325.

When total endosperm mRNA is translated and the products electrophoresed on SDS-polyacrylamide gels, a band can be seen which comigrates with purified sucrose synthetase. Anti-sucrose synthetase antibodies remove all of this band demonstrating that it is the only detectable translation product of this size. The recombinant clones were screened by positive hybridization-translation and one was found which gave a band corresponding to sucrose synthetase. This band could be specifically precipitated by sucrose synthetase antibodies (data not shown).

Three Ds-induced shrunken mutable alleles provided by Dr. McClintock were examined: Ds sh-m5933-1, Ds sh-m6233A2, Ds sh-m6258A. Also included was a Sh revertant of Ds sh-m5933-1 which we had generated. In contrast to the revertants studied by McClintock (1953), Ds is no longer detectable by its ability to cause chromosome breaks at the Sh locus in this stock. DNA was prepared from seedlings which did not carry Ac and all alleles were homozygous except for sh-m6258A which was heterozygous with the non-mutable recessive sh-std.

DNA was digested with a variety of restriction endonucleases, separated electrophoretically on agarose gels, blotted onto nitrocellulose sheets, and hybridized with the cDNA probe. The sucrose synthetase cDNA insert is only 285 nucleotides long but when used as a probe it detects only a single band in any homozygous maize strain. However, there seems to be significant restriction polymorphism at this locus among maize inbreds; therefore only _Sh_ DNA from the stocks with the short arm of chromosome 9 carrying _Ds_ in its original position was used as the standard for comparisons. The nonmutable _sh-std_, on the other hand, appears to be the same in all stocks investigated.

DNA digested with _Eco_ RI gives the most information in comparing the various alleles (Fig. 2). _Sh_ can be distinguished from _Ds sh-m6233A2_, the _Sh_ revertant of _Ds sh-m5933-1_, _Ds sh-m6258A_, and _sh-std_; although the latter two are not distinguishable from each other with this enzyme. The enzyme _Bst_ EII differentiates _Sh_, _Ds sh-m6258A_, and the _Sh_ revertant of _Ds sh-m5933-1_ (Fig. 3). Other experiments with this enzyme show that the lower band in 3C is due to the _sh-std_ allele. It is evident that more than a simple transposition event was necessary to create the _Sh_ revertant of _Ds sh-m5933-1_. A control that was employed to show that these differences were not due to partial cleavage was to hybridize the _Eco_ RI digested DNA seen in Figure 2 with labeled ribosomal RNA. The typical 9000 base pair repeat is seen in all samples (Fig. 4).

We conclude that there are differences at the _Sh_ locus due to _Ds_ intervention; but because of the small size of the probe nothing further can be said about the nature of these rearrangements. Without more data we cannot, for example, rule out the possibility that some of the differences observed are the result of deletions extending proximally from the site of _Ds_ into the _Sh_ locus.

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Figure 1: Schematic representation of a portion of the short arm of chromosome 9. I is a dominant inhibitor of color formation in the aleurone, Bz conditions the quality of aleurone pigmentation, and Wx is the locus for starch granule-bound ADP glucosyl transferase which is responsible for the formation of amylose. Ds was originally observed by McClintock between Wx and the centromere. In response to Ac elsewhere in the genome, it can transpose or cause chromosome breaks where it resides.

Figure 2: Maize DNA digested with Eco RI, blotted onto nitrocellulose, and hybridized with nick-translated cloned cDNA complementary to a portion of the sucrose synthetase mRNA. Molecular lengths in base pairs were determined by comparison with uncut and Pvu II cleaved T7 DNA. (These lengths are somewhat shorter than those found in earlier experiments.) A) sh-std, B) Sh, C) Ds sh-m6233A2, D) Ds sh-m5933-1, E) Sh revertant of Ds sh-m5933-1, F) Ds sh-m6258A/sh-std.

Figure 3: As in Figure 2 except DNA digested with Bst EII. A) Sh, B) Ds sh-m6233A2, C) Ds sh-m6258A/sh-std, D) Ds sh-m5933-1, E) Sh revertant of Ds sh-m5933.

Figure 4: Eco RI digested DNA as shown in Figure 2 (in a separate blot) hybridized with ^{32}P labeled maize ribosomal RNA. The major bands migrate with a molecular length of 9,000 base pairs. A) Sh, B) Ds sh-m6233A2, C) Ds sh-m5933-1, D) Sh revertant of Ds sh-m5933-1, E) Ds sh-m6258A, F) sh-std.

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