DNase I hypersensitivity and expression of the *Shrunken-I* gene of maize

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Abstract

The local chromatin structure of the *Shrunken-I (Sh)* gene of maize was probed by analyzing DNase I hypersensitivity. *Sh* encodes the gene for sucrose synthetase, a major starch biosynthetic enzyme, which is maximally expressed in the endosperm during seed maturation. In addition to general DNase I sensitivity, specific DNase I hypersensitive sites were identified in endosperm chromatin that mapped near the 5' end of the *Sh* gene. The pattern of hypersensitive sites and their relative sensitivity were altered in other non-dormant tissues that produce little or no enzyme. However, some changes in chromatin structure appear to be independent of *Sh* gene expression and may reflect general alterations associated with plant development. The chromatin structure of several *sh* mutations, induced by *Ds* controlling element insertions, was also analyzed. Although the insertions perturbed expression of the gene, there were no notable effects on local chromatin structure.

Introduction

An altered chromatin conformation, exhibiting hypersensitivity to nucleases such as DNase I, is associated with actively transcribed genes. Within regions of DNA that exhibit general sensitivity to DNase I, specific DNase I hypersensitive sites can be found predominantly near the 5' ends of transcribed genes. These findings are based on data accumulated from studies of a wide variety of animal genes (for reviews see [12] and [26]). There have been, on the other hand, few studies on the local chromatin structure of plant genes [24, 25]. Although general DNase I sensitivity has been observed previously, specific DNase I hypersensitive sites have only been reported for one other higher plant gene [29].

In order to investigate the local chromatin structure of a unique, higher plant gene, we focused on the *Shrunken-I (Sh)* gene of *Zea mays* (maize). *Sh* encodes the gene for the enzyme sucrose synthetase. The enzyme is made in abundant quantities in the endosperm and plays an important role in starch biosynthesis [7]. Expression of *Sh* is regulated for both tissue specificity and temporal specificity [11]. There are many controlling element-induced mutations of *Sh* [16, 17]. Although the mechanism(s) of the perturbation of gene expression by controlling elements is not evident in all cases, one possible explanation is that the presence of these elements within a gene might affect local chromatin structure.

A study of local chromatin structure in maize first required that the methodology for isolating nuclei from maize tissues be developed. DNase I treatment of these nuclear preparations was then used to demonstrate that the local chromatin structure of the actively expressed *Sh* gene in the en-
dosperm was sensitive. Specific DNase I hypersensitive sites were mapped to the 5' region of the gene in endosperm chromatin and compared with sites in tissues where the gene is inactive. Finally, the local chromatin structures of mutant sh alleles containing Ds controlling element insertions were compared to a reference Sh allele for possible alterations in hypersensitive sites.

Materials and methods

Materials

All strains used were homozygous for markers on the short arm of chromosome 9. The wild-type Sh used was CI-I Sh Bz Wx Ds, which is McClintock's reference stock, with Ds at the standard position, proximal to Wx. The mutants Ds sh5933, Ds sh6233, Ds sh6258, Ds sh6795, and sh bz-m4 were propagated from material originally obtained from Dr Barbara McClintock. Ds sh6795 was not investigated, but a revertant, Ds Sh6795 Rev, was; this revertant and two sh mutants that were subsequently obtained from the revertant were isolated in this laboratory. All the stocks, with the exception of the two sh mutants derived from Ds Sh6795 Rev, have been described in greater detail in a previous paper [3].

Western blot analysis

Maize tissues were homogenized directly in electrophoresis sample buffer and centrifuged at 15000 x g for 15 min. The supernatants were boiled for two min, then electrophoresed on a 10% polyacrylamide:0.1% SDS gel [2]. The gel-fractionated proteins were electrophoretically transferred to nitrocellulose and probed according to the method of Burnette [1] except that the electrophoresis transfer buffer was 25 mM Tris-HCl (pH 8.3), 192 mM glycine, and 20% methanol. The blots were probed with polyclonal antibody to purified sucrose synthetase [2] and 125I-labeled protein A (Amersham).

Northern blot analysis

Total maize RNA was isolated from developing kernels as described [2] except that the extraction buffer was supplemented with 4 µl/ml diethylpyrocarbonate. RNA was denatured with glyoxal, electrophoresed on a 1% agarose gel, transferred to nitrocellulose, and hybridized according to Thomas [27].

Isolation of nuclei

Ears were self-pollinated and fresh endosperms, embryos, or whole ovules were dissected from the kernels at the times specified in the figure legends. Dormant embryos were dissected from dried seed. All manipulations were carried out at 0-4 °C. Isolation buffers were modified from those described by Murray and Kennard [20]. All isolation buffers contained 10 mM PIPES (pH 7), 5 mM 2-mercaptoethanol, 10 mM NaCl, 250 mM sucrose, and 0.1 mM phenylmethanesulphonyl fluoride. Additional components were as follows: 'HB': 1.0 M hexylene glycol, 20% (v/v) glycerol, 10 mM MgCl2; 'WB': 0.5 M hexylene glycol, 3 mM MgCl2; 'Percoll solution': 81% (v/v) Percoll (Pharmacia) in WB.

Approximately 20 g fresh tissue were dissected directly into 10 ml HB buffer on ice, then homogenized with a cold mortar and pestle. Embryos dissected from dry seed were first vacuum infiltrated in HB on ice for 2.5 min. At the end of the evacuation period, the vacuum was slowly released before proceeding. After grinding, the volume of the homogenate was increased to 40 ml with HB and filtered through a piece of 50 µm Nitex screen (Tetko, Inc., Elmsford, N.Y.) with the aid of a camel hair paintbrush imbibed in HB. Twenty ml of filtrate were layered onto an 8 ml Percoll solution cushion and centrifuged in a Sorvall HB-4 rotor, 1500 rpm, 10 min. Most of the supernatant above the Percoll cushion was removed by aspiration. The nuclei were pipetted from the HB:Percoll interface, gently diluted with 1–2 volumes WB, and pelleted with a Sorvall SS34 rotor, 1500 rpm, 10 min. The pellet was resuspended in 0.5 ml WB, layered onto