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Genetics

The cloning, genetic mapping, and expression of the constitutive sucrose synthase locus of maize

(duplicate loci/sugar metabolism/anaerobic induction/monosomics/gene mapping)

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ABSTRACT **Two differentially expressed genes encode isoenzymes of sucrose synthase in *Zea mays*. A clone of the shrunken 1 (*Sh1*) locus, the structural gene for the major endosperm form of sucrose synthase, was used to isolate a genomic clone of constitutive sucrose synthase (*Css*), the structural gene for the isoenzyme expressed in embryo and other tissues. The *Css* clone was positively identified by RNA blot analysis of RNA from wild type and a *sh1* deletion stock and by analysis of the *in vitro* translation product of hybrid-selected mRNA. Southern blot analysis of DNA from monosomic plants derived from an *r-x1* stock, coupled with restriction fragment length polymorphism mapping, placed the *Css* gene 32 map units from *Sh1* on chromosome 9. In seedling tissues, Css mRNA is present at higher levels than Sh1 mRNA. Expression of both *Sh1* and *Css* in root tissue is enhanced by anaerobic conditions, although *Css* is induced to a lesser extent than is *Sh1*. Thus, *Css* appears to be expressed constitutively, whereas *Sh1* is expressed at high levels only in response to specific developmental and environmental stimuli.**

Starch biosynthesis in the maize seed has been the subject of extensive genetic and biochemical investigations. Many mutants are known that affect starch accumulation in the maize endosperm, and the structural genes for at least six major enzymes in the starch synthetic pathway of the endosperm have been identified (1–5). Biochemical studies, however, revealed that a genetically distinct isoenzyme form for each enzyme is expressed in the embryo (4–8). Hence, two differentially expressed sets of genes encode the starch biosynthetic pathway in the maize seed: one set is expressed only in the endosperm and a second set of isozymes is expressed in the embryo. These genes thus represent a model system for the study of tissue-specific gene expression in higher plants.

To identify regions of genes important in differential expression, those genes encoding the corresponding endosperm and embryo isozymes must be isolated and compared at the DNA sequence level. While the endosperm-specific genes shrunken 1 (*sh1*) and waxy (*wx*) have been cloned and characterized (9–14), little is known about the corresponding genes expressed in the embryo. Here we report the isolation of *Css*, the gene encoding the embryo form of the enzyme sucrose synthase.

Sh1 is the structural gene for the major endosperm form of sucrose synthase (3). This enzyme comprises 10% of the buffer-soluble endosperm protein (3) and is a homotetramer composed of 88-kDa subunits (15). The physiological function of the *Sh1* sucrose synthase during starch deposition in the endosperm is actually cleavage of sucrose (16, 17). *Sh1* has been cloned and its entire DNA sequence has been determined (9–11). Comparison of the DNA sequences of two *Sh1* wild-type alleles has revealed a remarkable degree of heterogeneity within the *Sh1* locus; many of these polymorphisms appear to be due to transposable element activity (11). Although *Sh1* had been originally thought to be expressed only in the endosperm, Springer *et al.* (18) recently reported that *Sh1* is expressed in roots and shoots and that the level of expression is increased by anaerobiosis.

Chourey and Nelson (3, 8) demonstrated the existence of a second sucrose synthase gene (designated here as constitutive sucrose synthase or *Css*) that is expressed in both embryo and endosperm tissues. The Css and Sh1 proteins are quite similar, although they differ somewhat in net charge and protease cleavage patterns (19). McCormick *et al.* (20) isolated an mRNA from a *sh1* deletion stock by hybrid selection to a Sh1 cDNA clone. This yielded an *in vitro* translation product that cross-reacted to Sh1 antiserum, suggesting that *Sh1* and *Css* share sequence homology at the nucleic acid level. We have used this homology to isolate a genomic clone of *Css* as described below. We further show that the structural genes for sucrose synthase 1 and sucrose synthase 2 are genetically linked and that these genes respond differently to anaerobic stress.

MATERIALS AND METHODS

Plant Material. The genotypes used were a W64A × 182E hybrid (*Sh1*); *Sh1 bz-m13* in W22; and *sh1 bz-m4* in W22. The *sh1 bz-m4* stock carries a deletion of most, if not all, of the *Sh1* locus (9, 21, 22).

Genomic Library. Maize DNA isolated from a single Black Mexican Sweet plant was digested with *Bam*HI using conditions recommended by the supplier EMBL-3 DNA was digested with *Bam*HI and *Eco*RI. A mixture of 2.5 μg of λ DNA and 1 μg of maize DNA was ligated at 10°C overnight with 1 unit of ligase in 10 μl of buffer. After monitoring for ligation, DNA was packaged using Packagene as recommended by Promega Biotec (Madison, WI) K-803 cells were then mixed with the phage using conditions described elsewhere (9). The library, consisting of 300,000 plaques, was screened before amplification with nick-translated DNA from the 3400-base-pair (bp) *Bgl* II fragment of the *Sh1* clone, p17.6 (9, 11). The probe begins in intron 2 of *Sh1* and ends in exon 14 (Fig. 1). Library screening and plaque purification (9) employed filter washes at 68°C in 3× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate) and 0.5% (wt/vol) $NaDodSO_4$. Filters were exposed to x-ray film for 48 hr at −80°C with two intensifier screens.

RNA Isolation. Total RNA was isolated from developing maize kernels harvested 22 days postpollination and from seedling tissues as described (23). Polyadenylylated RNA was enriched by a single passage through an oligo(dT) cellulose column (24).

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Abbreviations: bp, base pair(s); kb, kilobase(s).

RNA Blot Analysis. RNA samples (5–20 μg) were denatured in 2.2 M formaldehyde/50% formamide, subjected to electrophoresis in 1.5% (wt/vol) agarose containing 2.2 M formaldehyde, and transferred to nitrocellulose membranes (24). Membranes were prehybridized for 6 hr at 42°C in 10 ml of a solution containing 50% formamide, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 0.2% Ficoll, 50 mM Tris·HCl (pH 7.5), 1.0 M NaCl, 0.1% (wt/vol) sodium pyrophosphate, 1.0% (wt/vol) $NaDodSO_4$, 10% (wt/vol) dextran sulfate, and 100 μg of denatured salmon sperm DNA per ml. Nick-translated and denatured probe DNA (75–100 ng; specific activity, 10^8 dpm per μg) was added and the mixture was incubated for 16–24 hr at 42°C. The membranes were washed twice with 100 ml of 2× SSC at room temperature for 5 min, twice with 100 ml of 2× SSC and 1.0% $NaDodSO_4$ at 65°C for 30 min, and twice with 100 ml of 0.1× SSC at room temperature for 30 min. Membranes were exposed to x-ray film for 4–16 hr at −80°C with two intensifying screens.

Hybrid Selection. Sheared single-stranded p21.2 plasmid DNA was covalently linked to Sephacryl S-1000 (Pharmacia) by diazotization (25). Polyadenylylated RNA (120 μg) isolated from *sh1 bz-m4* kernels was incubated with 0.5 g of DNA-Sephacryl for 4 hr at 42°C in 0.5 ml of 50% formamide/20 mM Pipes, pH 6.4/0.6 M NaCl/0.2% $NaDodSO_4$. The matrix material was washed four times with 5 ml of 2× SSC/0.2% $NaDodSO_4$/50% formamide, followed by 5 ml of 10 mM Tris·HCl, pH 8.0/2 mM EDTA. The specifically bound RNA was eluted with 1.2 ml of water at 90°C. Seventy-five micrograms of calf liver tRNA was added, and the RNA was precipitated with ethanol.

***In Vitro* Translation.** Polyadenylylated or hybrid-selected RNA was translated *in vitro* (26) in a rabbit reticulocyte lysate system (obtained from Bethesda Research Laboratories). RNA (1–5 μg) was incubated for 90 min in a reaction mixture volume of 30 μl in the presence of 10 μCi of [^{3}H]leucine (1 Ci = 37 GBq) (New England Nuclear). Translation products were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (27) and fluorography (28). Alternatively, 10 μl of the translation mixture was added to 75 μl of an immunoprecipitation buffer containing 50 mM Tris·HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, and 2% (vol/vol) Triton X-100; followed by 10 μl of monospecific antiserum against Sh1 protein or a nonimmune control serum and 30 μl of a 10% (wt/vol) suspension of formalin-fixed *Staphylococcus aureus* cells (Bethesda Research Laboratories). The mixture was centrifuged for 5 min and the pellet was washed twice. The final pellet was dissolved in electrophoresis sample buffer and analyzed by electrophoresis and fluorography as described above.

RESULTS

Isolation of a *Css* Genomic Clone. As described above, the strategy adopted for isolating a clone of the *Css* locus was based on the apparent DNA sequence homology between *Sh1* and *Css* (20).

Nine strongly hybridizing plaques were selected, purified, and analyzed by restriction mapping. Eight clones contained inserts that corresponded to either of two wild-type *Sh1* alleles, indicating the heterozygous nature of the plant.

The ninth clone differed markedly from p17.6. *Bam*HI digestion revealed three inserted fragments of DNA. Only one of these, a 6.0-kilobase (kb) *Bam*HI fragment, hybridized to the 3400-bp *Bgl* II fragment of p17.6. It was subcloned into pUC9 and is designated p21.2.

Restriction maps of p21.2 and p17.6, the *Sh1* clone, are shown in Fig. 1. From Southern blot hybridizations containing digests of p17.6 probed with nick-translated p21.2 and from reciprocal Southern blots, the region of cross-homology was determined. This is summarized in Fig. 1. Relative orientation and position of the clones depicted in Fig. 1 have been confirmed by partial sequencing of p21.2. Thus, a genomic clone with partial homology to *Sh1* was isolated from a library in which both *Sh1* alleles had already been identified.

Identification of the *Css* Clone. Several explanations for the origin of p21.2 were considered: (*i*) p21.2 represented a *Sh1* rearrangement, (*ii*) p21.2 represented an inactive copy of *Sh1* or a pseudogene, or (*iii*) p21.2 was a clone containing all or part of the *Css* gene or some other functional gene.

The possibility that p21.2 arose from rearrangement of *Sh1* sequences was tested by Southern blot analysis of DNA from wild type and from plants carrying *sh1 bz-m4*, a deletion mutant that lacks most if not all of *Sh1* sequences (9, 21, 22). A *Bam*HI/*Hin*dIII digest of wild-type W22 DNA generated two hybridizing fragments 5.0 kbp and 3.2 kbp in size (Fig. 2, lane 1). Because of an internal *Hin*dIII site, the number of bands detected is consistent with p21.2 sequences being single copy. The pattern observed in the sh1 deletion mutant (also in W22) was similar except that the smaller fragment detected in wild type was replaced by a 3.3-kbp fragment in the deletion (lane 2). Thus, sequences homologous to p21.2 are clearly present in both the mutant and the wild type; therefore, p21.2 was not derived from rearranged *Sh1* sequences.

To determine whether p21.2 represented a gene active in the maize seed, total RNA and poly(A) RNA isolated from 22-day-old kernels of wild type and *sh1 bz-m4* were subjected to RNA blot analysis. Blots were hybridized with p17.6 (*Sh1*) or p21.2. The *Sh1* probe hybridized strongly to an abundant 2.75-kb mRNA, which was present in the wild type but absent in the deletion (Fig. 3, lanes A–D). A second, weakly hybridizing mRNA of slightly greater molecular weight could be identified in the deletion upon longer exposure. McCormick *et al.* (20) suggested that this second band might correspond to the *Css* transcript. The p21.2 clone hybridized strongly to an mRNA that had identical mobility to the mRNA that hybridized weakly to p17.6. The mRNA was

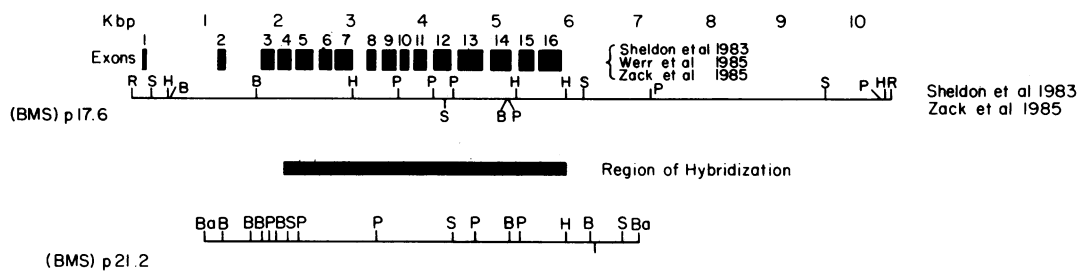

FIG. 1. Restriction maps of the two genes coding for sucrose synthase. The constitutive sucrose synthase (*Css*) clone, p21.2, was isolated by screening a genomic library with the 3400-bp *Bgl* II fragment of *Sh1*, which begins in intron 2 and ends in exon 14 (top line). The region of cross-homology between *Sh1* and *Css*, as judged by Southern blot hybridization, is indicated by the solid bar. Direction of transcription, as deduced from sequence comparison of *Sh1* and *Css*, is from left to right. Enzymes: R, *Eco*RI; S, *Sst* I; H, *Hin*dIII; B, *Bgl* II; P, *Pst* I; and B, *Bam*HI.

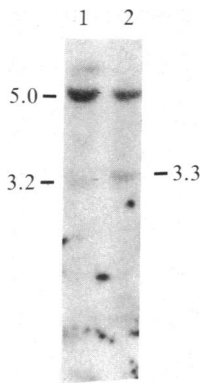

FIG. 2. Southern blot analysis showing that sequences homologous to clone p21.2 are present in the DNA from *sh1 bz-m4* plants. DNA was extracted from leaves of W22 wild type (lane 1) and W22 *sh1 bz-m4* (lane 2). DNA was digested with *Bam*HI and *Hin*dIII, electrophoresed, blotted, and probed with the nick-translated *Css* probe. Each lane contains 5 μg of DNA. Fragment sizes (in kb) are indicated in the margins.

present in equal abundance in wild type and in the deletion (lanes G and H). Cross-hybridization of p21.2 to the *Sh1* transcript was also evident. Therefore, p21.2 contains sequences from an active gene clearly distinct from *Sh1*.

To establish the identity of clone p21.2, hybrid selection from poly(A) RNA extracted from developing *sh1 bz-m4* kernels was performed with p21.2 DNA. When the hybrid-selected mRNA was translated *in vitro* (Fig. 4), a single translation product of 88 kDa, the size of the sucrose synthase 2 subunit, was observed. Furthermore, *Sh1* antiserum [antiserum to Sh1 protein cross-reacts with sucrose synthase 2 protein (19)] could be used to immunoprecipitate

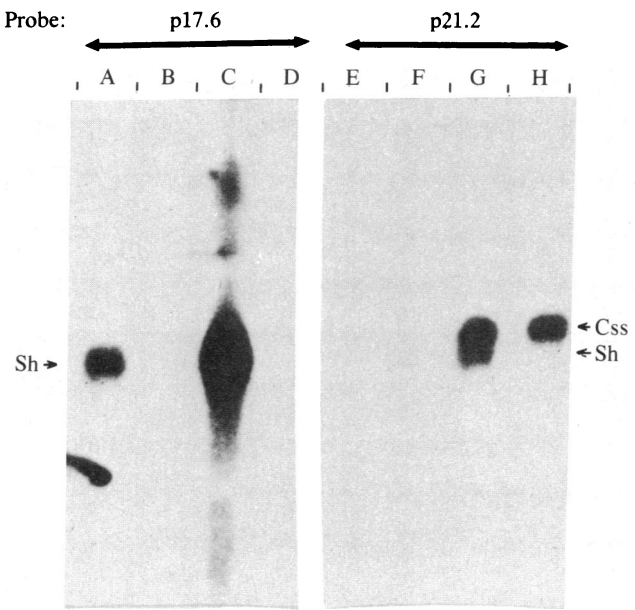

FIG. 3. RNA blot analysis showing that clone p21.2 corresponds to a functional gene. Total RNA and poly(A) RNA isolated from developing kernels of W64 × 182E wild type and the *sh1* deletion was analyzed by RNA blotting. Identical blots were probed with p17.6 (*Left*) and p21.2 (*Right*). Lanes A and E and B and F contain 20 μg of total RNA isolated from wild-type and *sh1 bz-m4* kernels, respectively. Lanes C and G and D and H contain 10 μg of poly(A) RNA from wild-type and *sh1 bz-m4* kernels, respectively. With longer exposure, cross-hybridization between p17.6 and the Css mRNA was detected in lane D.

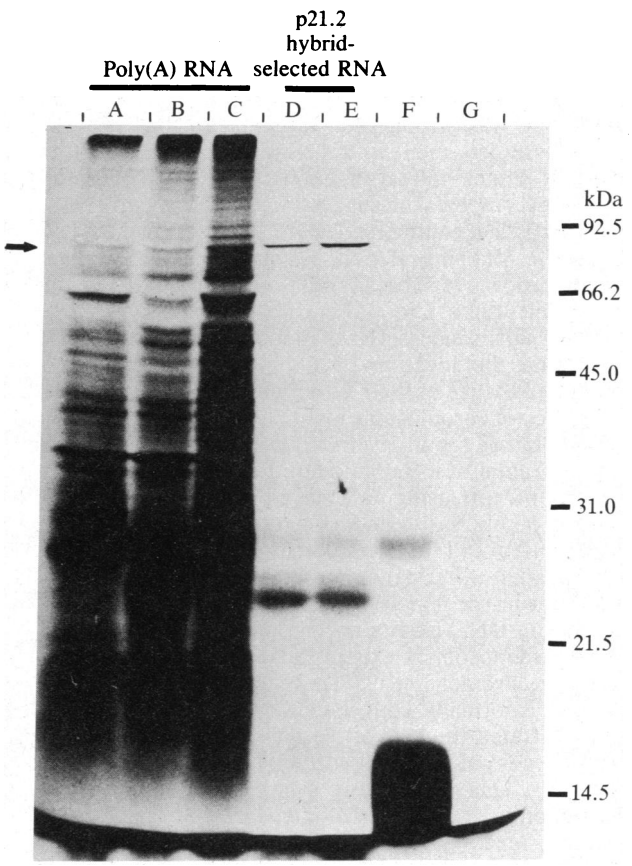

FIG. 4. *In vitro* translation products from hybrid-selected poly(A) RNA, showing that p21.2 selects an mRNA whose translation product is the size of sucrose synthase. Preparations of RNA were translated *in vitro* and the products were analyzed by $NaDodSO_4$ electrophoresis and fluorography. From left to right, the preparations are translation products of total poly(A) RNA from W22 wild type (lanes A and B), total poly(A) RNA from the deletion stock (lane C), 1 μl and 3 μl of mRNA selected from *sh1 bz-m4* developing seeds with p21.2. [RNA was selected from 120 μg of poly(A) RNA and suspended in 10 μl] (lanes D and E), globin mRNA control (lane F), and water control (lane G). In p21.2-selected mRNA produces a protein identical in size (88 kDa) to sucrose synthase. The origin of the smaller protein in lanes D and E is undetermined.

specifically the hybrid-selected translation product (Fig. 5). Therefore, p21.2 is a genomic clone containing *Css*.

DNA Sequence Homology Between *Sh1* and *Css*. The *Css* clone was partially sequenced. A 530-bp sequence beginning

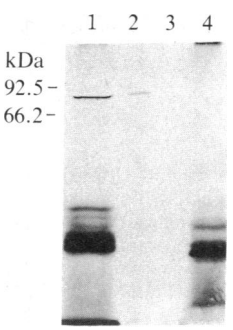

FIG. 5. Translation product of p21.2 hybrid-selected RNA cross-reacts with sucrose synthase antibody. Lane 1 contains the translation products of p21.2 hybrid-selected mRNA. Lane 2 contains the immunoprecipitate obtained with anti-Sh1 antiserum. Lane 3 is a nonimmune serum control. Lane 4 shows proteins synthesized *in vitro* in the absence of added mRNA.

at the *Sst* I site 2.6 kb from the left border of the p21.2 insert and extending to the right was used in a computer search for homology to p17.6 (*Sh1*). Substantial homology was detected in a region of *Sh1* extending 3′ from the *Sst* I site in exon 12. Homology was strongest in the exon regions (75–80%) of *Sh1*. These data confirm the relative orientation of the *Sh1* and *Css* clones shown in Fig. 1. The extent of sequence homology supports the suggestion that these two genes have evolved from a common ancestral gene by gene duplication.

Genetic Mapping of *Css*. Mapping was accomplished in collaboration with Scott Wright and Timothy Helentjaris at NPI (Salt Lake City, UT). Restriction fragment length polymorphism and a series of monosomic plants were used to determine the location of *Css*. Monosomic plants were generated by use of the *r-x1* deficiency, which causes a high frequency of chromosome loss (29, 30). Monosomic F_1 plants were obtained for all 10 maize chromosomes except 1 and 5. Hybridization of a genomic Southern blot from these plants and the parents using p21.2 as a probe indicated that *Css* is located on chromosome 9 (Fig. 6).

A 12.0-kb *Sst* I fragment that hybridized to Css DNA in the male parent (first lane) was replaced by a 4.7-kb *Sst* I fragment in the female (second lane). Both fragments were present in DNA from a normal diploid F_1 plant (third lane) and all monosomics except those that lacked a maternally derived chromosome 9. These plants lacked the 4.7-kbp fragment normally contributed by the female. This clearly demonstrated the loss of the female *Css* allele concomitant with the loss of chromosome 9 induced in the female gamete by *r-x1*. This assignment was further supported by the reduction in the intensity of the two 2.3-kb *Sst* I bands in the chromosome 9 monosomic plants. Comparable results were obtained for a *Hin*dIII restriction fragment length polymorphism (not shown).

The map position on chromosome 9 of *Css* was further resolved by screening 50 plants from an F_2 population segregating for the *Sst* I polymorphism and for other markers. As expected, *Css* showed linkage to chromosome 9 markers. *Css* was placed relative to two randomly cloned molecular markers (locus 80 and locus 14) that had been previously mapped at NPI (30). This places *Css* some 32 map units from *Sh1* and in the vicinity of the centromere on chromosome 9. Therefore, *Sh1* and *Css* are linked genetically, albeit loosely.

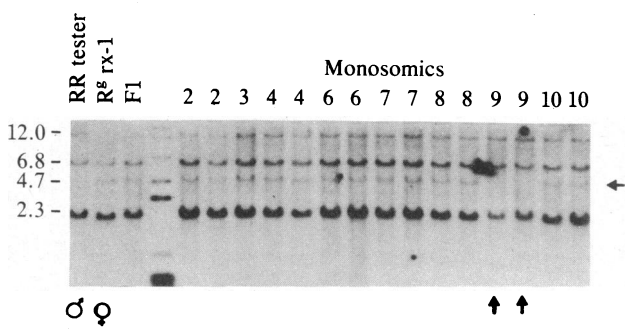

FIG. 6. Restriction fragment transmission analysis in monosomics showing that constitutive sucrose synthase is located on chromosome 9. DNA was extracted from plants monosomic for each of 8 of the 10 maize chromosomes, digested with *Sst* I, electrophoresed, blotted, and probed with the *Css* probe. First lane contains DNA from the male parent, the Mangelsdorf tester, and the second lane contains DNA from the monosomic-generating female parent, W22 *R-g/r-x1*. Banding pattern of a normal F_1 is shown in the third lane. The other lanes contain DNA from various monosomics. Numbers above the lanes indicate the monosomic chromosome. The unique 4.7-kb fragment contributed by the female parent is missing in plants lacking the chromosome 9 normally transmitted by the female. The intensity of the 2.3-kb doublet is also reduced in the chromosome 9 monosomics.

Differential Expression of *Sh1* and *Css*. *Sh1* and *Css* are clearly closely related genes, as evidenced by the enzymatic and structural similarities of their gene products (8, 19), cross-hybridization (this communication), and DNA sequence (unpublished data). Nonetheless, *Sh1* and *Css* differ markedly in their pattern of expression in tissues of the developing seeds. *Css* is expressed in embryo and endosperm, whereas *Sh1* is expressed only in endosperm. Recent studies indicate that both *Sh1* and *Css* are expressed in root and shoot tissues of maize seedlings (18, 19). Using probes specific for the *Sh1* transcript, Springer *et al.* (18) showed that *Sh1* is one of a small set of genes that are induced in root tissues under anaerobic conditions. However, the effect of anaerobiosis on *Css* expression has not been resolved. To assess directly the relative level of expression of *Sh1* and *Css* in normal and anaerobic seedling tissues, p17.6 and p21.2 were used to probe RNA blots of total RNA extracted from root and shoot tissues of 5-day-old seedlings and seedlings subjected to a 24-hr anaerobic treatment (Fig. 7 *a* and *b*). Under the conditions used, cross-hybridization between *Sh1* and *Css* was not detected. Fig. 7*a* clearly shows the induction of Sh1 mRNA in anaerobic root and shoot tissues. Transcript levels were quantified by determining the bound radioactivity via scintillation counting. *Sh1* was induced 7.5-fold in root tissues and 4.4-fold in shoot tissue of wild-type seedlings. Fig. 7*b* shows an identical blot probed with p21.2. The level of *Css* transcript was also enhanced in anaerobic wild-type root tissue, albeit less dramatically (2.0-fold). In wild-type and in *sh1 bz-m4* shoot tissue, Css mRNA actually decreased slightly during anaerobiosis. Css mRNA levels were 3–4 times higher than Sh1 mRNA levels in uninduced seedling tissues, while under anaerobic conditions Sh1 and Css mRNA levels were comparable (within 20%) in both root and shoot tissues. (This assumes that p21.2 includes the entire *Css* coding sequence.) Levels of mRNA

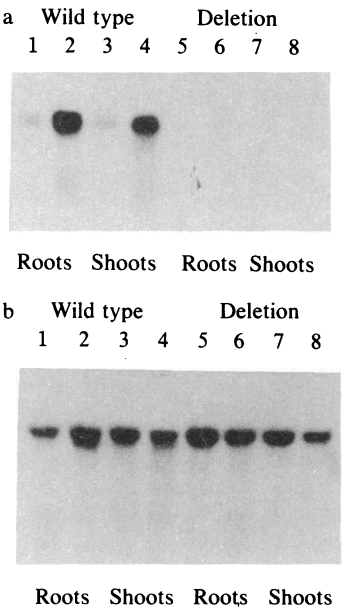

FIG. 7. RNA blot analysis of transcripts showing that *Css* is induced by anaerobic conditions, although not to the extent that *Sh1* is induced. Total RNA (16 μg per lane) was isolated from roots and shoots of W22 wild type and of the deletion *sh1 bz-m4*. Lanes 1, 3, 6, and 7 contain RNA from control tissues and RNA from anaerobically stressed tissue is in lanes 2, 4, 5, and 8 of each blot. (*a*) Probed with a *Sh1* plasmid; (*b*) probed with a *Css* plasmid. Seedlings were germinated on moist filter paper for 5 days at room temperature. Treated seedlings were submerged in 10 mM Tris·HCl (pH 8.0) for 24 hr, while control seedlings were exposed to air during this time period.

and patterns of induction of *Css* observed in the deletion stock are similar to those observed in wild type except that uninduced levels of mRNA were somewhat higher in roots of the deletion stock. Whether this represents compensation for the lack of Sh1 mRNA remains to be determined.

Thus, *Css* and *Sh1* differ in their level of expression in normal seedling tissues. However, under anaerobic conditions, expression of *Sh1* is enhanced to a level approximately equal to that of *Css*.

DISCUSSION

Through partial DNA sequence homology to *Sh1*, the structural locus for the major endosperm form of sucrose synthase in maize, we isolated the structural gene for another sucrose synthase isozyme, designated *Css*. RNA blot and hybrid-selection–translation techniques confirmed the identity of this clone as *Css*. *Css* was subsequently mapped to chromosome 9 ≈32 map units from *Sh1*.

Isolation of the *Css* locus is of particular interest for several reasons. It is the first member of the complement of starch biosynthesis genes expressed in the embryo of the developing maize seed to be identified and cloned. Comparison of the *Css* and *Sh1* gene, and eventually other differentially expressed genes of the starch pathway, will facilitate identification of structural features essential for tissue-specific gene expression in maize. Second, Css protein has been found in all maize tissues so far studied, and the results reported here show that mRNA levels are relatively high. These observations suggest that the promoter of this gene may have use in transformation work with maize.

Results reported here indicate that differential regulation of *Css* and *Sh1* not only occurs in the maize seed but also in seedling tissues. Css mRNA levels are 3- to 4-fold higher than Sh1 mRNA levels in normal seedlings. Although expression of both genes is apparently enhanced in anaerobic roots, *Sh1* is induced to a much greater extent than is *Css*. As a result, the amounts of the two mRNAs are nearly equal in anaerobic root and shoot tissues.

Css expression appears to be largely constitutive, with *Css* being the predominant sucrose synthase expressed in most plant tissues under normal conditions. The exception is developing endosperm tissue, where *Sh1* is predominant. *Sh1* might then be viewed as providing supplemental capacity that is expressed only in response to specific developmental and environmental signals—i.e., endosperm development and anaerobic conditions.

If *Css* functions in such a housekeeping role, *Css* null mutation might well be lethal. This could explain the absence of mutants in *Css* and perhaps other "embryo" starch genes. Alternatively, genetic loss of *Css* might be difficult to observe if, as suggested above, *Sh1* serves as a backup and can compensate for the loss of *Css* in those tissues in which *Css* normally functions. Mutants reduced in or completely lacking *Css* function would be of interest in resolving the two alternative explanations.

The physiological role of sucrose synthase in the anaerobic response in maize is not fully understood. The onset of fermentative metabolism, however, may lead to an increased demand for sugars (31), in which case newly synthesized sucrose synthase could provide an increased capacity for utilization of sucrose in anaerobic tissues. Whether *sh1* root tissues are less tolerant of an anaerobic environment than wild-type roots is not known.

We thank Scott Wright and Tim Helentjaris of NPI, who performed the hybridization depicted in Fig. 6 and who analyzed the subsequent F_2 families. We thank David Weber (Illinois State University) for generating the monosomics, Christine Chase and Eduardo Vallejos for their critical comments and suggestions for this manuscript, and Paul Still and Prem Chourey for providing the DNA used in the original library construction and for providing the *Sh1* antiserum. This is paper 7299 from the Florida Agricultural Experiment Station. This work was supported by grants from the National Science Foundation and the Department of Agriculture Competitive Grants Office.

1. Nelson, O. E. & Rines, H. (1962) *Biochem. Biophys. Res. Commun.* **9**, 297–300.
2. Tsai, C. Y. & Nelson, O. E. (1966) *Science* **151**, 341–343.
3. Chourey, P. S. & Nelson, O. E. (1976) *Biochem. Genet.* **14**, 1041–1055.
4. Pan, D. & Nelson, O. E. (1984) *Plant Physiol.* **74**, 324–332.
5. Pan, D. & Nelson, O. E. (1985) *Maize Genetics Cooperation Newsletter* **59**, 105–106.
6. Hannah, L. C. & Nelson, O. E. (1976) *Biochem. Genet.* **14**, 547–560.
7. Nelson, O. E., Chourey, P. S. & Chang, T. (1978) *Plant Physiol.* **62**, 383–386.
8. Chourey, P. S. (1981) *Mol. Gen. Genet.* **184**, 372–391.
9. Sheldon, E., Ferl, R., Fedoroff, N. & Hannah, L. C. (1983) *Mol. Gen. Genet.* **190**, 421–426.
10. Werr, W., Frommer, W. B. & Starlinger, P. (1985) *EMBO J.* **4**, 1373–1380.
11. Zack, C. D., Ferl, R. J. & Hannah, L. C. (1986) *Maydica* **31**, 5–16.
12. Shure, M., Wessler, S. & Fedoroff, N. (1983) *Cell* **35**, 225–233.
13. Schwarz-Sommer, Zs., Gierl, A., Klosgen, R. B., Wienand, U., Peterson, P. A. & Saedler, H. (1984) *EMBO J.* **3**, 1021–1028.
14. Wessler, S. R. & Varagona, M. J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4177–4181.
15. Su, J. C. & Preiss, J. (1978) *Plant Physiol.* **61**, 389–393.
16. Chourey, P. S. & Nelson, O. E. (1979) *Genetics* **91**, 317–325.
17. Cobb, B. G. (1982) Dissertation (Univ. of Florida, Gainesville, FL).
18. Springer, B., Werr, W. & Starlinger, P. (1985) *Maize Genetics Cooperation Newsletter* **59**, 32–33.
19. Echt, C. S. & Chourey, P. S. (1985) *Plant Physiol.* **79**, 530–536.
20. McCormick, S., Mauvais, J. & Fedoroff, N. (1982) *Mol. Gen. Genet.* **17**, 494–500.
21. Dooner, H. K. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 457–462.
22. Burr, B. & Burr, F. A. (1981) *Genetics* **98**, 143–156.
23. McCarty, D. R. (1986) *Maize Genetics Cooperation Newsletter* **60**, 61.
24. Maniatis, T., Fritsch, E. G. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
25. Buneman, H. & Westhoff, P. (1983) *Methods Enzymol.* **100**, 400–407.
26. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–251.
27. Laemmli, U. (1970) *Nature (London)* **227**, 680–685.
28. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
29. Weber, D. F. (1982) in *Maize for Biological Research*, ed. Sheridan, W. (Univ. of N. Dakota Press, Fargo, ND), pp. 79–83.
30. Helentjaris, T., Weber, D. F. & Wright, S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6035–6039.
31. Saglio, P. H., Raymond, P. & Pradet, A. (1980) *Plant Physiol.* **66**, 1053–1057.