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Structural Features of the Maize *sus1* Gene and Protein[1]

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Genomic clones, cDNA clones, and protein of the maize (*Zea mays* L.) *Suc synthase1* (*sus1*) gene were isolated and sequenced. Termini (5′ and 3′) of the transcribed unit were identified. The SUS1 protein was purified from tissue culture cells as a phosphorylated protein. The overall structure of *sus1* is virtually identical with that of the paralogous gene, *shrunken1* (*sh1*); however, the last intron of *sh1* is missing in *sus1*. This intron bears much sequence similarity with the adjacent exon, suggesting that the intron arose from an internal duplication. Although the placement of the other 14 introns is identical in both genes, the introns exhibit markedly greater differences in size and sequence relative to that shown by the exons. An explanation for the differential rate of divergence of exons and introns is selection pressure for gene function. Additionally, comparisons of coding regions of plant sucrose synthases show that *sh1*-like and *sus1*-like genes can be found in all monocots so far analyzed. These latter observations point to an important role played by both genes in this group of plants.

Suc synthase has been implicated in various roles in the synthesis and degradation of Suc as well as in the flow of carbon from one organ to another in maize (*Zea mays* L.). The gene *sh1* encodes the major endosperm Suc synthase and was one of the first plant genes cloned primarily because of interest in the biology of transposable elements (Burr and Burr, 1980; Fedoroff et al., 1983; Weck et al., 1984). The abundance of the protein and transcript in the endosperm and the collection of loss-of-function *sh1* mutants provided the necessary materials for the early cloning of the gene.

With the realization that not all Suc synthase activity in the endosperm is under the control of the *sh1* locus (Burr and Burr, 1980; Chourey, 1981), we (McCarty et al., 1986) used the sequence similarity between the two *sus* genes to isolate the second structural gene for this enzyme, now termed *sus1*.

Since the initial cloning of *sh1* and *sus1*, these genes have been used to isolate *sus* genes from other plants as well as to study the signal transduction pathways involving the response of the *sus* genes to various stimuli, including anaerobiosis (McCarty et al., 1986; Springer et al., 1986; McElfresh and Chourey, 1988), sugar levels (Koch et al., 1992), and genetically defined blocks in the synthesis of various storage products in the maize endosperm (Giroux, 1992). Furthermore, *sh1* and *sus1* likely arose via a gene duplication (McCarty et al., 1986). Hence, the fact that *sus1* is expressed in all tissues, including the pollen (Hannah and McCarty, 1988), whereas *sh1* is expressed in only a few tissues or only in response to certain signals, may make a comparative study of their respective regulatory sequences a productive exercise.

In contrast to other plants, maize contains mutants for both *sh1* and *sus1*, a fact critical in the establishment of the clone:gene:enzyme relationship. Loss of *sh1* function gives rise to a shrunken or collapsed kernel, because of the involvement of the *sh1*-encoded Suc synthase in starch biosynthesis. Furthermore, aspects of the loss-of-function *sh1*- phenotype are consistent with Suc synthase also playing a key role in cell wall synthesis.

Loss of *sus1* function is reported to have no phenotypic effect on the maize plant (Chourey, 1988; Chourey et al., 1988). This suggests then that, although *sus1* is expressed in all tissues of the maize plant, its function is dispensable. We have reexamined this point by determining the structure of maize *sus1* and comparing it to that of *sh1* as well as to closely related genes in other plants. We suggest that *sus1* is important to maize or at least to relatives of maize, because *sus1*-like genes can be found in all monocots so far examined and because of differential rates of divergence in introns and exons of *sus1* and *sh1*.

MATERIALS AND METHODS

Nomenclature

The nomenclature used follows the convention used in maize genetics and is in agreement with the recommendation of the Commission on Plant Gene Nomenclature (1994). Specifically, the *sh1* refers to the *shrunken1* gene of maize. The gene was named for the kernel phenotype conditioned by loss of this function. Recessive alleles of this gene are denoted *sh1*- and the particular allelic designation, when known or relevant, follows the dash. In the context of this

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Abbreviations: *bz-m4, bronze-mutable4;* RACE, rapid amplification of cDNA ends; *sh1, shrunken1;* SH1, protein encoded by *sh1*; *sus*, gene for Suc synthase; *sus1, sucrose synthase1;* SUS1, protein encoded by *sus1*.

work, *sh1* refers to the functional or wild-type form of the gene. SH1 refers to the protein produced by the *sh1* gene. SH1 is a subunit of the enzyme Suc synthase. The gene *sus1* also encodes a subunit of Suc synthase. The protein produced by *sus1* is SUS1. Allelic designations follow the system used for the *sh1* locus. The gene *sus1* was termed *Css* by us previously. Other laboratories have referred to this as *Ss2* and *Ssb*. These three latter symbols are now obsolete.

Following the suggestion of the Sucrose Synthase Subcommittee of the Commission on Plant Gene Nomenclature, members of the gene family containing the *sus1* gene of maize are also given the symbol *sus1*. Particular plant designations are given when needed. Furthermore, the *sh1* gene of maize is placed in the *sus2* plant gene family.

Plant Materials

Black Mexican Sweet corn (*Zea mays* L.) leaf DNA was the source of the *sus1* genomic clone p21.2 reported by McCarty et al. (1986) and the second genomic clone described here. Wild-type (W22) embryos and *sh1- bz-m4* (W22) kernels were used in the preparation of two cDNA libraries. The mutant *sh1- bz-m4* lacks the *sh1* locus.

Genomic and cDNA Libraries

Genomic DNA was digested with *Sst*I and fractionated on a 5 to 24% NaCl gradient. A portion of each fraction was electrophoresed, blotted, and probed with the 5′-most, 1.1-kb *Bam*HI-*Sst*I fragment of p21.2. Hybridizing fractions were ethanol precipitated and ligated to λ GEM-11 vector DNA (Promega) digested with *Sst*I and *Bam*HI. Ligations were packaged in Promega Packagene extracts, grown in K803, and probed with the 1.1-kb *Bam*HI-*Sst*I fragment of p21.2 using a minor modification of the procedure of Church and Gilbert (1984). Initial screening and subsequent plaque purifications included stringent washes in 0.2× SSC and 1% SDS.

cDNA clones of *sus1* were isolated from λ GT-10 libraries synthesized from poly(A)$^+$ RNA of developing *sh1- bz-m4* seeds or developing wild-type embryos (both at 20 d postpollination and in the W22 background) by screening with *sus1* genomic subclones using the methodology for the genomic library.

Subcloning

The resulting genomic and cDNA clones were subcloned into pUC19. Various restriction fragments of the genomic clones were further subcloned into M13 using protocols provided with Gibco-BRL's M13 cloning and sequencing systems.

Sequencing

Single-strand (M13) and double-strand (pUC19) sequencing used the dideoxy method and the large fragment of DNA polymerase I (Gibco-BRL sequencing system) or sequenase (United States Biochemical). Single-strand sequencing of deletion subclones or clones of overlapping restriction fragments from *Taq*I, *Sau*3AI, and other, similar enzymes yielded many of the data for the genomic clone. Deletion cloning in M13 was accomplished using the International Biotechnologies, Inc. (New Haven, CT) Cyclone System, which utilizes the 3′-to-5′ exonuclease activity of T4 DNA polymerase. The cDNA and some of the genomic sequencing was done using primarily plasmid clones containing large inserts (>1 kb) and specific primers synthesized by the University of Florida Interdisciplinary Center for Biotechnological Research DNA Synthesis Core. Reported data resulted from sequencing both strands of genomic and cDNA clones.

Determination of mRNA Termini

The 5′ terminus was identified by use of the Gibco-BRL 5′ RACE System and by primer extension, following the procedures by Ausubel et al. (1993). An oligonucleotide complementary to positions 2629 to 2742 of the *sus1* gene (these and all subsequent positions are based on the start of transcription as position 1) was used with Superscript RNase H- reverse transcriptase and 1 μg of total *sh1- bz-m4* RNA to obtain 5′ cDNA. This was tailed with dCTP and amplified for 40 cycles with 2.5 units of Taq DNA polymerase, using the anchor primer and a gene-specific oligonucleotide complementary to positions 2554 to 2578. Each of the four resulting bands seen on a 1.5% agarose gel was eluted and subcloned into pSPORT using an *Sal*I restriction site in the anchor primer and a *Pst*I site in *sus1* 5′ to the position of the gene-specific 3′ PCR primer. Several resulting clones were sequenced. Primer extension was accomplished using 9 μg of *sh1- bz-m4* poly(A)$^+$ RNA, a 35-bp oligonucleotide complementary to positions 112 to 1666 and avian myeloblastosis virus reverse transcriptase. The resulting products were separated on an 8% polyacrylamide sequence gel.

The 3′ termini were first defined by sequencing cDNA clones having poly(A) tails attached at two sites separated by 35 bp. Further characterization was accomplished using the Gibco-BRL 3′ RACE System. The poly(dT)-containing adapter primer in the 3′ RACE kit was used to prime first-strand cDNA synthesis. Two gene-specific primers, from positions 6165 to 6195 and 6329 to 6359, were used in separate amplifications. The universal amplification primer served as the 3′ primer in both cases. The resulting PCR products were electrophoresed, blotted, and probed with a 743-bp *Bgl*II-*Dde*I fragment from p21.2 from the last exon of *sus1* (position 5907–6650).

SUS1 Protein Purification

The cell culture used was P3377, kindly provided by Jack Widholm, University of Illinois. In vivo labeling of cell cultures was accomplished by adding 30 μCi of [γ-^{32}P]ATP (Amersham) directly to the culture medium for a 45-min incubation.

Purified SUS was isolated from four 50-mL culture flasks of cells. The cells were recovered and ground in a mortar and pestle with 20 mL of TEF buffer (10 mM Tris-HCl, 0.1 mM EDTA, 25 mM NaF). The slurry was centrifuged at 6000*g* for 10 min to remove insoluble material. The supernatant was then clarified by centrifugation at 100,000*g* for 30 min in a Beckman Ti75 rotor. The supernatant was precipitated with

40% (w/v) ammonium sulfate, and the pellet was discarded. After further addition of ammonium sulfate to 60% and centrifugation, the pellet was redissolved and dialyzed against TEF and then subjected to Mono Q fast protein liquid chromatography using a linear gradient of 0 to 1 M NaCl in TEF. The fractions containing the peak SUS protein were combined and applied to a Mono P fast protein liquid chromatography column for chromatofocusing using 0.025 M BisTris (pH 7.1) and 10% Polybuffer 74 (pH 4.0; Pharmacia). The peak Mono P fractions containing SUS were pooled and subjected to size chromatography on a Superose 12 column equilibrated with TEFD (TEF plus 5% glycerol). Molecular weight standards used to calibrate the Superose column were amylase, yeast alcohol dehydrogenase, and carbonic anhydrase (Sigma).

RESULTS AND DISCUSSION

Structure of *sus1*

The structure of *sus1* is presented in Figure 1, and the sequence, including 1152 bp upstream of the start of transcription, is in GenBank, accession number L29418. The structure was determined from primer extension analysis and the sequencing of conventional cDNA clones, genomic clones, and amplified termini of the *sus1* transcript as described below.

Three poly(A)-containing cDNAs from *sh1- bz-m4* developing seed (1.4, 2.0, and 2.1 kb) and one poly(A)-containing cDNA from the developing embryo (2.9 kb) were analyzed. The cDNA sequence was obtained primarily from the 2.0-kb seed and 2.9-kb embryo clones. Sequences were identical except for the difference noted below. The terminal sequences of all clones were determined.

The 5′ Terminus

The 5′ terminus of the *sus1* transcribed unit was identified by sequencing PCR-amplified cDNA from *sus1* mRNA. Primer extension analyses provided data consistent with those obtained from the conventional cDNA clones. Four PCR products resulting from 5′ RACE methodology were cloned, and the two largest ones were sequenced. Three clones containing the largest insert had identical sequences and were used to define the transcription start site. Transcription of *sus1* begins 15 nucleotides 5′ to the 5′ end of the 2.9-kb cDNA.

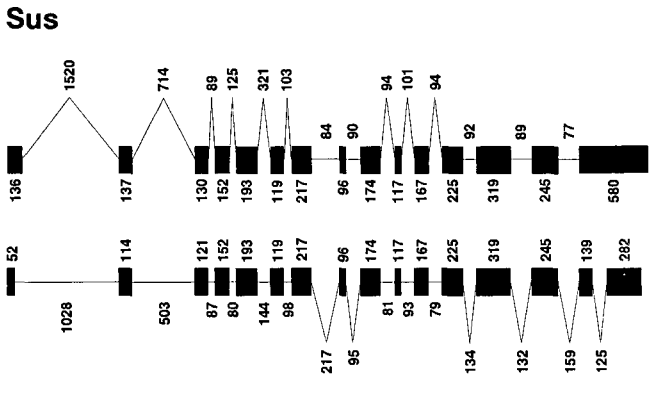

Figure 1. Structures of the *sus1* and *sh1* genes of maize. Exons are represented by boxes and introns are represented by lines. Numbers refer to the sizes of exons and introns.

The 3′ Termini

The 3′ termini of the four poly(A)-containing cDNAs were sequenced. The three seed cDNA clones contained a poly(A) tail starting at position 6600, whereas the embryo clone contained a poly(A) tail at position 6635. Accordingly, poly(A)$^+$ RNA from both developing endosperms and embryos was examined using 3′ RACE to determine whether different poly(A) addition signals were used in the two tissues. The sizes of the resulting bands suggest that the two polyadenylation signals identified in the cDNA clones (AATAATAAT at position 6563 and AAATAAG at position 6598) as well as possibly others close by are used in each of the two tissues.

The sequence of the transcribed region of *sus1* differs from that recently reported by Huang et al. (1994). The start of transcription reported by Huang et al. lies 137 nucleotides 3′ to the start site reported here. Since the tissues and genotype analyzed in the two studies are identical, differences in tissue-specific expression or genotype cannot be invoked as the cause of the difference. The additional 5′ sequences we report were derived from conventional as well as PCR-derived cDNA clones, and they are also present in the authentic *sus1* genomic clones. Interestingly, the reported transcription start site of Huang et al. lies exactly at the beginning of exon 2.

Two important differences within the protein-coding region distinguish the sequence reported here and that reported by Huang et al. (1994). First, 2 bp we report are missing between positions 2125 and 2129 (numbering of Huang et al., 1994) as are 2 bp between position 2235 and 2236. These alterations shift the reading frame, resulting in a truncated SUS1 protein, relative to the sequence reported here. We investigated this difference in two ways. First, we compared the region in question to sequences of nine other plant Suc synthases. The reading frame we report was found in all nine genes. Second, amino acid sequence data derived from a portion of the SUS1 protein distal to the point in question (see below) are identical with the sequence predicted by our sequence.

SUS1 Is a Phosphorylated Protein Expressed in Cultured Cells

We found that the SUS1 is a phosphorylated protein. Maize cells incubated with [γ-^{32}P]ATP for 45 min produced labeled SUS as a major phosphorylated protein. The ^{32}P-labeled SUS protein was purified to homogeneity, with the final purification step being size fractionation (Fig. 2A). Also shown is the electrophoretic analysis of the purified protein. The preparation contains only one protein as detected by protein staining (Fig. 2B). The protein is phosphorylated (Fig. 2C).

Because the purified SUS protein was blocked at the N

terminus, proteolytic fragments were partially sequenced. Amino acid sequences of 4 residues, 9 residues, 10 residues, and 21 residues were obtained from four peptides. Searches of protein data bases failed to reveal a perfect match, although the most similar alignment was found with SH1 of maize. The amino acid sequences were then compared to the SUS1 sequence derived from the DNA sequence presented here. We found that the amino acids could be aligned perfectly with positions 39 to 43, 456 to 464, 544 to 553, and 743 to 763 of the protein deduced from the *sus1* cDNA sequence reported here.

Interestingly, the purified, nondenatured, phosphorylated SUS1 protein exhibits a molecular mass between 150 and 205 kD upon gel chromatography (Fig. 2A). This size, coupled with the size of the *sus1* open reading frame, strongly suggests that the protein exists as a homodimer. Su and Preiss (1978) reported that the major maize endosperm Suc synthase, the product of *sh1*, is a tetramer, and Suc synthase has subsequently been shown to be a tetramer in other plant tissues. It is currently unknown whether the *sus1*-encoded Suc synthase exists as a homodimer in some but not all tissues or whether phosphorylation affects the state of aggregation.

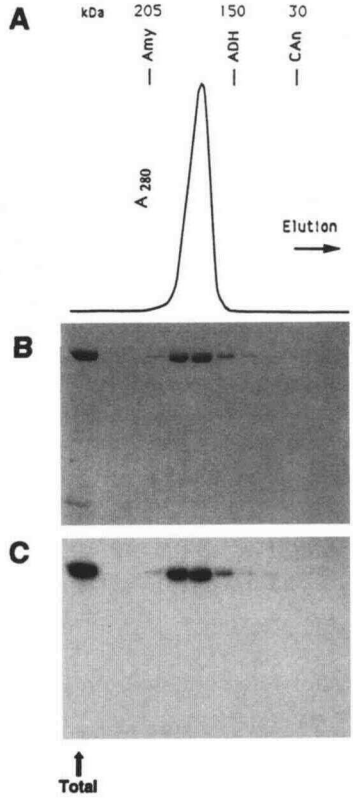

Figure 2. Purification of Suc synthase from Black Mexican Sweet corn cells. A, Size fractionation of purified SUS. Molecular mass markers and their masses are given. Fractions from A were separated by electrophoresis and stained for protein (B) and exposed to x-ray film for detection of ^{32}P (C). Electrophoresis was done at 30 mA constant current on a 10% polyacrylamide gel. Amy, Amylase; ADH, alcohol dehydrogenase; CAn, carbonic anhydrase.

Genomic Sequence of *sus1*

The isolation of the initial *sus1* genomic clone was reported previously (McCarty et al., 1986). Because the original clone lacked the 5′ upstream sequences, a 5′ 1.1-kb fragment of p21.2 (McCarty et al., 1986, fig. 1) was used to probe an enriched *Sst*I library of genomic Black Mexican Sweet corn DNA. This yielded a 13-kb λ clone containing the 5′ portion of *sus1*. A 4-kb *Sal*I to *Sst*I fragment of the original λ clone was subcloned and sequenced. This fragment includes sequences starting at 1152 bp before the start of transcription to the *Sst*I site in exon 4.

The *sus1*-containing region of the genomic subclone p21.2 and the further 5′ region isolated from the 13-kb *Sst*I genomic clone were sequenced. Comparison of the sequence of the genomic clones with the sequence of the full-length transcript gave rise to the structure presented in Figure 1.

The SUS1 and SH1 Proteins Are Quite Similar

The *sus1*-coding region contains 816 codons encoding a protein of 92,918 D. Maize *sh1* contains 802 codons, resulting in a protein of 91,707 D (Werr et al., 1985). The major differences are confined primarily to the termini, with SUS1 containing additional amino-terminal and carboxyl-terminal amino acids. The other major difference involves insertion of 3 amino acids in SUS1 approximately 60 amino acids from the amino terminus. Within the region common to both proteins, 80% of the amino acids are identical. The most divergent region lies in the amino termini. Less than 50% identity is found in the first 63 amino acids of SH1 (and 71 amino acids of SUS1). In contrast, Huang et al. (1994) reported that the carboxyl termini of the SUS1 and SH1 proteins were most divergent. We believe that this conclusion is based on two sequencing errors within the protein-coding region as discussed above.

Upstream Regions of *sh1* and *sus1* Show No Obvious Sequence Similarity

The genes *sh1* and *sus1*, although most likely the result of a gene duplication, exhibit different spectra of expression. Levels of *sh1* expression are highest in the endosperm and expression is inducible in other tissues. In contrast, *sus1* expression is found in most if not all tissues of the maize plant. As might be expected, 5′ sequences of *sus1* and *sh1* exhibit little similarity in comparison to that seen in their coding regions.

One noteworthy feature derived from sequence comparisons concerns signals possibly important in pollen expression. Dawe et al. (1993) showed that the sequence GGACTGA in the 5′ untranslated region of *adh1* was important for pollen expression. They further noted that other genes expressed in the pollen contain 5′ untranslated sequences similar to the one listed above, although the third nucleotide is variant. Of the two maize Suc synthase genes, only *sus1* is expressed in the pollen (Hannah and McCarty, 1988). *Sus1* contains the sequence GGcCTGA in the 5′ untranslated region, whereas *sh1* lacks these sequences.

The Genes *sh1* and *sus1* Most Likely Arose by a Gene Duplication

The structure of *sus1* is presented in Figure 1 along with a comparison with *sh1*. One notable feature is that the positions of the 14 introns in *sus1* are identical with those in *sh1*. The similarity in exonic sequences coupled to the identical placement of these 14 introns is most easily explained by a gene duplication giving rise to the two Suc synthase genes. These introns were most likely present before the duplication event.

The Last Intron of *sh1* Is Missing in *sus1*

Strikingly, the last intron of *sh1* (intron 15) is missing in *sus1*. Although the origin of this intron cannot be determined with certainty, sequence comparison highly suggests that it arose by an internal gene duplication. Sequences of intron 15 and exon 16 are shown in Figure 3. Identical sequences of 5, 6, 9, and 5 bp are found co-linearly in both the 125-bp intron 15 and the 5′ portion of exon 16. The perfect identity and co-linearity of these sequences highly suggest that they arose via internal duplication, followed by independent mutation.

sus1 or its predecessor may have served as the progenitor of *sh1*. We noted above that the simplest mechanism to account for the sequence similarity between intron 15 and exon 16 of *sh1* is an internal duplication within the gene, followed by independent mutation. The origin of this intron, then, would have occurred after the formation of the gene, in agreement with the intron-late hypothesis (Kuhsel et al., 1990) of gene evolution. The lack of this intron in *sus1* and its presence in *sh1* suggest that the internal duplication occurred after the duplication giving rise to these two genes. However, since these sequences represent a tandem duplication, nonhomologous recombination could remove this intron from *sus1*. On parsimonious grounds, however, it is simpler to consider *sus1* or its predecessor as the progenitor of *sh1*.

Exonic Sequences of *sh1* and *sus1* Exhibit Much Less Divergence Than Do the Introns

Another striking feature derived from a comparison of *sh1* and *sus1* sequences is the vast difference in the size and homology of their introns and exons. We compared the two genes and identified sequences exhibiting at least 70% similarity over a span of at least 30 bp. Sequences identified are shown in Table I. All such sequences lie in protein-coding regions of the two genes. Exonic sequences not encoding protein (exon 1 and most of exon 16 of *sh1* and corresponding sequences in exon 15 of *sus1*) do not exhibit this conservation. Homology was not found in any of the intronic sequences.

Table I. *Sequence similarity between sus1 and sh1 is found only in protein-coding regions*

Regions exhibiting at least 70% homology of at least 30 bp are listed in the first column. Positions given are those in *sus1*. Sequence positions of exon 2 to 15 of *sus1* are also listed.

Region of Homology	Exon	
	No.	Position
2877–2888	2	2809–2945
2901–2902		
2905–2935		
3680–3701	3	3660–3789
3883–4030	4	3879–4030
4159–4350	5	4156–4348
4665–4703	6	4670–4788
4723–4725		
4900–4938	7	4892–5108
4946–4951		
4962–5106		
5196–5295	8	5193–5288
5376–5549	9	5379–5552
5681–5759	10	5647–5763
5864–5920	11	5865–6031
5936–6033		
6115–6239	12	6126–6350
6250–6252		
6301–6351		
6444–6484	13	6443–6761
6589–6762		
6847–7001	14	6851–7095
7005–7010		
7017–7076		
7082–7093		
7170–7306	15	7173–7752

The high level of sequence divergence of the introns relative to that of the exons is most likely due to selection against mutations abolishing or greatly reducing function of either gene. Other than the sequences at exon/intron borders, nuclear introns exhibit little if any sequence similarity. These findings then suggest that mutations in introns that do not alter their ability to be recognized as introns would be selectively neutral. In contrast, a much smaller percentage of mutations in exonic sequences encoding proteins of importance to the organism would likely be selectively neutral. Because of the different rates of divergence exhibited by introns and exons, we conclude that both *sh1* and *sus1* must be important to the host, maize.

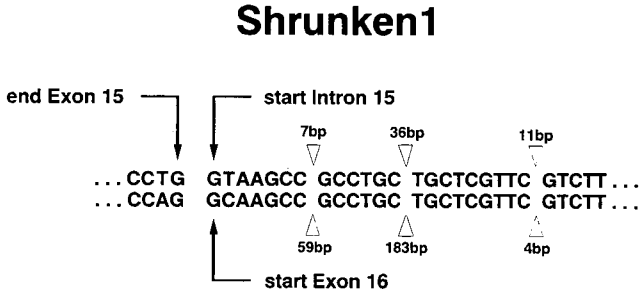

Figure 3. Sequence comparison of intron 15 and exon 16 of *sh1*. Sequences common to the exon and intron are given. Position and size of divergent sequences are depicted with triangles.

sh1 and *sus1*-like Genes Are Found in All Monocots Analyzed

Although the physiological importance of the *sh1* gene is clear, the relevance of *sus1* and its protein is equivocal, as noted above. Since a number of plant *sus* genes have been

cloned and sequenced, we asked whether both *sh1* and *sus1* counterparts could be found in all plants so far analyzed. A search of GenBank and relevant literature revealed 17 *sus* sequences. In four cases, the two sequences reported from one plant were identical or virtually so. Since these likely represent the same gene or alleles of the same gene, we removed these duplicates from the analysis below. We compared the derived amino acid sequence common to the remaining 13 clones. An evolutionary tree of these sequences initially was prepared using the UPGMA utility present in GeneWorks (Intelligenetics, Mountain View, CA) software (data not shown). This analysis revealed that *sh1* and *sus1* clearly fall into two separate branches of the tree, with each branch containing multiple members. Furthermore, *sus* genes from dicots defined a branch of the tree separate from the two containing the monocot *sus* genes.

A more exhaustive analysis was done using Phylogenetic Analysis Using Parisomy software (Swofford, 1993). In this analysis, the sequence from *Vicia faba* (Kuester et al., 1993) was omitted, since it is almost identical with that of *Vigna radiata* (Arai et al., 1992). The results of the Phylogenetic Analysis Using Parisomy analysis are shown in Figure 4. The data clearly show that plant Suc synthases can be placed into at least three major branches; one represented by all of *sus* genes from dicots and the other two composed of monocot genes. One multimembered branch contains the maize *sh1* gene, whereas the other contains maize *sus1*. The *sh1* branch is most divergent and can be viewed as containing two subbranches. One subbranch contains members from maize and sugarcane (*Saccharum officinarum*), whereas the other contains the *sus2* genes of rice (*Oryza sativa*), barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*). (Following the convention of the Sucrose Synthase Subcommittee of the Commission on Plant Gene Nomenclature, *sh1* of maize is placed in the *sus2* family of plant Suc synthases.)

Specific to the gene of study here, *sus1*, all four of the monocot sequences for which two or more genes are available (maize, wheat, rice, and barley) contain an easily recognizable *sus1* type of Suc synthase.

Interestingly, *sus* genes of dicots do not fall into the branches defined by *sus1* and *sus2* of monocots. Perhaps the duplication giving rise to *sus1* and *sus2* is specific to monocots. The presence of both *sus1* and *sus2* (*sh1*) types of Suc synthase genes in the various monocots and the fact that coding sequences of *sus1* and *sh1* of maize are conserved, whereas noncoding sequences are divergent, point to the physiological importance of both Suc synthase genes. In contrast, the studies from Chourey (1988) suggest an insignificant role for *sus1* in maize. A number of explanations are possible. (a) Perhaps the importance of *sus1* is seen only under certain growth situations, conditions not used in the previous studies. (b) Perhaps the detection methodology used in previous studies was not sensitive enough to detect low levels of *sus* gene expression conditioned by the *sus1*- mutant. (c) More intriguing is the possibility that *sus1* of maize is, in fact, now dispensable. If an additional enzyme having the ability to catabolize Suc has recently been added to the maize genome, it is possible that the exon/intron difference in sequence divergence and its presence in modern maize reflects selection pressure before the addition of this hypothetical third enzyme, whereas the phenotype of the mutant reflects the physiological importance of *sus1* in present-day maize. In this regard, it is interesting to note that Su (Wang et al., 1992b; Yu et al., 1992) recently reported the presence of a third Suc synthase gene in rice. A reexamination of our

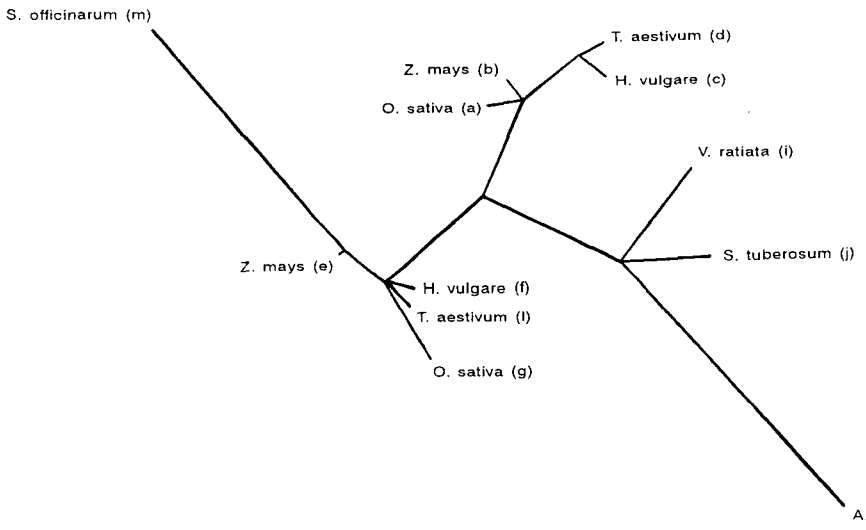

Figure 4. Unrooted tree of the plant Suc synthases. Lengths of the various lines are inversely proportional to the amount of relatedness. Letters denote references as follows: a, Wang et al. (1992a); b, this report; c, Martinez et al. (1993); d, Marana et al. (1988); e, Werr et al. (1985); f, Sanchez et al. (1992); g, Wang et al., (1992b); h, Kuester et al. (1993); h, omitted; i, Arai et al. (1992); j, *Solanum tuberosum*, Salanoubat and Belliard (1987); k, *Arabidopsis thaliana*, Chopra et al. (1992); l, Marana et al. (1988); m, GenBank accession number Z11532.

Southern blots, however, failed to reveal any hint of a third hybridizing fragment.

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