Plant Physiol. (1992) 98, 1214–1216
0032-0889/92/98/1214/03/$01.00/0

Received for publication October 16, 1991
Accepted November 24, 1991

Plant Gene Register

Genomic Nucleotide Sequence of a Wild-Type Shrunken-2 Allele of *Zea mays*[1]

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The maize (*Zea mays*) endosperm enzyme, ADP-glucose pyrophosphorylase, which produces ADP-glucose from ATP and glucose-1-phosphate, is an important enzyme in the synthesis of starch. ADP-glucose arising from the action of this enzyme is the major, if not sole, donor of glucose for starch biosynthesis. The enzyme is composed of two dissimilar subunits encoded by the two unlinked genes, *Shrunken-2* (*Sh2*) and *Brittle-2* (*Bt2*) (1, 2). The enzyme is allosterically activated by 3-phosphoglyceric acid and inhibited by phosphate (3). Although it remains an open question whether these allosteric properties are physiologically relevant or whether they simply reflect the evolutionary history of the two structural genes (4), there does exist, nevertheless, much interest in determining whether genetic modification of this enzyme could lead to increased rates of starch biosynthesis in the maize endosperm. Because endosperm starch content comprises approximately 70% of the dry weight of the seed, alterations in starch biosynthesis would clearly affect corn yield.

As a first step toward such experiments, we have isolated and sequenced genomic clones of *Sh2*. The structure of the gene (Table I, Fig. 1) is based on sequence analysis of three overlapping clones isolated from a Black Mexican Sweet genomic library. Exonic sequences were defined by comparison with the cDNA sequenced previously (2) and further sequencing of cDNAs subsequently isolated. These sequences correct for the fact that the original cDNA contained some non-*Sh2* sequences at its extremities. The exonic genomic and cDNA sequences are nearly 100% identical. The few differences may be due to DNA polymorphisms because these clones were isolated from different corn lines.

Placement of the start of the first exon is based on primer extension experiments. In the absence of dideoxynucleotides, four major bands, differing by one nucleotide, were observed. In the presence of dideoxynucleotides, the three large bands were seen in all four sequencing tracks. In each case, the largest band occurred at the same distance from the primer. It is unknown whether this reflects heterogeneity within the normal population of *Sh2* transcripts or some laboratory artifact. Nevertheless, the start of transcription can be placed within three base pairs.

[1] This work was supported by the U.S. Department of Agriculture competitive grants 88–37234–3917 and 90–37262–5638. This is University of Florida (Agricultural Experiment Station) Journal series No. R-01954.

ACKNOWLEDGMENTS

We would like to thank Jung-Myung Bae for the 5′ mRNA sequence. We thank the ICBR DNA Synthesis Core, University of Florida, for the oligonucleotides used for sequencing.

LITERATURE CITED

1. **Bae JM, Giroux M, Hannah L** (1990) Cloning and characterization of the *Brittle-2* gene of maize. Maydica **35:** 317–322
2. **Bhave MR, Lawrence S, Barton C, Hannah LC** (1990) Identification and molecular characterization of shrunken-2 cDNA clones of maize. Plant Cell **2:** 581–586
3. **Dickinson DB, Preiss J** (1969) Presence of ADP-glucose pyrophosphorylase in *Shrunken-2* and *Brittle-2* mutants of maize endosperm. Plant Physiol **44:** 1058–1062
4. **Hannah LC, Nelson OE** (1975) Characterization of adenosine diphosphate glucose pyrophosphorylase from developing maize seeds. Plant Physiol **55:** 297–302

Table I. *Characteristics of a Shrunken-2 Wild-Type Gene from Zea mays L.*

Organism:		
Zea mays L. var Black Mexican Sweet		
Location on Chromosome:		
3L		
Gene Product; Pathway:		
A subunit of ADP-glucose pyrophosphorylase; starch biosynthesis		
Gene Designation:		
Sh2		
Techniques:		
λEMBL3 *Sau*3A partial genomic library screened with *Sh2* cDNA resulting in three overlapping clones; restriction fragment subcloning into pUC19 and pSPORT; double-stranded plasmid dideoxynucleotide sequencing of the overlapping clones (both strands) using various subclones and synthetic oligonucleotide primers; primer extension with and without dideoxynucleotides to determine transcription start.		
Methods of Identification:		
Exon sequences nearly 100% identical to *Sh2* cDNA		
Expression Characteristics:		
Sh2 is expressed only in the endosperm of the maize kernel.		
Gene Structure:		
The coding region of 1913 bp[a] is interrupted by 15 introns ranging in size from 68 bp to 1821 bp		
Promoter Region:	putative TATA box	TATATAAA at −33 bp to −26 bp
	putative RY repeats	CATGCATG at −926 bp to −919 bp
		CATGCATA at −510 bp to −503 bp
	similarity to animal enhancer	GTGGAAAC at −911 bp to −904 bp
Intron Region:	similarity to animal enhancer	GTGGATAG in 5′ part of largest intron
Codon Usage:		
43.2% G + C in coding region		
No obvious bias in codon usage		
Protein Characteristics:		
The 1551 bp open reading frame gives rise to a 516 amino acid peptide; M_r 57,178.98; ADP-glucose pyrophosphorylase is allosterically regulated by phosphoglyceric acid (activation) and phosphate (inhibition).		
Antibodies:		
Antibodies made against fusion protein expressed in *Escherichia coli* recognizes an endosperm-specific protein. Size and amount of this protein is altered in *sh2* mutants (M. Giroux, L.C. Hannah, unpublished).		
Subcellular Localization:		
Reported to be in the amyloplasts.		
GenBank Accession No.:		
M81603		

[a] Base pairs.

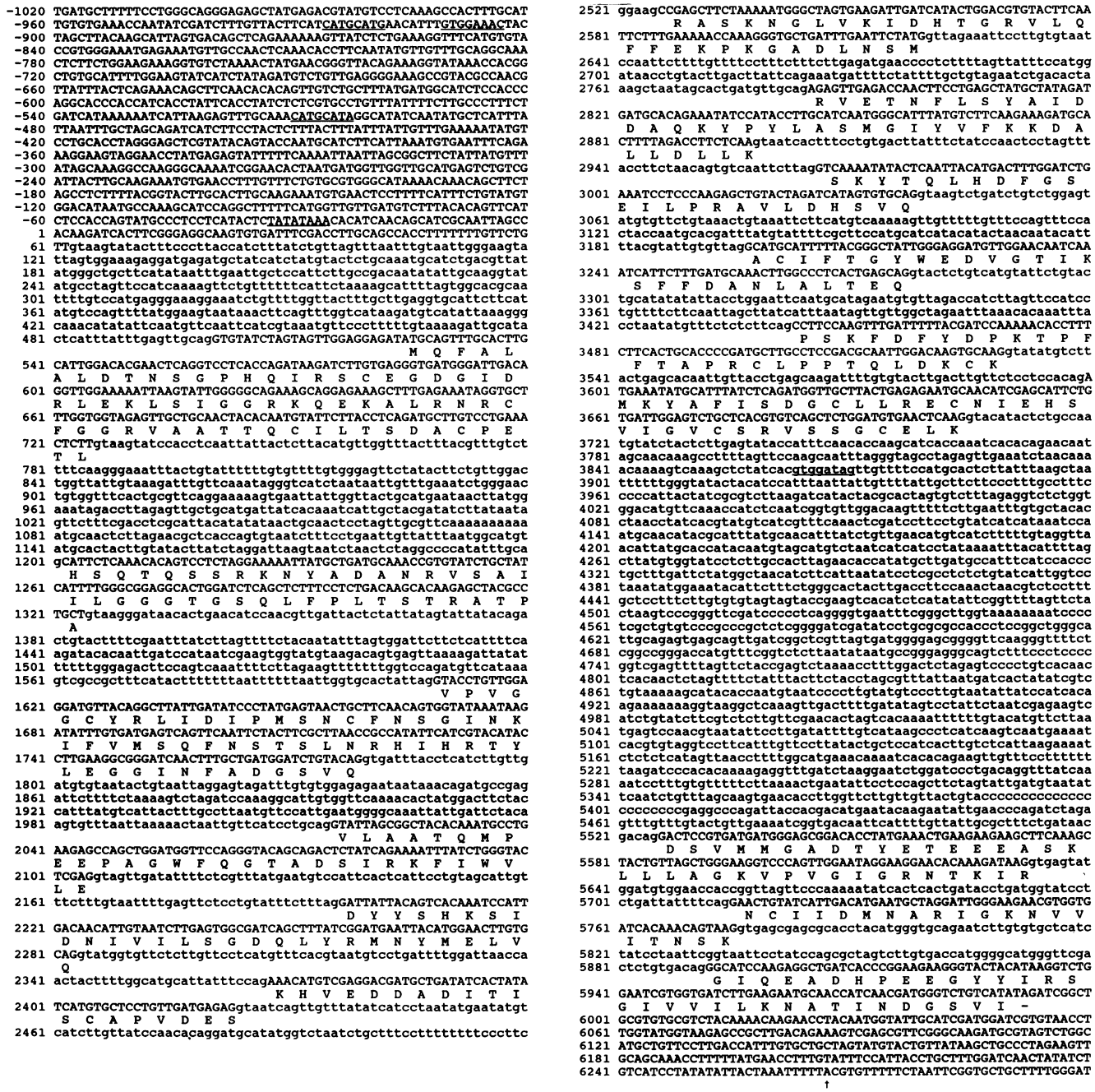

Figure 1. Nucleotide sequence and deduced amino acid sequence of a *Zea mays* wild-type *Sh2* allele. Base number 1 is the transcription start site. Introns are indicated by lowercase letters. Arrow indicates 3′ end of cDNA. Putative TATA, RY dyad, and enhancer sequences are underlined.