

# Expression of a Maize Sucrose Phosphate Synthase in Tomato Alters Leaf Carbohydrate Partitioning

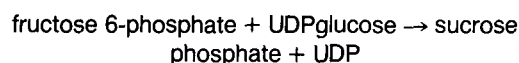
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**We isolated a complementary DNA sequence for the enzyme sucrose phosphate synthase (SPS) from maize utilizing a limited amino acid sequence. The 3509-bp cDNA encodes a 1068-amino acid polypeptide. The identity of the cDNA was confirmed by the ability of the cloned sequence to direct sucrose phosphate synthesis in *Escherichia coli*. Because no plant-specific factors were necessary for enzymatic activity, we can conclude that SPS enzyme activity is conferred by a single gene product. Sequence comparisons showed that SPS is distantly related to the enzyme sucrose synthase. When expressed from a ribulose biphosphate carboxylase small subunit promoter in transgenic tomatoes, total SPS activity was boosted up to sixfold in leaves and appeared to be physiologically uncoupled from the tomato regulation mechanism. The elevated SPS activity caused a reduction of starch and increase of sucrose in the tomato leaves. This result clearly demonstrates that SPS is involved in the regulation of carbon partitioning in the leaves.**

## INTRODUCTION

The disaccharide sucrose is the export form of the photoassimilate in most plants. Carbon fixed in the chloroplast exits as triose phosphates into the cytoplasm, where it enters the pathway of sucrose synthesis. Most of the sucrose formed in mature leaves (source) is exported by way of the phloem to the plant parts that are net consumers of photoassimilate. A key enzyme of the sucrose synthesis pathway, sucrose phosphate synthase (SPS, EC 2.3.1.14), catalyzes the following reaction:



SPS can be isolated from mature leaves as well as from germinating seeds. It is involved in the mobilization of the newly assimilated or stored carbon as sucrose (Stitt et al., 1987; Lunn and ap Rees, 1990). A growing body of work indicates that SPS, probably together with fructose 1,6-bisphosphatase, regulates the synthesis of sucrose and, therefore, may play a major role as a limiting factor in the export of photoassimilates out of the leaf (see Stitt and Quick, 1989, for a review). For example, in maize leaves the total extractable SPS activity is only threefold of the observed overall in vivo photosynthate export, and the total extractable SPS activity of different maize lines correlates with their respective growth rates (Rocher et al., 1989).

We have previously purified SPS from maize leaves to homogeneity and detected its distribution in the plant by immunoblots. The enzyme consists of two or four polypeptide subunits with a molecular mass of 138 kD and it comprises about 0.01% of total leaf protein (Bruneau et al., 1991). It appears that its activity is regulated by phosphorylation (Huber and Huber, 1991) and moderated by concentration of metabolites (Stitt et al., 1987) and light (Kalt-Torres et al., 1987b).

To date, a more detailed investigation of SPS and its potential role in the regulation of sucrose synthesis was limited by the lack of tools to dissect the complex network to which it is connected. Therefore, we set out to clone a cDNA of maize leaf SPS to allow direct manipulation of the SPS activity by way of transgenic plants. We were able to obtain limited peptide sequence from maize SPS and subsequently clone a full-length SPS cDNA. This cDNA, when expressed in *Escherichia coli*, exerts full enzyme activity. In transgenic tomato, the maize enzyme is active and influences carbon accumulation.

## RESULTS

### Protein Sequencing

Maize leaf SPS was purified by using a combination of conventional and immunoaffinity chromatography (Bruneau et al., 1991). The final preparation showed a

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multiband pattern with only very minor amounts of the 138-kD, full-length SPS polypeptide. The most prominent polypeptides migrated with an  $M_r$  of 95 to 105 (=SPS90) and an  $M_r$  of 35 to 40 (=SPS30). Antisera raised separately against SPS30 and SPS90 each recognized the full-length SPS polypeptide, which proved that these polypeptides were proteolytic breakdown products formed during the purification of the SPS enzyme (Bruneau et al., 1991). For protein sequencing, we used gel-purified SPS30 and SPS90. About 1 nmol of each was digested with trypsin, and the peptides were fractionated by reverse phase HPLC (data not shown). By automated Edman degradation of approximately 100 pmol of each peptide, we obtained the amino acid sequence from three SPS90 peptides (A8, Thr-Trp-Ile-Lys; B4, Tyr-Val-Val-Glu-Leu-Ala-Arg; B11, Ser-Met-Pro-Pro-Ile-Trp-Ala-Glu-Val-Met-Arg) and two SPS30 peptides (4K, Leu-Arg-Pro-Asp-Gln-Asp-Tyr-Leu-Met-His-Ile-Ser-His-Arg; 12N, Trp-Ser-His-Asp-Gly-Ala-Arg).

#### Generation of a Partial SPS cDNA by Polymerase Chain Reaction (PCR)

Our strategy for isolating an SPS cDNA was based on the limited amino acid sequence obtained from the tryptic peptides. As a first goal, we wanted to synthesize a partial SPS cDNA in a PCR reaction, which could serve as a probe for screening in DNA libraries (strategy of mixed oligonucleotide primed amplification of cDNA, Lee et al., 1988). For this purpose, oligonucleotides derived by reverse translation from two different SPS peptide sequences were synthesized. Because the location of the two peptides in the polypeptide sequence was not known, two sets of PCR reactions were required per peptide couple, covering both possibilities of relative order. Having obtained an amplified fraction of the SPS transcript, we would use this DNA fragment as a nondegenerate probe for library screening.

To apply this strategy, we used double-stranded cDNA, prepared from maize leaf poly(A) RNA, as a template in the PCR. Amplification could be achieved with several combinations of oligonucleotides and reaction conditions. The approach appeared to be very reproducible. In one case, using two degenerate oligonucleotides (20-mers), the sense primer derived from the SPS90 peptide B11 and the antisense primer derived from the SPS30 peptide 4K (see sequences in Methods), we amplified a 1200-bp cDNA connecting the coding sequences of the two peptides (data not shown). The complementary reaction, with the sense primer from peptide 4K and the antisense primer derived from the peptide B11, did not yield any amplified product. Oligonucleotides derived from areas of the 4K and B11 peptides not used for the design of the PCR primers confirmed in DNA gel blot hybridizations that indeed the amplified DNA was true SPS cDNA.

#### Maize SPS Is Encoded by a 3500-bp mRNA

The PCR-amplified fragment was used to screen conventional cDNA libraries derived from maize leaf poly(A) RNA. In summary, by using partial clones from several libraries, we were able to assemble an SPS cDNA of 3509 bp in length, as shown in Figure 1 (see Methods).

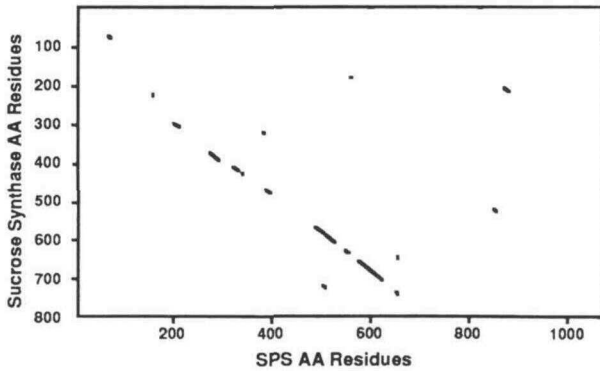
The cDNA contained one large open reading frame with a coding area of 3204 bp (assuming the first in-frame ATG is used for initiation). The derived 1068-amino acid polypeptide contained all 5 peptide sequences obtained by direct protein sequencing of the SPS30 and SPS90 tryptic peptides (Figure 1). This confirmed our immunological data that had indicated that SPS30 and SPS90 had arisen from 1 polypeptide. In addition, it showed that SPS90 is the N-terminal fragment and SPS30 the C-terminal fragment. The calculated molecular mass of the derived SPS polypeptide was 118.5 kD, which correlates reasonably well with the molecular mass of 138 kD as measured by SDS-PAGE. The 3509-bp cDNA contained at least the complete reading frame for the SPS polypeptide, as translational stop signals existed upstream of the first methionine codon. Also, primer extension experiments (data not shown) have indicated that the cap site of the native transcript was about 10 bases beyond the 5' end of our cDNA. The 190-bp 3' untranslated sequence contained two poly(A) addition signals (AATAA) at positions -140 and -90 relative to the poly(A) tail. This is relatively distant for the position of the classical poly(A) addition signal from the actual poly(A) tail in higher eukaryotes, so potentially non-consensus poly(A) sites are used (Mogen et al., 1990). The SPS cDNA was used as a probe to detect SPS transcripts. In RNA gel blots of maize leaf total RNA, a single hybridizing band migrating slightly slower than the 28S rRNA was apparent, which is consistent with the expected length for a 3500- to 3600-bp mRNA (data not shown).

When we searched current gene banks for sequences similar to the derived SPS amino acid sequence, we found weak but significant sequence identity with the published sequences of sucrose synthases from maize (Werr et al., 1985), wheat (Marana et al., 1988), soybean (Thummler and Verma, 1987), and potato (Salanoubat and Belliard, 1987). Figure 2 shows a dot matrix plot of maize SPS aligned with maize sucrose synthase. In the region of highest match, between SPS amino acids 488 to 686, maize sucrose synthase and maize SPS shared 25% sequence identity. The maize SPS sequence deviated slightly more from the other sucrose synthase sequences (data not shown).

#### Active SPS Enzyme Is Expressed from the Cloned cDNA

To prove that we had cloned a cDNA coding for a functional SPS enzyme, we expressed this cDNA in two heterologous systems. First, the SPS mRNA was transcribed from





**Figure 2.** Sequence Homology between SPS and the Related Enzyme Sucrose Synthase.

Dot matrix analysis comparing the derived amino acid sequences of maize SPS and maize sucrose synthase (Werr et al., 1985). A window of 9 amino acids is moved along the amino acid sequence. If the number of matching amino acids is equal or superior to the stringency (35%), a point is drawn in the corresponding position on the diagram.

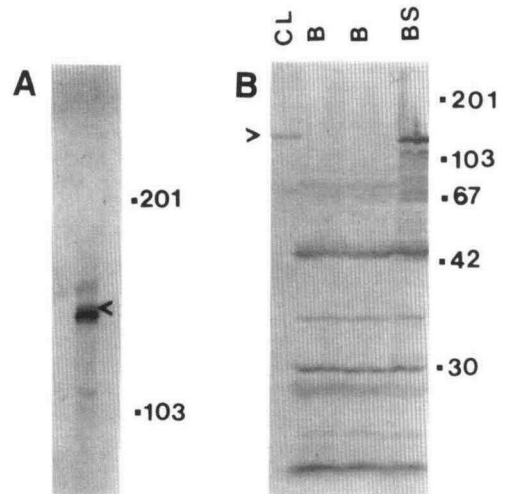
shown). Extracts with similar SPS activity had similar SPS immunoblot signals.

These results clearly prove that we have cloned a cDNA coding for the SPS enzyme. They also demonstrate that for its efficient functioning as a sucrose phosphate synthesizing enzyme, maize leaf SPS can be made from one single translational product. No other maize- or plant-specific factors and polypeptides appear to be necessary for sucrose phosphate synthesis. In addition, to obtain highest  $V_{max}$  (as observed with leaf SPS harvested from mature maize leaves at noon, Bruneau et al., 1991), no plant-specific post-translational modifications (phosphorylations, etc.) are needed.

**Expression of Active Maize SPS in Tomato Leaves**

Because we wanted to study the effect of added SPS on carbon partitioning in leaves, we expressed the maize SPS cDNA under the control of a promoter from a gene encoding the light-regulated and leaf-specific ribulose-1,5-bisphosphate carboxylase small subunit of tobacco (O’Neal et al., 1987; see Tobin and Silverthorne, 1985, for a review). The chimeric gene was transferred into tomato by cocultivation with *Agrobacterium* and 18 plants were regenerated. All of the transgenic plants were tested for the presence of the maize SPS by immunoblot of total leaf proteins, as shown in Figure 5. Tomato SPS did not cross-react with our polyclonal maize SPS antisera (Figure 5, lanes 1 and 10). With respect to maize SPS expression in the leaves of the tomato transformants, we found a wide

range of plant-to-plant variation, as is commonly observed with transgenic plants. The transformant with the highest expression level, plant SSU-11, had a maize SPS immunoblot signal twice as strong as extracts with equivalent amounts of protein from maize leaves (compare lanes 5 and 10 in Figure 5). We arbitrarily assigned the SPS signal in leaves of SSU-11 the value of 100% and ranked all of the other transformants relative to a dilution series of its extract (Figure 5, lanes 5 to 9); some representatives are shown in lanes 2, 3, and 4 of Figure 5, and their assigned percent values are given in Figure 6A.

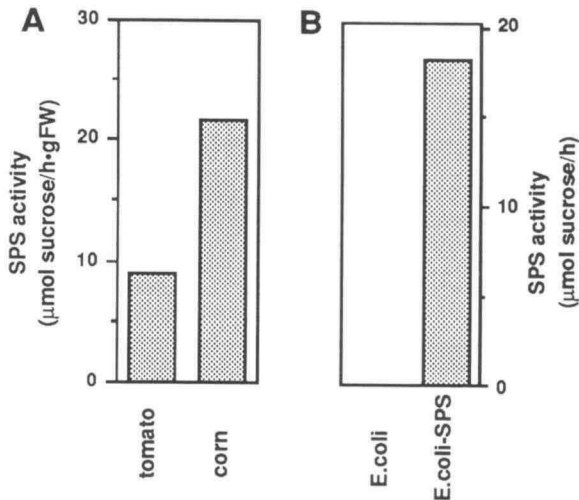


**Figure 3.** SPS Expression in Vitro and in *E. coli*.

(A) Autoradiogram of in vitro synthesized SPS. The plasmid pCGN3803 was transcribed in vitro by the T3 of bacteriophage RNA polymerase I, and the synthetic mRNA translated by rabbit reticulocytes ( $^{35}$ S-methionine) in vitro. An aliquot of the translation assay and maize leaf total protein extract were mixed into one sample. After SDS-PAGE, the proteins blotted to nitrocellulose, and the nonradioactive maize leaf-synthesized SPS was detected by immunoblot. After development of the immunosignal, the filter was processed for autoradiography. The single major band with a molecular mass of 135 kD represents the autoradiographic signal of the in vitro expressed SPS polypeptide. Exact alignment of the immunoblot with its autoradiograph allowed the precise band location of the maize leaf in vivo expressed SPS to be at a molecular mass of 138, indicated by the arrow.

(B) SPS cDNA expressed in *E. coli*. Immunoblot with SPS polyclonal antisera (Bruneau et al., 1991). Lane CL, 5  $\mu$ g of mature maize leaf extract; arrow depicts the SPS polypeptide band. Lanes B, 20  $\mu$ g of protein extract from *E. coli* transformed with vector alone. This control shows the *E. coli* protein background, which is intrinsic to the immunoblot procedure. Lane BS, protein extract from *E. coli* transformed with vector carrying the SPS cDNA insert (pCGN3803, protein extraction and immunological procedures, see Bruneau et al. 1991).

Molecular mass markers are given in kilodaltons.



**Figure 4.** Detection of SPS Activity in Plant Leaves and Transformed *E. coli*.

(A) Comparison of maize and tomato SPS levels. Tomato and maize leaves were harvested (noon) and assayed for extractable SPS ( $V_{\text{max}}$ ) for comparison.

(B) SPS activity extracted from *E. coli*. Transformed bacteria were grown on plates at 30°C, several colonies were harvested by scraping, and the cells were fragilized with lysozyme and opened by sonication. Proteins were extracted and desalted as described in Methods.

When tested for extractable SPS activity in the leaves of the transformants, we found that we had boosted it dramatically over the endogenous tomato leaf level. The extract with the highest SPS activity, SSU-9, had an SPS level sixfold over the tomato control (Figure 6B) and was twofold higher than a maize leaf extract (see tomato/maize comparison in Figure 4A). Other transgenic plants, like SSU-5, which had no detectable maize SPS immunoblot signal (Figure 4A), had an unaltered tomato-specific SPS activity level.

These data clearly demonstrated that maize SPS can be expressed in tomato leaves to levels higher than found in its origin, the mature maize leaves. In addition, it seems that in the transgenic cells the specific activity of maize SPS was high. Apparently, the tomato cell was unable to significantly down regulate this enzyme ( $V_{\text{max}}$ ) by protein modification.

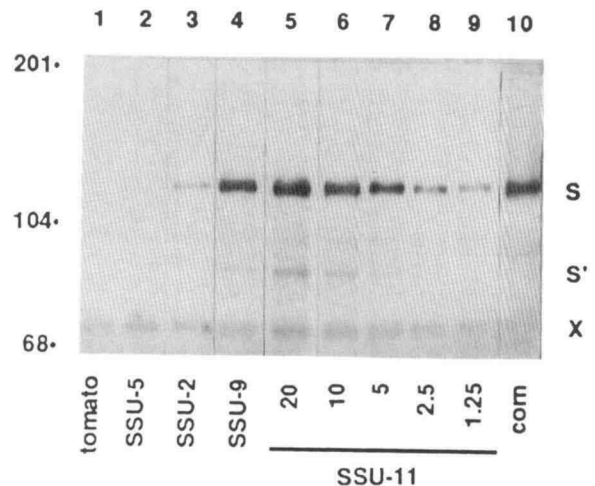
### Maize SPS in Tomato Leaves Is Uncoupled from the Diurnal Regulation

In maize leaves, SPS is diurnally regulated by protein modification (Huber and Huber, 1991), and the enzyme extracted from samples taken at midnight has about 25%

specific enzyme activity when compared to a leaf harvested at noon, as shown in Figure 7 (Bruneau et al., 1991). In tomato, the nightly reduction was not as pronounced (Figure 7). When we assayed several of our highest expressing transformants for day/night differences in extractable SPS activity, we found even less difference (Figure 7). This result indicated that, in the dicot tomato, maize SPS was uncoupled from the diurnal SPS modification. The tomato plant tolerated the drastically elevated enzyme levels. More research is necessary to establish the cause for the unregulated behavior of maize SPS in tomato.

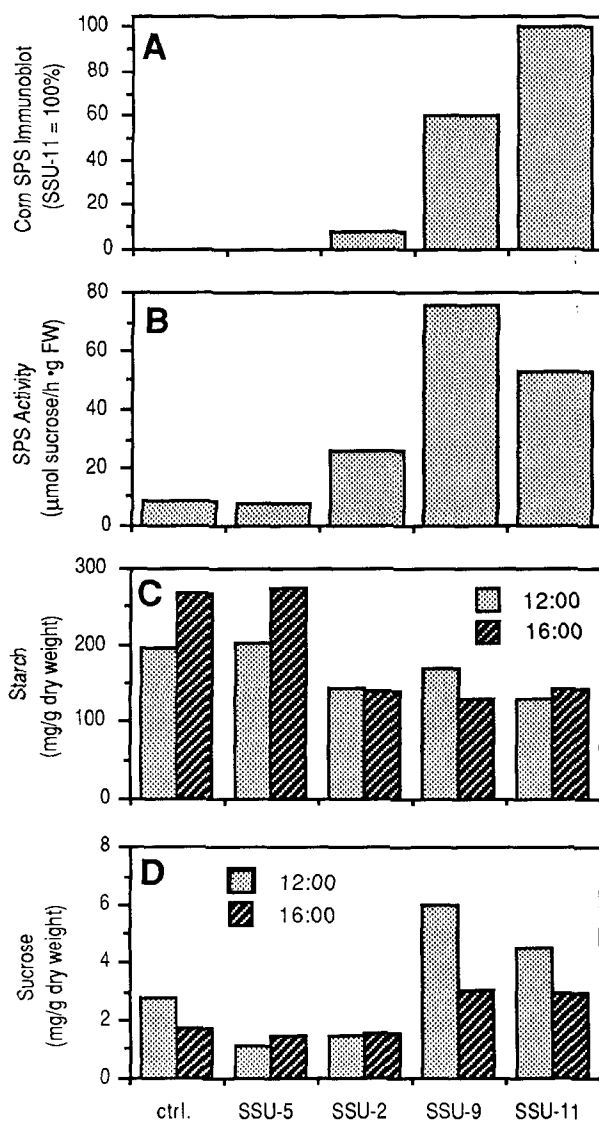
### Elevated Levels of SPS in Leaves Lead to a Reduction of Leaf Starch

During the photoperiod, tomato leaves accumulate a relatively high level of starch in the chloroplast and maintain a



**Figure 5.** Immunodetection of Maize SPS in Transgenic Tomatoes.

Mature leaves from greenhouse plants were harvested, and proteins were extracted and processed for immunoblot detection of maize SPS as described by Bruneau et al. (1991). For each lane, 20  $\mu\text{g}$  of total protein was loaded onto polyacrylamide gels. Source of protein is given under each lane. Polyclonal antiserum was used for maize SPS detection. Molecular mass reference scale is to the left (in kilodaltons). S, SPS full length polypeptide; S', putative SPS breakdown product; X, unrelated tomato leaf protein, nonspecific staining. To measure maize SPS accumulation levels, we used a dilution series of the protein extract from the transgenic tomato with the highest expression level (SSU-11) in extract from untransformed tomato. Numbers given refer to the amount of transgenic SSU-11 protein (micrograms) in the respective dilution sample. All other plants were compared to this reference series. Only a selection is shown.



**Figure 6.** Leaf Analysis of the Transgenic Tomatoes.

About 10 mature, expanded leaflets were harvested for each sample. We analyzed material from an untransformed tomato control (ctrl.) and four transgenic plants (SSU-5, SSU-2, SSU-9, and SSU-11) displaying very differing maize SPS expression levels.

**(A)** Maize SPS immunoblot signal. Total proteins were extracted, and 20  $\mu$ g of protein was separated by SDS-PAGE and maize SPS immunodetected as described by Bruneau et al. (1991). SPS immunoblot signals of all transformants were visually compared with a dilution series of the highest expresser, SSU-11, which was arbitrarily given the value 100%. The control tomato extract gave no signal at a molecular mass of 138 kD and was given the value 0% (see Figure 5).

**(B)** Extractable SPS activity. For SPS activity measurements, equal amounts of fresh weight tissue were analyzed.

**(C)** Total starch analysis. Tissue was lyophilized and soluble

low level of sucrose (Ho, 1979). Circumstantial evidence from a large body of physiological observations and experiments has led to the hypothesis that an elevated level of SPS should influence this balance in favor of sucrose and potentially lead to more export of sucrose from the leaf (Huber et al., 1985; Stitt and Grosse, 1988, for example). With our transgenic tomatoes, we were now in the position to test this hypothesis in the plant. Therefore, we analyzed mature source leaves from a selection of plants and looked for changes in carbon partitioning. In Figure 6C, the starch contents of leaves of different plants, harvested at noon and at 4 PM, are compared. Starch contents in the leaves of all the transformants that had detectable maize SPS were about 25% reduced as compared to the tomato control. This difference widens to about 50% reduction at 4 PM, when the tomato leaf starch levels were highest. This finding was complemented by our sucrose data (Figure 6D). In the leaves of both high-expressing transformants, SSU-9 and SSU-11, the sucrose levels had doubled relative to the tomato control.

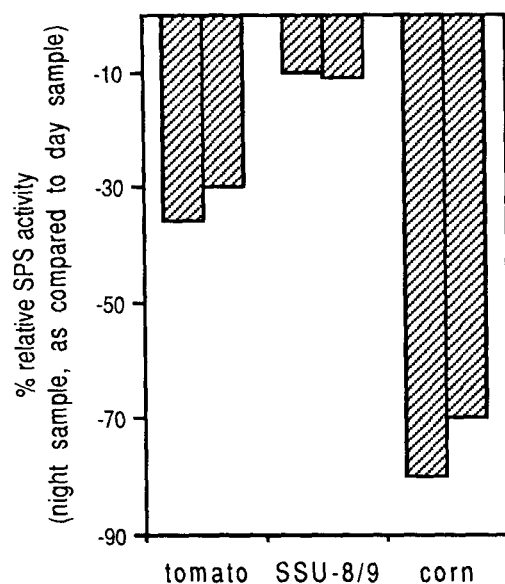
In summary, by boosting severalfold the SPS levels in tomato leaves, we were able to significantly change the balance between starch and sucrose. These data support the proposed role of SPS as a regulator in intracellular carbon partitioning. They give clear evidence that the cytosolic sucrose synthesis machinery can influence the accumulation of starch in the chloroplast.

## DISCUSSION

We have described the isolation and functional expression of a cDNA for SPS, a key plant enzyme in the sucrose biosynthetic pathway. Isolation of the cDNA required the purification of maize leaf SPS to homogeneity. Peptide sequences were used in the design of degenerate oligonucleotides that were successfully applied in a PCR scheme to synthesize a partial SPS cDNA (Lee et al., 1988). Subsequently, conventional library screening with the PCR-derived partial SPS cDNA allowed us to isolate a full-length 3509-bp SPS cDNA. Expression of the clone in

sugars were extracted. The insoluble residue was digested with amyloglycosidase, and starch was detected as newly generated glucose. Samples were taken at noon (12:00) and at 4 PM (16:00) and equal amounts of dry weight tissue were analyzed. All 16:00 values are the means of two independent experiments, variations observed were lower than  $\pm 10\%$ .

**(D)** Sucrose content of leaves. The soluble sugar fraction of lyophilized tissue sample of Figure 6C was assayed for glucose directly and a second aliquot after invertase treatment. Values obtained directly were taken off the values for the invertase samples. The difference was considered the sucrose content of the respective sample. All 16:00 samples are the means of two independent experiments.



**Figure 7.** Light Regulation of SPS in Tomato Leaves.

Leaves were harvested at noon and at midnight and assayed for SPS ( $V_{max}$ ) activity as described in Methods. The noon values obtained for each plant were set as 100%, and the difference between midnight samples as compared to the noon samples is shown in the graph. The results were obtained in duplicate for tomato and maize controls (see the double column). The SSU-8 and SSU-9 transformants were each measured once and serve as duplicates for each other.

*E. coli* confers SPS activity to the cell extracts, providing the proof of authenticity.

PCR proved to be a fast and powerful tool to build the bridge from the peptide sequences to the nucleic acids coding for them. We tested single-stranded and double-stranded cDNA as templates. In addition, we used primers that had full degeneracy or reduced degeneracy due to the use of the base analog deoxyinosine. All combinations appeared to be viable, but the lower degenerate primers produced more product.

By screening oligo(dT)-primed bacteriophage libraries, we were only able to isolate partial SPS cDNA clones. This has been shown to be common for the copying of very long mRNAs (Krupinski et al., 1989, for example). To reach the very 5' end of the transcript, we had to create special libraries primed from within the SPS coding sequence. Fortunately, DNA sequencing revealed complete sequence identity in the often several hundred base pair overlaps between the clones. This allowed us to splice the full-length 3509-bp molecule from partial representatives. We never found an SPS cDNA clone with deviating sequence in our search. This led us to the conclusion that there is only one predominant species of SPS transcript in maize

leaves. Therefore, the multiple forms of native SPS extractable from maize leaves (Kalt-Torres et al., 1987a) are caused by post-translational modification or are simply artifacts of the isolation procedures.

Maize SPS appears related to a different plant sucrose metabolizing enzyme, sucrose synthase. This enzyme catalyzes the reaction  $\text{sucrose} + \text{UDP} \rightarrow \text{UDP-glucose} + \text{fructose}$ . It is most active in the so-called sink tissues where this enzyme is responsible for the breakdown of the arriving sucrose (Chourey and Nelson, 1976). The overall sequence identity allows us to propose a common ancestor gene for SPS and sucrose synthase. Both types of enzymes share one metabolite, UDP-glucose. We speculate that the limited stretch of rather conserved sequence between SPS and sucrose synthases may indicate that this domain contains the UDP-glucose binding site. Looking at the SPS and sucrose synthase alignment, it is clear that the C-terminal 300 amino acids of SPS (from approximately residue 750 on) are unique, relative to the sucrose synthase. This C-terminal part represents about the extent of SPS30. One can speculate that a new domain had been added during evolution, and the observed proteolytic sensitivity of the full-length polypeptide (leading to SPS90 and SPS30) reflects the hinge area.

When the cDNA is expressed in vitro, a polypeptide with a molecular mass of 135 kD appears; its value is about 3 kD less than the plant-extracted protein. This slight divergence could mean that the SPS undergoes some post-translational modifications in the plant cell. It has been found by Huber and Huber (1991) that maize SPS is multiply phosphorylated. When our cDNA is expressed in *E. coli*, SPS activity accumulates. This clearly allows the conclusion that one type of subunit is sufficient to assemble SPS enzyme (homotetramer or dimer; see Bruneau et al., 1991). Obviously, for enzymatic function of the maize leaf SPS, no other plant- or species-specific polypeptide is necessary, and no plant-specific post-translational modification is required. Extractable SPS activity in plant tissue has been shown to be regulated by substrate concentrations and by the status of the respective leaf tissue (see Stitt et al., 1987; Huber et al., 1989, for a review). When SPS is synthesized in *E. coli*, it shows about the same specific activity as enzyme extracted from maize leaves (harvested in daylight, a stage with highest extractable  $V_{max}$  of SPS; see Bruneau et al., 1991). This observation leads us to the conclusion that most of the plant in vivo regulation can only be of a negative character. Phosphorylation of the maize leaf SPS protein (Huber and Huber, 1991) is not essential for enzyme function.

Introducing the maize SPS cDNA into the tomato genome and expressing it in leaves under the control of the light-regulated SSU promoter led to a drastic change of endogenous SPS levels. We were able to find transgenic plants that had an up to sixfold higher extractable level of SPS ( $V_{max}$ ). This drastic change in enzyme level resulted



in moderate change in the concentration of leaf carbohydrates and had generally no detrimental effect on the transgenic plants.

Classical physiological studies (see Stitt et al., 1987; Stitt and Quick, 1989, for recent reviews) have accumulated evidence that the de novo synthesis of sucrose in the cytoplasm is a regulator of the rate of photosynthesis, and SPS seems to be a limiting factor for net photosynthesis of carbon (Stitt and Grosse, 1988). The formation of sucrose by dephosphorylation of the product of SPS, the sucrose phosphate, releases inorganic phosphate. This inorganic phosphate then can be shuttled back into the chloroplast in a tightly coupled reaction by the triose-3-phosphoglycerate-phosphate translocator (Flügge et al., 1989) and allows more triose phosphates to be synthesized by the Calvin cycle. With our transgenic plants, we now have a system to study the respective balances of metabolites and enzyme interactions in detail. Our first set of data shows that, indeed, added SPS does influence the carbon partitioning in the leaf in a direction we expected. Clearly, most transformants do accumulate lesser amounts of starch in the chloroplast and have a higher level of sucrose in the leaves. This result clearly shows that the maize SPS, when expressed in tomato leaves, is active in plants.

Recently, von Schaewen et al. (1990) and Dickinson et al. (1991) have expressed apoplastic yeast invertase in transgenic tobacco and tomato. In both species, the plants are severely growth retarded, the rate of photosynthesis is inhibited, and starch accumulates in the leaves. These experiments demonstrated that the balance of carbon partitioning in the plant can be manipulated by the means of modern plant molecular biology. We now have taken a plant-derived enzyme essential for sucrose synthesis and have generated through over expression tomato plants with leaves low in starch and high in sucrose. Currently, we are investigating the effects of this manipulation on the rate of photosynthesis and the whole plant carbon partitioning between sources and sinks. We believe that this new avenue of research will allow us to investigate and ultimately find the control points of plant carbon partitioning.

## METHODS

### Sucrose Phosphate Synthase Extraction and Assay

SPS was assayed by colorimetric detection of F-6-P-dependent sucrose-phosphate synthesis from UDP-glucose (Kerr et al., 1987). Generated sucrose phosphate was measured by means of its fructose moiety with anthrone (van Handel, 1968). To extract total SPS activity from plant tissue, we followed the procedure of Kalt-Torres and Huber (1987). For enzyme extracts of *Escherichia coli*, we incubated cells at room temperature for 10 min with lysozyme (1 mg/mL) in 50 mM Tris, pH 8, 1 mM EDTA, 0.5%

BSA, and 100 mM NaCl. After lysis by sonication, debris was removed by centrifugation and the extract desalted and prepared for enzyme assay as described by Kalt-Torres and Huber (1987).

### Plant Material and Transformation

Maize leaf samples were taken from 30-day-old vegetative plants (*Zea mays* cv Pioneer 3184), frozen in liquid nitrogen, and stored at  $-70^{\circ}\text{C}$ . The tomato variety used for transformation was *Lycopersicon esculentum* var UC82B. Transformation followed essentially the protocol as described in Fillatti et al. (1987); transgenic plants were grown in a greenhouse.

### SPS Peptide Sequences

Immunoaffinity-purified SPS (Bruneau et al., 1991) was subjected to SDS-PAGE and proteins were visualized with KCl (Bergman, and Jörnvall, 1987). Bands were excised and electroeluted with the Isco electrophoretic sample concentrator (Isco, Lincoln, NE) in 4 mM sodium acetate, pH 8.6. One nanomole each of SPS30 and SPS90 was cleaved by trypsin and the peptides separated on reverse-phase HPLC as described by Sturm et al. (1987). Several peptides were sequenced by Edman degradation on a Perkin-Elmer automated sequencer.

### Cloning of SPS cDNA

Degenerate 20-mer oligonucleotides were designed by reverse translation. To obtain the DNA fragment between the two coding regions of the respective peptides, sets of two oligonucleotides (one each derived from a different peptide) were used as primers in PCR (Lee et al., 1988) with total double-stranded maize leaf cDNA. Poly(A) RNA had been reverse translated with oligo(dT) as a primer, second strand had been synthesized by the method of Gubler and Hoffman (1983), as modified by Promega. The temperature profile of the PCR cycle (30 cycles) was as follows: 1 min at  $94^{\circ}\text{C}$ , 1 min at  $50^{\circ}\text{C}$ , 2 min at  $72^{\circ}\text{C}$ . Reactions (100  $\mu\text{L}$ ) contained the standard buffer and nucleotide mix and Taq polymerase, according to Perkin-Elmer. Concentration of primers was kept usually in the range from 10 to 50  $\mu\text{M}$ , the template 10 to 50 ng DNA. In one PCR reaction, with the sense primer sequence reverse translated from the peptide B11 (in brackets are the degenerate positions, I stands for deoxyinosine), ATGCC(IC)CC-(IC)AT(ACT)TGGGC(IC)GA, and the antisense primer from peptide 4K, TGCAT(IC)AG(AG)TA(AG)TC(CT)TG(AG)TC, we could generate a 1200-bp PCR cDNA. This partial cDNA was first probed in DNA gel blots with short (11 to 14 bases) oligonucleotides reverse translated from both peptides, but from regions different from the PCR primers. The positive hybridization of an "inner" oligo nucleotide confirmed the authenticity of the cDNA as SPS related. This 1200-bp PCR product was directly used to screen conventional oligo(dT)-primed bacteriophage  $\lambda$  libraries (Stratagene, ZAPII) made from the mature maize leaf poly(A) RNA. In a first round, oligo(dT)-primed libraries were used and we could isolate up to 2.7-kb fragments; the 5' segment of the transcript was still missing. To overcome this problem, we primed reverse transcriptase with a short antisense primer derived from an area close to the 5' end of our longest clone. This cDNA was used for the construction of a SPS 5'-enriched library, and we were able



to isolate a series of SPS cDNA 5' fragments. The partial cDNAs were fused at shared restriction enzyme sites (all overlaps were completely identical). In total, more than 1 million plaques were screened to clone all necessary SPS cDNA fragments. The dideoxy chain termination method of Sanger et al. (1977) was employed. Sequence analyses were performed with the Intelligenetics package (University of Wisconsin, Madison, Genetics Computer Group) on a VAX computer; the dot matrix alignment was executed using the MacVector program on a Macintosh computer.

#### Expression of the SPS cDNA in Vitro and in *E. coli*

A fragment of the SPS cDNA, from position -7 (BamHI site at position 106; see Figure 1) relative to the proposed translational start codon up to the 3' terminal EcoRI site was cloned into the plasmid pBluescript SK- (Stratagene). This clone (pCGN3803) allowed the synthesis of a capped runoff transcript (Krieg and Melton, 1984) with the bacteriophage T3 RNA polymerase. The same pBluescript SK- SPS plasmid served as expression construct in *E. coli* (X11 Blue, Stratagene). The respective cloning steps placed the SPS cDNA into the *lacZ* gene and caused a translational fusion between the bacterial  $\beta$ -galactosidase and the SPS full-length polypeptide. The fusion protein contains at its N-terminal the 32 N-terminal residues of the modified  $\beta$ -galactosidase, 1 amino acid coded by the SPS 5' residual untranslated region followed by the proposed N-terminal methionine of SPS. Levels of uninduced expression in *E. coli* of this fusion protein are estimated between 0.1 and 1% of total (immunoblot, data not shown). It is noted here that the expression of SPS in *E. coli* seems to be detrimental to the host. Cultures harboring an SPS expression plasmid grow very slowly and tend to lose the plasmid after several days of culture. Induction with isopropylthiogalactoside causes the accumulation of SPS to higher levels and kills the cells.

#### RNA Extraction

Total plant RNA was extracted by a guanidinium thiocyanate method according to Turpen and Griffith (1986). Poly(A) fractionation of total RNA was achieved by two cycles of chromatography on oligo(dT)-cellulose.

#### Assembly of an SSU/SPS Gene

For expression in tomato, we constructed a transcriptional fusion between the 1.45-kb tobacco SSU 5' region of the gene TSSU3-8 (O'Neal et al., 1987; see Stalker et al., 1988, for its transgenic expression pattern) and the maize SPS cDNA. The fusion sequence area is as follows: -TGTAATTAACAGGGATCCGATG- (The first 11 bp are the last 11 bp of TSSU3-8 untranslated region, the bp 12 [boldface] comes from cloning. The remaining sequence is maize SPS cDNA, starting with bp 105 [Figure 1], with the initiation codon underlined). This construct (pCGN3809) creates a chimeric gene with a transcript containing an 81-bp 5' untranslated leader, 72 bp derived from the SSU gene. The 3' untranslated region is comprised of the complete original SPS cDNA, which contains poly(A) addition sites at positions 3418 and 3368 (Figure 1). This fusion construct was inserted into the binary vector pCGN1557 (McBride and Summerfelt, 1990), creating

pCGN3812, which was used for tomato transformation. All transformants labeled SSU- in this report are regenerated pCGN3812 plants.

#### Starch and Sucrose Analysis

One sample represents the tissue from five to 10 mature, expanded leaflets picked from several branches of a fruiting greenhouse plant. Leaflets were harvested at the respective time of day, lyophilized, and ground to powder. The material was analyzed for starch and sucrose according to the method of Haissig et al. (1979). In short, the lyophilized tissue is extracted with methanol/chloroform/water (12:5:3). Sucrose is detected after invertase treatment of the aqueous phase as glucose in a coupled enzyme system using glucose oxidase/peroxidase and *o*-dianisidine (values are corrected for endogenous glucose). Starch is detected as glucose after amyloglycosidase treatment of the insoluble fraction of the extract.

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