Plant Physiol. (1994) 104: 535–540

Temporal and Spatial Expression Pattern of Sucrose Synthase during Tomato Fruit Development[1]

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Sucrose synthase is proposed to play an important role in the early stages of tomato fruit (*Lycopersicon esculentum* Mill.) growth. In this work, the temporal and spatial expression patterns of sucrose synthase during tomato fruit development were investigated. Fruit contained the majority of the sucrose synthase protein and mRNA relative to other organs. Only trace levels of sucrose synthase protein and mRNA were detected in the stem, petiole, and roots. Sucrose synthase mRNA was detected in pistils prior to anthesis, reached peak levels in fruit 5 to 7 d after anthesis (DAA), and was not detectable after 35 DAA. Sucrose synthase protein levels reached a maximum at 20 to 25 DAA and then declined to nondetectable levels after 45 DAA. The lack of coordination between protein and mRNA levels suggests that sucrose synthase expression may be controlled at the levels of both transcription and translation. Sucrose synthase mRNA was differentially localized in the fruit, being most abundant in the mesocarp cells adjacent to the placenta, the columella, and the cells surrounding the vascular bundle. Except around the vascular tissue, the localization of sucrose synthase mRNA positively correlates with starch granule accumulation at the cellular level.

Sucrose synthase (UDP-Glc:D-Fru 2-glucosyl-transferase) catalyzes the reversible reaction Suc + UDP ↔ UDP-Glc + Fru. Its main physiological importance is to cleave Suc, providing UDP-Glc for starch, cellulose, hemicellulose synthesis, and respiration (Huber and Akazawa 1986; Sung et al., 1988; Chourey et al., 1991b).

Physiological and biochemical evidence indicates that sucrose synthase is an enzyme of importance to tomato (*Lycopersicon esculentum* Mill.) fruit growth and development. We have previously reported (Wang et al., 1993a) that sucrose synthase activity is positively correlated with fruit relative growth rate and starch accumulation. Inhibiting sucrose synthase activity but not acid invertase led to a reduction in the conversion of Suc into hexose and a reduction in starch synthesis in fruit 25 DAA relative to that of the control groups. These data imply that sucrose synthase, but not acid invertase, is the key enzyme initiating Suc metabolism in the early stages of tomato fruit development. Walker et al. (1978) reported that the rate of Suc import is regulated by the Suc concentration gradient between leaves and fruit and that there is an inverse relationship between Suc levels in the fruit and its import rate. Therefore, in addition to generating a pool of UDP-Glc for diverse cellular functions, sucrose synthase may contribute to fruit development through the regulation of the import of carbon to the fruit.

During tomato fruit development, sucrose synthase activity is very low in the first week after anthesis. It progressively increases to a maximum in fruit approximately 20 DAA and then steadily decreases, becoming undetectable in fruit at the onset of ripening (Robinson et al., 1988; Yelle et al., 1988; Wang et al., 1993a). When sucrose synthase activity in different organs was compared, the highest activity occurred in the sampled fruit at 13 DAA. This activity was approximately 10-fold higher than that of other organs (Wang et al., 1993a). These results suggest that sucrose synthase gene expression is regulated both developmentally and spatially.

To better understand the role and the regulation of sucrose synthase during the early stages of tomato fruit development, the current study examines the temporal expression patterns of tomato sucrose synthase mRNA and protein in developing fruit and the spatial expression pattern of sucrose synthase at the organ and cellular levels.

MATERIALS AND METHODS

Plant Material

Seeds of tomato (*Lycopersicon esculentum* Mill. cv UC82 B) were germinated on moistened filter paper in Petri dishes. After radicle emergence seedlings were transplanted to 15-cm pots with a soil mixture of peat moss, soil, and perlite (2:1:1). Plants were grown in a greenhouse and fertilized weekly with 200 ppm of 20:10:20. Fruit age measured as DAA was determined by tagging each flower at anthesis.

RNA Extraction and Northern Blot Analysis

Total RNA was isolated from pistils, fruit, petioles, base of stems (5 cm above the soil), roots, and leaves according to the method described by Rochester et al. (1986). RNA was electrophoresed on formaldehyde-agarose gels (1%) and transferred to GeneScreen membrane (Dupont, NEN). The membranes were prehybridized in a solution containing 50% formamide, 5× Denhardt's solution (1× Denhardt's solution is 0.02% Ficoll, 0.02% BSA, 0.02% PVP), 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM Na_3PO_4, pH 7.5, 1 mM EDTA), 1% SDS, and denatured single-stranded salmon sperm DNA (100

[1] Supported in part by the Minnesota Agricultural Experimental Station. Paper No. 20670, Scientific Journal Series.

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Abbreviation: DAA, days after anthesis.

μg/mL) at 42°C for at least 2 h. Sucrose synthase or acid invertase probes were added at approximately 1 μCi/mL in the same solution and hybridized at 42°C for 14 h. The blots were washed twice with 0.3× SSPE plus 0.1% SDS at room temperature for 15 min and then washed with the same solution at 65°C for 30 min.

The acid invertase cDNA clone (Klann et al., 1992) and a 1.5-kb *Eco*RI to *Hin*dIII fragment of potato sucrose synthase cDNA clone, λ 10a (Salanoubat and Belliard, 1987), were used as probes. It was subsequently determined that a tomato fruit sucrose synthase cDNA (Wang et al., 1993b) was very similar (97%) to the coding region of this potato sucrose synthase cDNA isolated from tuber tissue. ^{32}P-labeled probes were made using the random oligonucleotide priming method as described by the manufacturer (Boehringer-Mannheim). Probes were purified from free ^{32}P nucleotides by passing them through a Biogel P-60 (Bio-Rad) column.

Protein Extraction and Western Blot Analysis

Plant material (the same samples as used in RNA extraction except that pistils were omitted) was ground in liquid N_2 and proteins were extracted with buffer (precooled to 4°C) containing 100 mM Mes, 15% (v/v) ethylene glycol, 2% (v/v) 2-mercaptoethanol, and 100 mM Suc for 30 min at 0°C. The mixture was centrifuged at 8000*g* for 10 min. Protein was precipitated with saturated $(NH_4)_2SO_4$ solution (30–80% saturated fraction). Pellets were resuspended in the extraction buffer. The protein suspension was desalted by passing it through a Sephadex G-25 (Sigma) spin column. The concentration of the desalted protein solution was quantified as described by Bradford (1976).

Proteins were resolved by SDS-PAGE using a discontinuous Tris-Gly buffer system. Polyacrylamide concentration was 8 and 2.5% for the separating and stacking gels, respectively. Proteins were electrophoretically transferred to nitrocellulose membrane (BioTrace, Gelman Sciences, Ann Arbor, MI). Blots were blocked with 1% nonfat dry milk solution in PBS (10 mM K_3PO_4, 150 mM NaCl, pH 7.2) and 0.05% Tween 20. Polyclonal antibodies against maize sucrose synthase (Nolte and Koch, 1993) were used to react with sucrose synthase protein. Sucrose synthase and the antibody complexes were detected with goat antirabbit antibodies conjugated with ^{125}I.

In Situ Localization of Starch Granules and Tomato Sucrose Synthase mRNA

Cryosections were prepared from fruit at 6 DAA. It was found that fruit at this stage had high levels of sucrose synthase mRNA and contained many amyloplasts. Cryosections (8 μm) were air dried and placed on a 42°C slide warmer for 2 min. The dried specimens were fixed with ethanol:glacial acetic acid (3:1) for 15 min at 4°C, dehydrated in 70 and 95% ethanol for 10 and 5 min, respectively, and then air dried.

Starch granules were visualized by staining with KI/I_2 (2 g of KI and 1 g of I_2 in 300 mL of H_2O) for 5 min. Probes for the in situ hybridization were prepared using the 1.5-kb potato sucrose synthase *Eco*RI to *Hin*dIII insert (Salanoubat and Belliard, 1987), which was cloned into Bluscript KS$^+$. ^{35}S-labeled sense and antisense RNA probes were synthesized using T3 and T7 polymerases as described by the manufacturer (Promega Biotech CD). The sense strand probes were used to detect nonspecific binding. In situ hybridization was performed essentially according to the procedures of Smith et al. (1987). Labeled RNA probe (5×10^5 cpm) in 100 μL of hybridization solution was used for each slide. The slides were dipped in NTB-2 (Kodak) autoradiography emulsion and exposed at 4°C for 10 d before developing.

RESULTS

Sucrose Synthase Gene Expression in Tomato

Blots containing total RNA or protein from roots, stem bases, petioles, and 15-DAA fruit were hybridized with a DNA probe made from a potato sucrose synthase cDNA clone or polyclonal antibodies against maize sucrose synthase protein. One major band of approximately 2.8 kb was detected on the northern blots (Fig. 1A). The size of the major band was in agreement with a tomato sucrose synthase cDNA

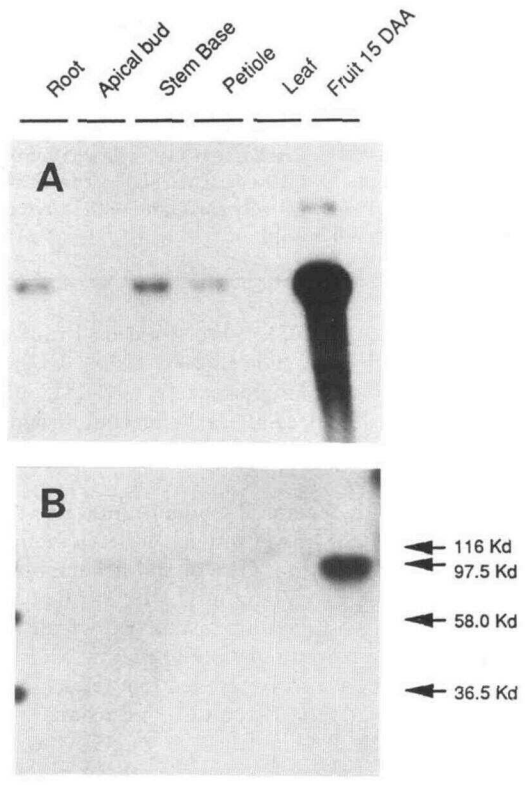


Figure 1. A, Northern blot analysis of the steady-state levels of sucrose synthase mRNA in different organs of tomato. Ten micrograms of total RNA were loaded into each lane. The filter was probed with a ^{32}P-labeled potato sucrose synthase cDNA clone (Salanoubat and Belliard, 1987). B, Western blot analysis of the steady-state levels of sucrose synthase protein in different organs of tomato. Fifteen micrograms of protein were loaded into each lane and separated by SDS-PAGE. The filter was probed with ^{125}I-labeled polyclonal antibodies against maize sucrose synthase (Nolte and Koch, 1993).

(Wang et al., 1993b) and was similar to that of the potato sucrose synthase transcript from tubers (Salanoubat and Belliard, 1987). Analysis of sucrose synthase copy number in maize (Echt and Chourey, 1985) and sorghum (Chourey et al., 1991b) indicates that there are two genes per haploid genome. Southern blot analysis of tomato genomic DNA hybridized under identical conditions as the northern blots indicated that the probe used in these studies hybridized to only one RNA species (data not shown).

Denaturing western blot analysis detected only one band with a molecular mass of approximately 97 kD (Fig. 1B). The molecular mass of this band was consistent with the size deduced from a tomato sucrose synthase cDNA clone (Wang et al., 1993b). Fruit at 15 DAA had much higher levels of sucrose synthase mRNA and protein relative to petioles, stem bases, and roots. No sucrose synthase mRNA or protein were detected in apical buds or leaves (Fig. 1).

Temporal Expression Pattern of Sucrose Synthase and Acid Invertase during Pistil and Fruit Development

Northern blot analysis (Fig. 2) and western blot analysis (Fig. 3) revealed that the steady-state levels of sucrose synthase mRNA and protein were developmentally regulated. Sucrose synthase mRNA was first detected in pistils of flower buds 5 to 6 mm in length, increased to high levels in pistils from flowers at anthesis, reached maximum levels in fruit 5 to 7 DAA, declined steadily in fruit 10 to 30 DAA, and was not detectable in fruit after 35 DAA (Fig. 2). Identical RNA samples were used to determine the temporal expression pattern of acid invertase. In contrast to that of sucrose synthase, low and relatively constant levels of acid invertase mRNA were detected in pistils and fruit 0 to 35 DAA (Fig. 2). Peak levels of acid invertase mRNA were detected in ripening fruit 45 and 55 DAA (Fig. 2). Only trace amounts of sucrose synthase protein were detected in fruit 0 to 4 DAA. The sucrose synthase protein level increased to a maximum at approximately 25 DAA and declined to nondetectable levels after 45 DAA (Fig. 3).

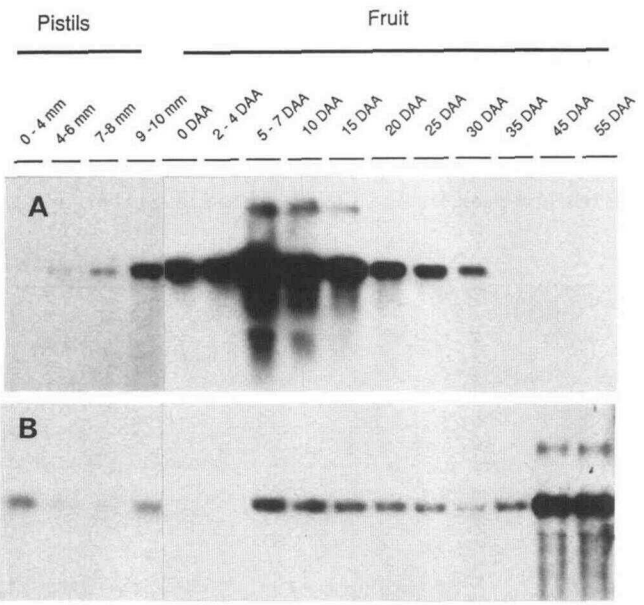


Figure 2. Northern blot analysis of the steady-state levels of sucrose synthase and acid invertase mRNAs in developing pistils and fruit. Ten micrograms of total RNA were loaded into each lane. The filters were probed with the ^{32}P-labeled potato sucrose synthase cDNA clone (A) (Salanoubat and Belliard, 1987) or with a ^{32}P-labeled tomato acid invertase cDNA clone (B) (Klann et al., 1992). Pistils and fruit were developmentally staged by sepal length and DAA, respectively, as indicated above the lanes.

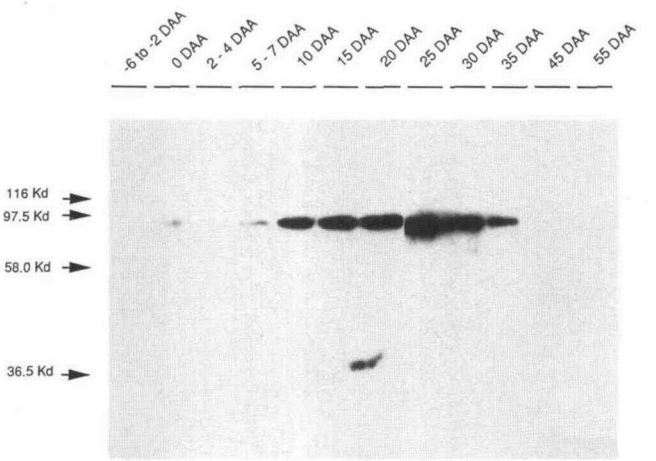


Figure 3. Western blot analysis of the steady-state levels of sucrose synthase protein in developing fruit. Fifteen micrograms of protein were loaded into each lane and separated by SDS-PAGE. The filter was probed with polyclonal antibodies against maize sucrose synthase (Nolte and Koch, 1993).

Sucrose Synthase Gene Expression and Starch Accumulation in Developing Fruit

The distribution of sucrose synthase mRNA in developing fruit was examined by in situ hybridization. Tomato fruit are composed of epidermis, pericarp (mesocarp, radial pericarp, and columella), vascular tissue, placenta tissue, and seeds (Fig. 4). Amyloplasts (plastids containing starch granules) accumulate differentially in the parenchymatous cells of fruit 6 DAA. Amyloplasts are in greater numbers in the inner mesocarp and radial pericarp cells adjacent to the placenta tissue and in the columella relative to other cells of the fruit (Fig. 5, A1, A2, A3). There was also a ring of amyloplasts in the placenta tissue surrounding the seed (Fig. 6B). In situ localization of sucrose synthase mRNA in fruit 6 DAA showed that there was a differential distribution of sucrose synthase mRNA that closely paralleled starch distribution (Fig. 5, B1, B2, B3; Fig. 6D). However, sucrose synthase mRNA was detected in cells surrounding the vascular bundles (Fig. 6C), where there was very little starch accumulation (Fig. 6A).

DISCUSSION

We previously reported that of all tomato organs, the developing fruit have the highest levels of sucrose synthase

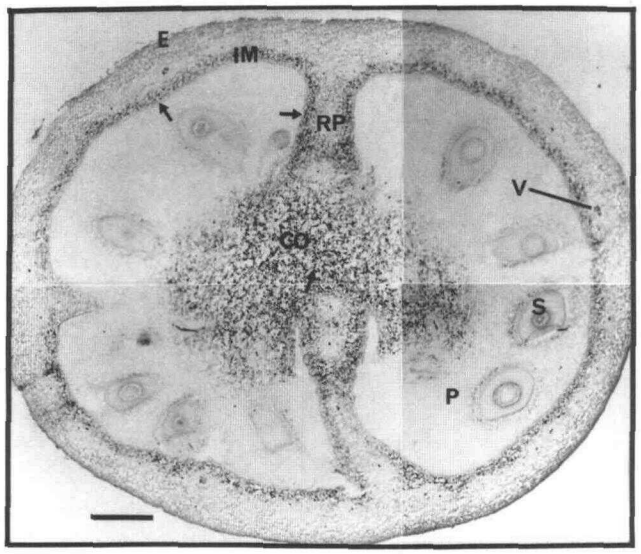


Figure 4. Anatomy and starch distribution of tomato fruit. Cryosections were prepared from fruit 6 DAA. The cryosection was stained with KI/I_2 and photographed using bright-field optics. E, Epidermis; IM, inner mesocarp; RP, radial pericarp; CO, columella; V, vascular bundles; P, placenta tissue; S, developing seeds. Arrows point to the areas where abundant starch granules were detected. The bar represents 1 mm.

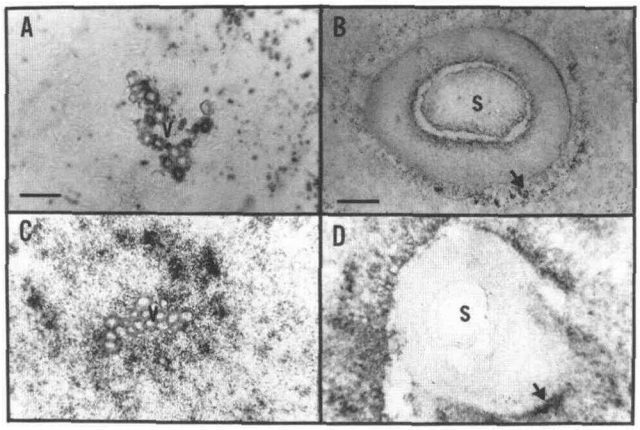


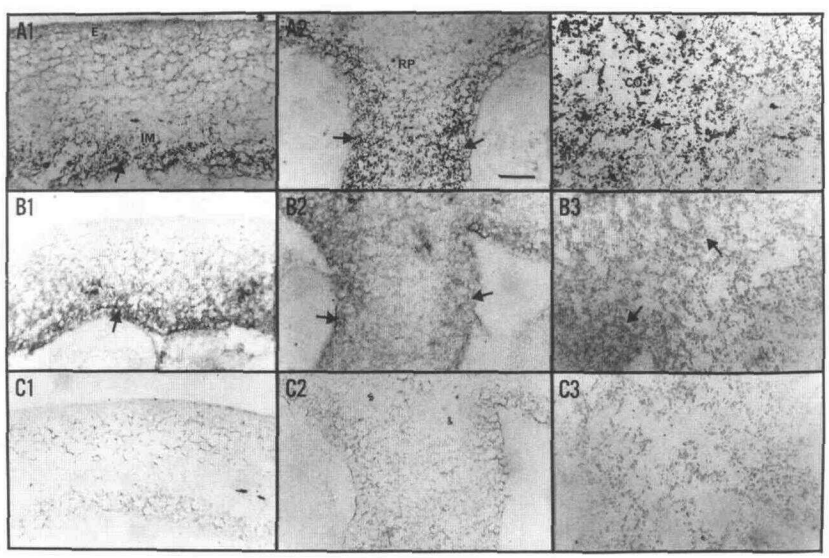
Figure 6. Sucrose synthase mRNA and starch granule localization around vascular bundles and developing seeds. Conditions for starch granule staining and sucrose synthase mRNA localization were the same as described in Figure 5. A and B, Starch localization; C and D, sucrose synthase mRNA localization. V, Vascular bundle; S, developing seeds. The bar in the left column (A and C) represents 50 μm. The bar in the right column (B and D) represents 200 μm.

activity (Wang et al., 1993a). Consistent with this observation, the highest levels of sucrose synthase mRNA and protein were detected in fruit (Fig. 1). Maximum levels of sucrose synthase mRNA occurred in fruit 5 to 7 DAA (Fig. 2). During this time period cell divisions continue to occur with the initiation of rapid cell enlargement and starch accumulation (Ho and Hewitt, 1986). In contrast to that of sucrose synthase, acid invertase transcript levels were low in developing fruit (0–35 DAA) and reached high levels in ripening fruit 45 to 55 DAA (Fig. 2). These results are consistent with the conclusion that sucrose synthase is the predominant Suc-cleaving enzyme during the early stages (0–35 DAA) of fruit development and acid invertase is more likely to be important during the fruit-ripening process.

Levels of Sucrose Synthase Protein and Activity Were Parallel

The steady-state levels of sucrose synthase protein paralleled its enzyme activity. Only trace amounts of sucrose synthase protein were detected in fruit 0 to 4 DAA (Fig. 3), corresponding to a trace level of sucrose synthase activity and no detectable fruit growth (Ho and Hewitt, 1986; Wang et al., 1993a). Sucrose synthase protein increased after 5 DAA and reached peak levels 20 to 25 DAA (Fig. 3). This is the

Figure 5. Sucrose synthase mRNA and starch granule localization in tomato fruit. The cryosections were either stained with KI/I_2 (row A) or hybridized with ^{35}S-labeled antisense (row B) or sense (row C) RNA probes prepared from the potato sucrose synthase cDNA. Row C represents hybridization background. Arrows point to examples of starch granule accumulation (row A) and sucrose synthase mRNA localization (row B). The left column represents sections from the pericarp tissue. E, Epidermis; IM, inner mesocarp. The middle column represents sections from the radial pericarp (RP). The right column represents sections in the center of the columella (CO). The bar represents 200 μm. All photos were taken using bright-field optics and are shown at the same magnification.



period when maximum sucrose synthase activity and fruit growth occur (Ho and Hewitt, 1986; Wang et al., 1993a). The steady-state levels of sucrose synthase protein declined in fruit after 25 DAA and became undetectable in fruit 45 DAA (Fig. 3). During this period sucrose synthase activity and fruit relative growth rate decrease progressively (Wang et al., 1993a).

The parallels among fruit growth, sucrose synthase activity, and sucrose synthase protein levels are expected. During the early stages of fruit development, sucrose synthase is the dominant enzyme in cleaving Suc (Wang et al., 1993a). Active cell division, cell enlargement, and starch accumulation during the early stages of fruit development (0–25 DAA) demand a constant supply of UDP-Glc, the product of sucrose synthase-catalyzed Suc cleavage. The lack of active sucrose synthase could lead to an arrest of fruit growth. Chourey et al. (1991a) hypothesized that sucrose synthase played a critical role in maintaining the cellular stability of developing maize kernels. They attribute the phenomena of early cell degeneration in *sh* mutant kernels to deficient cellulose and hemicellulose content due to reduced levels of UDP-Glc resulting from the mutation of the endosperm sucrose synthase gene.

Sucrose Synthase mRNA and Protein Levels Were Not Closely Coupled

Comparing the temporal patterns of sucrose synthase mRNA and protein accumulation shows that they are not synchronized. In pistils of flowers at anthesis sucrose synthase mRNA was abundant (Fig. 2), whereas there were only trace levels of sucrose synthase protein (Fig. 3). Sucrose synthase mRNA reached peak levels between 5 and 7 DAA, whereas sucrose synthase protein reached peak levels at 20 to 25 DAA. Sucrose synthase mRNA was not detectable at 35 DAA, when significant levels of sucrose synthase protein still existed. Therefore, both transcriptional and posttranscriptional regulation may contribute to the temporal expression pattern of sucrose synthase. McElfresh and Chourey (1988) reported that anaerobiosis caused the accumulation of the *Sh* mRNA, but not the corresponding protein. Further investigation showed that the SS1 protein was stable but the translation of the anaerobiosis-induced *Sh* mRNA was not efficient due to a block at steps beyond polyribosomal loading (Taliercio and Chourey, 1989). Similarly, regulation at the level of translation may be involved in sucrose synthase gene expression during fruit development.

Sucrose Synthase mRNA and Starch Granules Had Similar Distribution

In the previous study (Wang et al., 1993a), we observed that sucrose synthase activity positively correlated with starch levels in the pericarp as well as the stem base. In situ localization of sucrose synthase mRNA and starch accumulation demonstrates that, except in cells surrounding the vascular bundle, this positive correlation is consistent at the cellular level (Figs. 5 and 6). Cells of the inner mesocarp and radial pericarp adjacent to the placenta tissue, cells in the collumuna, and the placenta tissue surrounding the seeds accumulate many amyloplasts and have high levels of sucrose synthase mRNA. These results strengthen the conclusion that sucrose synthase plays an essential role in starch accumulation. The coordination of amyloplast development and sucrose synthase protein accumulation was also observed in cells of the maize kernel (Chen and Chourey 1989; Heinlein and Starlinger, 1989; Rowland and Chourey, 1990).

Sucrose Synthase mRNA Was Abundant in Cells Surrounding the Vascular Bundles

The association of sucrose synthase with vascular bundles has been reported (Claussen et al., 1985; Tomlinson et al., 1991; Nolte and Koch, 1993). Consistent with these reports, this study shows that sucrose synthase mRNA is localized in cells surrounding the vascular bundles (Fig. 6C). Several hypotheses for the physiological significance of sucrose synthase in companion cells have been proposed: maintenance of an equilibrium between phloem Suc and its breakdown products to prevent Suc leakage during transport, production of substrates for companion cell respiration, and providing precursors for the synthesis of complex carbohydrates, such as callose (Nolte and Koch, 1993).

How Are the Temporal and Spatial Regulation of Sucrose Synthase Gene Expression Achieved?

Yang and Russell (1990) transformed tobacco plants with a chimeric gene construct containing the *Sh1* 5′ end driving the expression of the β-glucuronidase reporter gene. Expression of this gene construct was found to be cell-specific, with abundant expression occurring in the endosperm tissue of immature seeds and in phloem tissues. These results suggest that the majority of the *Sh1* gene expression is regulated through the *cis*-acting element.

Koch et al. (1992) observed that sucrose synthase gene expression in corn roots was induced differently by different sugar levels. At a high sugar concentration (2% Glc), *Sus* gene expression was induced around the vascular bundles, whereas at a low sugar concentration (0.1% Glc), the *Sh* expression was induced in the parenchyma cells beneath the epidermis, which was in contact with the medium. The authors suggest that sugar levels could modify the expression of the *Sh* and *Sus* genes in a cell-specific manner. This suggestion is supported by the observations that high Suc concentrations induce potato sucrose synthase expression in leaves and petioles (Salanoubat and Belliard, 1989) and that rice sucrose synthase expression is induced by high levels (300 mM) of Glc, Fru, and Suc (Karrer and Rodriguez, 1992). Therefore, the organ-specific expression pattern of sucrose synthase can be modified by physiological factors such as sugar concentration.

The content of reducing sugars (Fru and Glc) increases from 0.1% of the pistil fresh weight to 2% of the fruit fresh weight during the first 2 weeks of fruit development (Ho and Hewitt, 1986). In fruit 20 to 40 DAA, levels of reducing sugars are relatively constant at 3.2% of the fruit fresh weight (Wang et al., 1993a). The increase of reducing sugars during the first 2 weeks is in agreement with reducing sugars being a major signal that controls sucrose synthase gene expression.

However, sugar levels cannot account for the decrease in sucrose synthase mRNA levels (Figs. 2 and 3) in fruit between 10 and 20 DAA, when high levels of reducing sugar are observed. Therefore, other factors may be involved.

Feedback regulation of sucrose synthase expression has been demonstrated. In a transient expression system, the inhibition of cellulose synthesis results in the suppression of expression from the *Sh* promoter (Mass et al., 1990). If this type of feedback regulation was acting through starch synthesis in the fruit, it might explain the decrease in sucrose synthase gene expression with the decreased starch synthesis and high reducing sugar content during fruit development. This mechanism is also consistent with the co-localization of sucrose synthase mRNA and starch granules at the cellular level.

In summary, sucrose synthase gene expression is under temporal and spatial regulation, with both transcription and translation implicated as control points. Multiple signals including cell differentiation, inducers, or inhibitors may be involved. Hence, the exact mechanisms that control this important temporal and spatial expression remain to be elucidated.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Salanoubat for the potato sucrose synthase cDNA clone, to Dr. Koch for the polyclonal antibodies against maize sucrose synthase, and to Dr. Bennett for the tomato acid invertase cDNA clone.

Received June 28, 1993; accepted September 25, 1993.
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