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Regulation of Sucrose Metabolism in Higher Plants: Localization and Regulation of Activity of Key Enzymes

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ABSTRACT: Sucrose (Suc) plays a central role in plant growth and development. It is a major end product of photosynthesis and functions as a primary transport sugar and in some cases as a direct or indirect regulator of gene expression. Research during the last 2 decades has identified the pathways involved and which enzymes contribute to the control of flux. Availability of metabolites for Suc synthesis and 'demand' for products of sucrose degradation are important factors, but this review specifically focuses on the biosynthetic enzyme sucrose-phosphate synthase (SPS), and the degradative enzymes, sucrose synthase (SuSy), and the invertases. Recent progress has included the cloning of genes encoding these enzymes and the elucidation of posttranslational regulatory mechanisms. Protein phosphorylation is emerging as an important mechanism controlling SPS activity in response to various environmental and endogenous signals. In terms of Suc degradation, invertase-catalyzed hydrolysis generally has been associated with cell expansion, whereas SuSy-catalyzed metabolism has been linked with biosynthetic processes (e.g., cell wall or storage products). Recent results indicate that SuSy may be localized in multiple cellular compartments: (1) as a soluble enzyme in the cytosol (as traditionally assumed); (2) associated with the plasma membrane; and (3) associated with the actin cytoskeleton. Phosphorylation of SuSy has been shown to occur and may be one of the factors controlling localization of the enzyme. The purpose of this review is to summarize some of the recent developments relating to regulation of activity and localization of key enzymes involved in sucrose metabolism in plants.

KEY WORDS: actin cytoskeleton, 14-3-3 proteins, invertase, SNF1 protein kinase, CDPK, regulatory protein phosphorylation, sucrose-phosphate synthase, sucrose-sensing, sucrose synthase, translocation.

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I. INTRODUCTION

Sucrose (Suc) plays a central role in higher plants as a transport sugar, nutrient, and potential signal molecule. As a result, considerable research interest has been focused on identifying the mechanisms that regulate Suc metabolism. Suc is synthesized in leaves as one of the primary end products of leaf photosynthesis. During the day, the substrate for Suc biosynthesis is triose phosphate (TP), released from the chloroplast on the triose phosphate translocator in exchange for inorganic phosphate (Pi). At night, starch mobilization provides the substrate for Suc biosynthesis probably in the form of Glc, derived from amylolytic breakdown of starch, released on the hexose transporter (Schleucher et al., 1998). Thus, Suc biosynthesis during periods of active photosynthesis involves cytosolic fructose-1,6-bisphosphatase (FBPase) and associated control by the Fru-2,6-P_2 system, whereas this control point is bypassed in the dark. The basic regulatory features of the sucrose biosynthetic pathway and the coordination with the rate of CO_2 assimilation and starch production have been reviewed recently (Stitt et al., 1987; Huber et al., 1993; Quick, 1996).

Regardless of the translocated sugar, it is now generally accepted that net sucrose synthesis can occur in some developing sink tissues (e.g., various fruits, including tomato and cucurbits, and sugar beet root; see Huber et al., 1993; Quick, 1996, and references therein) and nonphotosynthetic source tissues (e.g., germinating seeds). It appears that sucrose-phosphate synthase (SPS) is the enzyme responsible for sucrose synthesis in the non-green tissues, although the source of substrate, and hence pathways involved, will vary depending on whether translocated sugars are being utilized (as in developing fruit) or stored reserves (as in germinating seeds). In most sink tissues, of course, net sucrose degradation is occurring. Ultimately, hexose sugars or their derivatives are formed and metabolized for energy production or for C-skeletons for growth or storage product accumulation. Many details of this metabolism have been reviewed recently (Quick, 1996) as well as general principles of plant glycolysis (Plaxton, 1996).

This review focuses primarily on the recent progress on the enzymes directly involved in Suc biosynthesis (SPS) and degradation (SuSy and invertases). Cloning of the respective genes from several species and new developments relating to posttranslational regulation and compartmentation is presented. The models proposed are somewhat speculative and are intended to provide a framework for future studies in this important area.

II. SYNTHESIS OF SUCROSE AND ITS DERIVATIVES

A. Sucrose-Phosphate Synthase

The biosynthesis of Suc is catalyzed by the sequential action of SPS and sucrose 6′-phosphate phosphatase (SPPase):

$$\text{UDP-Glc} + \text{Fru-6-P} \leftrightarrow \text{Suc-6′-P} + \text{UDP} + \text{H}^+$$
$$\text{Suc-6′-P} + \text{H}_2\text{O} \rightarrow \text{Suc} + \text{Pi}$$

Rapid removal of Suc-6′-P by a specific and high-activity phosphatase displaces the reversible SPS reaction from equilibrium *in vivo* (Stitt et al., 1987), and thus it is thought that SPS activity contributes to the control of flux into Suc. In spinach leaves, SPS activity is regulated by the allosteric effectors, Glc-6-P (activator) and Pi (inhibitor), and effector sensitivity is altered by protein phosphorylation (discussed below). As a result, SPS activity is often measured under two different conditions: (1) the 'selective assay,' which contains rate-limiting substrate concentrations plus Pi (V_{SEL} activity), and (2) the 'nonselective assay,' which contains nearly saturating substrate concentrations in the absence of inhibitor (V_{MAX} activity). The 'activation state'

of the enzyme is defined as the V_{SEL} activity expressed as a percentage of the V_{MAX} activity, and often reflects the phosphorylation status of the enzyme.

The importance of SPS in sucrose biosynthesis has been confirmed by efforts to use molecular genetics to manipulate SPS. Overexpression of maize SPS in tomato results in increased sucrose synthesis, increased sucrose/starch ratios in leaves, and increased photosynthetic capacity, indicating that SPS is a major point of control of photosynthesis particularly under high CO_2 and saturating light (Galtier et al., 1993; Galtier et al., 1995; Micallef et al., 1995). Conversely, reduction in SPS expression in potato leaves via antisense repression resulted in inhibition of sucrose synthesis and increased flow of carbon into starch and amino acids (Krause, 1994). Studies with these transgenic plants produced a flux control coefficient of SPS in sucrose synthesis of 0.3 to 0.45 (Krause, 1994; Geigenberger et al., 1995), indicating that SPS is only one of the enzymes contributing to the control of sucrose synthesis. Interestingly, in some cases overexpression of SPS does not result in increased SPS activity because of posttranslational regulation of the enzyme (see below).

In the long term, the level of SPS protein is regulated by developmental, environmental, and nutritional signals. At least in some cases, for example, changes in irradiance, control appears to be at the transcriptional level, that is, transfer of plants from a low- to a high-light environment results in increased steady-state SPS-mRNA levels followed by a slower increase in SPS protein and activity (Klein et al., 1993). Superimposed on the regulation of SPS gene expression are several mechanisms that can rapidly regulate the catalytic activity of SPS. One such mechanism is allosteric control, with activation by Glc-6-P and inhibition by Pi (Doehlert et al., 1983; Doehlert et al., 1983; Doehlert et al., 1985). Thus, Suc biosynthesis is only promoted when metabolites are abundant. Another important mechanism involves reversible phosphorylation and has become a complex topic because of multiple phosphorylation sites (Huber et al., 1992). In the following sections, we review the current understanding of this mechanism and the physiological significance.

1. Fluctuation of SPS Activity in Leaves

a. Endogenous Rhythms

Diurnal changes in SPS activity were first observed in soybean plants and were of interest because activities of other enzymes involved in Suc biosynthesis did not fluctuate (Rufty et al., 1983; Huber et al., 1985). The diurnal pattern of SPS activity in greenhouse- or field-grown vegetative soybean plants typically exhibited a bimodal pattern, with one peak in the first part of the photoperiod followed by a second peak about 12 h later. Thus, SPS activity was often lowest during the afternoon hours. Interestingly, changes in SPS during the photoperiod generally paralleled changes in the rate of assimilate export (Huber et al., 1985). Diurnal changes in SPS activity have also been observed in fully expanded leaves of cotton, and, again, were correlated positively with assimilate export rate during the photoperiod (Hendrix et al., 1986). Collectively, these results lend strong support to the notion that SPS activity contributes to the control of C-flux into Suc, and thus can be taken as an indicator of the capacity for Suc synthesis (Huber et al., 1985).

A key feature distinguishing an endogenous rhythm in SPS activity from light/dark modulation (discussed below) is that in the former case changes in activity are not strictly aligned with light-dark transitions. In addition, an endogenous rhythm will also persist under continuous environmental conditions. The diurnal fluctuation in SPS activity in

soybeans (Kerr et al., 1985) and tomato (Jones et al., 1997) leaves has been demonstrated to persist under constant environmental conditions, proving control by the biological clock. In the case of soybean, SPS activity fluctuated with a period of about 12 h in either constant light or extended darkness, indicating an ultradian rhythm (Kerr et al., 1985). The basis for the change in SPS activity was suggested to involve the steady-state level of SPS protein or a posttranslational modification that affected only maximum catalytic activity and not regulatory properties or substrates affinities (Kerr et al., 1987). In contrast, SPS activity in leaves of tomato exposed to continuous low light fluctuated with a period of about 24 h, indicating an underlying circadian rhythm (Jones and Ort, 1997), and phosphorylation has been strongly implicated as the underlying biochemical mechanism. Specifically, the SPS-protein phosphatase(s) are thought to be controlled by the circadian clock at the level of gene expression (Jones and Ort, 1997). In tomato (unlike soybean), the rhythm in SPS activity was only observed with the V_{SEL} assay (i.e., V_{MAX} activity remained constant). The circadian rhythm controlling the activity of SPS in tomato can be delayed by overnight low-temperature exposure (chilling) (Jones et al., 1998), but similar experiments have not yet been done with soybean. While much remains unknown, it is clear that soybean and tomato are similar in that SPS activity is controlled by an endogenous rhythm, but specific characteristics and the underlying mechanisms *appear* to be different in the two species.

b. Light/Dark Modulation

In contrast to soybean and tomato (discussed above), in many species there are rapid and reversible changes in SPS activity that occur in response to light/dark transitions. In spinach leaves, the covalent modification of SPS (Walker et al., 1989), subsequently shown to involve reversible protein phosphorylation, affects affinities for the substrate Fru-6-P and the allosteric effectors, Glc-6-P (activator) and Pi (inhibitor) (Stitt et al., 1988). Thus, light/dark modulation of V_{SEL} activity can be observed, while V_{MAX} activity remains constant. Light modulation of SPS activity can be observed with a variety of species, but there are differences in the extent to which V_{SEL} and/or V_{MAX} activities are affected. For example, in a variety of C_4 plants, both activities are affected (Ohsugi et al., 1987; Huber et al., 1989; Huber et al., 1991). Light modulation of SPS activity is thought to be one mechanism to adjust the capacity for Suc biosynthesis in relation to the rate of photosynthesis, which is a function of irradiance, CO_2, and other factors.

c. Low-Temperature Stress

Plant adaptation to low (nonfreezing) temperatures often involves accumulation of soluble sugars, the most common and most abundant of which is Suc (Steponkus et al., 1968; Kaurin et al., 1981; Guy et al., 1992). In temperate grasses, low-temperature exposure can also induce fructan synthesis, which occurs in response to Suc accumulation (Pollock and Cairns, 1991). Similarly, some species synthesize raffinose family oligosaccharides in low-temperature environments and as a result show seasonal changes in tissue sugar composition (discussed further below).

The increased Suc accumulation in photosynthetic (Jeong et al., 1990; Tognetti et al., 1990; Guy et al., 1992; Holaday et al., 1992) and nonphotosynthetic tissues, for example, potato tuber (Geigenberger et al., 1995), has been linked with increased SPS activity. In both spinach leaves and potato tubers increased maximum SPS activity as a result of cold exposure was paralleled by an

increased steady-state level of SPS enzyme protein. In spinach leaves, the rate of SPS protein synthesis (measured as [^{35}S]-Met incorporation) was at least partially responsible and the SPS protein synthesized had an identical subunit M_r to that synthesized at normal temperatures (Guy et al., 1992). In contrast, the increased SPS activity in cold-exposed potato tubers was associated with the appearance of a new form of SPS (Reimholz et al., 1997). Potato plants can contain four forms of SPS that differ in subunit size: SPS-1a (125 kDa), SPS-1b (127 kDa), SPS-2 (135 kDa), and SPS-3 (145 kDa). The forms have different tissue distributions, but SPS-1a/b are generally the predominant forms. Cold exposure led to a reversible increase in SPS-1b that coincided with a change in the kinetic properties of SPS. Specifically, SPS activity in the selective assay increased while maximum activity remained constant (Hill et al., 1996). These changes in kinetic properties are thought to be essential for enhanced Suc synthesis that occurs in cold-stored tubers even though hexose-Ps are decreased (Hill et al., 1996). Because SPS is substrate limited *in vivo*, changes in kinetic properties are thought to provide an effective mechanism to alter the catalytic activity of the enzyme.

Antisense transformants with ~75% reduction in SPS expression had decreased accumulation (10 to 40%) of soluble sugars in response to low temperature (Krause et al., 1998), indicating that SPS expression per se is not the only factor controlling Suc accumulation. The increase of SPS-1b, in response to cold storage, was accompanied by a decrease of SPS-1a (Reimholz et al., 1997), consistent with the observation that maximum SPS activity was not increased by the stress. It is likely, but not yet proven, that SPS-1a and –1b are encoded by the same gene, because both forms are decreased in antisense transformants (whereas SPS-2 and –3 are not) (Krause et al., 1998). If SPS –1a and –2a are encoded by the same gene, the two forms could arise by alternate splicing or some posttranslational modification. However, phosphorylation does not appear to be the modification involved (Reimholz et al., 1997). It is also possible that they are encoded by different, but nearly identical, genes. The more important point may be that SPS-1b has altered kinetic properties, and thus can play an important role in regulation of Suc synthesis in cold-stored tubers. It is also worth noting that sugar accumulation in cold-stored tubers is also associated with the appearance of a new form of amylase, which may be critical for supply of substrates for Suc synthesis (Hill et al., 1996).

d. Osmotic Stress

When spinach leaves (Quick et al., 1989; Zrenner et al., 1991; Toroser et al., 1997) or potato tubers (Reimholz et al., 1994; Geigenberger et al., 1995; Geigenberger et al., 1997) are incubated in hyperosmotic solutions of mannitol or sorbitol to induce osmotic stress, activation of SPS occurs. Activity in the selective assay is increased, while maximum activity remains constant, resulting in increased activation state. In *Phaseolus vulgaris* plants, SPS activation state is also increased dramatically (Castrillo, 1992) even though osmotic stress leads to a significant reduction in maximum SPS activity (Vassey et al., 1989; Castrillo, 1992), suggesting that more than one process may be occurring simultaneously in the bean plants.

Phosphorylation of a unique site on SPS has been suggested to be responsible for that activation that occurs under osmotic stress (Huber and Huber, 1992; Huber et al., 1992). The site involved is thought to be Ser-424 and is discussed further below. Osmotic-stress activation requires transcription and translation (of unknown components). It is well known that the expression of certain genes responds to drought and salt stress, including genes

encoding Ca^{2+}-dependent protein kinases (Urao et al., 1994). As discussed below, a Ca^{2+}-dependent protein kinase also appears to be involved in the osmotic-stress activation process, but is probably distinct from the kinases that have been cloned thus far.

If osmotic-stress activation of SPS involves a signal transduction pathway, as is currently thought (see below), it is possible that it is interrelated with pathways mediating responses to other stresses. A particularly intriguing possiblity is that low temperature and osmotic stress (drought) may activate SPS by a common mechanism. It is known, for example, that the expression of a number of genes can be induced by both drought and low temperature, even though these stress conditions are quite different (Liu et al., 1998). Future studies will be necessary to determine whether the same molecular mechanism underlies the activation of SPS by osmotic stress and low temperature. Another possibility deserving future attention is that proline metabolism may interact with the osmotic-stress activation of SPS, because the intermediates of proline metabolism can influence the expression of certain genes (Iyer et al., 1998).

Osmotic stress generally results in increased soluble sugars, especially Suc, and decreased starch as a result of enhanced sugar synthesis and decreased starch synthesis. Accumulation of Suc could potentially play a direct role in osmoregulation and may also provide a rapidly metabolizable carbohydrate to energy production when carbon fixation is reduced and there is an increased diversion of carbon from growth to other functions (Hare et al., 1998).

2. Control by Reversible Protein Phosphorylation

Phosphorylation was originally discovered as the mechanism underlying light/dark modulation of SPS (Huber and Huber, 1992) and has more recently been implicated in the activation of the enzyme that occurs when leaf tissue is subjected to osmotic stress (Toroser and Huber, 1997). The enzyme is phosphorylated on multiple seryl residues *in vivo*, and the current view is that three of these sites may be of regulatory significance under different conditions. The three regulatory phosphorylation sites, which involve residues thought to be in loop regions of the protein (Salvucci et al., 1995) are discussed in more detail below (see Figure 1).

a. Ser158

In an unstressed leaf, SPS is phosphorylated on multiple seryl residues but only one is thought to function in light/dark-modulation of the enzyme (Huber and Huber, 1992). The phosphorylated residue involved in dark inactivation of the spinach leaf enzyme was subsequently shown to be Ser158 (McMichael et al., 1993), and this residue is conserved among species (Huber et al., 1996). Phosphorylation of Ser158 reduces SPS activity by altering affinities for substrates and effectors (V_{SEL} activity), without affecting maximum catalytic activity. Site-directed mutagenesis of Ser158 of spinach leaf SPS has confirmed its role in the modulation of enzyme activity *in vivo* and *in vitro* (Toroser et al., 1999). The S158A mutant enzyme, expressed in transgenic tobacco plants, had a relatively high activation state (activity assayed under 'selective' conditions expressed as a percentage of the maximum or 'nonselective' activity) and was not inactivated in the dark. In contrast, introduction of an acidic group at the 158 position in the double mutant S157F/S158E yielded a mutant enzyme that was not activated by light. The results demonstrated the regulatory significance of Ser158 as the major site responsible for dark inactivation

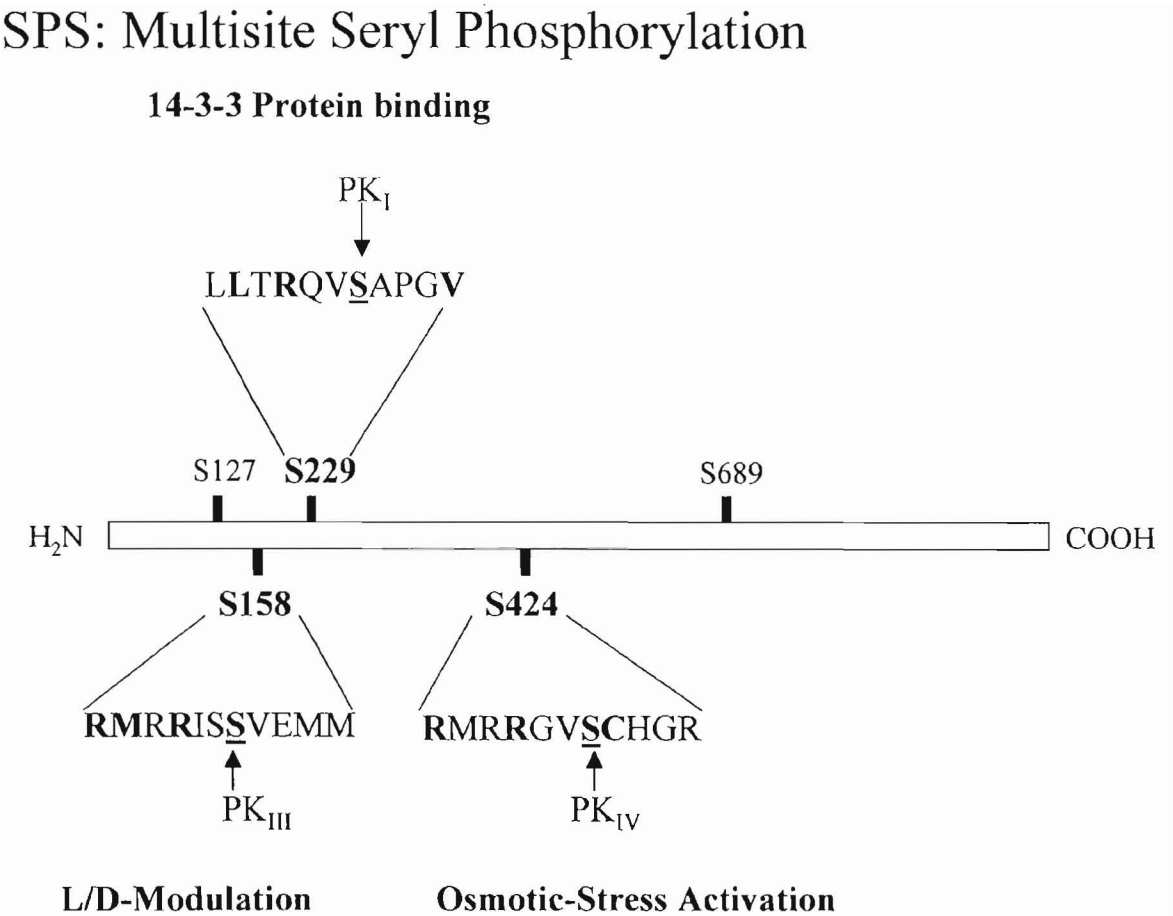

FIGURE 1. Multisite seryl phosphorylation of spinach SPS. Sequences of the three sites thought to be of regulatory significance are shown, and positive or negative recognition elements surrounding the phosphorylated seryl residue (underlined) are shown in boldface print. The primary protein kinase thought to phosphorylate each residue is identified using the nomenclature of Toroser and Huber (1997). (See text for discussion.)

of SPS *in vivo*, and suggest that the significance of phosphorylation is introduction of a negative charge at this position.

One of the major protein kinases that phosphorylates Ser158 in the native protein has been designated PK_{III} (see Figure 1) and tends to co-purify with SPS (McMichael et al., 1995). PK_{III} is Ca^{2+}-independent and has a native M_r of ~150,000. The catalytic subunit is ~60 kDa and is recognized by antibodies against RKIN1, a plant SNF1 homolog (Douglas et al., 1996). A characteristic property of the SNF1-like kinases is that they are often regulated by phosphorylation, and this has been demonstrated for PK_{III}. However, it is not known at present whether the phosphorylation state, and hence activity, of PK_{III} varies *in vivo*. If so, this could also contribute directly to the control of SPS activation state. The motif targeted by PK_{III} is a Ser residue flanked by basic residues at the –3 and –6 positions, and with a hydrophobic residue at the –5 position (McMichael et al., 1995; Toroser et al., 1998). These 'positive recognition elements' are shown in bold print in Figure 1.

Regulation of the PK_{III}-catalyzed phosphorylation/inactivation of SPS is thought to involve metabolites, in particular Glc-6-P (Weiner et al., 1992). The Glc-6-P could act by binding to SPS at its allosteric site, or by directly affecting the PK_{III} kinase, perhaps involving one of the putative regulatory subunits thought to associate with the ~60-kDa catalytic subunit to produce the native oligomeric protein. Metabolite regulation of the protein kinase(s) acting on SPS may explain the strong positive correlation observed between SPS activation state and the *in vivo* content of Glc-6-P in potato tubers (Giegenberger et al., 1998).

Phospho-Ser158-SPS can be dephosphorylated and activated *in vitro* by the catalytic subunit of the type 2A, but not type 1, protein phosphatase (PP2A) (Siegl et al., 1990). However, PP2As likely occur *in vivo* as oligomeric proteins, with the ~35-kDa catalytic subunit associated with one or more regulatory subunits. One of the interesting regulatory properties of the PP2As that act on SPS is inhibition by Pi (Weiner et al., 1992). Interestingly, the activity and regulatory properties of SPS-PP2A appear to change with light/dark treatment of leaves; the enzyme from illuminated leaves has a slightly higher activity and severalfold reduced sensitivity to Pi inhibition (Weiner et al., 1993). The molecular basis for the shift in properties is not known, but involves cytoplasmic protein synthesis either directly or indirectly as cycloheximide blocks both the light activation of SPS as well as the change in phosphate sensitivity (Weiner et al., 1992; Weiner et al., 1993).

The dephosphorylation of SPS by PP2As may also be influenced by amino acids. It has been observed that the light activation of SPS in detached leaves can be reduced by feeding amino acids via the transpiration stream and inhibition of SPS-PP2A activity by amino acids has been observed *in vitro* (Huber et al., 1996). This raises the possibility that the cytosolic concentration of amino acids may play some role in mediating source-sink interactions.

b. Ser424

Recent evidence suggests that there is a second regulatory phosphorylation site at Ser424 (see Figure 1) that is phosphorylated when leaf tissue is subjected to osmotic stress (Toroser and Huber, 1997). This site is also widely conserved among species. Phosphorylation of Ser424 activates the enzyme, perhaps by antagonizing the inhibitory effect of Ser158 phosphorylation and thereby allowing Suc synthesis to occur when it would otherwise be restricted (Huber et al., 1999). In spinach SPS, phosphorylation of Ser424 is increased severalfold in response to osmotic stress, which correlates with increased activation state of the enzyme, while maximum activity remains constant (Toroser and Huber,

1997). Control of the process apparently involves stress activation of the PK_{IV} kinase that phosphorylates Ser424 (via a presently unknown mechanism) and possibly elevation of cytosolic [Ca^{2+}], which may accompany the environmental stress.

Phosphorylation of this site may explain the observation that preincubation with ATP increases SPS activity in extracts from bundle sheath cells (BSC) of C_4 plants (Lunn et al., 1997). It is possible that the requisite kinase (perhaps analogous to the spinach leaf PK_{IV} kinase) is activated during cell isolation or that the kinase is always active in the BSC. In crude leaf extracts of the C_4 plant *Zea mays*, ATP-dependent inactivation of SPS can be clearly demonstrated (Huber and Huber, 1991), and *in vivo* dark inactivation of whole leaf SPS activity is correlated with increased phosphorylation of the enzyme (Huber et al., 1995). The results are consistent with Ser162 of maize SPS being the primary site responsible for dark inactivation of the enzyme. However, if the BSC enzyme were regulated differently *in vivo*, changes in SPS activity in whole leaf extracts may be dominated by the predominant enzyme from the mesophyll cells. Thus, it remains an intriguing possibility that the SPS in the two cell types of C_4 plants may be responding differently to phosphorylation. Indeed, slightly different diurnal changes in SPS activity between the two cell types of maize leaves have been observed (Ohsugi and Huber, 1987).

c. Ser229

Recent results demonstrate that 14-3-3 proteins can associate with spinach leaf SPS in the presence of Mg^{2+}, and the suggested site of interaction is phospho-Ser229 (see Figure 1) (Toroser et al., 1998). Evidence for an association between the two proteins was obtained by co-immunoprecipitation and co-elution during gel filtration. Binding of 14-3-3s to SPS requires phosphorylation of SPS (Huber et al., 1998), and the effect was to partially inhibit activity as measured both in the selective and nonselective assays. The site of interaction was suggested to be Ser229, based on two lines of evidence. First, the addition of a synthetic phosphopeptide based on SPS-229 was found to stimulate SPS activity, presumably by disrupting the SPS:14-3-3 complex. Second, the phosphorylated SPS-229 peptide was shown by surface plasmon resonance spectroscopy to bind a recombinant 14-3-3 protein (Toroser et al., 1998). This finding was significant, because the sequence surrounding Ser229 (RQVSAP) is a variant of the conserved motif RSXpSXP (where X is any amino acid and pS is phosphoserine) that serves as the binding site for 14-3-3s in many proteins (Muslin et al., 1996).

The physiological significance of the SPS:14-3-3 association is not yet clear. It may function to regulate SPS activity, but if so it is not clear under what conditions the association may be altered. Alternatively, the 14-3-3 protein may function as a scaffold protein (Jones et al., 1995) to facilitate the interaction between SPS and another protein (discussed further below). Clarification of these points awaits further experimentation.

Experimentally, the content of 14-3-3s associated with an SPS immunoprecipitate was specifically reduced when leaf tissue was pretreated with 5-aminoimidazole-4-carboxamide riboside (AICAR) (Toroser et al., 1998). The membrane permeable metabolite AICAR is phosphorylated in the cytosol by adenosine kinase, resulting in accumulation of the monophosphorylated derivative 5-aminoimidazole-4-carboxamide ribonucleoside monophosphate (ZMP), an analog of 5′-AMP (Vincent et al., 1992; Sullivan et al., 1994). AMP, and the analog ZMP, appear to interact with 14-3-3 proteins and directly reduce their binding to target ligands (Athwal et al., 1998). This appears to be the basis for AICAR activation of nitrate reductase (NR) (Huber

et al., 1996; Kaiser et al., 1997) and SPS (Huber et al., 1998; Toroser et al., 1998; Huber et al., 1999) in darkened spinach leaves.

3. Protein Complexes Involving SPS

There is increasing evidence from a variety of systems that soluble enzymes often occur in discrete complexes with other enzymes of the same pathway (Srere, 1987). In some cases, enzymes catalyzing sequential reactions are associated with the complex having modified physical and/or catalytic properties (Gaertner, 1978; Macioszek et al., 1987; Anderson et al., 1995). The concept of the 'metabolic unit' is becoming recognized in both prokaryotes and eukraryotes. Because of the high viscosity of the cytoplasm, estimates are that movement of metabolites by diffusion would be highly restricted (Clegg, 1992), and, thus, some 'ordering' of the macromolecules in the cytosol may be essential for efficient metabolism. With respect to SPS, there is some evidence for association with the PK_{III} protein kinase (Huber et al., 1991), which is often observed for proteins and their kinases. SPS may also form a complex with sucrose-phosphate phosphatase (SPPase). The occurrence of complexes is supported by both physical and kinetic evidence. Physical evidence for an interaction includes comigration of SPS and SPPase during native gel electrophoresis and results of isotope-dilution experiments that suggested channeling of Suc-6-P from SPS to SPPase (Echeverria et al., 1997). Kinetic evidence includes stimulation of SPS activity and reduced phosphate inhibition in the presence of SPPase (Salerno et al., 1996; Echeverria et al., 1997). Because SPS is a 14-3-3 binding protein (discussed above), and each subunit of the 14-3-3 dimer has the potential to bind a protein target (Braselmann et al., 1995), SPS could be complexed with another protein via a 14-3-3 scaffold. It is not known at present whether 14-3-3s are involved in the putative complex between SPS and SPPase, but certainly could be. Another logical possible binding partner for SPS would be UDP-glucose pyrophosphorylase (UGPase), which forms the UDP-Glc substrate for SPS. Indeed, inspection of the UGPase primary structure indicates a putative 14-3-3 binding site (Toroser et al., 1998), which raises the possibility of an SPS:UGPase complex that is bridged by a 14-3-3 protein. Further study is required to establish the existence of SPS complexes and to identify the physiological significance.

B. Sucrose as Substrate

In some species, Suc is metabolized further within the leaf to produce oligosaccharides of the raffinose family (RFO) as transport sugars, or fructans as soluble carbohydrate reserves within the vacuole. Each of these is a large topic on its own and is discussed briefly here to indicate that Suc can also serve as direct substrate for additional biosynthetic reactions.

1. Fructans

Fructans serve as reserve carbohydrates and are also thought to play at least some role in stress tolerance (Pollock and Cairns, 1991; Pilon-Smits et al., 1995). They occur in both source and sink tissues and are particularly important in grasses and cereals. It is well known that in leaves of perennial grasses such as Lolium, fructan accumulation is enhanced by environmental treatments that either increase CO_2 fixation or decrease export of assimilates (Pollock and Cairns, 1991). Suc is the substrate for fructan biosynthesis, which is formed by successive attachment of fructosyl residues to a Suc molecule. Early work established that fructans are synthesized and accumulate in vacuoles (Wagner et al., 1983),

and that induction of fructan accumulation required gene expression (e.g., excised leaves [Pollock and Cairns 1991]). Fructan synthesis is thought to occur via two steps as originally postulated (Edelman et al., 1968). The first step is irreversible and involves sucrose: sucrose 1-fructosyltransferase (1-SST), where Suc serves both as an acceptor and donor of a fructosyl residue to form the trisaccharide 1-kestose. The second step is catalyzed by fructan:fructan fructosyltransferase, which will reversibly transfer a fructosyl residue from one fructan (degree of polymerization ≥ 3) to 1-kestose or a higher-order fructan to produce a mixture of fructans with different chain lengths. In barley leaves, which accumulate branched fructans (the graminans) containing both β-2 $\rightarrow$ 1 and β-2 $\rightarrow$ 6 fructosyl linkages, the second step is thought to involve Suc:fructan 6-fructosyltransferase (6-SFT), which will only use Suc as fructosyl donor (Duchateau et al., 1995). The gene encoding 6-SST has been cloned (Sprenger et al., 1995) and was shown to be induced under conditions leading to fructan accumulation. Interestingly, the 6-SST gene is highly homologous to vacuolar invertases, suggesting that invertases were recruited, via minor amino acid changes, to function as fructan biosynthetic enzymes.

2. Raffinose Family Oligosaccharides

Raffinose saccharides are soluble galactosyl derivatives of Suc that occur widely in plants. Raffinose (trisaccharide) and stachyose (tetrasaccharide) are the most widely distributed members of this sugar family, but higher oligomers also occur but are more restricted. In many dicot species, raffinose and stachyose are found in the phloem sap (along with Suc) where they function as long-distance transport sugars (Huber et al., 1993). Raffinose and stachyose are synthesized by sequential transfer of galactosyl units from galactinol to sucrose. Galactinol is synthesized by galactinol (Gal) synthase, which catalyzes the first committed step in the pathway. The gene encoding Gal synthase has been cloned recently (Kerr et al., 1993), and the enzyme has been localized to the intermediary cells (Beebe et al., 1992), which are specialized companion cells linking mesophyll cells and sieve elements with high-density plasmodemata. Stachyose synthase has also been localized to the intermediary cells (Holtaus et al., 1991). Many of the symplastic loaders are known to translocate RFOs, which has led to the proposal that sucrose moves into the intermediary cells via plasmodesmata where it is converted to the larger RFOs that are too large to pass back through the plasmodesmata to the mesophyll cells. In this way, a concentration gradient of transport sugars can be developed to drive long-distance transport (Haritatos et al., 1995). It has also been shown that there are non-phloem-linked RFO pools in leaves of *Ajuga reptans* (Bachmann et al., 1994). There is a long-term storage pool in the mesophyl cells, which might function in frost resistance of this hardy perennial labiate, in addition to a transport pool in the phloem. Stachyose was the predominant RFO in the phloem while sugars with a higher degree of polymerization were found in the non-phloem pool. Activity of a novel galactinol-independent galactosyltransferase enzyme has been identified that may be involved in the synthesis of the higher-order RFOs (Bachmann et al., 1994).

III. TRANSPORT AND STORAGE OF SUCROSE

A. Compartmentation of Sucrose

Synthesis of Suc is restricted to the cytosol by the strict compartmentation of SPS and SuSy. However, because the vacuole is

10- to 20-fold larger than the cytosol, the vacuole represents the major diurnal storage pool for Suc in the mesophyll cell of species that accumulate Suc during the photoperiod (Gerhardt et al., 1987; Winter et al., 1994; Lohaus et al., 1996). The extent to which Suc accumulates in source leaf vacuoles has been correlated with its acid invertase activity (Huber, 1989) (see Section IV.B). Under physiological conditions sucrose is excluded from the chloroplasts and mitochondria (Heldt et al., 1971). Thus, it was surprising that the heterologeous expression of bacterial fructosyl transferase in the chloroplast resulted in accumulation of high levels of fructans, suggesting the presence of at least low concentrations of Suc in the chloroplast (Smeekens 1998).

The highest concentrations of Suc in a plant are found in the sieve tubes, where Suc is loaded for long distance transported from the source to the sink tissue driven by an osmotic system. Sieve tube Suc concentrations range from 200 to 1600 m*M* and exhibit diurnal changes with higher concentrations during the light period and lower concentrations during the night (Kallarackal et al., 1989; Lohaus et al., 1996; Winzer et al., 1996). The compartmentation of Suc requires both inter- and intracellular transport across cell membranes catalyzed by specific transport proteins.

B. Sucrose Transport Across Membranes

Intracellular transport of Suc occurs between the cytosol and vacuole. Biochemical studies of Suc uptake into isolated vacuoles and tonoplast vesicles from Suc-accumulating tissues such as red beet and sugar beet taproots (Briskin et al., 1985), sugarcane (Getz, 1991), and Japanese artichoke (Keller, 1992) indicate a carrier-mediated Suc uptake into the vacuole that likely involves a H^+-Suc antiporter. In contrast, studies on the vacuoles from barley mesophyll protoplasts, which do not accumulate Suc against a concentration gradient, strongly support facilitated diffusion as a mechanism of Suc transport across the tonoplast (Kaiser et al., 1984). No Suc transport protein of the tonoplast membrane has been cloned or purified to date.

Intercellular transport can occur via two different principle routes: carrier-mediated transport across the plasma membrane and diffusion through the aqueous phase of the cell wall (apoplastic route) or direct cell-to-cell transport via plasmodesmata (symplastic route). Intercellular Suc transport can occur within a plant via both routes, depending on the cell type and plant species. Mesophyll cells are interconnected by an extensive network of plasmodesmata, and fluorescent dyes with a molecular mass up to 1000 Da can move freely between mesophyll cells. Recently, it has been shown that even endogenous proteins such as transcription factors can move through plasmodesmata and mobilize the cell-to-cell transport of their own mRNAs (Lucas et al., 1995; Xoconostle-Cázares et al., 1999). A symplastic path of sucrose transport between cells could also occur between the sieve tube/companion cell complex (SE/CC), which are interconnected via multiple plasmodesmata. The density of these plasmodesmatal connections varies greatly among species (van Bel, 1993), and their occurrence is not sufficient to assume a symplastic transport of sucrose. Plasmodesmata are complex structures (Overall et al., 1996) and their permeability can be regulated by external and possibly endogenous factors (Lucas et al., 1993).

Whether phloem loading of sucrose occurs via an apoplastic or symplastic mode is still somewhat controversial. It has been difficult to understand how diffusion through plasmodesmata could generate the observed 10- to 50-fold sucrose concentration gradient between the sieve elements and the mesophyll cell cytosol in source tissue and maintain an osmotically insulated tissue, as would

be required for an osmotically driven phloem transport of sucrose. However, it is now recognized that many symplastic transporters translocate raffinose family oligosaccharides (see Section II.B.2) and intercellular compartmentation of synthesis appears to be a key feature to resolve this dilemma.

Overexpression of the *suc2* gene, which encodes a yeast-derived invertase, targeted to the apoplast in transgenic tobacco plants lead to an inhibition of carbohydrate export (von Schaewen et al., 1990; Heineke et al., 1992). This work strongly supported the involvement of an apoplastic step in the phloem loading process in tobacco. Apoplastic transport requires at least two different transport activities — one in the mesophyll cell plasma membrane for the release of sucrose into the apoplast, and one for the uptake of sucrose from the apoplast into the SE/CC via a H^+-Suc symporter (Bush, 1993). Complementation of a modified yeast mutant deficient in sucrose uptake was used to isolate sucrose transporter cDNAs from spinach and potato (Riesmeier et al., 1992). Heterologous screening was used to identify sucrose transporter genes (*sut*) from tobacco, tomato, arabidopsis, and plantago (Sauer et al., 1994; Stadler et al., 1995) and identified the presence of more than one sucrose transporter gene in a single species. The SUT1 protein has been shown to have proton/sucrose transport activities and kinetic characteristics similar to the ones described in isolated plasma membrane vesicles. Immunological detection of SUT1 protein (or its Arabidopsis homologue SUC2) showed its localization in the plasma membrane of companion cells in Plantago and Arabidopsis, while in tobacco, potato, and tomato it was detected in the membrane of sieve elements (Stadler et al., 1995; Stadler et al., 1996; Kühn et al., 1997) in all tissues. Antisense inhibition of SUT1 using a companion-cell specific promotor (*RolC*) or the unspecific CaMV 35S promoter led to an accumulation of Suc, hexose sugars, starch, and lipids in the leaves of the transgenic potato plants associated with a reduction in root and tuber growth, probably due to an inhibition of Suc export (Kühn et al., 1996; Schulz et al., 1998). A gene encoding a 62-kDa Suc-binding protein has been cloned from soybean (Overvoorde et al., 1996) and shown to mediate Suc transport across membranes independent of other proteins, such as SUT1. It catalyzed a nonsaturable Suc uptake that was not dependent on H^+ movement, and thus may be responsible for the 'nonsaturable' component of Suc transport often observed in plant tissues.

The expression of SUT1 is under diurnal, developmental and hormonal control (Harms et al., 1994; Kühn et al., 1997). Sucrose-mediated regulation of the sucrose transporter activity and mRNA levels has been shown to occur in faba bean cotyledons and sugar beet leaves fed with exogeneous sucrose (Weber et al., 1997; Chiou et al., 1998). However, studies of the expression pattern and sugar-inducibility of the *Suc2*-promoter with a reporter gene construct in Arabidopsis did not indicate sucrose-mediated regulation of expression (Truernit et al., 1995). Recent results also suggest the exciting possibility that the activity of the Suc transporter may be controlled by protein phosphorylation (Roblin et al., 1998).

C. Sucrose Signaling

Cells have to adjust their growth and metabolism to the availability and form of their carbon source. Most organisms have developed a sensing mechanism and signaling cascade to respond to changes in the availability of sugars. In some cases, sugar sensing is specific for Suc, while in other cases glucose or fructose can serve as signaling molecules. In response to sugar sensing, cells can adjust the amount of enzymes and proteins involved in metabolism and in patho-

gen defense, wounding response, cell cycle regulation, and senescence. For example, Suc hydrolysis in the cytosol has been identified as part of the signal-controling glycolysis and respiration in potato tubers (Trethewey et al., 1999). However, it was not possible to determine whether hexokinase activity (see below) or low cytosolic Suc levels was the actual signal.

A generalization to emerge is that carbohydrate depletion increases transcription of genes for photosynthesis, remobilization, and sucrose metabolism and decreases transcription of genes coding for proteins involved in storage and utilization. Abundant sugar supply, on the other hand, generally brings about the opposite effects (Koch, 1996). Recently it has been shown that even the proton/sucrose cotransporter is regulated in its mRNA level and activity when sucrose is supplied to excised sugar beet leaves (Chiou and Bush, 1998). Several reviews on plant sugar-sensing and sugar-induced signal transduction have been published recently (Jang et al., 1997; Smeekens, 1998; Lalonde et al., 1999).

Hexokinase has been identified as being at least part of a sugar-sensing mechanism by two different approaches. First, transient expression of sugar-responsive genes was monitored and only hexokinase substrates altered gene expression; nonmetabolizable substrate analogs, inhibitors, or products of hexokinase had no effect (Jang et al., 1997; Umemura et al., 1998). Second, antisense inhibition of hexokinase and overexpression in transgenic Arabidopsis plants showed corresponding changes in sensitivity to glucose. Interestingly, the expression of the hexokinase involved in sugar sensing in yeast was not functional in transmitting the signal in plant cells, and in fact led to hyposensitivity of the transgenic plants to glucose even though total hexokinase activity was increased (Jang et al., 1997). How hexokinase activity is involved in sugar sensing in plants (as well as in yeast and mammilian cells) is unclear. It is apparent that not all hexokinase enzymes can function in sugar sensing. In mammals, only one hexokinase isoform (hexokinase IV, a glucokinase) has a potential role in sugar sensing (Preller et al., 1992). This glucokinase was immunologically localized in the nucleus and translocated to the cytosol in response to glucose (Toyoda et al., 1994). Although there is no evidence connecting its localization with sugar sensing, it would be interesting to investigate a subcellular localization of hexokinase isoforms in plant cells.

There is also some notion that in plants a sugar sensor could be associated with the endomembrane system. This hypothesis resulted from the observation that heterologeous expression of a yeast invertase in transgenic tobacco plant caused alterations in gene expression and a chlorotic phenotype only when targetted to the apoplasmic space or the vacuole. Cytosolic expression of the invertase did not show these effects. Because hexokinase is a cytosolic enzyme, increased hexose concentrations by cleavage of the cytosolic sucrose would be expected to cause sugar-responsive changes in gene expression. It was suggested that either hexose transport or a membrane-associated hexose sensor are involved in the sugar sensing (Herbers et al., 1996). Pharmacological experiments indicate the involvment of Ca^{2+} and calmodulin and protein phosphatases in the signal transduction chain of sugar sensing (Lue et al., 1994; Takeda et al., 1994; Ohto et al., 1995). Further elements suggested to play a role in downstream sugar signaling are SNF-related protein kinases (see Section IV) (Halford et al., 1998) and putative sugar-sensing related DNA binding proteins that have been isolated from several plants (Ishiguro et al., 1992; Kim et al., 1994). More insight into signals and elements of the transduction of sugar sensing can be expected from the analysis of several Arabidopsis mutants that have been isolated by different laboratories and different screening methods.

IV. DEGRADATION AND UTILIZATION OF SUCROSE

The degradation of Suc can be catalyzed by at least two different classes of enzymes. Invertases catalyze the irreversible hydrolysis of Suc to Glc and Fru:

$$Suc + H_2O \rightarrow Glc + Fru$$

In contrast, a reversible cleavage of Suc is catalyzed by SuSy:

$$Suc + UDP \leftrightarrow UDP\text{-}Glc + Fru$$

Cleavage of Suc by SuSy conserves the binding energy of the glycosidic bond in UDP-Glc, which is the substrate for cellulose and callose synthesis, or can enter glycolysis via the combined action of UGPase and phosphoglucomutase:

$$UDP\text{-}Glc + PPi \leftrightarrow UTP + Glc\text{-}1\text{-}P \leftrightarrow Glc\text{-}6\text{-}P$$

In nongreen tissues from most plants, Glc-6-P or Glc-1-P are the preferred hexose-Ps taken up by amyloplasts for the synthesis of starch. A different mechanism for the regulation of starch synthesis exists at least in developing maize and barley endosperm. Here a cytosolic isoform of ADP-Glc pyrophosphorylase synthesizes ADP-Glc that is the most effective precursor for starch synthesis in isolated maize amyloplasts (Emes et al., 1997).

A. Sucrose Synthase

Intensive research over the last 10 years has revealed new insights in the regulation and function of SuSy in carbohydrate metabolism. In monocotyledonous species, SuSy is encoded by two differentially expressed nonallelic loci *sus1* and *sus2*. In rice plants even a third *SuSy* gene, *sus3*, has been identified recently (Huang et al., 1996). Most dicotyledonous species contain two nonallelic *SuSy* genes as well, which are functional analogs of the two classes of *SuSy* genes from monocotyledons (Fu et al., 1995). However, in legumes only single *sus* genes have been identified so far.

The isoenzymes exhibit different spatial and temporal expression (Chen et al., 1989) and are differentially regulated at the transcriptional and translational levels. SuSy protein has been shown to be subject to reversible protein phosphorylation occurs as a membrane-associated and a soluble form and can interact with the actin cytoskeleton (see Figure 2). Such an effort in regulating gene expression and the localization of an enzyme would suggest a crucial role in plant metabolism. Indeed, SuSy activity has been correlated with starch synthesis (Déjardin et al., 1997), cell wall synthesis (Chourey et al., 1998; Nakai et al., 1999), and overall sink strength (Sun et al., 1992; Zrenner et al., 1995).

Overexpression and antisense inhibition of SuSy protein in potato plants confirmed its crucial role in carbon partitioning and sink strength (Zrenner et al., 1995). The identification of maize mutants with loss of function for each *SuSy* gene (*sh1* and *sus1*) allowed detailed studies of their expression and physiological function. Loss of the *sus1* function is reported to have no phenotypic effect on the maize plant, but leads to ectopic expression of the *sh1* gene, suggesting that its function is dispensable and can be compensated for by increased expression of SS1 protein (Chourey et al., 1994). In contrast, mutants with a loss of *sh1* function developed a shrunken and collapsed kernel that was correlated with a loss in starch content (Chourey and Taliercio, 1994).

1. Transcriptional Control

The differences in expression of the two classes of *SuSy* genes (*sus1* and *sus2*) have been described in most detail for maize (Chen

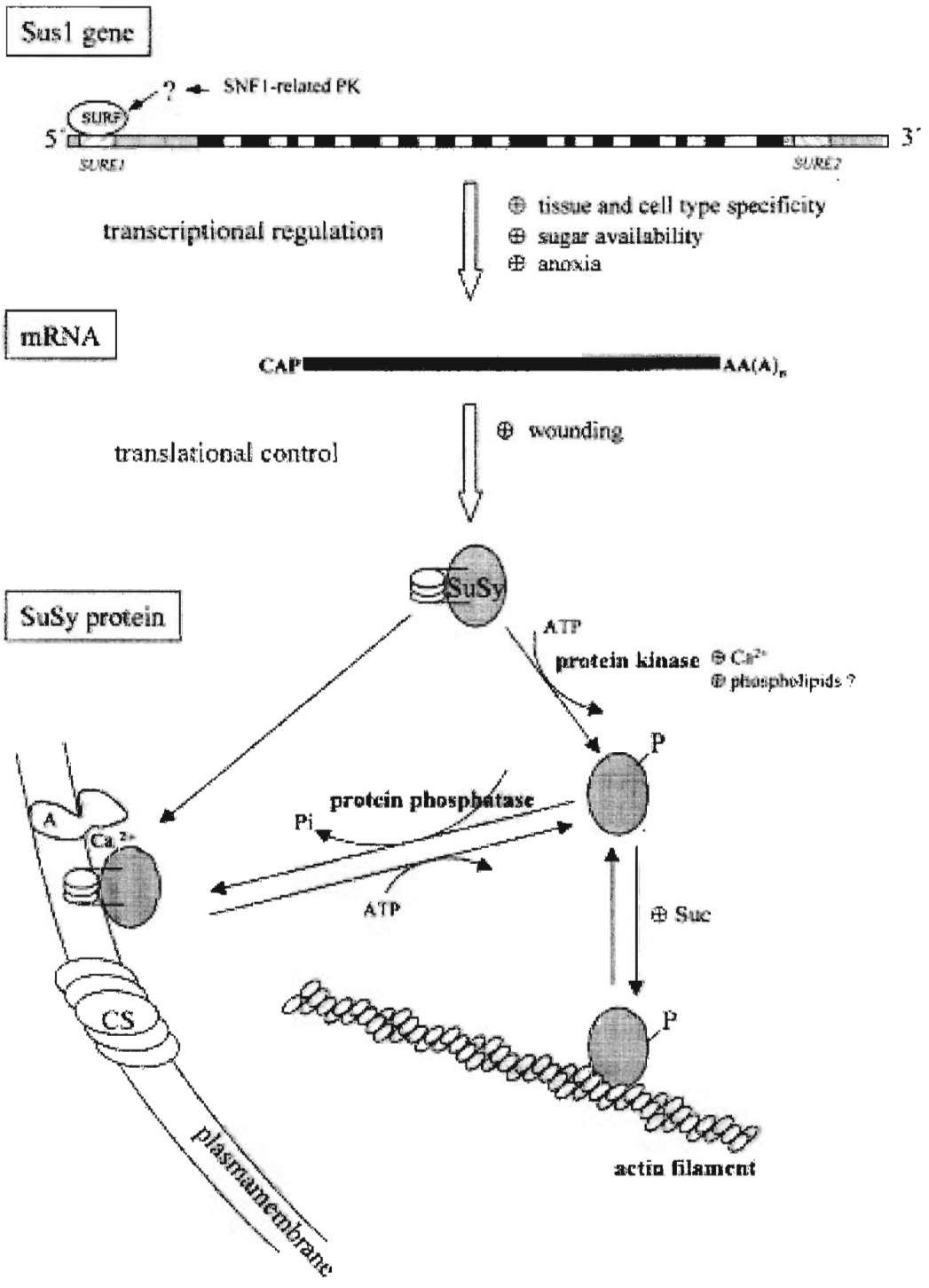

FIGURE 2. Speculative scheme depicting transcription, translation, posttranslational modification, and subcompartmental localization of SuSy (SS2) protein involving plasma membrane association and binding to the actin cytoskeleton. The 14 introns in the *sus1* gene are represented by white boxes and the 15 exons by black boxes. The protruding coil from the SuSy protein represents the hydrophobic domain(s) that are postulated to be surface exposed when the protein is dephosphorylated. (See text for discussion.)

and Chourey, 1989; Heinlein et al., 1989; Nguyen-Quoc et al., 1990; Koch et al., 1992; Koch 1996) and potato plants (Fu et al., 1995; Fu et al., 1995; Fu and Park, 1995). In maize plants, both *SuSy* genes coding for SS1 and SS2 protein are located on chromosome 9 and likely arose via gene duplication (McCarty et al., 1986). The proteins exhibit high sequence homology with 79% identity in the amino acid sequence; major differences in the protein coding regions are additional amino acids in the N- and C-termini of SS2. On the gene level, the positions of the 14 introns in *sus1* are identical with those in *sh1*. The last intron in *sh1*, intron 15, is missing in *sus1* and arose most likely from the last exon 16 by internal duplication followed by mutation (Shaw et al., 1994). The overall structure of the two *SuSy* genes isolated from maize (Shaw et al., 1994), rice (Yu et al., 1992), potato (Fu et al., 1995; Fu et al., 1995) and Arabidopsis (Chopra et al., 1992) are very similar. Expression of *sh1* and *sus1* shows different development and tissue specificity and differential modulation in response to sugar availability and anaerobiosis.

a. Tissue Specific Transcription

The highest levels of *sh1*-encoded SS1 protein is found in the endosperm, but the gene is also expressed at a low level in seedling roots and shoots. Reporter gene constructs with the maize *sh1* promotor demonstrated its tissue- and cell-type specificity in transgenic tobacco plants. The *sh1* promotor led to the expression of GUS activity in a few sieve tube/companion-cell complexes in the center of the phloem tissue bundles. Phloem specific expression of *sh1-GUS* was also observed in leaf, flower, and fruit tissues, and GUS activity was observed in the endosperm tissue. Anaerobic conditions enhanced the phloem specific *sh1*-driven *GUS* expression in the roots of the transgenic tobacco plants (Yang et al., 1990).

In root nodules a major nodule-enhanced protein (nodulin-100) has been identified as a SuSy protein. The levels of SuSy protein and RNA were higher in the N_2-fixing nodules than in the other tissues and organs of the legume plant. SuSy activity was shown to increase rapidly during nodule development and declines during senescence of the nodule (Thummler et al., 1987). Its enhanced expression during N_2 fixation in nodules would be consistent with a role in phloem unloading of sucrose and cleavage for the supply of the bacteriods with C-skeletons in the form of malate.

b. Sugar Modulation of Transcription

Tissue- and cell-specific transcription of *sh1* and *sus1* is differentially modulated by carbohydrate supply (Koch, 1996). Maximal transcription of *sh1* was observed under low concentrations of glucose supplied to excised root tips and increasing external glucose concentrations reduced the *sh1* RNA levels. The activity of the *sh1* promoter in maize protoplasts as monitored by transient reporter gene expression was repressed by high extracellular Suc concentrations but not by comparable concentrations of glucose or fructose (Maas et al., 1990). The *sus1* transcript level on the other hand was maximal under high external glucose supply to excised root tips (Koch et al., 1992). In detached potato leaves only transcription of the *sus4* gene was inducible after supply with sucrose, while expression of *sus3* remained unaffected (Fu et al., 1995).

Suc inducibility of potato *sus4* and maize *sus1* is encoded in the 5′ flanking region, a leader intron in this promoter region, and its 3′ flanking sequence. The 5′ flanking sequences contain an 18-bp motif with high homology to the 'Suc-responsive element' (*SURE1*) identified in the potato class I patatin promotor (Gierson et al., 1994). This sequence is a conserved motif in a number

of sucrose-responsive genes (Ishiguro and Nakamura 1992; Kim et al., 1994). Sequences highly homologous to *SURE1* are present in Arabidopsis *sus1* (Martin et al., 1993), maize *sus1* (Shaw et al., 1994), and rice *sus1* (Yu et al., 1992) promoter regions, but was missing in the non-sugar-responsive potato *sus3* gene (Fu et al., 1995). The *SURE* sequence is the binding site for a nuclear protein from potato tuber nuclear extracts named 'sucrose-responsive factor'(SURF), but sucrose is not directly required for the binding of SURF to *SURE* (Gierson et al., 1994).

New insight into the pathway mediating the sugar responsiveness of SuSy transcription recently came from the analysis of SNF-antisense repressed potato plants (Purcell et al., 1998). The SNF (sucrose-nonfermenting) kinases are members of a family of serine/threonine protein kinases required for the derepression of glucose-repressed genes in yeast and mammals. SNF1-related protein kinases (SnRKs) have also been identified with biochemical assays and genetic screens in higher plants (Halford and Hardie, 1998). SnRK1 was antisensed in potato plants, which led to the decrease of *SuSy* transcript levels below the detection limit by Northern blot analysis. SuSy activity in tubers was reduced by about 60%, but there was no effect on hexokinase or invertase activities or levels of soluble sugars and starch. In detached leaves, the induction of SuSy expression after supply of sucrose was suppressed (Purcell et al., 1998). This study clearly demonstrates the involvement of SnRKs in the sugar-modulated regulation of SuSy gene expression. Downstream elements (transcription factor) of the SnR-kinase-mediated transcriptional regulation of *SuSy* genes are currently unknown.

c. Anoxia

Tolerance of roots to anaerobic stress includes adequate sugar supply and higher rates of glycolysis and ethanol fermentation to maintain the energy status of the cells (Hole et al., 1992). While invertase activity is reduced during anoxic germination of rice, SuSy activity is enhanced, suggesting its role in phloem unloading and providing substrates for glycolysis while conserving energy (Ricard et al., 1991; Guglielminetti et al., 1995). Maize SuSy double mutants are highly sensitive to low O_2 availability (Ricard et al., 1998). Under anaerobic stress, *sh1* transcription in maize is increased. Higher levels of *sh1*-mRNA are detected in the vascular cylinder, pith, and epidermal cells of roots, and the most dramatic changes are in the root tip. Interestingly, the increase in *sh1*-mRNA level was not reflected to the same degree in SS1 protein levels. Only slight increases in SS1 protein were immunologically detected in these cell types. On the other hand, *Sus1* transcription was decreased during anaerobic stress as indicated by the slightly reduced amounts of *sus1*-mRNA in the root tissue, while SS2 protein actually increased in the root tip. It has been shown that the expression of both *SuSy* genes in maize roots under anaerobic conditions is not only regulated on the transcriptional but also on the translational level (McElfresh et al., 1988; Taliercio et al., 1989; Guglielminetti et al., 1996). Exact mechanisms for anaerobic control of SuSy transcription and translation are currently unknown, but sugar regulation of gene expression (see above) is likely involved because anoxia will affect tissue sugar content.

2. Protein Phosphorylation

On the protein level, SuSy is subject to posttranslational modification by reversible protein phosphorylation and possibly redox modification. The incorporation of ^{32}P into SuSy protein was first observed when cultured maize suspension cells were provided with [γ-^{32}P]ATP (Shaw et al., 1994), and excised root tips were provided with [^{32}P]Pi

(Koch et al., 1995). That ^{32}P-labeling of SuSy protein was caused by covalent phosphorylation of serine residues was shown by phosphoamino acid analysis of SuSy from the elongation zone of excised maize shoots provided with [^{32}P]Pi (Huber et al., 1996). Both SuSy isoforms in maize (SS1 and SS2) were phosphorylated *in vivo* and tryptic peptide mapping analysis suggested a single, similar phosphorylation site in both enzymes. The phosphorylation site on maize SS2 protein was identified as Ser15 (Huber et al., 1996) and in soybean nodule-enhanced SuSy protein its structural homolog Ser11 (Zhang et al., 1997). The phosphorylation site is conserved in sequences deduced for SuSy cDNAs cloned to date from mono- and dicotyledenous species with the exception of one isoform in arabidopsis (accession Q00917) and tomato (accession P49037). All other deduced SuSy sequences contain the phosphorylation site:

-L-[STA]-R-[LV]-H-S*-[VLQ]-R-

The sequence suggests that the Ser residue could be phosphorylated either by a calmodulin-like domain protein kinase (CDPK) (Bachmann et al., 1996) or a kinase similar to mammalian Protein Kinase C (PKC) (Kennelly et al., 1991). Endogenous SuSy kinases have been identified in extracts from elongating maize leaf tissue (Huber et al., 1996) and soybean nodules (Zhang and Chollet 1997) that were strictly Ca^{2+}-dependent with molecular weights of ~55 and 65 kDa, respectively. It has also been reported that a PKC-like enzyme (phospholipid stimulated and Ca^{2+}-dependent) can co-purify with and phosphorylate SuSy protein from maize seedlings (Lindblom et al., 1997). Although the residue phosphorylated in that study still remains to be determined, the existence of a plant analog to PKC and its involvement in Suc metabolism is an exciting possibility. In contrast to animal cells, where PKC is only active in its membrane associated form (Bell et al., 1991), this plant phospholipid-enhanced CDPK would have to be active in the soluble fraction. It has been demonstrated by site-directed mutagenesis that recombinant soybean SuSy contains a major phosphorylation site (Ser11) as well as a secondary site that was also phosphorylated by the CDPK, albeit weakly. The secondary site was suggested to reside between Glu14 and Met193 (Zhang et al., 1999). In cotton fiber cells, two protein kinases can phosphorylate SuSy protein *in vitro*. These two potential SuSy kinases differ in their Ca^{2+}-dependence and subcellular localization. SuSy from the soluble fraction of cotton fiber cells was phosphorylated by a soluble Ca^{2+}-dependent protein kinase, while in the membrane fraction *in vitro* phosphorylation of SuSy was Ca^{2+}-independent (Datcheva et al., 1998). If an enzyme is the substrate of two different protein kinases *in vivo*, its role in at least two different physiological functions, regulated by different signal transduction pathways, may be suggested. Assuming that phosphorylation of SuSy protein is a reversible process *in vivo*, the existence of a SuSy phosphatase would have to be postulated. There is currently no information about such a protein phosphatase.

The physiological significance of SuSy phosphorylation is still not clear. Pretreatment of desalted crude extracts with ATP showed a slight (ca. 25%) increase in SuSy cleavage activity, with no change in synthetic activity (Huber et al., 1996; Winter et al., 1997). Expression of recombinant mung bean SuSy in *E. coli* yielded active, tetrameric enzyme (Nakai et al., 1997; Nakai et al., 1998). The nonphosphorylated recombinant SuSy protein had similar V_{MAX} and K_m values for UDP-Glc and Fru to the native enzyme, but the K_m for Suc was tenfold higher than native (Nakai et al., 1997). The recombinant mung bean SuSy was phosphoryated *in vitro* on Ser11 with an exogenous protein kinase. The recombinant

phospho-SuSy, as well as the directed mutants S11E and S11D, had severalfold higher apparent affinities for Suc compared with the unphosphorylated recombinant protein (Nakai et al., 1997; Nakai et al., 1998). Collectively, the results suggest that phosphorylation affects SuSy by introduction of a negative charge at Ser11, which specifically activates cleavage without affecting synthetic activity (Nakai et al., 1998). Transformation of *Azetobacter xylylinum* with recombinant mung bean SuSy synthezised more cellulose than the wild type, and the amount of cellulose synthesized was enhanced further in transformants containing the S11E mutant SuSy. This increase in cellulose synthesis was attributed to an increased sucrose cleavage activity, and as a result reduced concentrations of UDP, which can inhibit cellulose synthase (Nakai et al., 1999). In contrast, the sucrose-cleavage activity and kinetic properties of the recombinant soybean nodule SuSy were not altered by *in vitro* phosphorylation on Ser11, N-terminal truncation, or mutagenesis of the phosphorylated serine to an acidic group (S11D) or a neutral amino acid (S11A or S11C) (Zhang et al., 199X). Thus, potential effects of phosphorylation on kinetic properties remains an important area for continued study.

Besides the effects on the activity, phosphorylation/dephosphorylation of SuSy seems to play a role in its distribution between the cytosol, plasma membrane, and actin cytoskeleton (Winter et al., 1997; Winter et al., 1998) (see Figure 2). The physiological conditions that lead to phosphorylation of the SuSy protein are not clear yet. A strict light-dependent regulation can probably be ruled out, because only under extended darkness was there an apparent decrease in SuSy phosphorylation *in vivo* in the maize leaf elongation zone (Huber et al., 1996).

Posttranslational modification of SuSy can also occur by reversible reduction/oxidation of thiol groups. Oxidation of purified SuSy protein from wheat by oxidized glutathione or thioredoxin caused an inhibition of cleavage activity, while synthetic activity was increased. This effect was reversible, but could be enhanced by the presence of sucrose during preincubation. At physiological pH (pH 7), preincubation with oxidized thioredoxin inhibited the cleavage reaction by 80% and the synthetic activity by 27% (Pontis et al., 1981). Whether redox-modulation of SuSy occurs *in vivo* remains to be shown. Regulation of enzyme activities by light-induced redox modulation has only been shown to occur in the chloroplast stroma (Scheibe, 1991).

3. Membrane Association

In a search for the catalytic subunits of cellulose synthase and callose synthase, plasma membranes from cotton fiber cells were labeled with [^{32}P]UDP-Glc, and SuSy was unexpectedly identified as the most abundant UDP-Glc binding protein on the plasma membrane (Amor et al., 1995). This was the first time that a plasma membrane-associated form of SuSy was described. This membrane-associated form of SuSy was active *in vitro* and the synthesis of cellulose and callose from [^{14}C]-Suc in digitonin-permeabilized cotton fiber cells suggested a potential role for SuSy in channeling UDP-Glc into glucan synthesis. The existence of plasma membrane-associated SuSy has also been shown in maize endosperm (Carlson et al., 1996), the stem pulvinus and leaf elongation zone of maize plants (Winter et al., 1998), and developing soybean seed cotyledons (Huber, J. L., Winter, H., and Huber, S. C., 1998, unpublished). Both SuSy isozymes in maize SS1 and SS2 were found to be capable of associating with the plasma membrane (Carlson and Chourey, 1996). The relative amounts of SuSy association with the plasma membrane are variable and dynamic *in vivo* due to the developmental state (Amor et al., 1995; Carlson and Chourey, 1996; Ruan et al.,

1997) and in response to enviromental factors, that is, gravity (Winter et al., 1997). Immunolocalization of SuSy in cotton fibers showed a labeling pattern very similar to the orientation of cellulose microfibrils (Haigler in Robinson, 1996). Localization of SuSy was also observed at the cell plate during the early stages of cell division, implying a possible role in channeling substrate for the synthesis of callose (K. Hassenstein, 1998, personal communication).

The association of SuSy with the membrane is relatively strong and can be released *in vitro* by strong detergents such as digitonin, CHAPS, and SDS (Amor et al., 1995), EGTA (Datcheva et al., 1998), or by phosphorylation of membrane vesicles (Winter et al., 1997). Although the mechanism of its membrane association is not well understood, it has been shown that phosphorylation of the SuSy protein itself could at least be part of the process. The association of SuSy with the membrane is a dynamic process *in vivo* and labeling experiments with ^{32}[P]Pi showed that in young maize leaves the membrane-associated form of SuSy had relatively less radioactivity incorporated than the soluble form (Winter et al., 1997). Dephosphorylating conditions increased the amount and activity of SuSy in the membrane fraction *in vitro*, while phosphorylating conditions *in vitro* with [γ^{32}P]ATP released ^{32}P-labeled SuSy protein from the membrane. On the other hand, SuSy labeling *in vivo* in cotton fibers showed the same relative incorporation of radioactivity into SuSy protein from the membrane fraction and the soluble fraction (Datcheva et al., 1998). The basis for these different results is not yet clear. There are examples in the literature protein phosphorylation is not involved in membrane association, for example, CTP-phosphocholine cytidylyltransferase (Houweling et al., 1994), and other cases where phosphorylation is the controlling mechanism, for example, MARCKS and PKC (Newton, 1993).

It is also unclear whether the membrane association of SuSy protein involves only an association of the protein with the lipid bilayer itself or also protein:protein interactions with one or more integral or peripheral membrane proteins (e.g., membrane protein "A" in Figure 2). A specific interaction between SuSy and cellulose/callose synthase in the membrane is appealing to 'target' SuSy to specific sites, but such interactions have not been shown to date.

At least part of the membrane association of SuSy may be achieved by its interaction with the lipid bilayer of the plasma membrane itself. This postulate is supported by three observations. First, when the deduced amino acid sequences for SS1 and SS2 protein from maize were analyzed with a transmembrane prediction program (TMpred), both protein sequences showed regions that strongly suggested possible transmembrane helices (Carlson and Chourey, 1996). In SS1, one strong candidate for a transmembrane helix was found between amino acids 266 and 286, while the SS2 protein showed two strong transmembrane helices (residues 274 to 294 and 679 to 699). These hydrophobic sequences almost certainly do not function as transmembrane helices, but if surface exposed could enable SuSy protein to associate with the hydrophobic environment of the lipid bilayer. An alignment (see Figure 3) of these hydrophobic domains from SuSy sequences of different plant species shows that in dicots (represented by potato) isoforms, an Arg replaces a Thr in the maize sequence, which might significantly reduce the extent of the hydrophobic stretch in domain I. All sequences contain a His (shown in bold italics in Figure 3), which raises the possibility that a reduction in pH might affect the overall hydrophobicity of domain I, and hence the association of SuSy with membranes. The second hydrophobic domain is relatively conserved between monocot and dicot species. However, domain II is not as pronounced in

Hydrophobic Domain I

Maize SS2	274-294	F L G T I P M V F N V V I L S P **H** G Y F A
Maize SS1	266-286	. . . . . M . . . . .
Potato SS2	269-290	. . . R . . . . .

Hydrophobic Domain II

Maize SS2	679-699	F G L T V V E A M T C G L P T F A T A Y
Potato SS2	673-693	. . . . . N H

FIGURE 3. Sequence alignment of hydrophobic domains in SuSy isoforms from monocots and dicots represented by maize and potato, respectively. The regions corresponding to putative transmembrane domains were identified by the Bioinformatics Group at ISREC, as originally pointed out by Carlson and Chourey (1996). Identical residues are indicated by dots.

the maize SS1 protein (Carlson and Chourey, 1996). If the hydrophobic domains shown in Figure 3 are found to be involved in the membrane association of SuSy, it is conceivable that the noted differences between monocots and dicots and between isoforms could be of significance *in vivo*.

The observation that SuSy protein purified from the soluble fraction of the elongation leaf zone in maize plants is stable in the soluble form in the cold for weeks, while the same purification under dephosphorylating conditions develops an insoluble white precipitate correlating with the loss of SuSy protein and activity from the soluble supernatant, indicates a conformational change due to dephosphorylation that results in a change in its solubility. That dephosphorylation of soluble SuSy protein caused a conformational change, thereby increasing its hydrophobicity was confirmed by monitoring changes in protein hydrophobicity with the fluorescent probe *bis*-ANS (4,4″-dianilino-1,1′-binaphthyl-5′5-disufonic acid). Fluorescence of this probe increases in a hydrophobic environment. It was shown that dephosphorylation of purified soluble SuSy resulted in an increase of its hydrophobicity, while phosphorylation in the presence of Suc led to a decrease in *bis*-ANS fluorescence, indicating a decrease in hydrophobicity. A hypothetical model based on the above-mentioned observations and experimental data would suggest that the soluble form of SuSy is phosphorylated. Dephosphorylation causes a conformational change that results in the exposure of the predicted transmembrane helices, enabling SuSy to associate with the membrane. Its specific localization on the membrane could require Ca^{2+} and may be determined by an interaction with other integral or peripheral proteins and/or by a specific lipid environment.

4. Actin Association

The recent finding that the soluble form of SuSy protein is capable of binding to the actin.cytoskeleton added a new and surprising aspect to its multifaceted regulation. When

SuSy protein was found in the detergent insoluble fraction of extracts from young maize leaves, it suggested the possibility of its association with the cytoskeleton. Further experiments showed that actin co-immunoprecipitated with SuSy from the soluble fraction. Tubulin was not found to co-immunoprecipitate and neither actin nor tubulin were found in immunoprecipitates of SuSy from the membrane fraction. When the actin cytoskeleton was stabilized *in situ* with phalloidin prior to extraction, SuSy was found to co-precipitate with the actin filaments. The portion of SuSy protein remaining in the supernatant was immunoprecipitated without a marked further increase in co-precipitating actin (Winter et al., 1998). These findings indicate that at least a portion of the soluble SuSy can be associated with the actin cytoskeleton in plants. A direct association between actin and SuSy is likely for two reasons. First, several *SuSy* clones were identified as actin-interacting peptides in a yeast two-hybrid system screening a maize endosperm cDNA library (Carneiro and Larkins, 1998, unpublished results). Second, maize SuSy binds to F-actin *in vitro* (Winter et al., 1998). Polymerization of rabbit muscle actin *in vitro* in the presence of purified SuSy showed a saturation of SuSy binding to actin at a monomer ratio of 1:5, respectively. Taking into account that the soluble form of SuSy was found to be a tetramer, the above ratio would suggest that one SuSy tetramer can maximally bind to an actin filament consisting of 20 actin monomers. This saturation stochiometry could simply be due to steric hinderance of SuSy tetramers (360 kDa) associated with filamentous actin (43 kDa/monomer). The specific sites of interaction on SuSy and actin are still unkown.

Sequence comparison of SuSy protein with known actin binding proteins indicate a region of high homology on SuSy with the actin binding site from mammalian aldolase (O'Reilly et al., 1993) (see Figure 4). Whether this sequence on SuSy is in fact involved in its association with F-actin will have to be investigated. No homologous sequences were found between SuSy protein and G-actin binding proteins or capping proteins (Winter and Huber, unpublished).

As only a fraction of SuSy seems to be associated with the actin cytoskeleton in the cell, it is possible that binding requires either another posttranslational modification to generate a binding site and/or the presence of cofactors or metabolic effectors to induce a conformational change that exposes the actin-binding site on SuSy. Preliminary results suggest that binding of SuSy to F-actin is strictly dependent on Suc, but trehalose or raffinose could substitute as well (H. Winter and S. C. Huber, unpublished data). Suc has been shown to induce a conformational change in phospho-SuSy, resulting in decreased surface hydrophobicity (Winter et al., 1998), which may be necessary for actin binding. While the K_m(Suc) of purified maize SuSy in the cleavage direction is about 8 m*M*, half-maximal stimulation

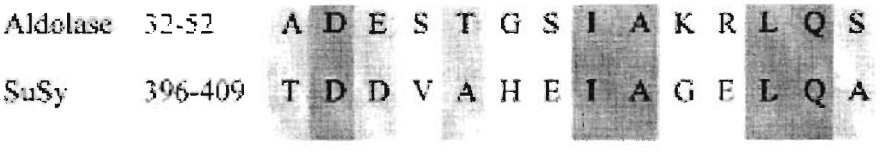

FIGURE 4. Identification of a region on SuSy that is highly homologous to the actin-binding site on mammalian aldolase. Identical and conserved residues are shaded dark and light, respectively. It is not known whether this site is involved in the interaction of SuSy with F-actin.

of actin binding is about an order of magnitude higher (~80 m*M*). This indicates that phospho-SuSy might have two different binding sites for sucrose: the catalytic site (high affinity) and a regulatory site (low affinity). The fact that raffinose can induce SuSy:actin association as well as Suc, but is itself not a substrate or does it compete with Suc for binding to the catalytic site (Wolosiuk et al., 1974) (H. Winter and S. C. Huber, unpublished) supports the concept of a regulatory sugar-binding site on SuSy. We postulate that high concentrations of Suc bind to the regulatory site, which causes a conformational change exposing or generating the actin-binding site. Clearly, these observations require additional confirmation *in vitro* and proof for occurrence *in vivo*. Because nothing is known about the physiological significance of the interaction of SuSy and actin, one can only speculate that a regulatory sugar-binding site on SuSy could be part of a "Suc-sensing" mechanism for the carbohydrate level in sink tissues. Cytosolic Suc concentrations range from 20 to 200 m*M* (Gerhardt et al., 1987; Winter et al., 1994), depending on the time of day and tissue. Under sufficient sucrose availability, SuSy could be recruited to the actin cytoskeleton as part of a control mechnism for the partitioning of carbohydrate toward cell wall or starch biosynthesis or to allow for accumulation of sucrose in the vacuole.

B. Invertases

In addition to SuSy, invertases can also catalyze the hydrolysis of Suc. The invertases are a group of β-fructosidases that differ in pH optimum for activity (acidic, neutral, and alkaline) and solubility (soluble vs. insoluble). The forms also differ in localization: soluble acid invertase is vacuolar, insoluble acid invertase is extracellular (cell wall associated), and neutral/alkaline invertases are cytosolic (Quick, 1996). Possible roles for the two soluble invertases were proposed in early studies (Richardo et al., 1970) and are still considered valid. It was suggested that vacuolar acid invertase cleaves Suc when there is a high demand for Suc hydrolysis (e.g., cell expansion). Cells containing high acid invertase cannot store Suc in the vacuole, and thus in these cells the need for Suc hydrolysis may be met by neutral/alkaline invertases. In contrast, the cell wall invertase is thought to play a role in assimilate uptake into some sink tissues by establishing a steep concentration gradient of Suc from source to sink (Escherich, 1980).

1. Soluble Acid Invertase

These invertases are encoded by a small gene family in maize (Xu et al., 1996), Arabidopsis (Haouazine-Takvorian et al., 1997), and carrot (Sturm et al., 1995). In maize, Northern blot analysis using *Ivr1*- and *Ivr2*-specific probes has shown that the genes are expressed in a developmental and tissue-specific manner. Of particular importance is the observation that *Ivr1* is upregulated by sugar depletion, whereas *Ivr2* is upregulated by an abundant sugar supply (Koch, 1996; Xu et al., 1996). Not enough is known about the enzymatic properties of the two invertases to ascribe some physiological significance to the differences in expression. However, upregulation of *Ivr1*, which is expressed primarily in root tips and reproductive structures, may confer 'import priority' to these essential tissues when assimilates are scarce (Koch, 1996).

In a given tissue, several forms of soluble acid invertase can often be chromatographically resolved. For example, in Arabidopsis leaves three forms were purified and are thought to be the result of posttranslational processing of the *Atβfruct4* gene (Haouazine-Takvorian et al., 1997). The vacuolar invertases are

N-glycosylated (Lauriere et al., 1988), but additional potential glycosylation sites exist and may explain the occurrence of proteins with similar molecular masses but different pIs. Little is known about the regulation of acid invertase activity, except that many are inhibited by hexose sugars, especially Fru (Walker et al., 1997). The importance of Fru inhibition as a regulatory mechanism *in vivo* is not known.

Recent molecular genetic approaches have confirmed the importance of soluble acid invertase in the regulation of tissue sugar composition in both source and sink tissues. Suppression of acid invertase activity by antisense RNA has been shown to increase the Suc content of tomato fruit (Ohyama et al., 1995; Klann et al., 1996) and to reduce hexose sugar accumulation in cold-stored potato tubers (Zrenner et al., 1996). Antisense repression of vacuolar invertase in tomato leaves also resulted in increased Suc content and reduced hexose sugar content (Scholes et al., 1996). These results are consistent with the earlier finding that considerable variation exists among species in the activity of soluble acid invertase in fully expanded leaves, and that only those species with low acid invertase activity accumulated Suc as an end product of leaf photosynthesis (Huber, 1989).

2. Neutral and Alkaline Invertases

The neutral/alkaline invertases are found in the cytosol and are considered 'maintenance' enzymes involved in Suc degradation when the activities of acid invertase and SuSy are low. The enzyme has been purified and characterized from a variety of sources, including soybean hypocotyl (Chen et al., 1992), developing *Vicia faba* cotyledons (Ross et al., 1996), *Chichorium* root (Van den Ende et al., 1995), and carrot (Lee et al., 1996). A gene encoding an enzyme with neutral/alkaline invertase activity has been cloned for the first time from *Lolium temulentum* (Gallagher et al., 1998). The deduced sequence shows little homology with those for acid invertases, but does contain the 'NDPN' invertase motif (Sturm et al., 1990). It is likely that the neutral/alkaline invertase umbrella covers more than one class of enzyme, but details await the cloning of additional genes from a range of species.

3. Cell Wall Invertase

Cell wall, or apoplastic, invertases are ionically bound to the cell wall matrix and are thought to play a role in phloem unloading by ensuring a steep concentration gradient from source to sink. Induction of cell wall invertase expression by Glc in suspension-cultured cells of *Chenopodium rubrum* is consistent with this notion, and it has been suggested that the enzyme may play a role in establishing metabolic sinks (Roitsch et al., 1995). Glc, but not Suc, induced expression of cell wall invertase in Arabidopsis roots clear specificity of sugar modulation. Moreover, the cell wall invertase of *C. rubrum* cultures was induced by Glc as well as 6-deoxyglucose (Roitsch et al., 1995), which is an analog that cannot be phosphorylated by hexose kinase. This observation is significant because the sugar regulation of many genes involved in photosynthesis requires Glc analogs that can be phosphorylated by hexose kinase, which has been suggested as the requisite 'sugar sensor' (Jang et al., 1997).

Another important development with cell wall invertases is the identification of an apoplasmic inhibitor protein (INH) that is present at certain stages of development. The INH of tobacco leaves is a 17-kDa nonglycosylated protein that has been purified to homogeneity, and the genes encoding the protein have been cloned from Arabidopsis and tobacco (Greiner et al., 1998). There are differences among organs in expression of the

INH relative to cell wall invertase, but these may be difficult to interpret because transcript level does not necessarily reflect protein amount (Greiner et al., 1998). The INH can also inhibit vacuolar acid invertase activity *in vitro*, but the protein is located in the apoplasm *in vivo*, and regulation of INH action by Suc is only observed with the cell wall enzyme (Sander et al., 1996). Although physiological significance is still speculative, one possibility is that the INH functions as a switch to modulate cell wall invertase activity under different conditions. Binding of INH to cell wall invertase is necessary but not sufficient for inhibition of catalytic activity. Thus, even though the two proteins are associated, for example, during a 40-d culture period of tobacco cells (Krausegrill et al., 1998), the inhibition of invertase activity is only apparent during the later stages of culture. The transition from the noninhibited to the inhibited complex may be induced by decreased Suc concentration and/or other, as yet unidentified, factors. Thus, the system may function to maintain Suc at some minimal but critical concentration during sugar starvation.

V. CONCLUSIONS AND FUTURE PROSPECTS

Work in the future will likely confirm and extend the concept that both Suc transport into cells and metabolism within cells are highly regulated processes that contribute to the control of 'Suc metabolism.' Of particular importance are the Suc and hexose transporters, and enzymes directly involved in Suc metabolism. Control of gene expression and enzyme (protein) activity are equally important, and in the future it will be necessary to fully integrate studies at different levels of control. One common biochemical mechanism that may regulate the activity of enzymes as well as transport proteins is reversible protein phosphorylation. The identification of target proteins, the effect(s) of phosphorylation of specific site(s), and the requisite protein kinases, will be important. With respect to SPS, where multisite phosphorylation has been documented (see Figure 1), it will be important to clarify the physiological significance and signals that control the phosphorylation of various sites. Moreover, possible hierarchical phosphorylation will need to be considered. With SuSy, its important physiological function in phloem transport and utilization of sucrose for synthesis of structural glucans or storage carbohydrate (starch) requires tight control of its expression, activity and localization within the cell (see Figure 2). There is a need for increased understanding of how Suc availability in different cell types and tissues controls the transcription, activity, and subcellular localization of the enzyme. It remains to be seen if the plasma membrane association of SuSy has a role in cellulose and/or callose synthesis and how targeting and association with the membrane are achieved. In plants, SuSy is the first metabolic enzyme to be shown to interact with the actin cytoskeleton, but the physiological significance (if any) of the association remains to be established. One exciting possibility is that it functions as one element of the 'Suc-sensing mechanism' in plants. The next 5 years will likely produce some answers as well as new questions.

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