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Sugar Sensing and Signaling in Plants

Filip Rolland, Brandon Moore,[1] and Jen Sheen[2]

Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02114

INTRODUCTION

In addition to their essential roles as substrates in carbon and energy metabolism and in polymer biosynthesis, sugars have important hormone-like functions as primary messengers in signal transduction. The pivotal role of sugars as signaling molecules is well illustrated by the variety of sugar sensing and signaling mechanisms discovered in free-living microorganisms such as bacteria and yeast (Stulke and Hillen, 1999; Rolland et al., 2001). For such unicellular organisms, nutrient availability is the main extracellular factor controlling growth and metabolism. The role of nutrients as regulatory molecules has come to be appreciated only recently in mammals despite extensive previous research on Glc homeostasis and diabetes (Hanson, 2000; Rolland et al., 2001).

In plants, sugar production through photosynthesis is a vital process, and sugar status modulates and coordinates internal regulators and environmental cues that govern growth and development (Koch, 1996; Sheen et al., 1999; Smeekens, 2000). Although the regulatory effect of sugars on photosynthetic activity and plant metabolism has long been recognized, the concept of sugars as central signaling molecules is relatively novel. Recent progress has begun to reveal the molecular mechanisms underlying sugar sensing and signaling in plants, including the demonstration of hexokinase (HXK) as a Glc sensor that modulates gene expression and multiple plant hormone-signaling pathways (Sheen et al., 1999; Smeekens, 2000). Analyses of HXK mutants will provide new evidence for distinct signaling and metabolic activities. Diverse roles of Snf1-related protein kinases (SnRKs) in carbon metabolism and sugar signaling also are emerging (Halford and Hardie, 1998; Hardie et al., 1998). In addition, Suc, trehalose, and other HXK-independent sugar sensing and signaling pathways add more complexity in plants (Goddijn and Smeekens, 1998; Lalonde et al., 1999; Smeekens, 2000).

[1] Current address: Department of Genetics and Biochemistry, Clemson University, 122 Long Hall, Clemson, SC 29631.

[2] To whom correspondence should be addressed. E-mail sheen@molbio.mgh.harvard.edu; fax 617-726-6893.

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Biochemical, molecular, and genetic experiments have supported a central role of sugars in the control of plant metabolism, growth, and development and have revealed interactions that integrate light, stress, and hormone signaling (Roitsch, 1999; Sheen et al., 1999; Smeekens, 2000; Gazzarrini and McCourt, 2001; Finkelstein and Gibson, 2002) and coordinate carbon and nitrogen metabolism (Stitt and Krapp, 1999; Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001). A number of reviews have appeared in the past few years emphasizing different aspects of sugar signaling and its interactions with other plant signal transduction pathways. In this review, the extent and impact of the sugar signaling network on plant life is illustrated. We explore diverse sugar responses, summarize biochemical and genetic evidence for different sugar sensing and signaling mechanisms, consider the extensive regulatory web that mediates sugar and hormone signaling, and suggest possible directions for future research.

SUGAR RESPONSES

Metabolism

Photosynthesis is active primarily in mature leaf mesophyll cells, and photosynthate is transported, primarily as Suc, to meristems and developing organs such as growing young leaves, roots, flowers, fruit, and seed. Light and sugars regulate these growth activities by a coordinated modulation of gene expression and enzyme activities in both carbohydrate-exporting (source) and carbohydrate-importing (sink) tissues (Figure 1). This ensures optimal synthesis and use of carbon and energy resources and allows for the adaptation of carbon metabolism to changing environmental conditions and to the availability of other nutrients (Stitt and Krapp, 1999; Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001; Grossman and Takahashi, 2001). In general, low sugar status enhances photosynthesis, reserve mobilization, and export, whereas the abundant presence of sugars promotes growth and carbohydrate storage (Figure 1) (Koch, 1996). The circadian clock can play an important role in carbon partitioning and allocation (Harmer et al., 2000). Several

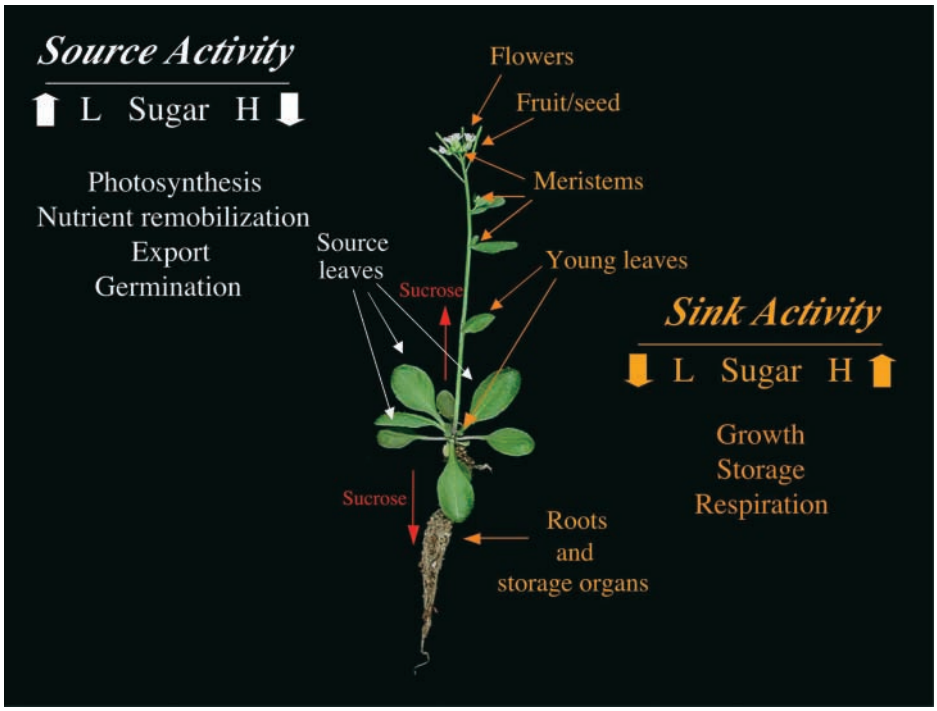

Figure 1. Differential Effects of Sugars on Plant Source and Sink Activities.

Suc is transported from photosynthesizing source leaves to sink organs such as roots, meristems, young leaves, flowers, fruit, and developing seed. Lowered (L) sugar levels can increase source activities, including photosynthesis, nutrient mobilization, and export. In contrast, higher (H) sugar levels in sink tissues stimulate growth and storage. Accumulation of higher (H) sugar levels in source tissues, however, is believed to downregulate photosynthesis, ensuring the maintenance of sugar homeostasis. The differential source-sink effects allow the adaptation of carbon metabolism to changing environmental conditions and to the availability of other nutrients.

photosynthetic genes, for example, peak in expression near the middle of the day, whereas a number of genes involved in sugar consumption, transport, and storage peak near the end of the day. During the night, genes involved in starch mobilization reach their highest expression levels (Harmer et al., 2000). However, although the circadian clock may allow plants to "anticipate" daily changes, the actual sensing of the quality and quantity of light and especially sugars (as the end products of photosynthesis) ensures an appropriate "response" of metabolism to specific situations. For example, variations in the environment can decrease photosynthetic efficiency and result in sugar-limited conditions in parts of the plant, which downregulate biosynthetic activity to conserve energy and protect cells against nutrient stress while upregulating starch degradation and protein and lipid catabolism to sustain respiration and metabolic activity (Yu, 1999; Fujiki et al., 2000). Photosynthetic acclimation to increased CO_2 is likely the result of sugar repression, especially under nitrogen deficiency, and sugar repression of photosynthetic gene expression, chlorophyll accumulation, and seedling development can be antagonized by nitrate signals (Moore et al., 1999; Stitt and Krapp, 1999). Moreover, several N-regulated genes are coregulated by sugars (Wang et al., 2000; Wang et al., 2001; Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001). In oxygen-limited environments (e.g., in flooded root systems), plant cells can shift metabolism to fermentation to sustain glycolysis by direct NAD^+ regeneration (Tadege et al., 1999), and interference between sugar and oxygen signals has been shown (Koch et al., 2000). Thus, plants can display photosynthesis, respiration, and fermentation at the same time in different tissues through a complex regulatory system that involves sugar signaling and integrates different metabolic, developmental, and environmental signals to control metabolic mode and activity.

Growth and Development

The effect of carbon allocation on organ and whole plant architecture is illustrated most dramatically by carbohydrate storage and the concomitant cell expansion in reserve organs such as roots, fruit, seed, and tubers. However, cell division and differentiation can be ascribed to both changes in

metabolic activity and sophisticated developmental switches (Jackson, 1999; Wobus and Weber, 1999a; Hajirezaei et al., 2000; White et al., 2000; Giovannoni, 2001). In *Vicia faba* embryos, gradients of sugars have been reported to correlate spatially with mitotic activity (Borisjuk et al., 1998). Consistently, Arabidopsis D-type cyclin gene expression is regulated differentially by sugars (Riou-Khamlichi et al., 2000). Therefore, sugars also could act as morphogens, providing positional information to the cell cycle machinery and different developmental programs. Remarkably, differential display analysis using portions of tomato meristems destined to form leaves revealed spatially regulated carbohydrate metabolism within the meristem and suggested the involvement of carbohydrate metabolism in organogenesis (Pien et al., 2001).

Sugar sensing and signaling are involved in the control of growth and development during the entire plant life cycle. During germination and early seedling development, sugars can repress nutrient mobilization, hypocotyl elongation, cotyledon greening and expansion, and shoot development (Yu et al., 1996; Dijkwel et al., 1997; Jang and Sheen, 1997; Perata et al., 1997; Kurata and Yamamoto, 1998; Arenas-Huertero et al., 2000; Gibson, 2000; Smeekens, 2000; Eastmond and Graham, 2001; Gazzarrini and McCourt, 2001). High sugar accumulation during early seedling development may reflect undesirable growth conditions at a crucial developmental period (Lopez-Molina et al., 2001), resulting in a reversible developmental arrest that acts as a protection mechanism. Sugar-dependent seedling phenotypes have been used extensively for the selection of sugar signaling mutants in Arabidopsis, including both sugar-insensitive and sugar-hypersensitive mutants (Table 1).

Based on a growth response in which a high level of Glc blocks the switch to postgermination development in Arabidopsis, both Glc-insensitive (*gin*) and Glc-oversensitive (*glo*) mutants have been isolated. Table 2 provides a list of *gin* mutants identified using the same genetic screen. Genetic analyses showed that multiple alleles of the *gin1*, *gin2*, *gin3*, *gin4*, *gin5*, and *gin6* mutants have been obtained (Table 2). Each *gin* mutant has been mapped to a different site on four chromosomes (Table 2). Some of the *gin* mutants are allelic to other sugar, abscisic acid (ABA), and salt mutants (Sheen et al., 1999; Gibson, 2000; Smeekens, 2000; Rook et al., 2001; W.-H. Cheng and J. Sheen, unpublished data; P.L. Rodríguez, personal communication) based on phenotype comparison, chromosomal location, and allelism tests (Table 2) (Zhou et al., 1998; Arenas-Huertero et al., 2000; W.-H. Cheng and J. Sheen, unpublished data; L. Zhou and J. Sheen, unpublished data).

The effects of sugars on floral transition have been studied in more detail recently and appear to be very complex. In Arabidopsis, increased leaf carbohydrate export and starch mobilization are required for flowering, suggesting that phloem carbohydrates have a critical function in floral transition (Corbesier et al., 1998). Interestingly, the addition of Suc can rescue the late-flowering phenotype of several mutants (Araki and Komeda, 1993; Roldan et al., 1999) and even promotes leaf morphogenesis and flowering in the dark (Roldan et al., 1999). However, high exogenous concentrations of sugars have been shown to delay flowering

Table 1. Arabidopsis Sugar Signaling Mutants

Name	Phenotype	Reference
Sugar insensitive		
cai (*carbohydrate insensitive*)	Growth on low nitrogen, 100 mM Suc	Boxall et al., 1997
gin (*Glc insensitive*)	Growth on 330 mM Glc	Zhou et al., 1998
isi (*impaired sugar induction*)	Reduced ApL3::P450 expression	Rook et al., 2001
lba (*low-level β-amylase*)	Low β-amylase activity on 175 mM Suc	Mita et al., 1997a
mig (*Man-insensitive germination*)	Germination on 7.5 mM Man	Pego et al., 1999
ram (*reduced β-amylase*)	Reduced β-amylase in *pgm* mutant	Laby et al., 2001
rsr (*reduced sugar response*)	Pat(B33)-GUS expression on 90 mM Suc	Martin et al., 1997
sis (*sugar insensitive*)	Growth on 300 mM Suc or Glc	Laby et al., 2000
sun (*Suc uncoupled*)	Expression of PC-LUC on 88 mM Suc	Dijkwel et al., 1997
sig (*Suc insensitive growth*)	Growth on 350 mM Suc	Pego et al., 2000
Sugar hypersensitive		
core (*conditional root expansion*)	Short, expanded roots on 4.5% Suc	Hauser et al., 1995
fus (*fusca*)	Growth arrest on 3% Suc	Castle and Meinke, 1994
glo (*Glc oversensitive*)	Growth arrest on 4% Glc	Sheen et al., 1999
gss (*Glc supersensitive*)	Growth arrest on 56 mM Glc	Pego et al., 2000
hba (*high-level β-amylase*)	High-level β-amylase on 175 mM Suc	Mita et al., 1997b
prl1 (*pleiotropic regulatory locus*)	Growth arrest on 175 mM Suc	Nemeth et al., 1998
sss (*Suc supersensitive*)	Growth arrest on 350 mM Suc	Pego et al., 2000

ApL3, ADP-Glc pyrophosphorylase; GUS, β-glucuronidase; Luc, luciferase; PC, plastocyanin; *pgm*, phosphoglucomutase.

Table 2. Arabidopsis *gin* Mutants

Mutant	Alleles	Other Names	Chromosome	6% Glc Resistance	Genetics	Molecular Identity
gin1	11	*aba2*, *sis4*, *isi4*, *san3*, *sre1*	1	Strong	Recessive	SDR1
gin2	2		4	Strong	Recessive	AtHXK1
gin3	2		1	Weak	Recessive	Unknown
gin4	8	*ctr1*, *sis1*	5	Strong	Recessive	Raf-like PK
gin5	4	*aba3*, *los5*	1	Strong	Recessive	Mo-cofactor sulfurase
gin6	8	*abi4*, *sun6*, *sis5*, *isi3*, *san5*	2	Strong	Recessive	AP2-like TF

aba2, *abscisic acid 2* (Leon-Kloosterziel et al., 1996; Schwartz et al., 1997); *abi4*, *aba insensitive 4* (Koornneef et al., 1984, 1998; Finkelstein et al., 1998); *ctr1*, *constitutive triple response 1* (Kieber et al., 1993); HXK, hexokinase (Jang et al., 1997); *isi*, *impaired sucrose induction 3* and *4* (Rook et al., 2001); *los5*, *low temperature and osmotic stress 5* (Xiong et al., 2001); Mo, molybdenum; PK, protein kinase; *san5*, *salobreno 5* (Quesada et al., 2000); SDR, short-chain dehydrogenase/reductase (W.-H. Cheng and J. Sheen, unpublished data); *sis*, *sugar insensitive 1*, *4*, and *5* (Laby et al., 2000); *sre1*, *salt resistant 1* (P.L. Rodríguez, personal communication); *sun6*, *sucrose uncoupled 6* (Huijser et al., 2000); TF, transcription factor.

significantly (Zhou et al., 1998). A recent report confirmed the pleiotropic effects of sugars on floral transition, depending on sugar concentration, vegetative growth phase, and genetic background. Sugars may control floral transition by positively and negatively regulating the expression of floral identity genes (Ohto et al., 2001).

Sugar sensing and signaling also are implicated in the regulation of leaf senescence that coincides with a decline in chlorophyll content and photosynthetic activity (Jiang et al., 1993; Bleeker and Patterson, 1997; Quirino et al., 2000). Sugars are known to repress photosynthetic gene expression, and in transgenic plants, HXK expression correlates well with the rate of leaf senescence (Dai et al., 1999; Xiao et al., 2000). Interestingly, the expression of phospholipase D, which is important for leaf senescence (Fan et al., 1997), is induced by Glc (Xiao et al., 2000). Leaf contents of Glc and Fru were shown to increase with leaf age, whereas starch content decreased (Wingler et al., 1998; Quirino et al., 2001), and the monosaccharide transporter homolog *SFP1* was induced during advanced leaf senescence (Quirino et al., 2001). However, although exogenously supplied sugars induce expression of the senescence-associated gene *SAG21* in a HXK-dependent manner (Xiao et al., 2000), another well-characterized senescence marker, *SAG12*, is repressed by sugars in senescing Arabidopsis leaves (Noh and Amasino, 1999). The regulation of different *SAGs* may be controlled differentially by other factors besides sugars, such as developmental state and hormones (He et al., 2001).

The recent demonstration of glucose oversensitive phenotypes of the Arabidopsis *hys1*/*cpr5* mutants, selected based on leaf hypersenescence and constitutive expression of pathogenesis-related genes, also suggests a role for sugar signaling in senescence and defense (Yoshida et al., 2002).

Stress

Abiotic and biotic stress stimuli, such as drought, salinity, wounding, and infection by viruses, bacteria, and fungi, can modulate source-sink activities. Because extracellular invertase, a key enzyme for hydrolyzing Suc (Sturm, 1999), is regulated by stress stimuli and hormones, it has been proposed to be a central modulator of assimilate partitioning, integrating sugar, stress, and hormone signals (Roitsch, 1999). Although stress may alter sugar levels, experiments with protein kinase (PK) inhibitors suggest that sugars and stress-related stimuli also may activate different signaling pathways independently (Ehness et al., 1997; Roitsch, 1999). It is intriguing that sugars regulate the expression of wound-inducible proteinase inhibitor II and lipoxygenase genes (Johnson and Ryan, 1990; Sadka et al., 1994), pathogenesis-related (*PR*) genes (Herbers et al., 1996; Xiao et al., 2000), and dark-inducible (*DIN*) genes (Fujiki et al., 2001). Some of the *DIN* genes also are activated by sugar starvation, pathogens, and senescence (Quirino et al., 2000; Fujiki et al., 2001; Ho et al., 2001), suggesting that a response to metabolic stress could be the underlying mechanism.

In addition, many jasmonate-, ABA-, and stress-inducible genes are coregulated by sugars (Reinbothe et al., 1994; Sadka et al., 1994). Further studies will be required to reveal the genetic and molecular basis of sensing and signaling pathways connecting sugar and stress in plants. Interestingly, an ancient regulatory system controlling metabolism, stress resistance, and aging appears to be conserved from yeast to mice (Kenyon, 2001). Both caloric restriction and increased oxidative stress resistance are able to increase life span. In yeast, Glc sensing and signaling pathways play a central role in longevity (Ashrafi et al., 2000; Lin et al., 2000; Fabrizio et al., 2001). The delayed senescence and increased stress resistance observed in Arabidopsis *HXK* antisense plants (Xiao et al., 2000) similarly connect plant sugar metabolism and sensing with the control of stress resistance and aging. Two Arabidopsis proteins, ZAT10 and AZF2, with similarities to the yeast zinc-finger transcription factors Msn2 and Msn4 have been isolated in a yeast (*snf4*Δ) suppressor screen (Kleinow et al., 2000). In yeast, Msn2 and Msn4 control growth-inhibitory genes (Smith et al., 1998). They are regulated positively by stress and nega-

tively by Glc through phosphorylation and cytosolic translocation (Gorner et al., 1998). Whether these factors are involved in Glc regulation in plants awaits further functional analysis.

Gene Expression

Research in plant sugar signaling has been focused largely on gene expression. A wide variety of genes are sugar regulated at the transcriptional level, including genes involved in photosynthesis, carbon and nitrogen metabolism, stress responses, and secondary metabolism in different plant species. However, little is known about the actual transcriptional machinery underlying these responses. Detailed analysis of different maize photosynthetic gene promoters did not reveal common regulatory elements for sugar regulation, suggesting the involvement of diverse transcription factors (Sheen, 1990, 1999). Examination of Glc-repressed rice α-amylase gene promoters has identified several *cis* elements required for sugar-regulated gene expression (Chan and Yu, 1998b; Hwang et al., 1998; Lu et al., 1998; Morita et al., 1998) and mRNA stability (Chan and Yu, 1998a). Specific regulatory elements involved in Glc repression also have been identified in the promotors of the cucumber malate synthase (Sarah et al., 1996) and bean *RBCS2* (Urwin and Jenkins, 1997) genes.

Currently, most progress has been made through the functional dissection of sugar-induced gene promoters. Suc-responsive elements are found in the patatin class I promoter. Similar *SP8* motifs are present in the promoters of the sweet potato sporamin and β-amylase genes, and interact with nuclear factors (Liu et al., 1990; Ishiguro and Nakamura, 1992, 1994; Grierson et al., 1994; Kim et al., 1994). Similar Suc-responsive sequences also are found in several Suc-inducible Suc synthase genes (Fu et al., 1995). A gene encoding a DNA binding protein that recognizes the *SP8* motif in the sweet potato sporamin and potato β-amylase gene promoters, *SPF1*, has been cloned. It encodes a negative regulator that is repressed transcriptionally by Suc (Ishiguro and Nakamura, 1994). *SPF1* has putative homologs in cucumber (Kim et al., 1997) and Arabidopsis that encode a WRKY domain transcription factor. The (T)TGAC(C/T) core sequence of WRKY binding elements (W-box) is found in the promoters of the wheat, barley, and wild oat α-*AMY2* gene (Rushton et al., 1995). W-boxes also are found in the promoters of an α-amylase gene homolog and many Arabidopsis genes possibly involved in plant defense (Du and Chen, 2000; Maleck et al., 2000).

Other motifs frequently found in several sugar-regulated promoters are the G-box and related sequences. The G-box motif (CACGTG) is involved in the transcriptional control of a variety of stimuli, such as phytochrome-mediated control of gene expression through binding of the PIF3 (Martinez-Garcia et al., 2000) and HY5 (Chattopadhyay et al., 1998) transcription factors. In addition, this motif is very similar to the CCACGTGG ABA-responsive element (Pla et al., 1993). Interestingly, β-amylase transcript is induced by ABA (Ohto et al., 1992), and induction of the β-phaseolin promoter by exogenous ABA in tobacco embryos is modulated by external Suc (Bustos et al., 1998). These data suggest that sugar, light, hormone, and defense signaling may converge in the transcriptional control of W- and G-boxes in diverse promoters. More thorough analysis is required to reveal the precise molecular basis of these interactions. The recent discovery of new sugar-responsive elements (Maeo et al., 2001) may stimulate the identification of specific transcription factors in sugar signaling.

As shown for the regulation of rice α-amylase transcript, sugars can repress gene expression by affecting mRNA stability through specific 3′ untranslated region sequences (Chan and Yu, 1998a). Also for the transcripts encoding rice ADH2 (alcohol dehydrogenase 2), G3PD (glyceraldehyde-3-phosphate dehydrogenase), and SSP2 (Suc synthase phosphate 2), sugars can enhance their stability (Ho et al., 2001). Other striking examples are the differential RNA processing and translation of the maize cell wall invertase transcripts mediated by 3′ untranslated region sequences (Cheng et al., 1999) and the 5′ untranslated region–dependent repression of translation of the Arabidopsis *ATB2* mRNA, encoding a bZIP transcription factor, by sugars (Rook et al., 1998).

SUGAR SENSING

Sugar Signals

Sugar control of metabolism, growth and development, stress, and gene expression has long been thought to be a metabolic effect. However, the control of gene expression observed with nonmetabolizable or partially metabolizable hexoses or hexose and Suc analogs clearly suggest the involvement of specific signal sensing and transduction mechanisms that do not require sugar catabolism (Figure 2) (Krapp et al., 1993; Graham et al., 1994; Jang and Sheen, 1994; Martin et al., 1997; Klein and Stitt, 1998; Roitsch, 1999; Sheen et al., 1999; Loreti et al., 2000; Smeekens, 2000; Brouquisse et al., 2001; Fernie et al., 2001). To study diverse sugar responses, sugars and sugar analogs have been applied exogenously to whole seedlings or plants, detached organs or tissues, and protoplasts or suspension-cultured cells. Manipulation of sugars in planta also has been accomplished by direct injection, petiole girdling, increasing CO_2, altering light intensity, or the genetic manipulation of invertase genes (von Schaewen et al., 1990; Sonnewald et al., 1991; Moore et al., 1999; Stitt and Krapp, 1999; Sturm and Tang, 1999; Tang et al., 1999; Pego et al., 2000).

Although hexoses are potent signals sensed in plants, Suc-specific (Chiou and Bush, 1998; Rook et al., 1998) and

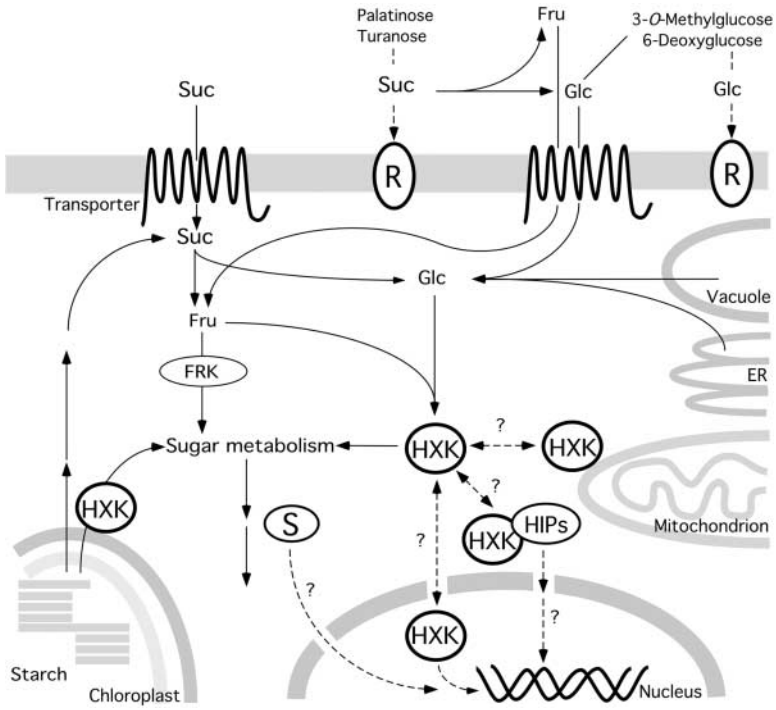

Figure 2. Possible Sugar Signals and Sensing Sites in Plant Cells.

Glc (and Fru) can be transported into the cell by hexose transporters or mobilized from cytosolic and vacuolar Suc and plastid starch. Glc then enters metabolism after HXK-catalyzed phosphorylation. The HXK sugar sensor, as a cytosolic protein or associated with mitochondria or other organelles (see text), then could activate a signaling cascade through HXK-interacting proteins (HIPs) or affect transcription directly after nuclear translocation. Possibly, different HXK (and fructokinase [FRK]) isoforms and HXK-like proteins have distinct metabolic and signaling functions. Metabolic intermediates could trigger signal transduction by activating metabolite sensors (S). Negative regulation of SnRK activity by Glc-6-phosphate, for example, suggests that SnRKs might act as sensors of metabolic activity. Finally, sugars, including Suc and hexoses and non-metabolizable sugars and sugar analogs, also could be sensed at the plasma membrane by sugar transporters or transporter-like proteins or by specific sugar receptors (R). Solid lines represent transport and enzymatic reactions involved in sugar sensing and signaling, and dashed lines represent putative interactions and translocations. ER, endoplasmic reticulum.

trehalose-mediated (Goddijn and Smeekens, 1998) signaling pathways also play important roles in regulating development and gene expression. In developing seed, it has been suggested that Suc regulates differentiation and storage, whereas hexoses control growth and metabolism (Weber et al., 1997; Wobus and Weber, 1999b). The ability of both 3-*O*-methylglucose and 6-deoxyglucose to regulate gene expression indicates the presence of HXK-independent pathways through novel sensors in plants (Martin et al., 1997; Roitsch, 1999). Recently, new sugar sensing mechanisms also have been revealed through the use of disaccharide analogs (palatinose and turanose) that are not even membrane permeable (Loreti et al., 2000; Fernie et al., 2001).

Sugar Sensing Mechanisms

To activate signal transduction pathways, sugars first have to be sensed. The sugar's dual function as a nutrient and a signaling molecule, however, significantly complicates analysis of the mechanisms involved (Rolland et al., 2001). Even in yeast in which downstream components of sugar signaling pathways have been characterized in detail, elucidation of the initial Glc sensing and activation mechanisms has been difficult. Only recently, the involvement of transporter-like Glc sensors (Snf3 and Rgt2) and a G protein–coupled receptor (Gpr1) and new evidence for Hxk2 function in Glc signaling have been reported (Johnston, 1999; Rolland et al., 2001). It has been proposed that similar transporter-like Glc sensors and receptors could function in yeast, mammals, and plants (Lalonde et al., 1999; Barker et al., 2000; Williams et al., 2000; Rolland et al., 2001). Interestingly, mammalian sweet taste receptors are encoded by *GPR* genes and can distinguish Suc from Glc (Nelson et al., 2001). Despite initial concerns, compelling evidence indicates that HXK also functions as a sugar sensor in plants (Moore and Sheen, 1999).

A regulatory role for HXK in plant hexose sensing was suggested by testing the effects of a variety of sugars, Glc

analogs, and metabolic intermediates on photosynthesis and glyoxylate cycle gene repression in *Chenopodium* (Krapp et al., 1993) and cucumber (Graham et al., 1994) cell cultures and in a maize protoplast transient expression system (Jang and Sheen, 1994). Sugars that are substrates of HXK, including Man and 2-deoxyglucose, which are phosphorylated but inhibit Glc-6-phosphate and ATP production (Klein and Stitt, 1998), cause repression of photosynthetic gene expression at low physiological levels (1 to 10 mM in maize mesophyll protoplasts) (Jang and Sheen, 1994). The repression is blocked by the HXK-specific competitive inhibitor mannoheptulose (Jang and Sheen, 1994). L-Glc (not transported), 6-deoxyglucose and 3-*O*-methylglucose (transported but not phosphorylated), and sugar phosphates (delivered into the protoplasts by electroporation) do not trigger the same repression. Possible depletion of Pi and ATP as a cause of reduced gene expression has been excluded by the inability of Pi and ATP to relieve repression (Graham et al., 1994; Jang and Sheen, 1994). Consistently, 2-deoxyglucose and Man can arrest seed germination by a mechanism that affects sugar signaling independently of hexose metabolism or depletion of seed ATP and Pi levels. Mannoheptulose also overcomes this inhibition (Pego et al., 1999). Glc repression of the α-amylase gene in rice embryos (Umemura et al., 1998) and Arabidopsis *DIN* genes is likely mediated by HXK (Fujiki et al., 2000). It is clear that caution must be taken when poorly or nonmetabolizable sugar analogs such as Man, 2-deoxyglucose, and 3-*O*-methylglucose are used, because they often produce toxic side effects and eventually can result in cell death (Stein and Hansen, 1999; Brouquisse et al., 2001).

Experiments with transgenic Arabidopsis plants further indicate the uncoupling of HXK catalytic and regulatory functions (Jang et al., 1997). Overexpression of the sense and antisense *AtHXK1* or *AtHXK2* gene results in sugar hypersensitivity or hyposensitivity, respectively, as demonstrated by altered sugar responses in seedling development and gene expression. On the contrary, overexpression of the yeast *HXK2* gene reduces sugar sensitivity in transgenic plants despite a marked increase in kinase activity (Jang et al., 1997). Definitive proof for the dual function of HXK obviously must come from mutant analysis. In yeast, Hxk2 is implicated in the Glc repression of genes involved in the respiration and metabolism of alternative carbon sources (Rolland et al., 2001). Mutations that uncouple HXK's signaling function from its catalytic activity have been isolated (Hohmann et al., 1999; Kraakman et al., 1999; Mayordomo and Sanz, 2001a). However, the most striking example of such a dual function for a sugar kinase comes from the Gal induction pathway in yeast. Gene expression involved in Gal metabolism is controlled by the transcriptional activator Gal4, which is associated with and inhibited by the Gal80 repressor. In the presence of Gal and ATP, galactokinase (Gal1) or its catalytically inactive homolog Gal3 binds to Gal80, thereby destabilizing the Gal4-Gal80 complex and enabling transcription by Gal4 (Bhat et al., 1990; Zenke et al., 1996; Sil et al., 1999).

In the Arabidopsis genome, there are six *HXK* and *HXK*-like (*HXKL*) genes (Arabidopsis Genome Initiative, 2000), three fructokinase (*FRK*) genes, and several *FRK*-like genes (Arabidopsis Genome Initiative, 2000; Pego and Smeekens, 2000). Mutational and functional analyses of Arabidopsis HXKs and HXKLs suggest their involvement in sugar sensing and signaling (B. Moore and J. Sheen, unpublished data). Importantly, two null mutants of *AtHXK1* (*gin2-1* and *gin2-2*) have been isolated and show Glc insensitivity in gene expression and development (Figure 3). The isolation of *AtHXK1* mutants with distinct catalytic and regulatory activity would provide invaluable tools to elucidate broad sugar responses at the cellular and whole plant levels. In tomato, transcripts of both *FRK1* and *FRK2* are induced by exogenous application of Glc, Fru, and Suc (Kanayama et al., 1998). Although it is generally believed that FRKs serve important metabolic functions, the identification of an *frk2* null mutation in a Man-insensitive *mig* mutant (Pego and Smeekens, 2000) suggests that FRK also may be involved in sugar sensing.

HXK sensing and signaling function likely are dependent on HXK's subcellular localization, translocation, and/or interactions with downstream effectors. A model summarizing possible sugar sensing sites in plant cells is presented in Figure 2. Yeast Hxk2, for example, has been shown to interact with the protein phosphatase (PP) complex that modifies Snf1 kinase activity, thereby stimulating Glc repression (Alms et al., 1999; Sanz et al., 2000). Yeast Hxk2 also is found in the nucleus, participating directly in regulatory DNA-protein complexes with *cis*-acting regulatory elements of a Glc-repressed gene (Herrero et al., 1998; Randez-Gil et al., 1998). Mammalian HXK activity is known to be regulated by protein interactions that ensure proper subcellular localization and function. Mammalian HXKI, for example, is bound to the outer mitochondrial membrane, which enables direct access to the ATP generated by oxidative phosphorylation (Adams et al., 1991; BeltrandelRio and Wilson, 1992a, 1992b). In hepatocytes and pancreatic β-cells, glucokinase (HXKIV)-interacting proteins control sugar-regulated nucleocytoplasmic glucokinase translocation and activity (Van Schaftingen et al., 1994; Farrelly et al., 1999; Munoz-Alonso et al., 2000; Shiraishi et al., 2001). Interestingly, human pancreatic glucokinase complements the Glc signaling defects of yeast *hxk2* mutants (Mayordomo and Sanz, 2001b).

In plants, HXK has long been considered a cytosolic protein involved in glycolysis (Plaxton, 1996). However, the occurrence of noncytosolic, organelle-associated HXK has been demonstrated (Borchert et al., 1993; Galina et al., 1995; Wiese et al., 1999). In maize roots, most of the noncytosolic HXK is bound to mitochondria (Galina et al., 1995, 1999), whereas a substantial portion appears to be associated with the Golgi apparatus and other cellular membranes (da-Silva et al., 2001). Possibly, HXK is involved directly in UDP-Glc synthesis and Golgi glycosylation (Galina and da-Silva, 2000; da-Silva et al., 2001). A substantial fraction of spinach leaf HXK1 is located at the outer envelope membrane of plastids via its N-terminal membrane anchor (Wiese et al., 1999).

HXK associated with chloroplasts and plastids from

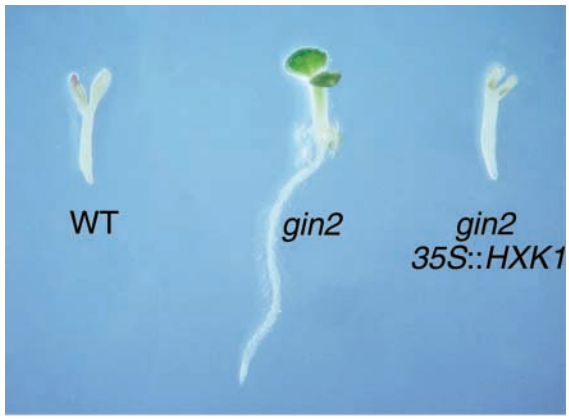

Figure 3. *gin2* Mutant Phenotype and Complementation by *35S*::*AtHXK1*.

Plants were grown on 6% Glc Murashige and Skoog (1962) medium for 5 days under light. WT, wild type.

nongreen tissues could be involved in the direct phosphorylation of Glc when it leaves the organelles as a product of starch hydrolysis (Focks and Benning, 1998; Wiese et al., 1999). However, in maize, the ADP inhibition of noncytosolic HXK isoforms suggests that these HXKs may not be involved in glycolysis and thus could play a regulatory role in sugar sensing (da-Silva et al., 2001). Induction of systemic acquired resistance and repression of photosynthetic gene expression in transgenic tobacco plants expressing yeast-derived invertase in the apoplast or vacuole suggest that hexose sensing somehow may involve the secretory pathway (Herbers et al., 1996). In Arabidopsis, HXK1 is found associated with mitochondria (B. Moore and J. Sheen, unpublished data), but the physiological and biochemical function of this interaction remains to be elucidated.

Sugar Metabolites

Because sugars are nutrients and metabolized extensively, their presence also could be sensed through downstream metabolites. Sugar induction of *PR1* and *PR5*, for example, appears to depend on HXK catalytic activity but not on its signaling function, suggesting the involvement of one or more catabolites (Xiao et al., 2000). The identification of Glc-6-phosphate as a regulator of SnRK activity provides a possible mechanism underlying some Glc responses in plants (Toroser et al., 2000). Acetate and other respiratory intermediates have been shown to affect gene expression (Sheen, 1990; Koch, 1996; Koch et al., 2000), possibly indirectly through pH effects. In addition, changes in energy status also can affect gene expression and enzyme activities. In mammals, for example, high ATP levels generated by Glc catabolism in pancreatic β-cells are known to activate ATP-sensitive K^+ channels and eventually stimulate insulin release (Matschinsky et al., 1998). Glc depletion, on the other hand, causes a high AMP/ATP ratio and can activate AMP-dependent protein kinases (Hardie et al., 1998).

Trehalose

The disaccharide trehalose has been shown to affect plant metabolism and development. The presence of trehalose as an endogenous compound has long been thought to be confined to resurrection plants, in which it serves as a stress protectant, but the Arabidopsis genome sequence and yeast mutant complementation have revealed the presence of functional plant genes encoding enzymes involved in trehalose synthesis and hydrolysis (Goddijn and Smeekens, 1998; Goddijn and van Dun, 1999; Leyman et al., 2001). At least 11 putative *TPS* genes (encoding trehalose-6-phosphate synthase) are present in the Arabidopsis genome, one of which (*TPS1*) was shown to encode a functional TPS (Blazquez et al., 1998; Leyman et al., 2001). In yeast, this reserve carbohydrate and stress protectant has been implicated in the control of glycolytic flux and sugar signaling (Thevelein and Hohmann, 1995). Trehalose-6-phosphate is a potent inhibitor of yeast HXK, and there is evidence for the possible involvement of the yeast Tps1 protein itself in controlling glycolytic flux (Bonini et al., 2000; Van Vaeck et al., 2001). Proper control of Glc influx into glycolysis apparently is required for a wide range of Glc signaling effects in yeast (Van Aelst et al., 1993).

In Arabidopsis, inhibition of trehalase causes the accumulation of trehalose and a strong reduction in starch and Suc contents, suggesting a role for trehalose and trehalase in carbon allocation (Müller et al., 2001). In addition, trehalose has been shown to inhibit Arabidopsis seedling root elongation and cause starch accumulation in shoots. Furthermore, trehalose increases AGPase (ADP-Glc pyrophosphorylase) activity and induces *APL3* gene expression (Wingler et al., 2000; Fritzius et al., 2001). In soybean, trehalose also affects Suc synthase and invertase activities (Müller et al., 1998). How trehalose affects plant gene expression, enzyme activities, photosynthetic activity, and carbon allocation is not clear, but trehalose-6-phosphate does not appear to have any effect on plant hexose phosphorylation (Wiese et al., 1999). However, transgenic tobacco plants expressing *Escherichia coli* homologs of TPS and trehalose-6-phosphate phosphatase show a positive correlation between trehalose-6-phosphate levels and photosynthetic activity, suggesting a regulatory role for trehalose-6-phosphate in plant carbohydrate metabolism (Paul et al., 2001).

BIOCHEMICAL DISSECTION OF SUGAR SIGNALING

In contrast to the situation in microorganisms, most downstream components in plant sugar signaling cascades are

not well characterized. Some clues to which molecules might be involved come from biochemical and pharmacological studies as well as from obvious analogies to the yeast system.

Protein Kinases and Phosphatases

One of the most common mechanisms in signal transduction is protein phosphorylation and dephosphorylation, and the use of specific inhibitors has indicated the involvement of a variety of PKs and PPs in plant sugar signaling. Extensive pharmacological experiments have been performed in the maize protoplast system to investigate the underlying sugar signaling mechanisms (Sheen, 1993, 1999; J.-C. Jang and J. Sheen, unpublished data). Inhibitors of PP1 and PP2A mimic the sugar repression of photosynthesis gene promoters (Sheen, 1993). This result is consistent with the role of the yeast PP1 (Glc7) in Glc repression (Ludin et al., 1998; Alms et al., 1999). However, broad-spectrum PK inhibitors and calcium also can block photosynthetic gene expression, suggesting a complex interaction of PKs and PPs (Jang and Sheen, 1997). PP inhibitors display a similar effect on photosynthetic genes in photoautotrophic cultures of *Chenopodium rubrum* (Ehness et al., 1997). In the latter system, PP inhibitors as well as Glc- and stress-related stimuli also trigger the activation of stress-inducible invertase and Phe ammonia-lyase gene expression and the rapid and transient activation of putative mitogen-activated protein kinases (Ehness et al., 1997). Differential effects of the PK inhibitor staurosporine, however, suggest the involvement of different PKs in sugar and stress signaling pathways. In sweet potato, on the other hand, PP inhibitors block the Suc induction of genes encoding sporamin and β-amylase (Takeda et al., 1994), indicating the involvement of different phosphorylation mechanisms in Glc activation. Using a similar pharmacological approach, genes induced by dark and by sugar starvation are controlled differentially by distinct PPs (Fujiki et al., 2000). However, these results can be explained by the differential potency of the PP inhibitors used (Sheen, 1993).

SNF1, AMP-Activated Protein Kinase, and SnRK in Yeast, Mammals, and Plants

The yeast Snf1 Ser/Thr PK is well characterized as one of the major components in yeast sugar signaling and is required for derepression of a large number of Glc-repressed genes upon sugar starvation (Carlson, 1999). Snf1 phosphorylates the transcriptional repressor Mig1, causing its translocation to the cytoplasm and derepression of target genes (De Vit et al., 1997; Treitel et al., 1998). In addition, Snf1 directly affects the transcription machinery through interactions with the Srb/mediator complex of RNA polymerase II (Kuchin et al., 2000) and histone phosphorylation (Lo et al., 2001). Snf1 itself is activated by phosphorylation and Glc7 PP1 dephosphorylates and inactivates Snf1 by inducing an autoinhibitory conformational change in the Snf1 complex (Jiang and Carlson, 1996). Although yeast Snf1 is not activated by AMP, it is a conserved member and prototype of a family of AMP-activated protein kinases (AMPKs). In mammals, these kinases are involved in protection against environmental and nutritional stresses through signaling of altered cellular AMP/ATP ratios (Hardie and Carling, 1997; Hardie et al., 1998; Kemp et al., 1999). Through derepression of genes involved in the metabolic conversion of alternative carbon sources, Snf1 similarly ensures sufficient ATP synthesis in yeast in the absence of Glc. In addition, Snf1 activation presumably also mediates yeast aging by triggering a shift toward gluconeogenesis and energy storage (Lin et al., 2001).

In recent years, biochemical and molecular analysis has revealed the existence of a large family of SnRKs in plants, classified in subgroups SnRK1, -2, and -3 on the basis of amino acid and sequence similarities (Halford and Hardie, 1998). Several SnRKs (all SnRK1 class) have been shown to complement the yeast *snf1*Δ phenotype (Alderson et al., 1991; Muranaka et al., 1994; Bhalerao et al., 1999). Although functional characterization of these proteins is still in an early stage, it is proposed that they act as global regulators of carbon metabolism in plants (Halford and Hardie, 1998). Existing data support complex and distinct functions of SnRKs in plants. For example, expression of an antisense *SnRK* in potato prevents transcriptional activation of a Suc-inducible Suc synthase gene, suggesting its involvement in sugar activation but not repression (Purcell et al., 1998). The Arabidopsis pleiotropic regulatory locus (*prl1*) mutant exhibits transcriptional derepression of a variety of sugar-regulated genes but a sugar-hypersensitive growth phenotype (Nemeth et al., 1998).

It has been shown that PRL1 is an inhibitor of the Arabidopsis SnRKs (AKIN10 and AKIN11), and in yeast, its interaction with AKIN10 and AKIN11, based on the two-hybrid assay, is regulated negatively by Glc. However, the *prl1* mutation does not seem to affect the regulation of SnRK activity by sugars (Bhalerao et al., 1999). The complex interactions of SnRK with PRL1, SKP1/ASK1 (ubiquitin ligase), and a subunit of the 26S proteasome (α4/PAD1) reveal another aspect of SnRK function in protein degradation (Farras et al., 2001), which may explain the seemingly contradictory phenotypes in the *prl1* mutant (Nemeth et al., 1998).

In plants, SnRKs also play an important role in carbon metabolism by directly phosphorylating and inactivating the biosynthetic key enzymes 3-hydroxy-3-methyl glutaryl CoA reductase, nitrate reductase (NR), and Suc phosphate synthase, as shown in vitro (Sugden et al., 1999). Phosphorylation of a Suc phosphate synthase peptide by Arabidopsis AKIN kinase complexes also is stimulated by Suc (Bhalerao et al., 1999). Interestingly, a SNF1-like gene, together with genes encoding enzymes in primary sugar metabolism (AGPase and Suc synthase), was shown to be expressed asymmetrically in tomato apical meristems, with higher expression levels in the parts destined to form leaves (Pien et al., 2001). Like yeast

Snf1, plant SnRKs do not seem to be activated directly by AMP. The evidence that some plant SnRKs are activated by sugars and involved in sugar-activated gene expression implies an opposite regulation from that of mammalian AMPKs and yeast Snf1. However, Glc-6-phosphate might act as a negative regulator of SnRK activity (Toroser et al., 2000), and AMP appears to inhibit dephosphorylation and the concomitant inactivation of spinach SnRK activity at physiological concentrations (Sugden et al., 1999). Therefore, the exact nature of SnRK activation in response to sugars remains unclear. The result that the tobacco SnRK NPK5 is constitutively active in yeast (Muranaka et al., 1994) underscores the differential regulation of SnRK activity in yeast and plants.

In both yeast and mammals, Snf1-related PKs are implicated in a variety of distinct regulatory and developmental processes (Hardie and Carling, 1997; Carlson, 1999; Ashrafi et al., 2000; Cullen and Sprague, 2000). In plants, several Arabidopsis, wheat, maize, and rice SnRKs from different subfamilies are regulated differentially by light, temperature, cytokinin, developmental stage, and sugars (Takano et al., 1998; Ikeda et al., 1999; Ohba et al., 2000; Chikano et al., 2001). Interestingly, the ABA-induced barley SnRK PKABA1 mediates the ABA suppression of GA-induced α-amylase gene expression in aleurone cells (Gomez-Cardenas et al., 2001). Expression of antisense SnRK1 in barley anthers causes abnormal pollen development and male sterility (Zhang et al., 2001). Finally, a *Chlamydomonas* SnRK (Sac3) was shown to regulate responses to sulfur limitation (Davies et al., 1999).

Further functional analysis of SnRKs can be complicated by redundant functions and multiple interactions with regulatory proteins (Bouly et al., 1999; Kleinow et al., 2000; Ferrando et al., 2001). Yeast Snf1 and mammalian AMPK function in heterotrimeric complexes, consisting of a catalytic protein kinase α-subunit and regulatory β- and γ-subunits. While the regulatory γ-subunit activates the PK by inhibiting the autoinhibitory conformation, the β-subunit acts as an adaptor protein and confers substrate specificity to the complex (Jiang and Carlson, 1996, 1997; Vincent and Carlson, 1999). Although similar complex compositions exist in plants, a novel class of plant SnRKs was identified recently that contains only two components: a Snf1-related kinase subunit and a unique regulatory βγ-subunit, which appears to have developed by domain fusion during plant evolution (Lumbreras et al., 2001).

Interestingly, expression of an Arabidopsis cDNA library in yeast has identified several heterologous multicopy suppressors of the *snf4* γ-subunit mutant with remarkable functional or structural similarity to yeast suppressor genes (Kleinow et al., 2000).

14-3-3 Proteins

The evolutionarily conserved 14-3-3 proteins bind specifically to phosphorylated substrates, thereby controlling enzyme activities, subcellular location, and protein–protein interactions required for signal transduction (Finnie et al., 1999; Sehnke et al., 2002). A diverse family of 14-3-3 proteins in Arabidopsis interacts with cytosolic enzymes involved in primary nitrogen and carbon metabolic pathways (Bachmann et al., 1996a, 1996b; Moorhead et al., 1996, 1999; Toroser et al., 1998), with plasma membrane H^+-ATPase (Jahn et al., 1997), and with the transcriptional machinery (Lu et al., 1992). Inactivation of NR by spinach SnRK phosphorylation, for example, requires the presence of a 14-3-3 protein (Bachmann et al., 1996a, 1996b; Moorhead et al., 1996), and in wheat, the WPK4 SnRK both phosphorylates and transfers a 14-3-3 protein to NR (Ikeda et al., 2000).

Recently, it was found that 14-3-3 proteins globally regulate the cleavage of their binding partners in sugar-starved Arabidopsis cells. It is proposed that the loss of 14-3-3 protection and the resulting proteolysis underlie the major metabolic shift to reduced nitrate assimilation and sugar synthesis upon sugar starvation (Cotelle et al., 2000). Overexpression of 14-3-3 proteins also is associated with enhanced cell survival and delayed senescence, whereas antisense expression results in opposite phenotypes (Markiewicz et al., 1996; Wilczynski et al., 1998). However, 14-3-3 proteins also were reported to accelerate the degradation of phosphorylated NR (Weiner and Kaiser, 1999; Kaiser and Huber, 2001), suggesting that the underlying mechanisms might be more complex. Plant trehalose-6-phosphate synthase also interacts with 14-3-3 proteins (Moorhead et al., 1999), supporting a role for trehalose-6-phosphate in the starvation response. Loss of 14-3-3 binding might release trehalose-6-phosphate from the trehalose synthesis complex under conditions of low carbon supply (Paul et al., 2001).

Like that of NR, the binding of 14-3-3 to Suc phosphate synthase also is regulated by SnRK (Bhalerao et al., 1999; Sugden et al., 1999). The collaboration of SnRKs and 14-3-3 proteins, therefore, might be more general.

Ca^{2+} as a Second Messenger

A role for Ca^{2+} in sugar signaling is suggested by the isolation of a sugar-induced, plasma membrane–associated, calcium-dependent protein kinase in tobacco leaf tissues (Iwata et al., 1998). Pharmacological studies with Ca^{2+} channel blockers ($LaCl_3$), EGTA, and calmodulin inhibitors provide additional evidence for the involvement of Ca^{2+} signaling in sugar induction of sporamin and β-amylase gene expression in sweet potato (Ohto and Nakamura, 1995) and of anthocyanin biosynthesis in *Vitis vinifera* cell suspension cultures (Vitrac et al., 2000). Suc-induced increases in cytosolic levels of free Ca^{2+} have been shown in transgenic tobacco leaf discs expressing apoaequorin (Ohto and Nakamura, 1995). In aequorin-transformed Arabidopsis plants, the moving rate of ^{14}C-Suc (fed to the roots) was reported to be approximately comparable to that of the observed lumi-

nescence (Furuichi et al., 2001). In the latter study, it was suggested that increases in free cytosolic Ca^{2+} concentrations could be attributable to membrane depolarization caused by sugar-proton symport. To further elucidate the precise role of Ca^{2+} in sugar signaling, the development of cellular and genetic tools is necessary (Sheen, 1996; Allen and Schroeder, 2001).

GENETIC DISSECTION OF SUGAR SIGNALING

Although the biochemical approach has revealed the involvement of PPs and PKs, including PP1, SnRK, calcium-dependent protein kinase, mitogen-activated protein kinase, and the second messenger Ca^{2+}, in plant sugar signaling, the targets of these regulatory molecules and their physiological functions remain elusive. A genetic approach using Arabidopsis as a model plant offers distinct strategies to dissect the complex mechanisms that underlie sugar sensing and signaling in plants. Based on either sugar-regulated gene expression or sugar-insensitive or sugar-oversensitive phenotypes during germination and seedling development (Figure 4), a large collection of sugar signaling mutants has been isolated in Arabidopsis (summarized in Tables 1 and 2) (Sheen et al., 1999; Gibson, 2000; Smeekens, 2000; Rook et al., 2001).

Although the designs for genetic screens are different, many independently isolated sugar-insensitive mutants are allelic, suggesting the use of conserved mechanisms in plant sugar responses. For example, the Glc-insensitive *gin1* mutant is allelic to *sis4* (*sugar insensitive*) and *isi4* (*impaired sugar induction*), *gin4* is allelic to *sis1*, and *gin6* is allelic to *sun6* (*Suc uncoupled*), *sis5*, and *isi3* (Table 2) (Sheen et al., 1999; Gibson, 2000; Smeekens, 2000; Rook et al., 2001). With the exception of the *prl1* mutant, most mutants that show hypersensitivity to Glc (*glo* and *gss*) or Suc (*sss*) have not been well characterized. Further phenotypic analyses of these sugar-hypersensitive mutants and the unique sugar-insensitive mutants, and molecular cloning of their corresponding genes, will reveal new mechanisms of sugar regulation.

The most surprising and exciting outcome of the genetic, phenotypic, and molecular characterization of Glc-insensitive mutants is the extensive and direct interactions between sugar and hormonal signaling (Figure 5). The observation that the "*gin*" phenotype can be mimicked by 1-aminocyclopropane-1-carboxylic acid (the immediate ethylene precursor) treatment of wild-type seedlings on 6% Glc Murashige and Skoog (1962) plates prompted the investigation of interactions between sugar and ethylene signaling (Zhou et al., 1998). The constitutive ethylene biosynthesis (*eto1*) and constitutive ethylene signaling (*ctr1*) mutants are insensitive to Glc repression of cotyledon and shoot development, similar to *gin1*. These findings have been further confirmed by the isolation of new alleles of *ctr1*, including *gin4* and *sis1*

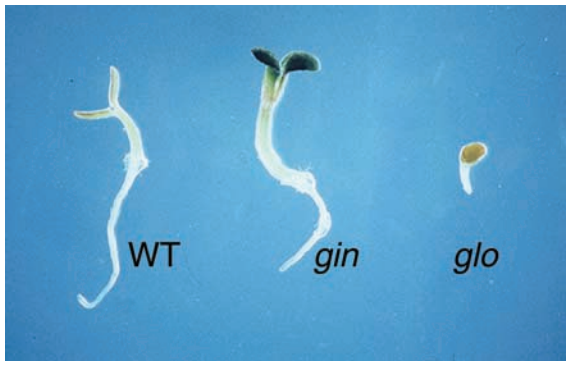

Figure 4. Arabidopsis Seedling Phenotypes on High-Glc Medium.

Wild-type (WT), Glc-insensitive (*gin*), and Glc-oversensitive (*glo*) plants were grown on 6% Glc Murashige and Skoog (1962) medium for 5 days under light.

(Table 2) (Gibson et al., 2001; W.-H. Cheng and J. Sheen, unpublished data). However, unlike *eto1* and *ctr1*, *gin1* does not display the triple response phenotype in the dark, a typical ethylene response (Roman et al., 1995), suggesting that the *gin* phenotype and the triple response can be uncoupled (Figure 5).

Interestingly, overexpression of the C terminus of EIN2 (ethylene insensitive) shows many constitutive ethylene phenotypes but not the triple response (Alonso et al., 1999). Many (but not all) ethylene-insensitive mutants, including *etr1*, *ein2*, *ein3*, and *ein6*, also exhibit Glc hypersensitivity (Zhou et al., 1998; W.-H. Cheng and J. Sheen, unpublished data). Double mutant analyses suggest that GIN1 acts downstream of the ethylene receptor ETR1 and HXK (Figure 5) (Zhou et al., 1998; W.-H. Cheng and J. Sheen, unpublished data). Future research should focus on understanding the precise molecular link between Glc and ethylene signaling through the analysis of more double mutants and the biochemical characterization of known signaling components (Sheen et al., 1999; Bleecker and Kende, 2000; Stepanova and Ecker, 2000).

The HXK-mediated Glc signaling pathway is connected not only to the ethylene pathway but also to the ABA pathway. The *gin1* mutant is known to be allelic to *aba2* (Laby et al., 2000; Rook et al., 2001; W.-H. Cheng and J. Sheen, unpublished data; P.L. Rodríguez, personal communication), a classic Arabidopsis mutant with deficiency in ABA biosynthesis (Schwartz et al., 1997; Koornneef et al., 1998). The *ABA2* gene was found recently to encode a short-chain dehydrogenase/reductase (SDR1) that catalyzes the second to last step of the major endogenous ABA biosynthesis pathway (W.-H. Cheng and J. Sheen, unpublished data). This surprising result indicates that Glc can modulate ethylene signaling through the ABA pathway. This finding also is consistent with the recent discovery that the *gin6* mutant is allelic to *abi4* (Arenas-Huertero et al., 2000), an AP2 domain

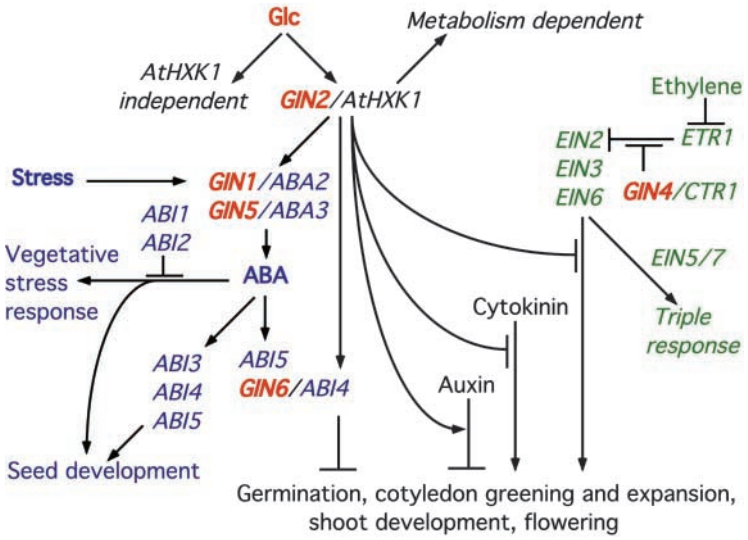

Figure 5. Genetic Model of Interactions between Sugar and Hormone Signaling in Arabidopsis.

The *gin* phenotype (shown in Figure 4) is mimicked by ethylene precursor treatment of wild-type plants and is displayed in constitutive ethylene biosynthesis (*eto1*) and constitutive ethylene signaling (*ctr1*) mutants, whereas the ethylene-insensitive mutants *etr1-1* and *ein2* exhibit the *glo* phenotype (shown in Figure 4). Epistasis analysis with the *gin1 etr1* and *gin1 ein2* double mutants puts GIN1 downstream of the ETR1 receptor and EIN2 (Zhou et al., 1998; W.-H. Cheng and J. Sheen, unpublished data). Thus, Glc and ethylene signaling pathways antagonize each other (Zhou et al., 1998; W.-H. Cheng and J. Sheen, unpublished data). However, the triple response is not affected by Glc. The *gin1*, *sis4*, and *isi4* mutants are allelic to *aba2* (Laby et al., 2000; Rook et al., 2001; W.-H. Cheng and J. Sheen, unpublished data). *ABA2* encodes a short-chain dehydrogenase/reductase (SDR1) that is involved in the second to last step of ABA biosynthesis (Rook et al., 2001; W.-H. Cheng and J. Sheen, unpublished data; P.L. Rodríguez, personal communication; Seo and Koshiba, 2002) and is controlled directly by Glc (W.-H. Cheng and J. Sheen, unpublished data). Other ABA-deficient mutants (*aba1-1*, *aba2-1*, and *aba3-2*) also are Glc insensitive (Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000). *ABA1* and *ABA3* are important for ABA biosynthesis and are regulated directly by Glc (W.-H. Cheng and J. Sheen, unpublished data). Characterization of the *gin5* mutant shows the requirement of Glc-specific ABA accumulation for HXK-mediated Glc signaling (Arenas-Huertero et al., 2000). In addition, *gin6*, *sun6*, *sis5*, and *isi3* are allelic to *abi4*, an ABA-insensitive mutant (Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000; Rook et al., 2001). Glc activation of *ABI4*, which encodes an AP2 domain transcription factor (Finkelstein et al., 1998), requires ABA, although *ABI4* is not induced by ABA directly (Arenas-Huertero et al., 2000; Soderman et al., 2000; W.-H. Cheng and J. Sheen, unpublished data). The *abi5* mutant also is Glc insensitive. Glc activates *ABI5* directly (W.-H. Cheng and J. Sheen, unpublished data), encoding a basic Leu zipper transcription factor (Finkelstein and Lynch, 2000b). However, other ABA-insensitive signaling mutants (*abi1-1*, *abi2-1*, and *abi3-1*) do not exhibit the *gin* phenotype, as do *abi4* and *abi5* mutants (Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000), suggesting that a distinct ABA signaling pathway is involved in Glc signaling. In summary, Glc activates ABA biosynthesis and ABA signaling, and both antagonize ethylene signaling (W.-H. Cheng and J. Sheen, unpublished data). It remains possible that Glc also inhibits ethylene signaling directly. The *AtHXK1* mutant (*gin2*) affects ABA and ethylene signaling but also displays reduced sensitivity to auxin and increased sensitivity to cytokinin (L. Zhou and J. Sheen, unpublished data).

transcription factor (Finkelstein et al., 1998). Independent genetic, phenotypic, and molecular analyses of the *sun6* (Huijser et al., 2000), *sis5* (Laby et al., 2000), and *isi3* (Rook et al., 2001) sugar signaling mutants have revealed their allelism to *gin6*/*abi4*. Similar studies also have confirmed that *gin1*/*aba2* is allelic to the sugar signaling mutants *sis4* (Laby et al., 2000) and *isi4* (Rook et al., 2001) (Table 2). In addition, overexpression of the Arabidopsis ABA-responsive element (ABRE) binding factors ABF3 or ABF4 confers both ABA and glucose oversensitive phenotypes, supporting an intimate interaction between glucose and ABA signaling (Kang et al., 2002).

It is now clear that HXK-mediated Glc signaling is connected to ethylene and ABA signaling. Further analyses show that Glc modulates genes involved in ABA biosynthesis and signaling (Figure 5) (Arenas-Huertero et al., 2000; W.-H. Cheng and J. Sheen, unpublished data) as well as ethylene signaling (W.-H. Cheng and J. Sheen, unpublished data). Moreover, a sugar-induced increase in ABA synthesis may be required for HXK-dependent sugar signaling (Figure 5) (Arenas-Huertero et al., 2000). A connection between ABA and ethylene signaling is supported further by the recent isolation of alleles of *ctr1* and *ein2* as enhancer and suppressor mutations of *abi1*, respectively (Ghassemian et al., 2000). In addition, the *era3* mutant (enhanced response to ABA) is allelic to *ein2* (Beaudoin et al., 2000). These studies dramatically illustrate the fact that ABA and ethylene signaling pathways antagonize each other during germination and seedling development. At lower sugar concentrations, the ABA inhibition of germination is reduced by sugars

(Finkelstein and Lynch, 2000a). Thus, hormone and sugar signaling networks may have different links depending on cell types, developmental stages, physiological state, and environmental cues.

The isolation of the Arabidopsis *gin2* mutant provides genetic evidence for a direct link between AtHXK1 and sugar sensing and signaling, suggested previously by biochemical, molecular, and transgenic analyses (Jang and Sheen, 1994, 1997; Pego et al., 1999) (Figure 3). Phenotypic analyses of the *gin2* mutant, in addition, reveal a new connection between sugar and auxin/cytokinin signaling that is independent from ethylene and ABA signaling (Figure 5). Further characterization of the auxin and cytokinin signaling components (Guilfoyle et al., 1998; Gray and Estelle, 2000; Hwang and Sheen, 2001; Mok and Mok, 2001) should reveal the molecular mechanisms that underlie their interactions with the sugar signal transduction pathway. The characterization of enhancer and suppressor mutations of *gin2* also will be a useful approach to further elucidate HXK action.

CONCLUSIONS AND PERSPECTIVES

Consistent with the pleiotropic effects of sugars on plant metabolism, growth and development, stress response, and gene expression, a complex picture of sugar-controlled regulatory networks and interactions with multiple signaling pathways is emerging. Although biochemical studies provide evidence for the involvement of a variety of protein kinases, protein phosphatases, 14-3-3 proteins, and Ca^{2+} as a second messenger, and although several transcription factors and regulatory *cis* elements have been found to mediate sugar control of gene expression, their precise roles in sugar signal transduction pathways require further investigation. Significant progress has been made with the identification of AtHXK1 as a sugar sensor, the possible involvement of SnRKs in sugar and metabolite signaling, and the identification of sugar signaling mutants as components in plant hormone biosynthesis and signal transduction.

Elucidation of the complete signaling circuitry that underlies the complex biological responses to changing sugar levels is challenging for a number of reasons. First, there is the intrinsic complexity of a multicellular photosynthetic organism with both source and sink tissues and with sugar producing and consuming activities. As a consequence, research is focused on multiple model systems and different cell types, further complicating direct comparison of experimental results. Different species also display distinct source-sink relationships and nutrient uptake, transport, and utilization mechanisms. For example, potato plants have a strong tuber sink and generally show less prominent sugar-related symptoms in source leaves. Sugar-related symptoms usually are also less pronounced in Arabidopsis leaves than in tobacco or tomato leaves, perhaps as a result of differences in plant architecture and leaf nitrogen allocation (von Schaewen et al., 1990; Dai et al., 1999). In addition, plants can display different sensitivities to endogenous and external signals at different developmental stages. Therefore, mesophyll cells of young and old leaves respond to sugars differently (Sheen, 2001).

Finally, genetic analysis in plants has been far more difficult than in the simple unicellular yeast system. However, the establishment of Arabidopsis as a plant model with the availability of the complete genome sequence, knockout lines, and microarray technology has boosted genetic research in plant signal transduction (Arabidopsis Genome Initiative, 2000; Bleecker and Kende, 2000; McCarty and Chory, 2000; Richmond and Somerville, 2000; Sussman et al., 2000; Zhu and Wang, 2000; Schroeder et al., 2001). The comparison of global gene expression profiles between sugar mutant and wild-type plants will reveal novel signaling mechanisms and facilitate the dissection of downstream interacting pathways mediating ABA, auxin, cytokinin, ethylene, and nitrogen regulation. The design of more specific genetic screens, such as the isolation of suppressor and enhancer mutations of the *gin2* mutant, may lead to new mechanisms of HXK action in the protein complexes and in interacting proteins. Genetic evidence for the functions of PKs, PPs, Ca^{2+}, G proteins, and sugar sensors other than HXK will be required to fill in the gaps in the sugar signaling network. Further development of physiological cell systems will power the analyses of molecular and biochemical mechanisms underlying interactions between signaling pathways. Because of its central role in plant signal transduction, insight into sugar sensing and signaling and the control of carbon allocation offer the possibility of important biotechnological applications.

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