Sugar Sensing and Signaling in Plants: Conserved and Novel Mechanisms

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Abstract

Sugars not only fuel cellular carbon and energy metabolism but also play pivotal roles as signaling molecules. The experimental amenability of yeast as a unicellular model system has enabled the discovery of multiple sugar sensors and signaling pathways. In plants, different sugar signals are generated by photosynthesis and carbon metabolism in source and sink tissues to modulate growth, development, and stress responses. Genetic analyses have revealed extensive interactions between sugar and plant hormone signaling, and a central role for hexokinase (HXK) as a conserved glucose sensor. Diverse sugar signals activate multiple HXK-dependent and HXK-independent pathways and use different molecular mechanisms to control transcription, translation, protein stability and enzymatic activity. Important and complex roles for Snf1-related kinases (SnRKs), extracellular sugar sensors, and trehalose metabolism in plant sugar signaling are now also emerging.

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INTRODUCTION

Life on earth largely depends on the photosynthetic fixation of carbon and light energy in energy-rich sugar molecules and the concomitant production of oxygen. Consistent with their importance as the prime carbon and energy sources for most cell types, sugars, in addition, have acquired important regulatory functions early in evolution, controlling metabolism, stress resistance, growth, and development in bacteria, yeasts, plants, and animals. The regulatory roles of sugars (and nutrients in general) are most explicit in free-living microorganisms that are challenged by a constantly, often dramatically changing environment. The yeast *Saccharomyces cerevisiae* (baker's or brewer's yeast) is a particularly well-studied eukaryotic model system for sugar sensing and signaling, not in the least because of the numerous applications of alcoholic fermentation. In multicellular organisms, maintenance of nutrient and energy homeostasis within cells and tissues is of vital importance and requires the constant monitoring and adjusting of nutrient availability. Failure to do so can have dramatic consequences that cause life-threatening diseases, such as diabetes, in mammals. In photosynthetic, sugar-producing, and sessile organisms like plants, maintenance of energy homeostasis requires even more sophisticated and flexible regulatory mechanisms to account for the amazing physiological and developmental plasticity seen in plants. In recent years, a pivotal role of sugars as signaling molecules and their dramatic effects on plant growth and development have become apparent. Still, a great deal remains to be learned about the precise molecular mechanisms involved. There have been comprehensive reviews on various aspects of sugar regulation in plants (43, 67, 76, 111, 123). This review intends to provide an overview of the latest evidence supporting the central roles of sugar signals and signaling in plant life. A comparison of conserved and novel sugar regulation mechanisms in the yeast and plant model

systems is presented. Promising future research directions and emerging new pathways and mechanisms are discussed.

YEAST AS A MODEL SYSTEM

In general, yeast has proven very useful as a model and experimental tool for reverse genetics approaches of eukaryotic cell biology. *Saccharomyces cerevisiae* is a facultative anaerobic organism but, even in the presence of oxygen, prefers the fermentation of sugars like glucose, fructose and sucrose to far more energy-efficient respiration. Rapid proliferation and reusable ethanol production during fermentation apparently offers a selective advantage over less ethanol-tolerant microorganisms. This yeast therefore has developed a whole array of glucose sensing and signaling pathways to enable the optimal and exclusive use of this carbon source. In view of the ancient and possibly conserved nature of cellular sugar sensing and signaling mechanisms in eukaryotes, the pathways elucidated in yeast are introduced concisely (**Figure 1**) (reviewed in 112 and 113).

An important pathway, responsible for transcriptional repression of a large number of genes involved in respiration, gluconeogenesis and the uptake and metabolism of alternative carbon-sources, is the "main glucose repression pathway" (**Figure 1*a***). Glucose activation of this pathway involves the glycolytic enzyme and sensor Hexokinase2 (Hxk2), which interacts with the Glc7-Reg1 protein phosphatase 1 (PP1) complex that dephosphorylates and inactivates a key protein kinase (PK), Sucrose nonfermenting1 (Snf1). In addition, Hxk2, in response to glucose, translocates to the nucleus, where it interacts with Mig1 [a zinc-finger DNA-binding transcription factor (TF)] to form a stable complex that recruits co-repressor proteins (91). Based on extensive random mutagenesis, it was concluded that the role of Hxk2 in glucose repression was tightly associated with its catalytic activity. However, a regulatory role for Hxk2 as a glucose sensor is recently substantiated by the isolation and characterization of new *hxk2* mutants with uncoupled catalytic and signaling activities (112, 113).

The Snf1 PK, an ortholog of mammalian AMP-activated PK (AMPK), is required for derepression of gene expression under low glucose and starvation conditions through phosphorylation of Mig1, which causes Mig1 to dissociate from the repressor complex and subsequently undergo nuclear export.

Due to low energy efficiency, fermentative metabolism requires a high metabolic flux through glycolysis. Therefore, in the presence of glucose, the expression of hexose transporters (HXTs) with appropriate affinity and capacity is upregulated through the action of a second glucose signaling pathway (**Figure 1*b***). Two catalytically inactive Hxt homologs, Snf3 and Rgt2, function as high- and low-affinity sensors, respectively, for extracellular glucose and activate casein kinase 1 (Yck1). This HXT-induction pathway inactivates the Rgt1 transcription repressor through SCF^{GRR1} (ubiquitin E3 ligase)- and proteasome-mediated degradation of the Rgt1-interacting and -regulating proteins Std1 and Mth1. Yck1 presumably phosphorylates Std1 and Mth1, which are tethered from the nucleus to the C-terminal tails of the glucose sensors, thereby targeting them for ubiquitination and degradation (61).

The third sugar-regulatory pathway involves cAMP-PKA signaling, which enables the fast growth rate on fermentable carbon sources through a phosphorylation cascade (–). Remarkably, glucose activation of cAMP synthesis by adenylate cyclase (AC) involves a dual mechanism. Extracellular glucose or sucrose is sensed by the G-protein coupled receptor (GPCR) system (75), consisting of the Gpr1 receptor, Gpa2 (a heterotrimeric Gα-protein), and Rgs2, a negative regulator of G-protein signaling. However, activation also strictly depends on glucose uptake and phosphorylation (but no further metabolism). Some evidence again suggests a rather regulatory role for multiple hexose kinases [Hxk1 and 2 or Glucokinase1 (Glk1)], possibly

HXK: hexokinase

PK: protein kinase

TF: transcription factor

GPCR: G-protein coupled receptor

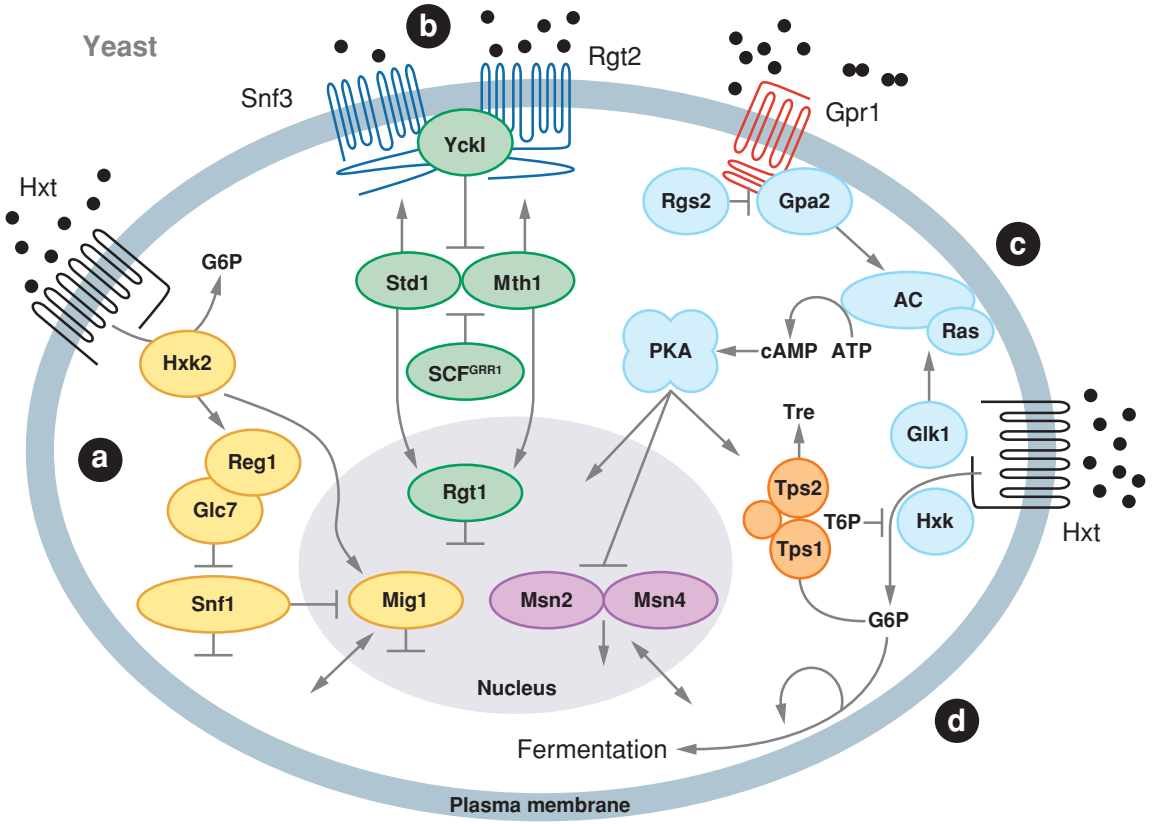

Figure 1

Glucose sensing and signaling pathways in yeast. (*a*) The main glucose repression pathway. Hexokinase 2 (Hxk2) acts as a sensor and translocates to the nucleus in response to glucose. Sucrose nonfermenting 1 (Snf1) is required for derepression of gene expression under starvation conditions. (*b*) The HXT induction pathway. Two hexose transporter homologs, Snf3 and Rgt2, function as glucose sensors at the plasma membrane, where they activate Casein kinase I (Yck1). (*c*) The cAMP-dependent Protein Kinase A (PKA) pathway with a dual sensing system for glucose activation: extracellular glucose (and sucrose) sensing by the G-protein coupled receptor (GPCR) system and intracellular hexose phosphorylation. (*d*) Glycolytic activity and gene expression are controlled by metabolic intermediates, including trehalose-6-P (T6P), which inhibits Hxk2 activity. High and low levels of glucose (*black dots*) and sucrose (*double dots*) are indicated. See text for details.

through activation of the small Ras G-proteins, which are required for (basal) AC activity. In addition to stimulating glycolytic activity and mobilization of reserve carbohydrates and ribosomal protein gene expression, PKA activity also dramatically reduces stress resistance during growth on glucose through inactivation (phosphorylation and cytoplasmic translocation) of the Msn2 and Msn4 TFs. These TFs, isolated as multicopy-suppressors of the *snf1* mutant phenotype, activate gene expression in the absence of high glucose levels by binding to stress response elements (STRE) in the promoters of stress-regulated genes. PKA also integrates signaling by other essential nutrients such as phosphate, sulphate, and nitrogen sources.

Finally, metabolic intermediates are involved in expression and (allosteric) activity regulation of glycolytic enzymes (**Figure 1*d***). Unexpectedly, mutant alleles conferring a general glucose-sensing defect are affected in

the trehalose-6-P (T6P) synthase gene *TPS1*. Trehalose has a dual function as a storage carbohydrate and stress protectant in microorganisms and is accumulated mainly during starvation conditions. In addition, trehalose metabolism plays a vital role in controlling yeast glycolysis, at least in part through the allosteric inhibition of T6P on HXK activity.

Numerous regulatory interactions between these different pathways enable exquisite fine-tuning of the cell's response to glucose availability. Interestingly, Hxk2, PKA, and Snf1 signaling have also been implicated in yeast longevity control, and analogous ancient pathways appear to exist in mammals and plants.

SUGAR SIGNALS IN PLANTS

Sugar regulation is necessarily far more complex in plants. First, multicellular organisms need both long-distance and tissue- or even cell-type-specific signaling mechanisms and coordination with both development and physiological and environmental changes. As autotrophic, photosynthetic organisms, plants are made up of sugar exporting (source) and sugar importing (sink) tissues and organs, and sugar signals are generated from different sources at different locations (**Figure 2**). Sugar metabolism is a very dynamic process, and metabolic fluxes and sugar concentrations alter dramatically both during development and in response to environmental signals such as diurnal changes and biotic and abiotic stress (11, 12, 108, 124, 146). Integration of environmental signals with metabolism is particularly important for sessile organisms. Not surprisingly, intricate regulatory interactions with plant hormones are an essential part of the sugar sensing and signaling network. Finally, photosynthesis and carbon metabolism and allocation are themselves subject to rigorous feedback regulation and a prime target of sugar signaling.

In general, source activities like photosynthesis, nutrient mobilization, and export are upregulated under low sugar conditions,

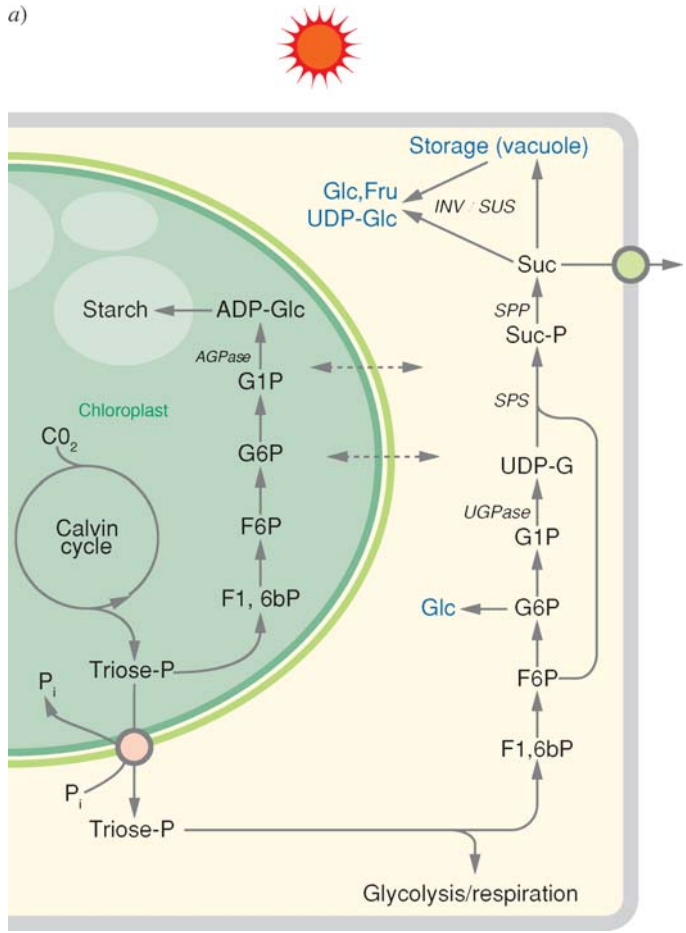

Figure 2

Sugar signals in source and sink cells. Simplified model of the major carbon fluxes and sugar signal generation by photosynthesis, transport and hydrolysis in photosynthetic source cells during the day (*a*) and night (*b*) and in sink tissue (*c*). See text for details. SPS, sucrose-P synthase; SPP, sucrose-P phosphatase; AGPase, ADP-glc pyrophosphorylase; UGPase, UDP-glucose pyrophosphorylase; INV, invertase; C-INV, cytosolic INV; CW-INV, cell wall INV; V-INV, vacuolar INV; SUS, sucrose synthase

whereas sink activities like growth and storage are upregulated when carbon sources are abundantly available. Photosynthesis and sink demand need to be rigorously coordinated, and this coordination involves both metabolic (substrate and allosteric) regulation and specific sugar-signaling mechanisms. Although sucrose is the major photosynthetic product and transport sugar in plants, many sugar-signaling effects on growth and metabolism

T6P: trehalose-6-P

TPS: trehalose-6-P synthase

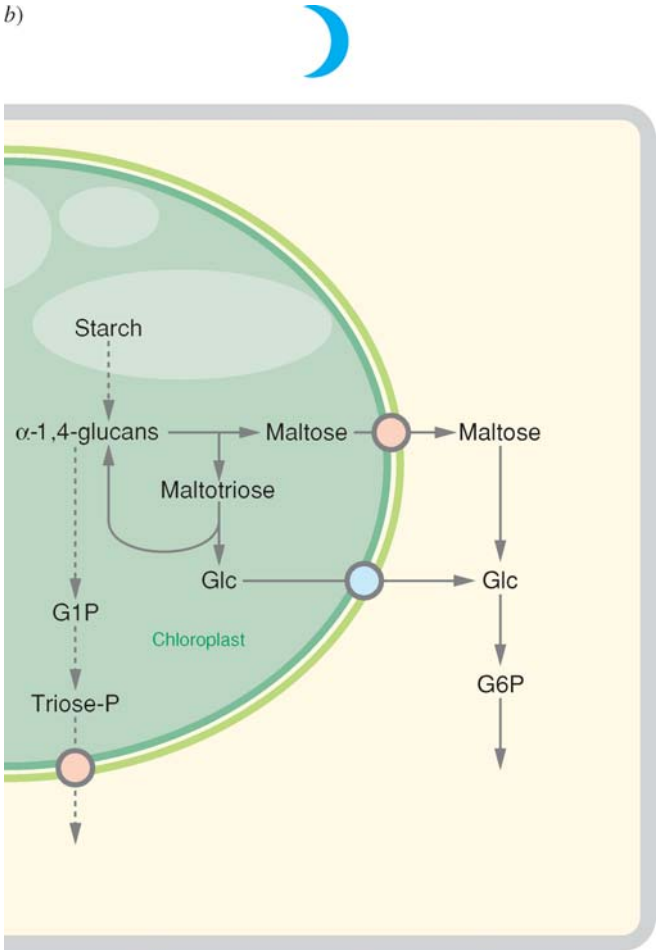

Figure 2
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CW-INV: cell wall invertase

AGPase: ADP-glc pyrophosphorylase

C-INV: cytosolic invertase

SUS: sucrose synthase

V-INV: vacuolar invertase

G6P: glucose-6-P

can be attributed to the action of its hydrolytic hexose products, glucose and fructose (or their downstream metabolic intermediates). However, recent studies suggest that sucrose and trehalose (or T6P) regulate specific responses that are not affected by hexoses (see below).

A current, simplified model of the major carbon fluxes in plants shows where sucrose and hexose signals can be generated and perceived in source and sink cells (**Figure 2**). In photosynthetic (source) cells (**Figure 2*a***), photosynthate generated in the Calvin cycle is exported, mainly as triose-phosphates, from the chloroplast to the cytosol, where it is used in glycolysis (and subsequently in respiration or biosynthesis) or converted to sucrose for local use or export to sink tissues. Net export or import of sucrose depends on the source or sink status of the leaf cells. Biotic or abiotic stress and hormonal signals can also induce cell wall invertase (CW-INV) expression and sink formation in leaf tissue (7, 109). Excess photosynthate is transiently stored as starch in the chloroplast during the day. ADP-glucose pyrophosphorylase (AGPase), a key enzyme in starch synthesis, is highly regulated by sugars (28, 42, 69). A major source for glucose signals is transitory starch breakdown from chloroplasts in leaf cells during the night (mainly via maltose and glucose export; **Figure 2*b***) and from plastids (amyloplasts) in starch-storing organs (124, 145).

In sink tissues (**Figure 2*c***), sucrose can be imported into cells through plasmodesmata (symplastic transport) or the cell wall (apoplastic transport). Intracellular sucrose is cleaved by cytoplasmic INV (C-INV), generating glucose and fructose, or by sucrose synthase (SUS) producing fructose and UDP-glucose. Sucrose can also be imported and stored in the vacuole, and vacuolar INV (V-INV) is a major intracellular source of hexoses in expanding tissues. In the apoplast, extracellular sucrose is hydrolysed by CW-INV, a major driving force in sugar unloading and gradient maintenance and therefore sink strength. These enzymes generate high levels of extracellular glucose and fructose that are taken up by hexose transporters, which are coexpressed and coordinatedly regulated with CW-INV (109).

It is clear that sucrose transport and hydrolysis play key regulatory roles in carbon allocation and sugar signal generation. The extensive feedback regulation of the INVs and SUS by sugar signaling generates a very sensitive self-regulatory system (67). The actual situation is more complex with transport of sugars and intermediates in and out of plastids (145) and vacuoles. Interestingly, substantial direct glucose-6-P (G6P) to glucose conversion by glucose-6-phosphatase activity is also observed in maize root tips (2). Besides photosynthesis and breakdown of sucrose and starch, the hydrolysis of cell wall

polysaccharides also likely generates sugar signals. Several cell wall glycosyl hydrolases are upregulated under stress conditions such as dark, sugar depletion, senescence and infection (23, 40, 74; J. Sheen, unpublished observations). Sugar signaling also needs to be integrated with the availability of other essential nutrients, such as nitrogen, phosphate and sulfate. Diurnal changes in nutrient availability are anticipated by the plant, and circadian regulation of enzymes involved in carbon allocation contributes significantly to an optimal use of the available resources. Moreover, recent studies have offered strong evidence for the critical role of sugar signaling in the actual regulation of diurnal gene expression (11, 33).

SUGAR CONTROL OF GROWTH AND DEVELOPMENT

Seed Development and Germination

Our understanding of sugar regulation of growth and development has benefited from studies on (mainly legume and especially bean) embryo and seed development. These processes are characterized by well-defined developmental and metabolic transitions (146). For example, during early seed development, high maternal CW-INV activity generates high hexose levels that promote embryo growth by cell division. High-resolution histographical mapping reveals a clear correlation between free glucose concentrations (present in spatial gradients) and mitotic activity in developing cotyledons (13). This role for glucose as a developmental trigger or even "morphogen" is possibly mediated by sugar (and cytokinin) control of cyclinD gene expression (107). D-type cyclins are involved in the G1/S transition, which in yeast and mammals is also controlled by nutrient availability. CYCD3;1 expression appears to be associated primarily with proliferating tissues and downregulation of CYCD3;1 might be an important factor in mitotic cell cycle exit and

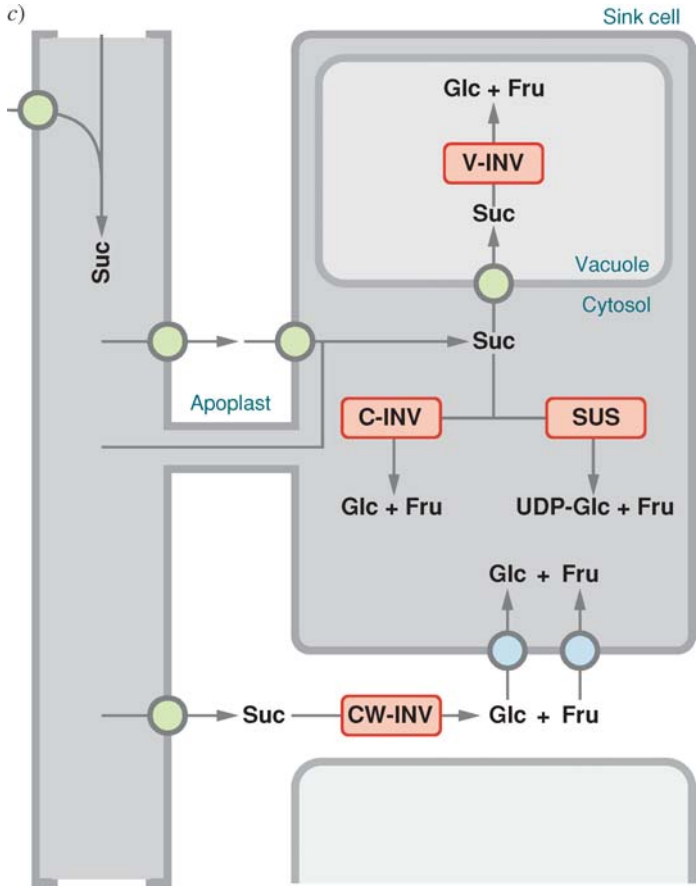

Figure 2
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the onset of cellular expansion and differentiation (31). In the moss *Physcomitrella patens*, targeted *cycD* gene knockouts exhibit developmental progression independent of sugar supply but have no obvious morphological phenotype (83). During the so-called transition phase, the embryo switches from a mainly mitotic growth to differentiation and growth driven by cell expansion. This switch is associated with a strong transient increase in sucrose uptake and the establishment of embryo sink strength. During this phase, free hexose levels decrease dramatically and the metabolic flux is redirected to storage product accumulation (mainly starch and nitrogen in the case of pea seeds). Sucrose, in general, appears to be rather associated with the regulation of storage- and differentiation-related

processes, in part through the regulation of metabolic enzyme gene expression and activity (146). The transition phase is also marked by a shift from high maternal CW-INV activity to high filial SUS activity.

Arabidopsis seed development follows a similar format with the major difference that it stores mainly lipids. A recent microarray study has initiated the dissection of the contrapuntal networks of gene expression during seed filling (115). Interestingly, the *wrinkled1* mutant, that has a severely reduced seed oil content, is deficient in a putative APETALA2 (AP2)-type TF that controls glycolytic gene expression and activity necessary for the conversion of sucrose into triacylglycerol biosynthesis precursors (16). Remarkably, loss-of-function mutations in the AP2 TF itself, best known for its involvement in flower development, increase seed mass and yield (60, 94). The increased cell size and number in the mutant embryos is associated with an increased hexose to sucrose ratio throughout embryo development (94); this observation suggests that this TF exerts its effect by modulating sugar metabolism and thereby signaling and development. In the storage stage of *Arabidopsis* embryogenesis, trehalose metabolism also plays an essential role (35; see below).

Although supplementation of exogenous sugars relieves the inhibition of germination by added ABA or mannose, glucose inhibits *Arabidopsis* seed germination (30, 105). Glucose-delayed seed germination is ABA-dependent but not caused by an increase in cellular ABA concentrations. This delay is rather associated with a slowing down of the decline in endogenous ABA important for the last stage of seed maturation and dessication. It appears that glucose and ABA interactions vary when their concentrations change. In addition, some sugar and ABA signaling mutants display normal germination kinetics (105), suggesting the involvement of specific signaling pathways in germination and differential responsiveness to sugars depending on the developmental stage.

ABA: ABSCISIC ACID (SYNTHESIS)

GIN: GLUCOSE INSENSITIVE

Sugar and Hormone Signaling in Early Seedling Development

Positive interactions between sugar and ABA signaling are more obvious during early seedling development (43, 76). ABA mediates a postgermination developmental arrest checkpoint that enables the germinated embryos to cope with new, adverse growth conditions (82). During *Arabidopsis* early seedling development, high levels of exogenous sugars similarly repress hypocotyl elongation, cotyledon greening and expansion, and shoot development.

The developmental arrest phenotype has enabled different groups using somewhat different screening conditions to isolate a number of sugar-insensitive and sugar-hypersensitive mutants in *Arabidopsis*. These mutants are often allelic, and their characterization has revealed extensive and intimate connections between sugar and plant hormone signaling pathways (**Figure 3**) (reviewed in 43, 76, and 111). Notably, several of the sugar mutants isolated turned out to be allelic to known *ABA synthesis* (*aba*) and *ABA insensitive* (*abi*) mutants. A central role for ABA in plant sugar signaling was substantiated by the characterization of *glucose insensitive5* (*gin5*) and *gin6/sucrose uncoupling6* (*sun6*)/*sugar insensitive5* (*sis5*) as mutant alleles of *ABA3* and the gene that encodes the AP2-type transcription factor ABI4, respectively (3, 43, 76). The glucose-insensitive *sis4/gin1* mutants are allelic to *aba2*, which is deficient in a short-chain dehydrogenase/reductase (SDR1) required for ABA synthesis (21, 43, 76). Exogenous glucose specifically increased both (*a*) expression of ABA synthesis and signaling genes and (*b*) endogenous ABA levels (21). This suggests that glucose-specific accumulation of ABA is required for glucose signaling during early seedling development. The fact that not all *abi* mutants are glucose insensitive during early seedling development indicates that there are multiple pathways for glucose and ABA signaling (3).

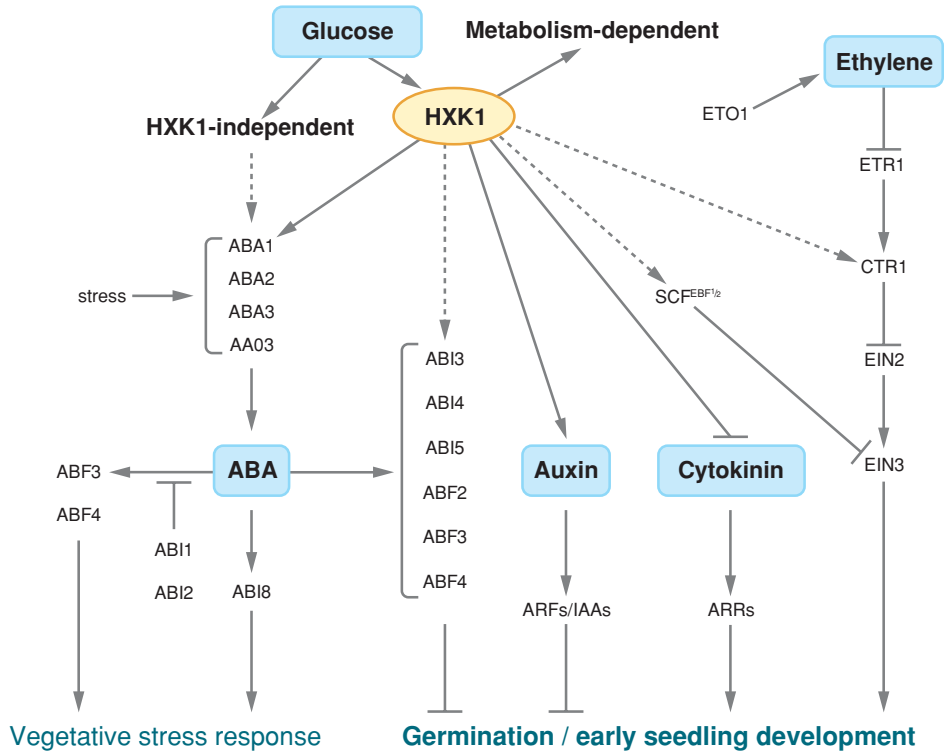

Figure 3

Model of genetic interactions between sugar and hormone signaling. HXK1-mediated glucose signaling that controls seedling development involves an increase in ABA and induces both ABA synthesis and ABA signaling gene expression. Glucose and ethylene signaling converge on the ETHYLENE INSENSITIVE3 (EIN3) TF to differentially regulate its protein stability. Finally, HXK-signaling interacts positively and negatively with auxin and cytokinin signaling, respectively. Hypothetical connections are shown (*dashed lines*). See References 43, 76, 111, and text for more details.

Another hormone that clearly interacts with sugar signals in controlling seedling development is ethylene (**Figure 3**). Treatment of wild-type seedlings with the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) copies the *gin* phenotype, and epistatic analysis puts GIN1/ABA2 downstream of the ethylene receptor ETR1 (156). Whereas ethylene insensitive mutants, *etr1-1* and *ethylene insensitive2* (*ein2*), exhibit glucose hypersensitivity (156), *gin4* and *sis1* are mutant alleles of *CONSTITUTIVE TRIPLE RESPONSE1* (*CTR1*), a negative regulator of ethylene signaling (21, 43, 76). A molecular link between glucose and ethylene signaling is provided by the finding that glucose and ethylene antagonistically regulate protein stability of the EIN3 TF (152).

Mutant screens for altered sugar responsiveness of germination and seedling development are not saturated, and new mutants are still being identified. The *glucose hypersensitive1* (*ghs1*) mutant contains a T-DNA insertion in a plastid ribosomal protein gene (93). However, because of the extensive interactions between sugar and other pathways, it is expected that more components will be identified in screens and studies of other responses. The *bls1* mutant, for example, was isolated in a screen for photomorphogenic mutants and uncovers interactions between brassinosteroid, light and sugar (bls) responses (73).

SnRK: Snf1-related kinase

Still, many sugar mutants identified by other screens are involved in the ABA pathway. Several sugar-insensitive and salt, low temperature, and osmotic stress-resistant mutants, for example, are allelic to *aba3* or *abi4* (reviewed in Reference 111). In addition, ABF2, an ABA response element (ABRE) binding basic leucine zipper (bZIP) TF, is an essential component of glucose signaling (65), and overexpression of ABF3 and ABF4 also increases glucose sensitivity (64). Whereas most ABA response mutants have only subtle defects in the absence of stress, the glucose-insensitive *abi8* mutant displays severely stunted growth and male sterility (14). Interestingly, the mutant has reduced expression of *V-INV*, *C-INV*, and *SUS* genes, and glucose supplementation improves viability and root growth. Consistent with the predominant expression of ABI8 in the root elongation zone, *abi8* is allelic with two dwarf mutants defective in root meristem maintenance and cell elongation (14).

Vegetative and Reproductive Development

Local sink establishment, carbon metabolism and sugar accumulation appear to play important roles in vegetative plant growth and development, presumably in part through sugar signal generation (**Figure 2**). Several lines of evidence point to a crucial role for carbon metabolism and especially the sucrose-cleaving enzymes in plant growth and development (109). A particularly remarkable finding is the spatially regulated expression of genes that encode carbon metabolic proteins like SUS, AGPase, and Snf1-related kinases (SnRK) in the tomato apical meristem; these genes serve as markers for early leaf development (101). Transgenic expression of the *Arabidopsis thaliana* homeobox leucine zipper transcription factor ATHB13, also revealed a sugar-dependent control of cotyledon and leaf shape through the specific modulation of lateral expansion of epidermal cells (51). These observations support a role for sugar metabolism and signaling in vegetative organogenesis. Interestingly, source strength appears to determine the timing of fixed developmental programs. Decreased photosynthetic rates by antisense suppression of the RUBISCO Small subunit (RBCS) in tobacco, for example, specifically delayed early shoot morphogenesis and increased the shoot/root ratio. These results suggest that plants have a source strength threshold for full, adult shoot morphogenetic growth (137). However, metabolic control of cell growth also allows remarkable flexibility in the response to changing growth conditions, as exemplified by the gravity response of maize internodal pulvinal cells. Auxin redistribution asymmetrically increases invertase expression and activity, and this increase results in the asymmetrical accumulation of hexoses and differential cell elongation across the pulvinus (80). In dark-grown *Arabidopsis* seedlings, exogenous sucrose can induce adventitious root formation (128). Many *Arabidopsis* sugar mutants, such as *gin1*, *gin2* and *gaolaozhuangren2* (*glz2*), exhibit abnormal growth and development (20, 21, 89). Recent exciting studies have also demonstrated the critical role of *Arabidopsis* TPS1 and T6P in vegetative growth and flowering (6, 117, 118, 141).

In addition to vegetative development, carbon allocation and sugar signals also control reproductive development. Induction of flowering is associated with starch mobilization and a transient increase in leaf carbohydrate export to the shoot apical meristem, suggesting that phloem carbohydrates are a critical factor. More specifically, the C/N ratio of the phloem sap increased markedly and early during induction (24). Sucrose availability on the aerial part of the plant promotes morphogenesis and flowering of *Arabidopsis* in the dark, and supplementation with 1% exogenous sucrose rescues the late-flowering phenotype of several mutants (110). However, higher concentrations of exogenous sugars delay the transition in both wild-type and late-flowering mutants by extending the late-vegetative phase. The concomitant delay in

activation of LEAFY suggests that sugars can control the expression of floral meristem identity genes (95). Interestingly, a glycosyl hydrolase, SUS, and asparagine synthase are putative direct targets for the LEAFY TF, which is essential for flower development. Expression of a CW-INV under a meristem-specific promoter causes accelerated flowering and enhanced branching of the inflorescence and seed yield, whereas the C-INV causes delayed flowering and both reduced seed yield and branching. These results emphasize the importance of the exact source, nature, and location of the sugar signals (55). More evidence for an essential role of accurate carbon allocation in reproductive development comes from the male-sterility phenotypes in tobacco with tissue-specific antisense suppression of a CW-INV (48) and antisense *SnRK* transgenic barley (155). The *Arabidopsis* sugar-insensitive mutant *glz2* shows delayed flowering, aberrant flowers and fruits, and completely sterile gynoecium (20).

Senescence and Stress

After reproduction, the final stage in plant life is senescence, which is a highly regulated stepwise process controlled by complex developmental programs and environmental signals. Leaf senescence typically coincides with a decline in chlorophyll content and photosynthetic activity. The repressive effect of sugars on photosynthetic gene expression and activity and the correlation between HXK expression and the rate of leaf senescence (29, 89, 151) are indicative of an important role for HXK-dependent sugar signaling in leaf senescence. Although this is consistent with observations in other eukaryotic organisms, there has been some controversy about the senescence-inducing effect of sugars. This controversy is mainly due to the observation that dark-treatment of leaves, and concomitant chlorophyll breakdown and starvation, can induce senescence. However, dark incubation of whole plants delays senescence, and, although some senescence-associated genes (*SAG/SEN*) are induced by dark-incubation and repressed by sugars, a recent microarray analysis found significant differences in gene expression between dark/starvation-induced and developmental senescence (15). Moreover, leaf senescence is induced by high light and long days and is associated with hexose accumulation (32). More research is needed to explain the apparent contradiction between starvation responses and sugar accumulation during senescence.

The exact source of the sugar accumulation during senescence is not clear. In castor bean leaves, sieve tube occlusions and carbohydrate back-up seem to precede chlorophyll degradation during natural senescence (62). This observation suggests that phloem blockage by stress-induced callose deposition is the cause there. However, senescence is generally associated with massive nutrient (especially nitrogen) remobilization and export from deteriorating leaves, implying that basic cellular metabolism and phloem transport remain functional until the later stages of senescence. Although it is possible that a high sugar-to-nitrogen ratio is the trigger for senescence and nitrogen remobilisation from older leaves (103, 150), analysis of *Arabidopsis* recombinant inbred lines shows that late-senescing lines appear to mobilize glutamine, asparagine, and sulfate more efficiently than early-senescing lines (32).

Although ABA is known to promote senescence, a recent study (103) suggests that ABA is not required for the sugar-dependent induction of leaf senescence. Cytokinins, on the other hand, can delay plant senescence, and studies with *gin2* show that sugars and cytokinins work antagonistically (89). Interestingly, cytokinin-induced CW-INV expression is an essential downstream component of cytokinin-mediated local delay (green islands) of leaf senescence (7).

Senescence is also associated with the expression of *pathogenesis-related* (*PR*) genes. Both exogenous sugars and overexpression of a yeast INV in the plant vacuole or cell wall can induce *PR* gene expression (54, 130, 151).

In addition to regulating carbon partitioning, plant development, and hormone responses, INVs have an important role in stress responses as central signal integrators and modulators (109). CW-INV is induced by both abiotic stress and pathogen infection to locally increase respiratory sink activity and can be regarded as a PR protein. However, PK inhibitor studies indicate that sugars and stress regulate source and sink metabolism and defense responses through different pathways (36, 108). The *Arabidopsis* hypersenescing mutant *hys1* provides the clearest link between sugar, senescence, and stress signaling. This mutant is not only hypersensitive to sugar inhibition of seedling development and gene expression, but also allelic to *constitutive expressor of pathogenesis-related genes5* (*cpr5*) (153).

Sugar Starvation

As well as being able to sense and optimally exploit carbohydrate availability, plants need to be able to cope with carbohydrate depletion. In addition to natural diurnal fluctuations, variations in other environmental conditions can result in sugar starvation conditions. In general, plants deal with such conditions by arresting growth and by redirecting cellular activity towards basic metabolism and respiration based on protein, amino acid, and lipid catabolism rather than glycolysis. Energy-consuming biosynthetic processes, including protein synthesis, are switched off (11, 23, 58, 154). As in yeast and animal cells, starvation conditions also trigger proteolysis and autophagy in plants. *Arabidopsis* orthologs of the yeast AUTOPHAGY (ATG) protein system are induced by sucrose starvation and have been shown to be essential for nutrient recycling and senescence (23, 132).

Starvation conditions are, however, difficult to manipulate experimentally in whole plants. Cell cultures or excised roots and leaves have often been used to study starvation effects, including derepression of α-amylase gene expression (in rice suspension-cultured cells), the coordinated induction of the glyoxylate cycle (malate synthase and isocitrate lyase) gene expression (in cucumber cell cultures), activation of a β-methylcrotonyl-coenzyme A carboxylase (MCCase) subunit gene (in sycamore cell suspension cultures), increased mitochondrial fatty acid β-oxidation, and increased proteolysis and nitrogen distribution (in excised maize root tips) (reviewed in Reference 154). Interestingly, induction of a number of *DARK INDUCED* (*DIN*) genes in detached leaves is inhibited by sucrose supplementation. This indicates that sugar deprivation is the key factor in the induction of these genes. Consistent with this hypothesis, *DIN* gene expression is induced by addition of a photosynthesis inhibitor and in sucrose-depleted *Arabidopsis* cell culture and tobacco BY-2 cells (40). Most *DIN* genes encode proteins involved in amino acid and carbohydrate catabolism, and some are associated with leaf senescence (40). *DIN6/ASPARAGINE SYNTHASE1* (*ASN1*), which encodes a glutamine-dependent asparagine synthase, appears to be a particularly good reporter gene for sugar starvation conditions (11, 23, 71, 104, 134). In *Arabidopsis* culture cells, sucrose deprivation leads to structural changes in mitochondria, a decrease in mitochondrial volume, a reduction in the rate of cellular respiration, and global gene expression changes (23, 45). Recent detailed analysis of the molecular events of dark-induced leaf senescence also revealed a prominent increase in asparagine levels and *ASN1* gene expression, and mitochodrial amino acid catabolism (58, 78). The clever use of an *Arabidopsis* starchless *phosphoglucomutase* (*pgm*) mutant with larger diurnal changes of endogenous sugar levels has facilitated the identification of genes controlled by low sugar conditions and the circadian clock in whole plants (11, 131).

SUGAR SENSORS

The Roles of the Hexokinase Glucose Sensor

Recent isolation and characterization of the *Arabidopsis gin2* mutants clearly identify

hexokinase (AtHXK1) as a core component in plant sugar sensing and signaling (89, 152). Initial evidence for a function of plant HXK as a glucose sensor came from studies with different sugars, sugar analogs, and metabolic intermediates in a mesophyll-protoplast transient expression system and phenotypic analyses of transgenic *Arabidopsis* (59). HXK genetically functions upstream of GIN1/ABA2 in the glucose-signaling pathway (156). In order to study the function of AtHXK1 in a more physiological context, plants were grown under various light intensities that altered endogenous sugar levels and signals. Whereas increased energy supply under high light accelerated wild-type plant growth, *gin2/hxk1* mutant plants remained small with reduced cell expansion. In addition to modulating developmental arrest in the presence of high exogenous glucose, AtHXK1 has an important role in growth promotion as well. Analyses of a possible link with growth hormones revealed that *gin2* mutant hypocotyl explants are relatively insensitive to auxin-induction of cell proliferation and root formation, but hypersensitive to shoot induction by cytokinin. Consistent with this observation, seedling development of the auxin-resistant mutants *auxin resistant1* (*axr1*), *axr2*, and *transport inhibitor response1* (*tir1*), and plants with a constitutive cytokinin response or supplemented with exogenous cytokinin is insensitive to high glucose levels (**Figure 3**). The *gin2* mutant plants also display a clear delayed-senescence phenotype and reduced fertility. These effects parallel the effects of calorie restriction and mutations in signaling components on longevity in other eukaryotes (89).

Most interestingly, the *gin2* mutants still have 50% of the wild-type glucose kinase activity and accumulate normal sugar phosphate levels. Moreover, there is no clear correlation between glucose kinase activity and glucose reduction of chlorophyll content and photosynthetic gene expression. Uncoupling of metabolic and signaling activity is confirmed by the construction and analysis of two catalytically inactive *AtHXK1* alleles. Although deficient in ATP binding and phosphoryl transfer, respectively, these alleles sustain wild-type growth, repression of photosynthetic gene expression, and auxin and cytokinin responsiveness when expressed in a *gin2* background (89). Future functional characterization of the high-molecular-weight protein complexes that harbor the AtHXK1 protein will shed light on the molecular details of its regulatory interactions (Y. Cho & J. Sheen, unpublished observations).

The glucose kinase activity still present in the *gin2* mutants is probably not solely due to the presence of AtHXK2. *Arabidopsis* encodes four more hexokinase-like (AtHKL) proteins, one of which has detectable kinase activity (therefore dubbed AtHXK3) (B. Moore & J. Sheen, unpublished observations). HXK and HXL protein localization is expected to play an important role in their functions (**Figure 4*a***). A completely functional glycolytic metabolon is found on the outside of the *Arabidopsis* mitochondrial membrane (44). This enables both optimal substrate availability and coordination of glucose metabolism with cellular energy demand. AtHXK1 protein indeed appears to be predominantly associated with mitochondria (B. Moore & J. Sheen, unpublished observations). A regulatory role for HXK in metabolic control of cell death similar to the situation in mammals is therefore also possible. HXK activities have also been detected in the cytosol and associated with plastids (148). Consistent with the fact that photosynthetic cells generate glucose mainly from starch breakdown, the major glucose-phosphorylating enzyme in the moss *Physcomytrella patens* is a novel type of chloroplast stromal kinase (96). Such an inner-plastidic HXK has also recently been identified in tobacco (46). Plants in general appear to contain two types of HXKs: type A kinases (such as PpHxk1 and two *Arabidopsis* HKLs), which have a predicted chloroplast transit peptide, and type B kinases (such as AtHXK1 and AtHXK2), which have a membrane anchor (96; B. Moore & J. Sheen, unpublished observations).

HKL: hexokinase-like protein

Metabolon: an enzyme complex enabling transfer of biosynthetic intermediates between catalytic sites without diffusion, thereby maximizing metabolic flux and avoiding interference

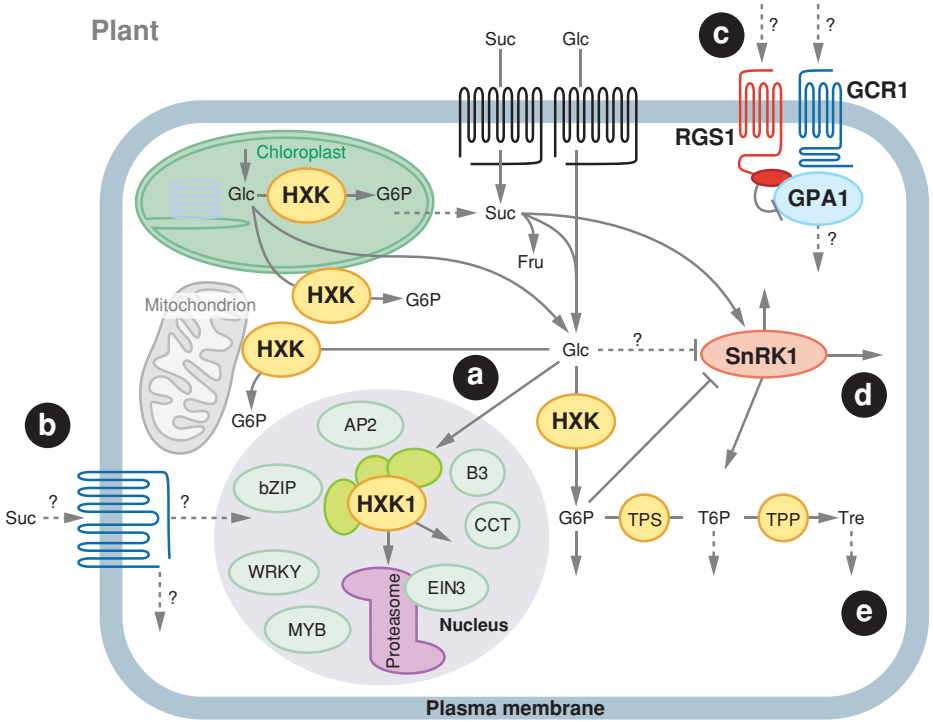

Figure 4

Model of sugar-sensing mechanisms in plants. (*a*) The HXK1 glucose sensor is mainly associated with mitochondria, possibly as part of a glycolytic metabolon. In addition, HXK1 is found in high-molecular-weight complexes in the nucleus where it controls transcription and proteasome-mediated degradation of the EIN3 TF. Other HXK and HKL proteins are also associated with the outer membrane of plastids, including chloroplasts, or cytosol. HXK can also be found in the chloroplast stroma. (*b*) Sucrose (and other disaccharides) appears to be sensed at the plasma membrane, possibly by transporter homologs. Monosaccharide transporters might have similar functions as membrane sensors. (*c*) G-protein coupled receptor signaling by RGS1 and GPA1 is involved in glucose control of seed germination and seedling development, possibly in a hexokinase-independent way. (*d*) SnRK1 proteins play an important role in plant sugar and starvation signaling, although the significance of the regulation of these proteins by sucrose (*Suc*) and G6P is still unclear. (*e*) Important regulatory effects are reported for trehalose (*Tre*) and T6P, apparently downstream of SnRK1. In the nucleus, several types of transcription factors are involved in sugar-regulated transcription. See text for more details.

Surprisingly, AtHXK1 can translocate to the nucleus as well (**Figure 4*a***) (152). More complex functions of HXK are anticipated in rice, in which ten functional HXK homologs have been identified (J.S. Jeon, personal communication). In addition to HXKs, plants also contain several fructokinases, some of which might also be involved in sugar sensing (98). Surprisingly, the *gin2* mutants are insensitive to glucose but still sensitive to fructose and sucrose (W. Cheng & J. Sheen, unpublished observations).

Cell Surface Receptors

In yeast, extracellular glucose and sucrose are detected by the Gpr1-Gpa2 system, one of only two GPCR systems, the other one being involved in pheromone detection. Animals use different GPCR combinations to

function as sweet taste receptors. Remarkably, proteins of the Type 1 Receptor (T1R) family are also expressed in the intestinal tract and enteroendocrine cells (34), where they could be involved in sugar sensing. In striking contrast to animals, where GPCRs constitute one of the major mechanisms for extracellular signal detection, plants apparently contain only one canonical G-protein α-subunit (encoded by *GPA1* and *RGA1* in *Arabidopsis* and rice, respectively). These proteins and the associated β and γ subunits have been implicated in a wide variety of developmental, light, phospholipid, and hormone responses (100), oxidative stress response, and fungal disease resistance. As the yeast proteins are involved in sensing the most vital signals (sugar and sex), one is intuitively tempted to speculate about the involvement of hetero-trimeric G-proteins in plant sugar sensing (**Figure 4*b***). Interestingly, GPA1 interacts with two putative receptor proteins: G-protein coupled receptor1 (GCR1), a seven-transmembrane domain protein with some homology to classical GPCRS, and Regulator of G-protein signaling1 (RGS1), an unusual hybrid seven-transmembrane domain protein with a C-terminal RGS-box (19). RGS proteins typically negatively regulate heterotrimeric G-proteins by accelerating their intrinsic GTPase-activity. Consistent with such a function, loss of RGS1 increases GPA1 activity, which results in increased cell elongation in hypocotyls in darkness and increased cell production in roots grown in light (19). The *rgs1* mutant seedlings display insensitivity to 6% glucose, whereas RGS1 overexpressors are hypersensitive (18, 19). Based on the use of different sugars and sugar-analogs, it is suggested that AtRGS1 functions in an HXK-independent glucose signaling pathway (18). The *gpa1* mutants are hypersensitive to ABA and sugar inhibition of germination (139). Glucose addition also causes a rapid transient increase in the interaction of AtRGS1 with AtGPA1 at the plasma membrane and its subsequent internalization, possibly as a desensitization mechanism (P. Taylor & A. Jones, personal communication). It will be interesting to identify the targets and processes downstream of RGS1 and GPA1.

Another potential extracellular glucose or sucrose detection system in plants may involve proteins analogous to the yeast glucose transporter-like sensors, Snf3 and Rgt2 (**Figure 4*c***) (70). More specifically, the *Arabidopsis* and tomato SUT2 sucrose transporter homologs, which do not have detectable transport activity, are characterized by a long central cytoplasmic loop reminiscent of the Snf3/Rgt2 structure. This observation suggests that these proteins may have a role in sucrose sensing (8). However, conclusive evidence for a membrane sugar sensor probably requires extensive mutant analysis. The use of nonmetabolizable disaccharides has provided useful information. For example, structure-function analysis indicates that the fructose moiety is needed for sensing nonmetabolized lactulose, palatinose, and turanose disaccharide and α−amylase repression in barley embryos (84). In addition, monosaccharide transporters with extended cytoplasmic loops are encoded in the *Arabidopsis* genome, and the monosaccharide sugar transporter STP13 acts as a heterologous multicopy suppressor of the yeast *snf4*Δ mutant growth phenotype (66).

MOLECULAR MECHANISMS OF SUGAR REGULATION

Distinct Sugar Signaling Pathways Regulate Gene Expression

Based on the role of HXK1, three distinct glucose signal transduction pathways are defined in plants (151). In the first HXK1-dependent pathway, gene expression is correlated with the HXK1-mediated signaling function. A major effect of this pathway is the repression of photosynthetic gene expression. Target genes in this pathway can now be defined genetically by the *gin2* mutants and catalytically inactive alleles (89, 151; E. Baena-Gonzalez & F. Rolland, unpublished results). A second

pathway is glycolysis-dependent and can also be sustained by heterologous yeast Hxk2 activity. An example is the glucose induction of *PR1* and *PR5* gene expression (151). Finally, there is evidence for HXK1-independent signaling pathways. Glucose induction of *CHS*, *PAL1* and genes encoding AGPase as well as glucose repression of *ASN1* are observed independent of sense and antisense overexpression of *Arabidopsis* HXK1 or overexpression of yeast Hxk2 (151). Transcriptional responses to nonphosphorylated glucose analogs have been observed in *Chenopodium* cell suspension cultures, *Chlorella*, and transgenic *Arabidopsis*, although one should be cautious about possible nonspecific effects of such chemicals. The glucose analog 3-OMG, not perceived as a sugar signal, is still phosphorylated by HXK with low catalytic efficiency, and the product, 3-OMG-6-phosphate, accumulates in these cells (25). Conversely, none of the 200 glucose-responsive *Arabidopsis* genes identified in a recent study responds to 3-OMG or 6DOG (142).

There are a number of selected genes [e.g., a gene that encodes a sugar beet proton-sucrose symporter (141a)], whose expression is regulated by sucrose but not glucose or fructose; this observation points to an HXK-independent, sucrose-specific signaling pathway. Interestingly, nonmetabolizable sucrose-analogs such as palatinose and turanose can also affect carbohydrate metabolism and gene expression. This observation suggests the existence of a di-saccharide sensing system at the plasma membrane (**Figure 4**) (5, 38, 84, 135). However, such analogs again have no physiological relevance and can elicit distinct responses consistent with their perception as stress-related stimuli.

Transcription Control

Over the years, a large number of plant genes have been found to be transcriptionally regulated by sugars, consistent with the coordinated regulation of source and sink activities. Importantly, several genes that encode metabolic proteins involved in sugar signal generation undergo transcriptional feedback-regulation by their own products. Repression of photosynthesis gene promoters, for example, has been studied in mesophyll protoplasts and transgenic seedlings. As well as photosynthesis genes, the *INV* and *SUS* genes are also extensively regulated by sugar availability (67). Also, when sugar levels are high, carbohydrate storage through starch synthesis is upregulated by the induction of genes that encode AGPase (28).

Many sugar-regulated genes and promoters have been used to screen for *Arabidopsis* mutants with potential defects in transcription control (reviewed in References 43 and 111). A screen using the regulatory sequences of the sugar-inducible AGPase large subunit (*APL3*) gene fused to a negative selection marker has identified several *impaired sucrose induction* (*isi*) mutants. Another screen based on the activity of a luciferase (LUC) reporter gene under the control of the *APL3* promoter yielded *high sugar-response* (*hsr*) mutants that exhibited elevated LUC activity and *APL3* expression in response to low sugar concentrations. The screen using sugar-regulated expression of an *Arabidopsis* β-amylase generated *low beta amylase* (*lba*) and *high beta amylase* (*hba*) mutants with altered sugar-regulation of a subset of genes. *Arabidopsis reduced sugar response* (*rsr*) mutants were selected using the sucrose-activated promoter of patatin, a potato tuber storage protein. Molecular analysis of the mutants will bring new information on the mechanisms underlying sugar-mediated transcription control.

Genome-Wide Expression Analyses

From the examples described above, it is clear that our knowledge about sugar-regulated gene expression largely comes from data from a variety of species, mutants, tissues, developmental stages, and treatments. The new microarray technologies now enablE genome-wide expression analyses of *Arabidopsis* sugar and starvation responses. Soil-grown adult

Arabidopsis phosphorus-deficient3 (*pho3*) mutant plants are used specifically to study the genomic response to sugar accumulation (79). This mutant is affected in the SUCROSE TRANSPORTER2 (SUC2), and therefore accumulates soluble sugars, starch, and anthocyanins. High expression levels of genes that encode sucrose phosphate synthases (SPS), the plastid glucose G6P/phosphate translocator (characteristically expressed only in heterotrophic tissues), and the AGPase large subunits are consistent with the starch accumulation in the mutant. Also consistent with the phenotype, there is a large increase in the expression of TFs and enzymes involved in anthocyanin biosynthesis. Apparently, secondary metabolism is also an important target for transcription regulation by sugars. Using a more comprehensive approach, the short-term effects of glucose and nitrogen in global gene expression in the dark have been studied in liquid-grown *Arabidopsis* seedlings (104). The use of the protein synthesis inhibitor cycloheximide shows that glucose repression is a more direct process than glucose induction, which often requires de novo protein synthesis. TFs with sugar-regulated expression profiles are likely regulators of the broad transcriptional response to sugars.

Several global gene expression studies have been published on sugar starvation responses. Using cDNA macroarrays and seedlings grown in the presence or absence of sucrose, a small number of (mostly carbohydrate and amino acid metabolism) genes were shown to be upregulated in concert during sugar depletion (74). A more detailed analysis of nutrient mobilization in response to sucrose starvation in *Arabidopsis* cells cultured in suspension has been carried out using the ATH1 GeneChip (23). Consistent with extensive nutrient recycling for cell survival, genes that were upregulated are involved in carbohydrate, amino acid, protein and lipid catabolism and autophagy. Although these cultures were nonphotosynthetic, several photosynthesis-associated genes were also upregulated upon starvation. Genes that were downregulated are involved in metabolism (biosynthesis), protein synthesis, and cell division. Similar expression profiles were observed in the responses of *Arabidopsis* rosettes to an extended night period and a starchless *pgm* mutant at the end of the night (11, 131). These studies also introduce the use of MAPMAN, a practical and informative tool to display complex genomic data in diagrams of metabolic and regulatory pathways. Interestingly, the molecular events in dark-induced senescence of *Arabidopsis* leaves (analyzed using a combination of cDNA microarray and biochemical analyses) exhibited extensive similarities with the sugar starvation response. Many TF genes were identified as putative regulators (78). However, a comparative microarray study reveals significant differences in gene expression and signaling pathways between developmental and dark/starvation-induced senescence (15).

Extended dark treatment causes a starvation condition that overrides the transcriptional regulation by circadian rhythm. However, in addition to energizing sugar production and (re)setting the clock, light can also directly affect gene expression through light-specific mechanisms. In a recent study, the effects of both light and sugar were examined. The results reveal that the majority of affected genes are co-regulated by both stimuli (133, 134). More extensive time-course gene expression analyses using wild-type and the *pgm* mutant plants under a 12 h photoperiod provide a clear picture of the essential roles of sugar signals for a large set of circadian regulated genes (11).

Coordination between sugar and other nutrient metabolic pathways is essential to optimize the use of energy resources. A number of genes involved in N-assimilation are co-regulated by sugars, and N-availability extensively regulates carbon-metabolic-gene expression (26, 116, 144). Sugar responses in general depend significantly on the N-status of the plant. Sugar repression of photosynthetic gene expression, chlorophyll accumulation and seedling development are

antagonized by nitrate (89). Complex interactions are observed between C and N signaling (116, 144). The effects of nitrogen and a combination of both glucose and nitrogen have been recently analyzed (104). Interestingly, most of the nitrogen responses seem to require the presence of a carbon source. A combination of microarray and extensive informatics analyses, classification, and modeling provides evidence for combined carbon and nitrogen signaling, especially in the control of metabolism and energy and protein synthesis, even suggesting the existence of a single CN-responsive regulatory cis-element for a subset of genes (97).

Oxygen availability also affects sugar signaling, especially the regulation of sucrose metabolism (68). A recent microarray analysis provides more insights into the effects of sucrose on gene expression in *Arabidopsis* seedlings under anoxia conditions (85).

Promoter Elements and Transcription Factors

The large genomic datasets generated in microarray experiments provide an excellent opportunity to identify conserved DNA elements in the promoters of co-regulated genes. Currently, most information on regulatory cis-elements involved in sugar signaling comes from a few selected genes, encoding sweet potato tuber and cereal seed proteins, and proteins involved in maize photosynthesis.

Studies on sugar activation of sweet potato tuber class I *patatin*, *SUS*, *sporamin* and *β-amylase* promoters identified several sucrose-responsive cis-elements, including the Sucrose-responsive element (SURE), A- and B-boxes, the TGGACGG element, an SP8 motif, and an SP8-binding protein, SPF1 (reviewed in Reference 111). SPF1 is a WRKY -type sucrose-repressed negative regulator with putative orthologs in other species, including *Arabidopsis*. These factors typically bind to (T)TGAC(C/T) W-boxes, also found in defense-related gene promoters. A sugar-induced WRKY-type TF, SUSIBA2 that is expressed in barley endosperm binds to the SURE and W-box, but not the SP8a element, to activate the barley *isoamylase1* (*iso1*) promoter (127). In addition, a novel DNA-binding protein, designated STOREKEEPER (STK), specifically recognizes the B-box motif to control sucrose-induced patatin expression in potato tubers (157). A more recent dissection analysis of the sugar/ABA-induced sweet potato *sporamin A* promoter in transgenic tobacco has yielded a minimal promoter (Spo^{min}) that contains negatively acting regions and two carbohydrate metabolite signal responsive elements (CMSRE), CMSRE-1 (TGGACGG) and CMSRE-2, in addition to the SP8a motif (92).

The most recent and fruitful studies of transciption control have been obtained by analyzing the sugar-inducible promoter of a sporamin gene that encodes the most abundant protein in sweet potato storage roots. Two putative TFs, WRI1 (activator of Spo^{min}::LUC1; ASML1) and a novel CCT-domain protein (ASML2) were isolated recently by enhancer activation-tagging of an *Arabidopsis* line carrying the LUC reporter under control of a short, minimal sugar/ABA-inducible *sporamin* promoter. Several sugar-regulated genes, including *βAMY* and, in the case of ASML2, *APL3*, are activated in the transgenic lines (87, 88). Both TF genes are also specifically induced by high sugar concentrations. Apparently, the WRI1 TF plays an important role in directing the carbon flow towards storage when sugar levels are high. The *hsi2* mutant displays high Spo^{min}::LUC1 reporter activity even in noninducing conditions and is deficient in a novel B3 domain transcriptional repressor (138).

Sugars also modulate hormone signaling at the transcriptional level. Most obviously, glucose induces *ABA* and *ABI* gene expression as a core mechanism of its signal transduction (4, 21). A detailed analysis of three factors involved in sugar signaling, ABI4, ABI5, and CTR1, documents their specific and

differential regulation by glucose, ABA, stress, and developmental stage (4). Glucose repression of several ethylene biosynthesis and signal transduction genes suggests that interactions between sugar and ethylene signaling take place in part at the transcriptional level (104). Transcriptional regulation of other hormone signaling components by sugars is also likely.

Studies with several maize photosynthetic gene promoters (119, 120) suggest the involvement of different regulatory elements in sugar repression and negative control of positive cis-elements. Extensive studies of sugar repression and starvation induction of transcription have also been carried out on the promoters of rice genes that encode α-amylases (*αAMY*), involved in seed starch degradation. In a study with a minimal *αAMY3* promoter, a sugar response sequence (SRS) was identified with three essential elements for high sugar starvation-induced expression: the GC-box, the G-box, and the TATCCA element. Interestingly, three novel MYB proteins with a single DNA-binding domain (OsMYBS1-3) specifically bind to the TATCCA element to regulate *αAMY* expression (86).

The identification of G-box cis-elements provides a link between nutrient stress and other environmental stress responses. The G-box motif (CACGTG) is, for example, involved in phytochrome-mediated light control of gene expression and is very similar to ABRE (CCACGTGG). The ABRE-binding factors ABF2, ABF3 and ABF4 have also been implicated in sugar signaling (64, 65). Analysis of a conserved minimal light-responsive module (CMA5) recently revealed an ABI4-dependent sugar and ABA repression mechanism involving a novel element conserved in several RBCS promoters (1). This S-box element (CACCTCCA) is an ABI4-binding site and is typically closely associated with the G-box in light-regulated promoters. Novel bioinformatics and experimental approaches will be required to use fully the large number of publicly available microarray data to uncover new regulatory elements and TF functions in sugar regulation.

Transcript Stability and Processing

The abundance of mRNA is not only the result of transcription control. Several important regulatory effects of sugars appear to operate at the post-transcriptional level. Sugar repression of rice *αAMY3* involves control of both transcription and mRNA stability. Specific sequences in the 3′ untranslated region (UTR) of the transcript can control sugar-dependent mRNA stability (17). Using the transcription blocker actinomycin D to study mRNA half-life, several other growth- and stress-related genes have been shown to be controlled by sugars at the level of mRNA stability (56). Expression of the maize CW-INV gene *Incw1* is also differentially regulated by sugars in a complex manner. In a maize cell suspension culture, both metabolizable and nonmetabolizable sugars induce *Incw1* expression. However, only the sucrose- or glucose-induced increase in steady state abundance of a smaller transcript (divergent in the 3′UTR) results in increased protein expression and enzyme activity (22). Although the exact mechanisms are not clear, the 3′UTR of the *Incw1* gene can be considered a sensor for sugar starvation that links sink metabolism to cellular mRNA processing and translation (22).

Translation

Another level of expression regulation controlled by stress and nutrient starvation conditions is selective mRNA translation. One such mechanism involves the presence of micro-open reading frames (μORFs) in the 5′UTR; these μORFs positively or negatively affect the translation efficiency of the downstream coding sequence (CDS)/ORF. For example, transcription of the *Arabidopsis* S-class bZIP TF ATB2/bZIP11 is stimulated by light and sugars, but its subsequent translation is repressed by higher levels of sucrose. Interestingly, specific sucrose-induced repression

of translation (SIRT) is dependent on the unusually long 5′UTR of the ATB2/bZIP11 transcript (114). Detailed analysis has now identified four μORFs in the 5′UTR, one of which (μORF2) is necessary and sufficient for translational regulation (147). Consistent with the differential expression regulation of ATB2/bZIP11 by sugars and its specific expression pattern in vascular tissues of fertilized ovules (funiculi), seedlings, and young vascular tissues, a regulatory role for ATB2/bZIP11 in resource allocation to newly established sinks has been proposed (114). The sucrose control μORF (SC-μORF) is also conserved in four other *Arabidopsis* and several more (often stress- and hormone-induced) mono- and dicot S-class bZIP TF UTRs. In at least one other case (AtbZIP2) the SC-μORF is essential for SIRT. This suggests that the use of μORFs is a general regulatory feature for a subset of plant bZIP TFs (147). The molecular details of this type of regulation and its exact physiological importance, however, are still unclear.

Protein Stability

Once a protein is synthesized, its activity can still be regulated in many ways. Recent bioinformatic analyses suggest that over 5% of the *Arabidopsis* proteome may be involved in ubiquitin-and 26S proteasome-dependent protein degradation. Consistently, protein stability and selective proteolysis have emerged as major regulatory mechanisms in plant signaling and development, rivaling transcription control and protein phosphorylation (122). It is not surprising that sugar signaling pathways also make use of these mechanisms. Consistent with the glucose oversensitive (*glo*) phenotype for the ethylene insensitive (*etr1*, *ein2* and *ein3*) mutants and the *gin* phenotype for constitutive ethylene signaling (*ctr1/gin4*) mutants (**Figure 3**), glucose antagonizes ethylene signaling by enhancing proteasome-dependent degradation of the key downstream transcriptional regulator EIN3 in the nucleus (152). Ethylene on the other hand, enhances EIN3 stability (152, 41). Interestingly, the glucose response is dependent on AtHXK1, which can also be found in the nucleus. Interactions with auxin and even cytokinin signaling could involve similar mechanisms. Two specific EIN3-binding F-box proteins, EBF1 and EBF2, that form SCF complexes to repress ethylene action and promote growth by directing EIN3 degradation, have been identified (41). The precise molecular link of these proteins with the sugar signaling pathway remains to be elucidated. Interestingly, EBF1 and EBF2 are most related to the yeast F-box protein Glucose repression resistant1 (Grr1), which has been implicated in controlling and possibly coupling sugar sensing and the cell cycle. Many key components in light, biotic and abiotic stress, and hormone responses, as well as developmental programs (such as flowering and senescence) and cell cycle control are indeed well-known targets for controlled proteolysis in plants (122). As in other eukaryotes, the half-life of many plant cyclin-dependent kinase (CDK) modulators is regulated by the proteasome (122). Interestingly, the D-type cyclin CYCD3;1, which is transcriptionally upregulated by sucrose or glucose and cytokinins to enable the G1/S transition (107), appears to be a highly unstable protein and is degraded by a proteasome-dependent mechanism upon sucrose depletion (102). Moreover, CYCD3;1 is phosphorylated in sugar starvation conditions, and a hyperphosphorylated form accumulates in the presence of a proteasome inhibitor. These observations suggest that phosphorylation is involved in targeting CYCD3;1 for destruction (102). Changes in the expression and the enzymatic properties of the 20S proteasome mediated by oxidation have been observed in sugar-starved maize roots (9).

SNF1-RELATED PROTEIN KINASES

A Large Superfamily of CDPK-SnRK

Protein phosphorylation and dephosphorylation are key regulatory mechanisms in

controlling protein function and activity. Experiments with specific inhibitors indicate the involvement of a variety of different PKs and protein phosphatases (PPs) in plant sugar signaling. Higher plants encode a particularly large superfamily of calcium-dependent PKs (CDPKs) and SnRKs. Several CDPKs are specifically induced by sucrose, and both pharmacological studies and observations of sugar-induced Ca^{2+}-fluxes have suggested the involvement of Ca^{2+} as a second messenger in sugar signaling.

The SnRK family consists of three subgroups, based on sequence similarity and domain structure. The SnRK1 proteins are most closely related to yeast Snf1 and mammalian AMPK (50). There are three members in *Arabidopsis*, only two of which, AKIN10 and AKIN11, are expressed (10; F. Rolland & E. Baena-Gonzalez, unpublished observations). The SnRK2 and SnRK3 (also termed CBL-interacting PK or CIPK) groups are probably unique to plants (50). SnRK1 homologs from various plant species can complement the yeast *snf1*Δ mutant phenotype, suggesting an evolutionary conservation in function. However, the best defined SnRK1 regulation and functions are mostly plant-specific and include activation by sucrose, phosphorylation of plant enzymes, and activation of starch synthesis in potato tubers (50). Possibly because of a key role in starch accumulation, SnRK1 silencing by DNA bombardment causes abnormal pollen development and male sterility in transgenic barley (155). Remarkably, significant differences appear to exist between the activation mechanisms for plant SnRKs, yeast Snf1, and mammalian AMPKs (50).

Modulation of Enzymatic Activity and Protein Degradation

Two SnRKs from spinach leaf can, in vitro, phosphorylate and inactivate 3-hydroxy-3-methylglutaryl-CoA reductase, nitrate reductase (NR), and SPS, enzymes involved in isoprenoid synthesis, nitrogen assimilation, and sucrose biosynthesis, respectively (126). The activation state of NR is associated with photosynthetic activity and sugar availability. This observation offers a mechanism for SnRKs to coordinate carbon and nitrogen metabolism. SnRK1s have overlapping substrate specificities with CDPKs, and detailed phosphorylation studies with synthetic peptides have defined the minimal recognition sequence and the differential effects of specific residues and their positions on activity and specificity (57). Phosphorylation by SnRKs or CDPKs is, however, not always sufficient for enzyme inactivation. Phosphorylation of NR creates a phosphopeptide motif for 14-3-3 protein binding. This motif is responsible for the actual reversible inhibition of enzyme activity under stress conditions. Several other metabolic enzymes have been shown to bind 14-3-3 proteins, including a TPS (90), and 14-3-3 proteins have been implicated in cell survival under stress conditions. Several key metabolic enzymes, like NR, have rather short half-lives and phosphorylation and 14-3-3 protein binding appears to be important in controlling protein degradation as well. However, there are contradictory results and interpretations as to the exact function of 14-3-3 protein binding in protein degradation (63). Although some evidence indicates that 14-3-3 protein binding initiates and/or accelerates NR degradation, selective loss of 14-3-3 protein binding appears to regulate cleavage of their binding partners, including NR and SPS, in sugar-starved *Arabidopsis* cells (27).

In addition to phosphorylation or dephosphorylation and protein stability or breakdown, redox regulation is emerging as another important post-translational mechanism in sugar control of plant metabolism. This mechanism is well known to be involved in reversible light-activation of key photosynthetic enzymes, but is now also found to regulate plastid enzymes in nongreen heterotrophic organs as well. Studies with potato tuber AGPase demonstrated that redox activation of the enzyme (by reducing a disulphide bond between two subunits of the

Redox regulation: signals generated by the photosynthetic electron transport chain, transmitted to thioredoxins, can modify target enzymes by disulphide bond reduction

tetrameric protein) regulates starch synthesis in response to sucrose import (135). Redox activation of AGPase is also observed after supplying exogenous sucrose to *Arabidopsis* leaves in the dark, or in a sugar accumulating *pgm* mutant (53). Two different signaling pathways have been proposed for sucrose and glucose, involving SnRK1 and HXK, respectively (42, 135). Glucose feeding, through HXK–dependent metabolism (e.g., the oxidative pentose-P cycle), increases the overall NADPH/$NADP^+$ ratio, which then most likely increases the reduction state of the plastid thioredoxins. Unlike glucose, sucrose activation of AGPase is strongly attenuated in SnRK1-antisense potato tubers and can be mimicked by the nonmetabolizable sucrose-analog, palatinose. Although the exact signaling mechanism is not clear, this phenomenon appears to be another sucrose-specific signaling effect.

Gene Expression Regulation

SnRK1, like Snf1 and AMPK, also affects gene expression. Antisense knockdown of SnRK1 in potato, for example, causes a significant reduction in *SUS4* gene expression in tubers and loss of *SUS4* sucrose-inducibility in leaves (106). This result is, however, not consistent with a role for SnRK1 in sugar starvation. It is proposed that SnRK1 can be activated by high cellular sucrose and/or low cellular glucose levels (50), although sucrose is hydrolyzed to glucose and fructose in plant cells. Expression of the wheat *αAMY2*, is induced by carbon starvation in cultured embryos, and SnRK1 antisense silencing represses transient *αAMY2* promoter activity (72). Although a prominent role for SnRK1 in plant metabolic signaling is now generally accepted, there are often seemingly conflicting results. Possibly, SnRK1 regulation and function differ depending on the cell or tissue type, developmental stage, and on the interactions with other signaling mechanisms.

Regulation of SnRK1

SnRK1 kinase activity is controlled by phosphorylation of a conserved threonine in the so-called activation or "T-loop" of the catalytic subunit. No upstream kinases or phosphatases have been identified in plants yet. Unlike AMPK, SnRK1 is not allosterically activated by AMP. However, T-loop dephosphorylation and consequent inactivation is inhibited by binding of low, physiological concentrations of 5′-AMP to SnRK1 (125). Also consistent with a role in sugar starvation conditions, sugar phosphates, especially G6P, can inhibit SnRK1 activity (136). Similar to the yeast Snf1 and mammalian AMPKs, SnRK1s are heterotrimeric proteins. The association of the catalytic α-subunits in complexes with different regulatory β- and γ-subunits, differentially regulated by hormonal and environmental signals, cell and tissue type, and developmental stage, offers another mechanism for complex and dynamic activity regulation and signal integration.

In plants, SnRK1s interact with several more proteins. Pleiotropic Regulatory Locus1 (PRL1) is a nuclear WD (Trp Asp) repeat protein that interacts with the *Arabidopsis* SnRK1s (10). The *prl1* mutant displays complex phenotypes, including transcriptional derepression of glucose-responsive genes but hypersensitivity to sugar and multiple hormones as well as hyperaccumulation of free sugars and starch. In a kinase assay using immunoprecipitated protein complexes from *Arabidopsis* and an SPS peptide substrate, both sucrose and the *prl1* mutation increased SnRK1 activity. These data again challenge the idea for a role of SnRK1 in sugar starvation conditions. An in vitro assay with purified proteins confirms that PRL1 indeed acts as a negative regulator of SnRK1. However, although low glucose levels enhance the yeast two-hybrid interaction with PRL1, the sucrose regulation of SnRK1 activity is unaffected in *prl1* mutant plants (10). Apparently, other factors or regulatory mechanisms are also involved.

Partly explaining the complex and pleiotropic phenotypes, and possibly providing a direct mechanistic link with metabolic regulation of protein degradation, the *Arabidopsis* SnRK1 is found to interact with both the SCF ubiquitin E3 ligase subunit SKP1/ASK1 and the SKP1/ASK1-binding 26S proteasome subunit α4/PAD1 (37). SKP1/ASK1 is also found in SCF complexes involved in the regulation of auxin and jasmonate signaling and senescence (122). In vitro, binding of SKP1/ASK1 to SnRK1 increases under low glucose conditions and competes with PRL1 binding to the same regulatory domain of SnRK1. In vivo, however, they do not seem to occur in common SnRK1 complexes (37). Further experiments confirm that SnRK1 associates with the 26S proteasome. The exact relevance of this interaction is not clear.

Diverse SnRK1-interacting proteins (SnIPs) have been identified using yeast two-hybrid screening. It remains to be learned whether these SnIPs are regulators or targets. A novel protein tyrosine phosphatase (PTP), dubbed PTPKIS, has been shown to interact with SnRK1 via a kinase interaction sequence (KIS) domain (39). The barley endosperm class I heat shock protein BHSP17 is a phosphorylation substrate of spinach leaf and barley endosperm SnRK1 (121), providing an obvious link with a general stress response. More specifically, the geminivirus proteins AL2 and L2 interact with and inactivate tobacco SnRK1. The metabolic alterations mediated by SnRK1 may be a component of the plant's antiviral defense mechanism (52).

In a screen for heterologous multicopy suppressors of the yeast *snf4* (Snf1 regulatory protein) deficiency, several proteins including a plant casein kinase I ortholog and two Msn2/4-type zinc-finger factors, AZF2 and ZAT10, involved in stress responses, were isolated in addition to the *Arabidopsis* Snf4 ortholog (66).

The moss *Physcomitrella patens* (*Pp*) is an excellent model system for functional genomics based on targeted gene knockouts. A moss *Ppsnf1a Ppsnf1b* double knockout mutant, which lacks all SnRK1 activity, displays abnormal development with premature senescence, hypersensitivity to auxin, and hyposensitivity to cytokinin. The mutant is unable to grow in low light or day/night light cycles, but the growth defect can be partially rescued by supplementation of an external carbon source, indicating that the moss SnRK1 is required for survival under low-energy conditions (129). The function of SnRK1 in legume seeds is also being characterized by gene silencing and microarrays (146). Recent analysis of *akin10 akin11* double knockout in *Arabidopsis* leaves has revealed a central role of SnRK1 as a master regulator in the stress and starvation signaling network (F. Rolland, E. Baena-Gonzalez & J. Sheen, unpublished observations). Although a conserved function for Snf1/AMPK/SnRK1 in eukaryotic nutrient stress signaling appears to be established, their regulation, downstream targets, and interactions with other pathways are likely more divergent. More research is needed to resolve the complex issues of SnRK1 regulation and functions in flowering plants.

TREHALOSE

It is often difficult to determine at which level sugar metabolism affects signal transduction (112). In plants, as in yeast and mammals, metabolic intermediates or alterations in cellular energy or redox state can also act as signals.

The ample examples of substrate and allosteric feedback and feed-forward regulation of carbon metabolism by metabolic intermediates, although important, are not a topic of this review. However, trehalose metabolism, a small side-branch of the major carbon flux in bacterial, yeast, and plant cells, has recently drawn a lot of attention because of its intriguing regulatory effects on plant growth, development, and stress resistance. The disaccharide trehalose is typically synthesized in a two-step reaction: T6P is first synthesized from G6P and UDP-Glc by TPS, and then

TPP: trehalose-6-P phosphatase

dephosphorylated to trehalose by a T6P phosphatase (TPP). T6P levels are tightly regulated in yeast by a complex of the TPS (Tps1) and TPP (Tps2) proteins, together with a regulatory protein (redundantly encoded by *TSL1* and *TPS3*). In *Arabidopsis*, addition of even fairly low amounts of external trehalose to the growth medium results in a significant inhibition of seedling root elongation (149). Although its specificity is not well defined, the potent trehalase inhibitor validamycin has been used to exclude possible effects of trehalose hydrolysis to glucose. The growth defect on external trehalose is associated with a strong induction of the AGPase gene *APL3*, increased AGPase activity, and concomitant hyperaccumulation of starch in the cotyledons. This suggests that a failing allocation of photosynthate to the roots is causing the growth defect (149). Consistent with that hypothesis, addition of metabolizable sugars can suppress the growth inhibition by trehalose.

In contrast to microorganisms, and with the exception of some desert resurrection plants like the pteridophyte *Selaginella lepidophylla* [in which the high trehalose concentrations (up to 15% of the dry weight) have been associated with extreme drought tolerance], plants in general do not accumulate trehalose at all. This can be explained partly by the high trehalase activity that is likely also involved in exogenous trehalose breakdown during symbiotic and pathogenic interactions with microorganisms. However, introduction of yeast and bacterial trehalose synthesis genes can improve abiotic stress resistance significantly, albeit without increasing endogenous trehalose concentrations to the extend found in microorganisms; thus, this result is inconsistent with a function for trehalose as a compatible solute/stress protectant (reviewed in Reference 99). In addition, whereas heterologous alteration of trehalose metabolism typically leads to clear morphological changes, regulated bacterial TPS-TPP coexpression or expression of a bacterial TPSP (TPS-TPP) fusion construct abolishes these morphological side effects (99). These observations all point to an important regulatory function.

Current attention has shifted to endogenous plant trehalose metabolism and its role in growth and development, and heterologous yeast *tps1*Δ mutant complementation has identified functional plant TPS genes Remarkably, *AtTPS1* is essential for embryo maturation. *Arabidopsis tps1* knockout mutants are developmentally arrested in the torpedo stage, a phase in embryo development that is generally associated with an increase in sucrose levels and initiation of storage reserve accumulation (35). It has been proposed that trehalose metabolism may be important for the regulation of storage reserve accumulation. However, using reserve protein promoter-reporters and transcriptomic, metabolite, and microscopic analyses, it is found that the actual cellular differentiation of the torpedo stage *tps1* embryos resembles that of a equally old, cotyledon-stage wild-type embryo. This observation indicates that morphogenesis (cell growth and division) is affected but uncoupled from differentiation (49). Expression of a bacterial TPS (OtsA) but not the addition of trehalose could rescue the embryo-lethal mutants (117), pointing to the importance of the T6P intermediate in development.

Controlled alterations of T6P levels by expressing combinations of *E. coli* trehalose metabolism genes clearly demonstrate that T6P is indispensable for carbohydrate utilization for growth in *Arabidopsis* (117). Moreover, dexamethasone-induced expression of TPS1 allows recovery of mature homozygous *tps1* mutant plants, showing that T6P is essential for both normal vegetative growth and the transition to flowering (141). Trehalose-mediated growth inhibition of seedlings is also likely due to T6P accumulation (118). The exact mechanism of T6P action, however, is still unclear, since, unlike in yeast, T6P is not an inhibitor of plant HXK activity (35).

Surprisingly, but consistent with an important regulatory role in growth and development, a plethora of trehalose metabolism

genes is now being uncovered in plants (77, 118, 143). There are four *TPS1* (class I *TPS*; *AtTPS1-4*) and seven TPS2 (class II *TPS* with two C-terminal phosphatase boxes; *AtTPS5-11*) homologs in *Arabidopsis*. No TPS or TPP activity has been detected yet for the class II proteins. Ten putative T6P phosphatase (*AtTPPA-AtTPPJ*) genes are annotated that basically contain only the phosphatase box domain. Although some have been shown to complement a yeast *tps2*Δ mutant, it is not clear how specific they are. Plants do not appear to have a TSL1-TPS3-like regulatory subunit, required for complex formation in yeast. Interestingly, expression of the trehalose metabolism genes is differentially regulated during embryo development and senescence and by nitrogen and sugar availability, hypoxia, circadian rhythm, ABA, and external trehalose. A microarray analysis of the plant's response to exogenous trehalose (118) has identified target genes mostly involved in stress signaling. *AKIN11* is also upregulated by trehalose, and its expression seems to correlate with T6P levels. Interestingly, T6P regulates starch synthesis via redox activation of AGPase downstream of SnRK1 (69). Several *Arabidopsis* TPS proteins possess multiple SnRK1 phosphorylation sites revealed by a recent study using a novel multiparallel kinase target assay (47). *AKIN10* overexpression also induces class II *TPS* gene expression (F. Rolland & E. Baena-Gonzalez, unpublished observations).

TPS and *TPP* expression occurs in a wide range of tissues. Remarkably, the *Arabidopsis* class II *TPS* genes show a cell layer-specific expression in root and shoot apical meristems (M. Ramon, personal communication). This suggests a prominent role in growth regulation. It remains unclear how trehalose metabolism affects growth and development and stress resistance. As in yeast, trehalose metabolism likely interferes with sugar signaling in plants. *Arabidopsis* plants overexpressing the yeast *TPS1* are drought tolerant and insensitive to sugar and ABA, suggesting a role for *TPS1* or its product in downregulating HXK-dependent signaling (6). In addition to trehalose, some plant and bacterial species accumulate fructose oligomers and polymers, called fructans, as a reserve carbohydrate that can enhance plant cold and drought tolerance. The occurence of fructan exohydrolases (FEHs) in non-fructan-accumulating plants such as *Arabidopsis* similarly suggests a defense-related role for these enzymes (by acting on bacterial fructans) or the presence of undetected low amounts of endogenous fructans with a role as signaling molecules (140).

CONCLUSIONS AND PERSPECTIVES

Sugars are finally being recognized as important regulatory molecules with signaling functions in plants and other organisms. Whereas the power of yeast genetics has enabled the rapid and detailed elucidation of diverse sugar sensing and signaling pathways, plant sugar signaling has proven more difficult to study due to the complexity of source-sink interactions, responses to diverse sugar signals and metabolites, and the intimate integration of a web-like signaling network governed by plant hormones, nutrients, and environmental conditions. The use of different experimental systems, including isolated cells, excised tissues, cell cultures, whole plants, and mutants under different environmental and nutrient conditions at various developmental stages is critical in dissecting the plethora of sugar responses and their connections in plants. Microarray and clustering analysis are new, powerful genomic tools to provide a global view on the transcript dynamics controlled by different sugar responses and identify novel regulatory components and target genes. The sharing of the massive data sets is beginning to provide new insights into the extent and mechanisms of sugar-regulated gene expression and interactions with other signals. The molecular details of signal transduction pathways and their crosstalk with other pathways will be revealed by using a combination of genomic proteomic

and genetic approaches. Current technology limits the ability to visualize and quantify the precise location and concentration of various sugar molecules and metabolites in living cells. Novel molecular sensors and fluorescence resonance energy transfer (FRET)-based imaging (81) will hopefully circumvent this limitation and provide critical information to facilitate the elucidation of intracellular sugar signal transduction pathways.

SUMMARY POINTS

1. Yeast is an excellent model and tool to study the conserved mechanisms of eukaryotic sugar sensing and signaling.
2. In plants, sugars control metabolism, growth, stress responses, and development from embryogenesis to senescence.
3. Plant sugar regulation is mediated by diverse sugar signals, which are generated at different locations depending on environmental conditions and developmental stage. Sucrose transport and hydrolysis play key regulatory roles in sugar signal generation.
4. Plant-specific sugar signaling mechanisms involve extensive interactions with plant hormone signaling.
5. HXKs are evolutionarily conserved eukaryotic glucose sensors. Plants may also use membrane receptors for extracellular sugar sensing.
6. Sugars regulate cellular activity at mutiple levels, from transcription and translation to protein stability and activity.
7. SnRK1s appear to play a conserved role in starvation responses, but are likely regulated differently in yeast, mammals and plants. Future studies will clarify the unique regulation of SnRK1s by sucrose and their critical role in cellular stress signaling, as well as novel functions in the regulation of the daily cycle of carbon metabolism in plants.
8. Trehalose metabolism is emerging as a novel, important regulator of plant growth, metabolism, and stress resistance.

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This study uncovered an intriguing novel mechanism of sucrose-specific translation control, involving a conserved upstream open reading frame.

This paper describes the molecular link between glucose and ethylene signaling and the remarkable observation that plant HXK1 can translocate to the nucleus.

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