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Authors: Ramon, Matthew, Rolland, Filip, and Sheen, Jen

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Sugar Sensing and Signaling

Matthew Ramon,[a] Filip Rolland,[b] and Jen Sheen[a,1]

[a] Department of Molecular Biology, Massachusetts General Hospital, Department of Genetics, Harvard Medical School, Boston, Massachusetts 02114
[b] Department of Biology, Institute of Botany and Microbiology, K.U. Leuven, Kasteelpark Arenberg 31, 3001, Heverlee, Belgium
[1] Address correspondence to sheen@molbio.mgh.harvard.edu

Plants, restricted by their environment, need to integrate a wide variety of stimuli with their metabolic activity, growth and development. Sugars, generated by photosynthetic carbon fixation, are central in coordinating metabolic fluxes in response to the changing environment and in providing cells and tissues with the necessary energy for continued growth and survival. A complex network of metabolic and hormone signaling pathways are intimately linked to diverse sugar responses. A combination of genetic, cellular and systems analyses have uncovered nuclear HXK1 (hexokinase1) as a pivotal and conserved glucose sensor, directly mediating transcription regulation, while the KIN10/11 energy sensor protein kinases function as master regulators of transcription networks under sugar and energy deprivation conditions. The involvement of disaccharide signals in the regulation of specific cellular processes and the potential role of cell surface receptors in mediating sugar signals add to the complexity. This chapter gives an overview of our current insight in the sugar sensing and signaling network and describes some of the molecular mechanisms involved.

INTRODUCTION

Since their appearance, plants have played a crucial role in the evolution of life on earth through the production of energy-rich sugar molecules and oxygen by photosynthetic carbon fixation. Sugars are the prime carbon and energy source to build and fuel cells, and early in evolution also acquired important regulatory functions in controlling metabolism, stress resistance, growth and development. For free-living microorganisms, nutrient availability is a major factor controlling growth in a constantly changing environment, while multi-cellular organisms need to maintain nutrient and energy homeostasis within cells and tissues. Sugars can complement and interact with a variety of hormone and growth factor signaling mechanisms to modulate metabolism and growth in complex systems. In sugar-producing and sessile plants, monitoring the sugar and energy status is particularly critical, enabling the integration of metabolic, environmental and developmental cues required for their physiological and developmental plasticity. Unlike hormones and growth factors, however, sugars are present in the millimolar range and actively participate in metabolism, making the investigation of their dual functions in metabolism and signaling very challenging. Still, in recent years, the pivotal role of sugars in plant growth and development and key players in the sugar signaling network have been uncovered using Arabidopsis as the prime model system. Several comprehensive reviews have been published on the subject (Koch, 1996; Roitsch, 1999; Yu, 1999; Smeekens, 2000; Rolland et al., 2002; Leon and Sheen, 2003; Gibson, 2005; Rolland et al., 2006; Smith and Stitt, 2007). This chapter presents a primer of this rapidly evolving field, with an integrated view on the plant sugar signaling network, and an update on the most recent literature.

GENERATING SUGAR SIGNALS

As photoautotrophic organisms, plants generate their own sugars through the process of photosynthesis. During the day, photosynthetic source tissue converts CO_2 and water to carbohydrates and oxygen, using sunlight as an energy source. Carbohydrates, generated in the chloroplasts, are then exported to the cytosol, mainly as triose-phosphates, where they can be converted to hexose-phosphates or sucrose for local use or storage in the vacuole (Fig. 1). In addition, sucrose is transported to non-photosynthetic sink tissues. There, sucrose is taken up and converted to different hexoses by invertases and sucrose synthases or stored in vacuoles and in amyloplasts as starch for longer term storage (Rolland et al., 2006). To ensure a continuous supply of sugars, chloroplasts also store excess photosynthate as transitory starch during the day to be remobilized and exported as maltose and glucose during the subsequent night (Fig. 1) (Smith and Stitt, 2007). Thus, a variety of sugars are produced by plant metabolism at different times and locations. In order to coordinate all these processes and respond appropriately to changes in the environment (affecting energy metabolism) and altering metabolic and energy demands during development, plants have developed an array of mechanisms to specifically sense diverse 'sugar signals'. An efficient communication between activities in different organs, tissues and subcellular compartments is crucial for an optimal use of the available resources for growth and development.

CARBON REGULATION OF SOURCE-SINK STATUS

Interactions between source tissues (producing and exporting sugars, e.g. rosette leaves) and sink tissues (importing sugars, e.g.

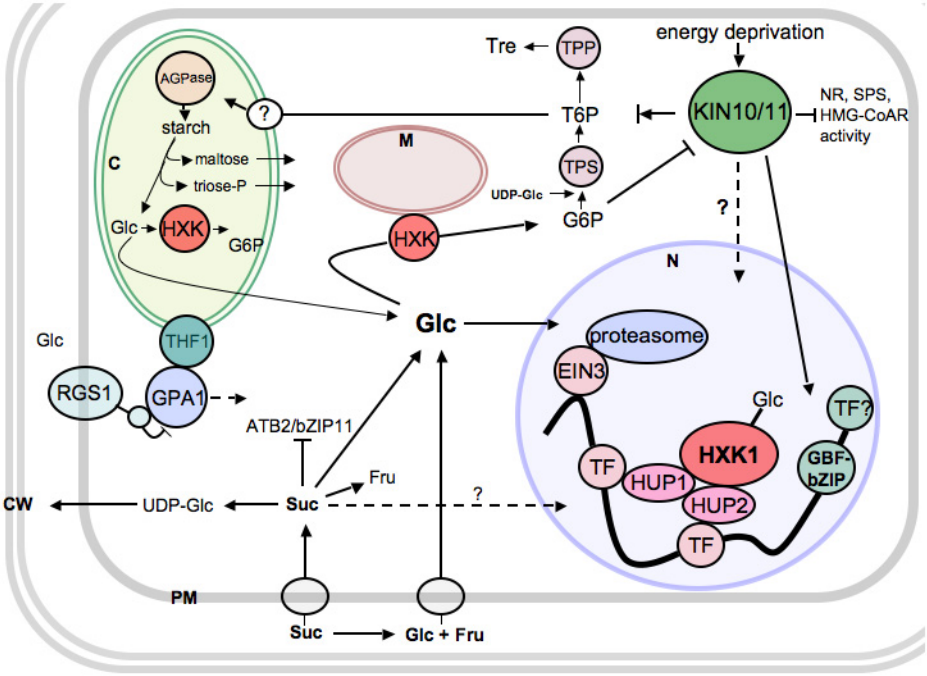

Figure 1. Summary of sugar and energy sensing and signaling models in Arabidopsis.

Glc and Fru can be transported into the cell by hexose transporters or mobilized from starch and cytosolic and vacuolar Suc. Glc then enters metabolism after phosphorylation by HXK. HXK1 is mainly associated with mitochondria and a specific isoform is also found in plastids. In addition, HXK1 is present in high-molecular-weight complexes with HXK unconventional partners (HUPs) and transcription factors (TFs) in the nucleus where it controls transcription and modulates proteasome-mediated degradation of the EIN3 TF. KIN10/11 play a key role in plant energy signaling, mediating massive reprogramming of transcription (in part through bZIP TFs) and controlling enzymes post-translationally. Sugar phosphates (especially G6P) inhibit KIN10/11 activity. An important regulatory role is emerging for trehalose (Tre) metabolism, mediated by Tre-6-P (T6P) synthase (TPS) and T6P phosphatase (TPP) enzymes. T6P has been proposed to be a regulatory signaling molecule. KIN10/11 control the expression and phosphorylation status of several of the class II TPS proteins with unknown activity and function. G-protein coupled receptor signaling by RGS1 and GPA1 has been implicated in sensing extracellular glucose and signaling through THF1, located in the plastids. Sucrose, the main transported sugar in Arabidopsis, is found to have specific effects, not triggered by its hydrolysis products Glc and Fru. The ATB2/bZIP11 TF is subject to sucrose-induced repression of translation. C: chloroplast; CW: cell wall; M: mitochondrion; N: nucleus; PM: plasma membrane; NR, nitrate reductase; SPS, sucrose phosphate synthase; HMG-CoAR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase.

roots) are an important regulatory mechanism in plants (Roitsch, 1999). Feedback inhibition of photosynthetic carbon assimilation as a result of decreased sink demand or nitrogen limitation has been investigated extensively and different experimental approaches have established the pivotal role of sugars in the repression of photosynthetic gene expression (Sheen, 1994). Conversely, sugar depletion results in the activation of photosynthetic gene expression and increases photosynthetic capacity (Krapp et al., 1993).

Invertases, converting sucrose to glucose and fructose, are key players in the regulation of carbon allocation and different isoforms in distinct subcellular compartments are differentially controlled by sugar signals (Ehness et al., 1997; Sturm and Tang, 1999; Andersen et al., 2002; Koch, 2004; Roitsch and Gonzalez, 2004). Stress and hormones play important roles in fine-tuning sink-source interactions. For example, invertase expression can be induced by microbial elicitors and the growth stimulating cytokinin and brassinosteroid hormones (Ehness and Roitsch, 1997; Roitsch and Gonzalez, 2004). In contrast, ethylene represses the expression of extracellular invertase (Linden, 1996).

When assimilates are generated in excess of sink demand by high rates of photosynthesis, sugar accumulation in leaves has direct effects on ADP-glucose pyrophosphorylase (AGP) activity, the first committed step in starch synthesis, and other carbohydrate-responsive enzymes of sucrose and starch metabolism (Koch, 1996; Gibon et al., 2004). For instance, sucrose can induce AGP large subunit (APL3) gene expression (Rook et al., 2001) and redox-dependent activaton of AGP in Arabidopsis rosette leaves (Hendriks et al., 2003; Lunn et al., 2006). During the night, reduced leaf sucrose content can trigger starch degradation (Paul and Foyer, 2001; Sharkey et al., 2004; Smith and Stitt, 2007). In addition, sucrose feeding in sugar beet leaves represses the expression of a sucrose symporter gene in the phloem companion cells, which may provide a regulatory mechanism in the coordination between source photosynthetic activity and sink utilization of photoassimilate (Chiou and Bush, 1998; Vaughn et al., 2002). These examples illustrate the enormous complexity of source-sink regulation, the generation and sequestration or storage of sugars and sugar signals, and the delicate balance required for optimal growth.

SUGAR SENSING

HXK1 as a Glucose Sensor

Glucose is a universal carbon and energy source, by far preferred by organisms ranging from unicellular microbes to plants and animals. While sucrose is the most important transport sugar in plants, most regulatory effects can be ascribed to glucose. In many organisms, HXKs and other sugar kinases are the most ancient, conserved sugar sensors (Johnston, 1999; Stulke and Hillen, 1999; Rolland et al., 2001; Rolland et al., 2006; Claeyssen and Rivoal, 2007). A regulatory role for plant HXKs in plant sugar sensing was first suggested by the marked effects of various sugars on photosynthetic and glyoxylate cycle gene expression in *Chenopodium* and cucumber cell cultures (Krapp et al., 1993; Graham et al., 1994) and maize mesophyll protoplasts (Sheen, 1990). The reduction in photosynthesis related gene expression by low amounts of glucose (1-10 mM) but not glucose-6-phosphate (G6P) in a maize protoplast transient expression system, supported a signaling role for HXK (Jang and Sheen, 1994). Other substrates for HXK phosphorylation, like fructose, mannose and 2-deoxyglucose (2-dGlc) also trigger repression. Moreover, the observed repression can be blocked by the HXK-specific competitive inhibitor mannoheptulose (Jang and Sheen, 1994). Conversely, sugars that cannot be transported into the cell (L-glucose), or that are taken up but cannot be phosphorylated by HXK (6-deoxyglucose and 3-O-methylglucose), as well as intermediates of glycolysis and sugar phosphates (delivered into the protoplasts by electroporation), do not cause the same repression (Jang and Sheen, 1994). The possibility that depletion of Pi and ATP caused the reduced gene expression is excluded by the inability of P_i and ATP to relieve the glucose repression (Graham et al., 1994; Jang and Sheen, 1994).

The hypothesis that HXK acts as a glucose sensor was further substantiated by the characterization of transgenic Arabidopsis *HXK* sense and antisense lines (Jang et al., 1997). Wild type Arabidopsis seedlings grown under continuous light on MS medium containing 6% (approximately 333 mM) glucose exhibit developmental arrest with repression of cotyledon greening and expansion, leaf development, and hypocotyl and root elongation. Plants carrying the antisense construct of *HXK1* are hyposensitive to glucose repression, while plants overexpressing *HXK1* are hypersensitive (Fig. 2). No apparent differences are observed with the same concentration of mannitol or 3-O-methylglucose as osmotic controls (Jang et al., 1997). Further evidence for HXK1 as a sugar sensor is provided by the isolation of two *gin2* (*glucose insensitive 2*) mutants mapped to the *HXK1* gene (Moore et al., 2003). Importantly, two catalytically inactive HXK1 alleles (G104D and S177A) can mediate developmental arrest on 6% glucose and glucose repression of chlorophyll accumulation and photosynthetic gene expression in the *gin2* mutant background. These results unambiguously show that HXK1 is a true glucose sensor in plants and that glucose signaling can be uncoupled from glucose metabolism (Moore et al., 2003).

The *gin2* mutants provide a valuable tool to investigate the physiological importance of glucose sensing and signaling during normal plant growth, without the manipulation of exogenous sugars. In wild type plants, high light conditions (200-300 µE) can boost photosynthesis and sugar production, leading to accelerated growth with earlier leaf senescence. However, *gin2* plants remain small and dark green and show little cell expansion, supporting the role of HXK1 in promoting cell expansion and growth in roots, leaves and inflorescences under enhanced light conditions (Moore et al., 2003). In transgenic tomato plants, displaying higher sugar sensitivity than Arabidopsis plants, *HXK1* overexpression inhibits growth and chlorophyll accumulation and promotes senescence (Dai et al., 1999; Granot, 2007). Thus, HXK1-mediated signaling can promote or repress growth, depending on the intrinsic glucose levels and glucose sensitivity of the plants.

In the Arabidopsis genome, six HXK and HXK-like (HKL) genes can be found, serving a variety of physiological functions and likely controlled by tissue-specific expression patterns, subcellular localization and protein complex formation (Claeyssen and Rivoal, 2007; Granot, 2007; Karve et al., 2008). Proteomic and GFP fusion analyses indicate that several plant HXKs are located on the outer mitochondrial membrane, where a completely functional glycolytic metabolon can be found (Giege et al., 2003; Kim et al., 2006; Balasubramanian et al., 2007; Damari-Weissler et al., 2007; Granot, 2007; Karve et al., 2008). In addition, plant HXKs are found in plastids (Wiese et al., 1999; Olsson et al., 2003; Giese et al., 2005; Cho et al., 2006a; Kandel-Kfir et al., 2006; Granot, 2007). Unique in moss, the chloroplast stromal HXK accounts for 80% of the total hexose kinase activity and is responsible for glucose-mediated growth and formation of caulonemal filaments (Olsson et al., 2003). Several recent papers also report diverse functions for mitochondrial HXKs in processes ranging from programmed cell death to actin filament reorganization and glucose-mediated gene expression (Kim et al., 2006; Balasubramanian et al., 2007; Damari-Weissler et al., 2007). It will be interesting to determine how plant HXKs integrate glycolysis and cell death without the proapoptotic protein, associated with the mitochondrial glucokinase in mammalian cells (Danial et al., 2003). The functional significance of HXK interactions with porin, actin and voltage-dependent anion channels deserves further investigation (Kim et al., 2006; Balasubramanian et al., 2007).

HXK1 is also found in high molecular weight complexes in the nucleus (Cho et al., 2006b). Apparently, HXK1 regulates transcription by direct binding to the promoter of glucose-repressed genes.

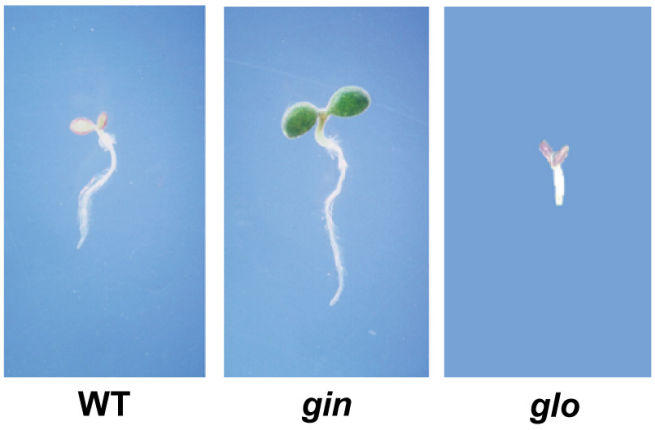

Figure 2. Arabidopsis WT, *glucose insensitive* (*gin*) and *glucose oversensitive* (*glo*) seedling growth phenotypes on MS medium containing high (6%) exogenous glucose levels. Wild type seedlings are affected in cotyledon greening and expansion and show reduced hypocotyl and root elongation.

Chromatin immunoprecipitation experiments with HXK1 show that this complex specifically binds to *cis*-regulatory elements upstream of the *CAB2* (chlorophyll a/b binding protein 2) and *CAB3* coding regions. This nuclear activity requires two HXK1 unconventional partners, HUP1 and HUP2, components shown to be part of the plant vacuolar H^+-ATPase (VHA-B1) and the 19S regulatory particle (RPT5B) of the proteasome complexes, respectively (Sze et al., 2002; Yang et al., 2004a; Cho et al., 2006b). Interestingly, both interaction partners have closely related homologues that do not interact with HXK1 in Arabidopsis (Cho et al., 2006b). In addition, *vha-b1* and *rpt5b* mutants display similar seedling and adult plant phenotypes as observed for *gin2* plants (Moore et al., 2003), confirming the vital role of their interaction with HXK1 in glucose signaling (Cho et al., 2006b)(Fig. 1).

The HXK-Independent Pathway

Analysis of sugar-regulated gene expression and growth of wild type, *35S-HXK1* and *35S-antiHXK1*, and *35S-ScHXK2* (yeast *Saccharomyces cerevisiae HXK2*) Arabidopsis plants confirms the existence of both HXK1-dependent and -independent signaling pathways (Xiao et al., 2000). Interestingly, the effect of glucose on the expression of genes encoding AGP, CHS (chalcone synthase), PAL (phenylalanine ammonia-lyase) and ASN (asparagine synthetase) is similar in wild type plants and transgenics, indicating that this effect is independent of HXK1 (Xiao et al., 2000). Furthermore, seed germination can be inhibited by glucose in concentrations below those known to inhibit early seedling development. This inhibition operates on imbibed seeds and is independent of HXK function (Dekkers et al., 2004). Interestingly, the HXK-dependent and HXK1-independent pathways can cooperate in glucose regulation, acting at different levels. For example, glucose repression of the grape monosaccharide transporter *VvHT1* expression is dependent on HXK1, while its post-transcriptional glucose regulation is not (Conde et al., 2006).

Since sugars are extensively metabolized, downstream metabolites are also potential signaling molecules. Glucose induction of two pathogenesis-related genes (*PR1* and *PR2*) is higher in plants overexpressing *HXK1* but absent in *35S-antiHXK1* plants, consistent with the requirement of HXK for signaling. However, this induction is enhanced both in plants overexpressing *HXK1* and plants overexpressing yeast Sc*HXK2*, suggesting that the induction of *PR1* and *PR2* is dependent on HXK catalytic activity rather than on its regulatory function. This indicates that the regulatory signal is possibly generated by downstream metabolic intermediates (Xiao et al., 2000). Genome-wide expression analyses with wild-type and *gin2* mutant plants will reveal more genes regulated by HXK1-dependent and HXK1-independent sugar signaling under various growth conditions.

Disaccharide Sensing

Sucrose is the main transported sugar in plants. Although sucrose can be substituted by the hexose products in many cases of sugar effects, a number of studies have provided compelling evidence for sucrose-specific regulation in gene expression and growth. Sucrose-induced expression has been reported for genes encoding UDP-glucose pyrophosphorylase, anthocyanin biosynthesis enzymes, and a putative chloroplast glucose-6-phosphate/phosphate translocator (Wenzler et al., 1989; Yokoyama et al., 1994; Ciereszko et al., 2001; Teng et al., 2005; Gonzali et al., 2006; Solfanelli et al., 2006). Conversely, sucrose-specific repression has been shown for the transcription of a proton-sucrose symporter gene in sugar beet (Chiou and Bush, 1998; Vaughn et al., 2002) and for carrot somatic embryo radicle elongation (Yang et al., 2004b). Also, the marked effects on leaf shape, caused by the overexpression of the homeodomain leucine zipper transcription factor gene *ATHB13*, are specifically induced by sucrose (Hanson et al., 2001).

Specific disaccharide signaling, independent of metabolism, is also demonstrated using non-metabolizable sucrose analogs, such as palatinose and turanose. These analogs affect the expression of α-amylase in barley embryos and AGP in potato (Loreti et al., 2000; Tiessen et al., 2003), and induce starch synthesis and sucrose degradation in potato tubers (Fernie et al., 2001). However, they may activate novel pathways different from sucrose-signaling as they could be perceived as a more general stress-related signal rather than a specific non-metabolizable sucrose substitute (Sinha et al., 2002; Roitsch et al., 2003).

Another line of fascinating research suggests that sucrose regulates the translation of the ATB2 protein. The *ATB2* gene encodes a group S basic region leucine zipper transcription factor (bZIP11), characterized by an unusually long 5' untranslated region (UTR). Point mutations and deletions in this 5' UTR have shown that a highly conserved small upstream open reading frame (uORF) encoding 42 amino acids is responsible for sucrose induced repression of translation. This uORF is also conserved in the 5' UTRs of other S-class bZIP transcription factors of Arabidopsis and many other plant species. Expression of a 5' UTR-GUS (β-glucuronidase) fusion construct throughout the whole plant, indicates that this sucrose regulation mechanism is functional in all plant tissues (Rook et al., 1998; Wiese et al., 2004, 2005). To demonstrate the specificity of sucrose in its repression mechanism, several other sugars, such as glucose, fructose, galactose, maltose and raffinose, were tested, but none induces repression of translation to the same extend as sucrose. Intriguingly, *ATB2* transcription is induced both by sucrose and glucose (Wiese et al., 2004), providing a mechanism of rigorous fine-tuning of ATB2 protein levels in response to sucrose (Fig. 1). Sucrose may also act as a signal, controlling specific developmental processes not affected by hexose sugars. In *Vicia faba* embryos, high hexose levels are associated with active division during early embryo development, while sucrose accumulation is correlated with the switch from active cell division to cell expansion, differentiation and reserve (starch) accumulation (Weber et al., 1996). It will be important to uncover how the sucrose signal is perceived and transduced to regulate the diverse responses.

Cell Surface Receptors

In yeast and mammals, extracellular sucrose and glucose can be perceived by G-protein-coupled-receptors (GPCRs) linked to heterotrimeric guanine nucleotide-binding proteins (G-proteins) (e.g. Lemaire et al., 2004; Chandrashekar et al., 2006). In such

a system, the extracellular signal molecule (ligand) specifically binds to the GPCR, resulting in a conformational change that stimulates the exchange of GTP for GDP at the guanine-nucleotide binding site on the interacting G-protein. This initiates intracellular signaling by dissociation of the α- and the β/γ-subunits and their interaction with a variety of downstream effectors. G-proteins act as molecular switches and intrinsic GTPase activity catalyses the return to the inactive heterotrimer. In many cases, GTPase activity and desensitization is accelerated by a new class of regulators of G-protein signaling (RGS) proteins (Srinivasa et al., 1998).

One Arabidopsis gene encodes an unusual hybrid seven-transmembrane domain protein with a C-terminal RGS domain, RGS1 (Chen et al., 2003). The *rgs1* mutants are insensitive to 6% glucose, while overexpressors are hypersensitive. Growth assays with different sugars and sugar analogs suggested that RGS1 may function as a glucose sensor, independently of HXK (Chen and Jones, 2004)(Fig. 1). Glucose appears to alter the interaction of RGS1 with GPA1 (the only Gα subunit in Arabidopsis) *in vivo*, leading to an increased GTP hydrolysis activity of GPA1 and sugar-specific signaling events (Johnston et al., 2007). In addition, Arabidopsis *gpa1* mutants are hypersensitive to inhibition of germination by sugar and ABA, confirming their potential role in sugar signaling (Ullah et al., 2002; Pandey et al., 2006). Interestingly, THF1 (THYLAKOID FORMATION1), a protein found in the outer membrane and stroma of plastids, interacts with GPA1 in root hair cells when plasma membrane and plastid membrane are in close proximity (Huang et al., 2006). The *thf1* mutant seedlings show altered sensitivity to glucose and THF1 protein levels are rapidly degraded by glucose, suggesting a role for this protein in the sugar response (Huang et al., 2006)(Fig. 1).

Besides GPA1, three Arabidopsis genes encode one Gβ (AGB1) and two Gγ subunits (AGG1 and AGG2)(Weiss et al., 1994; Mason and Botella, 2000; Jones and Assmann, 2004). A loss of function mutation in the Gβ subunit (*agb1*) affects multiple processes, including hypersensitivity to glucose. Although the *gpa1* and *agb1* mutants often have opposite phenotypes, they seem to share the same function in glucose signaling (Ullah et al., 2003; Wang et al., 2006; Temple and Jones, 2007). Interestingly, a mutation in a Golgi-localized hexose transporter (*sgb1, suppressor of G beta1*) can reverse glucose hypersensitivity in the *agb1* mutants (Wang et al., 2006). In addition, *agg1* and *agg2* mutants are also hypersensitive to glucose (Trusov et al., 2007). As *agg1* mutants are sensitive to mannitol and more susceptible to infection, these proteins are likely involved in different cellular processes (Trusov et al., 2007).

In budding yeast, novel hexose transporter-like genes, *SNF3* and *RGT2*, encode extracellular glucose sensors that are responsible for the regulation of functional hexose transporter gene expression. These catalytically inactive transporters possess a long cytoplasmic domain for interaction with downstream signaling components (Johnston, 1999; Johnston and Kim, 2005). The plant disaccharide transporter SUT2, containing a similar extended cytoplasmic N-terminal and central loop domain, has been proposed to be a putative sucrose sensor (Lalonde et al., 1999; Barker et al., 2000). In addition, some monosaccharide transporters also have extended cytoplasmic loops (Kleinow et al., 2000). Further research is required to demonstrate that these putative receptors are actively involved in sugar sensing.

SUGAR SIGNALING MECHANISMS

After sugars have been detected by sensors at the plasma membrane or a specific intracellular location, the information is passed on through signal transduction and amplifying cascades, ultimately resulting in the appropriate responses. These responses vary from changes in gene expression to altered enzyme activities through the activity of protein kinases, protein phosphatases and other signal transduction mediators such as Ca^{2+} and calmodulin. The characterization of different sugar-signaling mutants isolated in diverse screens has also revealed extensive interactions with hormone biosynthesis and signaling pathways. An overview of the different screens used to select sugar-response mutants is given in Table I. More detailed mutant information is also organized and presented in Table II and Table III.

Sugar-Signaling Mutants

Two major strategies have been used to screen for sugar signaling mutants in Arabidopsis. A simple approach is based on the effect of high levels of external sugars on seedling growth and development. For example, *gin* (*glucose insensitive*) mutants develop normally on MS medium supplemented with 6% (approximately 333 mM) glucose (Zhou et al., 1998), while *sis* (*sugar insensitive*) and *sig* (*sucrose insensitive growth*) mutants are selected on media containing 300 mM and 350 mM sucrose, respectively (Laby et al., 2000; Pego et al., 2000)(Fig. 2). Conversely, *glo* (*glucose oversensitive*), *gss* (*glucose-supersensitive*), and *sss* (*sucrose-supersensitive*) mutants show developmental arrest in the presence of otherwise non-inhibiting sugar concentrations (Table I) (Smeekens, 2000; Rolland et al., 2002; Leon and Sheen, 2003; Gibson, 2005). Remarkably, many mutants isolated in the different screens are allelic to mutants in hormone biosynthesis and signaling. Since these screens are done with seedlings, the information on sugar-hormone interactions in the signaling networks is necessarily limited to the early developmental stages.

To ensure uniform sugar responses, the initial mutant isolation screens were mostly performed on media containing high glucose or sucrose concentrations, raising concerns about the physiological relevance of sugar regulation. Moreover, the phenotypes could also be influenced by osmotic stress and other nutrients in the media. The analyses of wild-type and *gin2* seedlings show that the presence of high nitrate concentrations (40 mM nitrate) in full MS medium can antagonize the glucose effects. When the nitrate effect can be minimized, the sugar effect can be observed at more physiological concentrations (Martin et al., 2002; Moore et al., 2003; Cho et al., 2006b). Indeed, 2% (110 mM) glucose without MS medium is sufficient to cause glucose repression of *RBCS*, *CAB* and *CAA* (carbonic anhydrase) gene expression and chlorophyll accumulation in whole seedlings (Moore et al., 2003; Cho et al., 2006b). For HXK1-mediated growth promotion in seedlings, 0.2% (11 mM) glucose in a 0.1x MS medium is sufficient to provide the glucose signal requirement *in vivo* (Moore et al., 2003; Cho et al., 2006b). In isolated maize and Arabidopsis mesophyll protoplasts, concentrations between 1 to 20 mM are sufficient to cause specific glucose but not sucrose repression in various assays (Jang and Sheen, 1994; Moore et al., 2003; Yanagisawa et al., 2003). Recent microarray studies show that 15 mM sucrose can

Table I. Sugar-signaling mutants isolated with sugar-specific screens

Name	Screen and conditions	Reference
Sugar insensitive		
cai (carbohydrate insensitive)	Growth on low nitrogen, 3.4% (100 mM) Suc	(Boxall et al., 1997)
gin (glucose insensitive)	Growth on 6% (333 mM) Glc	(Zhou et al., 1998)
glz2 (gaolaozhuangren2)	Insensitivity to 6% (333 mM)	Glc (Chen et al., 2004)
isi (impaired sugar induction)	Reduced *APL3::P$_{450}$* expression on 3.4% (100 mM) Suc	(Rook et al., 2001)
lba (low levels of β-amylase)	Low β-amylase activity on 6% (175 mM) Suc	(Mita et al., 1997b)
mig (mannose-insensitive germination)	Germination on 0.14% (7.5 mM) mannose	(Pego et al., 1999)
rsr (reduced sugar response)	*Pat(B33)-GUS* expression on 3% (90 mM) Suc	(Martin et al., 1997)
sig (sucrose insensitive growth)	Growth on 12% (350 mM) Suc	(Pego et al., 2000)
sis (sugar insensitive)	Growth on 10% (300 mM) Suc or 5.4% Glc (300 mM)	(Laby et al., 2000)
sun (sucrose uncoupled)	Expression of PC-LUC on 3% (88 mM) Suc	(Dijkwel et al., 1997)
Sugar hypersensitive		
core (conditional root expansion)	Short, expanded roots on 4.5% (132 mM) Suc	(Hauser et al., 1995)
fus (fusca)	Growth arrest on 3% (88 mM) Suc	(Castle and Meinke, 1994)
glo (glucose oversensitive)	Growth arrest on 4% (222 mM) Glc	(Sheen et al., 1999)
gss (glucose supersensitive)	Growth arrest on 1% (56 mM) Glc	(Pego et al., 2000)
hba (high level of β-amylase)	High-level β-amylase on 6% (175 mM) Suc	(Mita et al., 1997a)
hsr (high sugar-response)	Enhanced *ApL3::LUC* expression on 1% (30 mM) Suc	(Baier et al., 2004)
osu (oversensitive to sugar)	Increased anthocyanin accumulation and reduced root length on imbalanced C:N ratios	(Gao et al., 2008)
sss (sucrose supersensitive)	Growth arrest on 12% (350 mM) Suc	(Pego et al., 2000)
uns (unusual sugar response)	Altered amylase levels and anthocyanin accumulation in leaf petioles supplemented with 5% (146 mM) Suc	(Mita et al., 1995; Ohto et al., 2006)

APL3, ADP-Glc pyrophosphorylase; Glc, glucose; GUS, β-glucuronidase; Luc, luciferase; PC, plastocyanin; *pgm*, phosphoglucomutase; Suc, sucrose

modulate the expression of a large number of Arabidopsis genes in carbon-deprived seedlings grown in liquid culture (Osuna et al., 2007). Interestingly, changes in light intensity are correlated with endogenous glucose levels and plant growth phenotypes (Moore et al., 2003). The use of mutants (e.g. *pgm, phosphoglucomutase*) with different endogenous sugar levels has further substantiated the physiological relevance of the sugar sensing and signaling network in Arabidopsis (Bläsing et al., 2005; Gonzali et al., 2006). It would be interesting to examine the effects of *pgm* at various endogenous sugar levels without the presence of high nitrate (Bläsing et al., 2005).

Other more elaborate mutant screens are based on altered responses to sugar repression or activation of gene expression in transgenic reporter lines. For example, the *sun* (*sucrose uncoupled*) mutants show high activity of a sucrose-repressed plastocyanin (*PC*) promoter fused to a luciferase reporter in the presence of 3% sucrose (Dijkwel et al., 1997). The *isi* (*impaired sucrose induction*) mutants loose their ability to induce *ApL3* gene expression on sucrose-containing medium. The regulatory sequence of the *ApL3* gene was fused to a negative selection marker. Hence, seedlings surviving this screen are impaired in sucrose induction of the starch biosynthesis gene promoter (Rook et al., 2001). Both sugar insensitive and hypersensitive mutants are also obtained based on the sugar-inducible expression of a β-amylase gene (Mita et al., 1997a; Mita et al., 1997b). Other screens include the use of the sugar inducible patatin promoter in front of a GUS reporter gene (*rsr*, *reduced sucrose response* mutants) (Martin et al., 1997) or the ApL3 promoter in front of a luciferase reporter gene (*hsr, high sugar response* mutants) (Baier et al., 2004) (Table I).

Complex sugar signaling mutants are also identified in unrelated genetic screens. For example, the *hypersenescence1* (*hys1*) mutant was isolated based on an early senescence screen and displays a sugar hypersensitive phenotype (Yoshida et al., 2002). Analyses of the double mutant *hys1 gin2* reveal HXK1-dependent action of HYS1 in glucose-mediated developmental arrest but HXK1-independent function of HYS1 in senescence. The *hys1* mutant is allelic to *cpr5* (*constitutive expression of pr genes5*) with elevated salicylic acid (SA) levels. HXK1 and HYS1 act synergistically in the negative regulation of SA accumulation (Aki et al., 2007). Another unique sugar mutant, *prl1* (*pleiotropic regulatory locus1*), displays complex hormone and sugar response phenotypes and is recently found to exhibit severe defects in plant defense (Nemeth et al., 1998; Palma et al., 2007). PRL1 interacts with the C-terminal region of the Arabidopsis SnRK1 protein kinases KIN10 and KIN11, acting as a negative regulator (Bhalerao et al., 1999). This C-terminal part of KIN10 and KIN11 also interacts with both the SCF ubiquitin E3 ligase subunit SKP1/ASK1 and the SKP1/ASK1-binding 26S proteasome (Farras et al., 2001). In addition, PRL1 is associated with CUL4, a subunit of the E3 ligase, *in vivo*. KIN10 degradation is slower both in the *prl1* and *cul4cs* mutants, suggesting a link between SnRK1 and the ubiquitin/26S proteasome complex (Lee et al., 2008). Intriguingly, the glucose hypersensitive phenotypes found in the dark-grown *hsr8* (*high sugar response 8*) mutant are suppressed by *prl1*, indicating that PRL1 acts as a positive regulator for the specific glucose effects observed in etiolated *hsr8* seedlings. Under light, *hsr8* does not show overt phenotypes whereas *prl1* displays hypersensitive glucose repression in developmental arrest (Li et al., 2007), suggesting complex effects of glucose regulation in different growth

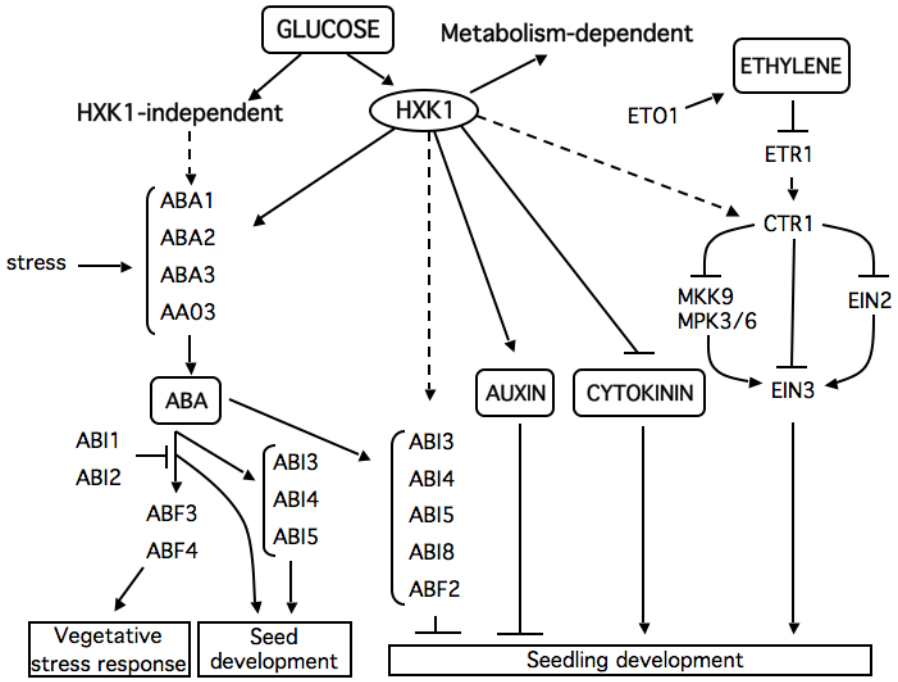

Figure 3: Overview of the elaborate genetic interactions between glucose and hormone signaling in *Arabidopsis thaliana*.

Glucose signaling mediated by HXK1 leads to increased ABA levels and induces both ABA synthesis (ABA1-3 and AAO3 gene expression) and ABA (ABI3-5, ABI8, ABF2-mediated) signaling to control seedling development. Other ABA-insensitive signaling mutants (*abi1-1*, *abi2-1*) do not exhibit the gin phenotype, suggesting distinct ABA signaling pathway for Glc signaling and the vegetative stress response. The glucose insensitive (*gin*) phenotype is mimicked by ethylene precursor treatment of wild-type plants and is also displayed in constitutive ethylene biosynthesis (*eto1*) and constitutive ethylene signaling (*ctr1*) mutants, whereas the ethylene-insensitive mutants *etr1-1*, *ein2*, *ein3* as well as *mkk9* exhibit glucose hypersensitivity (*glo* phenotypes). Glucose and ethylene signaling converge on the regulation of the ETHYLENE INSENSITIVE3 (EIN3) TF to differentially modulate its protein stability. The bifurcate and antagonistic CTR1 and MKK9 pathways are both critical in determining ethylene-signaling specificity through two MAPK phosphorylation sites with opposite effects on EIN3 stability. In addition, HXK-signaling positively and negatively interacts with auxin and cytokinin signaling, respectively. More details on hormone and sugar interactions are presented in the text.

conditions. Map-based cloning reveals that *HSR8* is allelic to *MUR4*, encoding an enzyme involved in arabinose synthesis. The studies indicate that failure of arabinose synthesis and consequent changes in cell wall composition could have a strong impact on sugar signaling (Li et al., 2007). It remains an important challenge to link complex sugar phenotypes to specific protein-protein interactions and signal transduction pathways.

Interplay Between Abscisic Acid and Sugar Signaling

A first indication for crosstalk between sugar and ABA came from the observation that several glucose insensitive mutants (*gin1/isi4/sis4* and *gin5/isi2/sis3*) (Table II) are allelic to ABA biosynthesis mutants (*aba2* and *aba3*) with lower endogenous ABA levels and reduced seed dormancy (Arenas-Huertero et al., 2000; Laby et al., 2000; Rook et al., 2001; Cheng et al., 2002) (Table II). The extensive crosstalk is further corroborated by the inability of glucose to repress the *CAB1* and plastocyanin (*PC*) genes in the *gin1/aba2* and *gin5/aba3* backgrounds. Also, overexpression of *HXK1* in the *gin1* and *gin5* mutants does not alter glucose-insensitivity, indicating that ABA acts downstream of HXK1 (Zhou et al., 1998; Arenas-Huertero et al., 2000)(Fig. 3). Interestingly, ABA accumulation and transcript levels of several ABA biosynthesis genes are significantly increased by glucose, providing a mechanistic link at the molecular level (Cheng et al., 2002). Except for *ABA2*, which is specifically upregulated by glucose but not by ABA (Seo and Koshiba, 2002), several ABA biosynthesis genes (*ABA1*, *ABA3*, *NCED3* and *AAO3*) show a synergistic effect of glucose and ABA in their induction (Cheng et al., 2002). While expression of the *APL3* gene is not induced by ABA (Rook et al., 2001), its sucrose-induction is significantly enhanced by ABA, indicating that sugar responses are dependent on an ABA-controlled responsiveness of the tissue (Rook and Bevan, 2003). It is suggested that, through induction of ABA synthesis, high concentrations of sugars would make the seedlings also more responsive to the sugar signal (Rook and Bevan, 2003). More studies are needed to reveal whether ABA biosynthesis is directly involved in the sugar signal transduction cascade or indirectly stimulated by sugars, and modulating sugar-responsiveness. Since many of

Table II. Allelism between sugar and hormone signaling mutants in Arabidopsis

Name	Allelic with	Molecular	Identity Reference
gin1	*aba2/isi4/sis4*	SDR1	(Laby et al., 2000; Rook et al., 2001; Cheng et al., 2002)
gin2		HXK1	(Moore et al., 2003)
gin4	*ctr1/sis1*	Raf-like MAPKKK	(Zhou et al., 1998; Cheng et al., 2002; Gibson et al., 2001)
gin5	*aba3/isi2*	Mo-cofactor sulfurase	(Arenas-Huertero et al., 2000; Rook et al., 2001)
gin6	*abi4/isi3/sis5/sun6*	AP2-like TF	(Arenas-Huertero et al., 2000; Rook et al., 2001; Laby et al., 2000; Huijser et al., 2000)

ABA: abscisic acid, HXK: Hexokinase, Mo: molybdenum, SDR: short-chain dehydrogenase/reductase, TF: transcription factor

these mutants were isolated in screens on high amounts of glucose, glucose-specific (likely ABA-mediated) osmotic effects should also be considered.

In addition to mutants in ABA biosynthesis, several sugar-response mutants are affected in ABA signaling. The *gin6* mutant contains a T-DNA insertion in the promoter of the *ABI4* (*ABSCISIC ACID INSENSITIVE 4*) gene (Arenas-Huertero et al., 2000). In screens for mutants insensitive to sucrose, *sun6*, *sis5* and *isi3* were found to be allelic to *ABI4* (Huijser et al., 2000; Laby et al., 2000; Rook et al., 2001) (Table II). *ABI4* encodes a transcription factor of the APETALA 2 (AP2) domain family, involved in seed development and germination (Finkelstein et al., 1998). *ABI4* expression is induced by high glucose levels and this induction is abolished in the *gin6* mutant (Arenas-Huertero et al., 2000; Arroyo et al., 2003). The *gin6* mutant also shows insensitivity to ABA during seed germination, suggesting that cis-acting elements are required for the ABA and high glucose regulation of the *ABI4* gene expression. Interestingly, the glucose-insensitive *abi4* phenotype cannot be rescued by the addition of ABA, suggesting that ABI4 acts downstream of the ABA-mediated glucose signaling cascade (Arenas-Huertero et al., 2000)(Fig. 3). However, detailed analysis of glucose effects on seed germination shows that there is a differential sensitivity to different glucose levels at distinct developmental stages (Price et al., 2003). The same low glucose levels are inhibitory for seed germination but stimulatory for seedling growth and development (Yuan and Wysocka-Diller, 2006). In addition, ABA is not required for sugar-dependent induction of leaf senescence, although leaf senescence can be triggered independently by application of ABA and sugars (Pourtau et al., 2004). The complexity of these data suggests caution when analyzing tissue- and developmental stage-specific crosstalk between sugars and hormones.

In addition to *abi4*, mutation of *ABI5*, encoding a transcription factor belonging to a large basic leucine zipper (bZIP) gene family, similarly confers a glucose-insensitive phenotype, albeit more moderate (Arenas-Huertero et al., 2000; Finkelstein and Lynch, 2000; Laby et al., 2000). Conversely, overexpression of *ABI5* and some of the closely related bZIP transcription factors (*ABF2*, *ABF3* and *ABF4*) confers hypersensitivity to sugars (Brocard et al., 2002; Kang et al., 2002). All of these transcription factors bind to the ABA responsive cis-element ABRE (Finkelstein et al., 2005). However, *abf3* and *abf4* loss-of-function mutants are not insensitive to glucose, but display defects in salt and dehydration responses, suggesting that they may only play a minor role in glucose-mediated signaling (Kim et al., 2004a). Interestingly, the ABI5 protein is only expressed in vegetative tissue shortly after seed germination, consistent with a role in monitoring environmental conditions during early seedling development (Lopez-Molina et al., 2001; Brocard et al., 2002). Furthermore, *ABI5* gene expression is activated by glucose in an ABA-dependent manner (Cheng et al., 2002), and, unlike *ABI4*, highly inducible by ABA and mannitol (Brocard et al., 2002). Still, both *ABI4* and *ABI5* respond to the glucose analog 2-deoxy-glucose, suggesting a common HXK-dependent expression regulation (Arroyo et al., 2003)(Fig. 3).

Two other ABA signaling components may also be involved in the sugar response. Overexpression of the transcription factor *ABI3* leads to glucose hypersensitivity at the seedling stage (Finkelstein et al., 2002). Expression of *ABI3* is induced by 6% glucose in an ABA-dependent manner, albeit to lower levels than *ABI4* and *ABI5* (Cheng et al., 2002), and loss-of-function *abi3* mutants are insensitive to the glucose-induced inhibitory effect on seed germination (Yuan and Wysocka-Diller, 2006). Based on glucose-induced *ABI3* expression, altered glucose-mediated gene expression in the *abi3* mutants and glucose insensitive phenotypes of *abi3* mutant alleles, it is concluded that ABI3 is an essential component in the regulation of glucose insensitivity (Dekkers et al., 2008)(Fig. 3). In addition, loss-of-function mutation of *ABI8*, encoding a small plant-specific protein with unknown function, confers highly stunted root growth, which can be partially rescued by glucose (Brocard-Gifford et al., 2004) (Fig. 3).

While several ABA-signaling transcription factors appear to be involved in sugar-mediated responses of early seedling development, two dominant ABA-insensitive mutants, *abi1* and *abi2*, respond normally to high concentrations of external glucose (Arenas-Huertero et al., 2000). Considering the complex nature of ABA signaling (Finkelstein and Rock, 2002) and the ABA-glucose interactions (Leon and Sheen, 2003; Price et al., 2003; Yuan and Wysocka-Diller, 2006), more detailed molecular analyses will be necessary to determine the genetic and spatio-temporal network linking ABA with the mechanisms conducting sugar signaling.

Interaction with Ethylene Signaling

Interactions between the ethylene and sugar-signaling pathways were first suggested by the finding that the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) prevents inhibition of cotyledon greening and expansion at high concentrations of glucose in wild type seedlings (Zhou et al., 1998; Gibson et al., 2001). In addition, the ethylene insensitive mutants *etr1*, *ein2* and *ein3* display glucose hypersensitivity (Zhou et al., 1998; Cheng et al., 2002; Yanagisawa et al., 2003). Conversely, the ethylene overproduction mutant (*eto1*) and the constitutive ethylene triple response mutant (*ctr1/gin4/sis1*) are glucose insensitive (Zhou et al., 1998;

Gibson et al., 2001; Cheng et al., 2002)(Table II). However, consistent with the idea that the crosstalk between sugar and hormone-signaling pathways is strongly dependent on glucose levels and developmental stage, the *ctr1* mutant is sensitive to low glucose-mediated seed germination (Price et al., 2003).

To further elucidate the role of *GIN1* (later identified as *ABA2*) in the ethylene signal transduction cascade, *etr1 gin1* and *ein2 gin1* double mutants were generated and analyzed. These double mutants display a glucose insensitive phenotype, indicating that ethylene affects glucose signaling partially through ABA to promote germination and seedling development (Zhou et al., 1998; Ghassemian et al., 2000; Cheng et al., 2002). Moreover, protein stability of the transcription factor EIN3 (ETHYLENE INSENSITIVE 3), a key regulator in ethylene signaling acting downstream of ETR1, EIN2 and MKK9, is differentially regulated by glucose and ethylene (Chang et al., 1993; Alonso et al., 1999; Yanagisawa et al., 2003; Yoo et al., 2008). In maize and Arabidopsis mesophyll protoplasts, glucose enhances the proteasome-dependent degradation of EIN3 proteins in the nucleus. This glucose-induced degradation is dependent on HXK1, as it is no longer detected in *gin2* mutants. Transgenic plants overexpressing EIN3 are insensitive to glucose, whereas *ein3* mutants are hypersensitive (Yanagisawa et al., 2003). The degradation of EIN3 proceeds much faster in seedlings treated with glucose and the presence of the ethylene precursor ACC delays EIN3 degradation in Arabidopsis plants. The glucose hypersensitivity observed in *etr1*, *ein2*, *ein6* and *mkk9* mutants and the allelism of *gin4* with *ctr1* can also be explained by these findings (Rolland et al., 2002; Leon and Sheen, 2003; Yoo et al., 2008) (Fig. 1 and 3).

Other Interactions between Sugar and Hormone Signaling

The observation that *gin2* mutants show growth defects under high light suggests a link between glucose signaling and hormones important in cell elongation, such as auxin, gibberellins and brassinosteroids (Moore et al., 2003). Tissue cultures assays show that *gin2* mutants are indeed defective in auxin-induced cell proliferation and root formation. Since auxin concentrations are similar in wild type and *gin2* hypocotyl, and exogenously applied IAA (indole-3-acetic acid) does not induce cell proliferation in *gin2* hypocotyls, *gin2* mutants are likely deficient in auxin signaling and/or uptake (Moore et al., 2003). Furthermore, the auxin-resistant mutants, *axr1*, *axr2* and *tir1*, are insensitive to growth inhibition on 6% glucose. Remarkably, the catalytically inactive HXK1 alleles restore auxin-mediated cell proliferation in *gin2* tissue cultures, demonstrating that glucose sensing by HXK acts synergistically with auxin signaling (Moore et al., 2003). Another aspect of sugar and auxin interactions is revealed by the *hookless1* (*hls1*) mutant that enhances sugar-induced gene expression, which can be partially eliminated by IAA (Ohto et al., 2006).

The delayed leaf senescence found in *gin2* plants links sugars with another plant hormone, cytokinin (Moore et al., 2003). In tissue culture, *gin2* mutants are hypersensitive to cytokinins for shoot regeneration. The addition of cytokinins to seedlings grown on 6% glucose also eliminates the developmental arrest (Moore et al., 2003). Because cytokinins promote ethylene biosynthesis and ethylene counteracts the effects of glucose, the effect of cytokinins could be indirect. As cytokinins still promote greening in the ethylene insensitive mutants *etr1* and *ein2*, ethylene and cytokinin act independently in response to glucose. Furthermore, constitutive cytokinin signaling transgenic plants can overcome the glucose repression response, supporting the antagonistic relationship between cytokinin and sugar signaling (Moore et al., 2003). A recent analysis of the *hys1* mutant reveals that its hypersensitivity to glucose is HXK1-dependent but cannot be prevented by adding ethylene or cytokinin (Aki et al., 2007), suggesting broad HXK1 effects beyond ethylene and cytokinin signaling.

Several studies support a close link between sugars and hormones in the control of cell division and growth. Cyclin D3 (CycD3), important in the control of G_1 to S-phase transition during the cell cycle, is induced by cytokinins in the presence of sucrose (Riou-Khamlichi et al., 1999). In the absence of sucrose, addition of auxin or cytokinin no longer induces its expression. In contrast, sucrose alone is able to induce expression of this cyclin gene, suggesting that sucrose acts upstream of hormones or synergistically with hormones in regulating CycD3 expression (Riou-Khamlichi et al., 2000; Hartig and Beck, 2006).

Another hormone that interacts closely with sugars is gibberellin (GA). Glucose can affect GA-mediated α-amylase expression in barley embryos (Perata et al., 1997). Interestingly, several studies show that glucose and GA responses share the same promoter elements in rice (Morita et al., 1998; Chen et al., 2002). Three MYB transcription factors from rice bind the glucose and GA response element TATCCA, present in all promoters of α-amylase genes isolated from cereals, have been identified. All three MYBs are involved in the glucose-regulation and cooperate with a GA-regulated transcription factor to regulate expression in the absence of GA. This suggests that these three MYB transcription factors play roles in both glucose- and GA-mediated regulation of α-amylase gene expression (Lu et al., 2002). In addition, RGL2 (RGA-like) and SPY (SPINDLY), two negative regulators of GA signaling may be involved in glucose repression of seed germination as *rgl2* and *spy* mutants germinate normally in the presence of inhibiting glucose concentrations (Yuan and Wysocka-Diller, 2006). Recently, it is shown that GA represses the sucrose activation of anthocyanin biosynthesis genes, whereas ABA and jasmonate promote the effect of sucrose (Loreti et al., 2008).

Finally, several recent studies also link SA and brassinosteroids (BR) with sugar signaling. The sugar hypersensitivity of the *bls1* (*brassinosteroid, light* and *sugar1*) mutant can be repressed by exogenous BR application, suggesting crosstalk between BR and glucose signaling (Laxmi et al., 2004). The sucrose- and glucose-induced expression of the *PR2* and *PR5* genes (Xiao et al., 2000; Thibaud et al., 2004) are abolished in NahG mutants with reduced SA levels, suggesting a role for SA in sugar-induction of *PR2* and *PR5* expression (Thibaud et al., 2004).

Interactions between Sugars and Other Nutrients

Carbon and nitrogen metabolism are tightly linked and need to be coordinated in order to maintain proper plant growth and development. Carbohydrates provide energy and the carbon backbone for nitrate and ammonium assimilation in the biosynthesis of amino acids. Nitrogen is essential for incorporation in nucleic acids, amino acids and chlorophyll. The integration of sugar and nitrogen

metabolism as well as sensing and signaling enables plants to respond to acute changes in internal and external nitrogen concentrations or carbon supplies by regulating the expression of genes involved in nitrogen transport, assimilation and metabolism (Coruzzi and Zhou, 2001; Lejay et al., 2003).

It has been clearly illustrated that Arabidopsis seedling growth, lipid mobilization and photosynthetic gene expression is profoundly influenced by the availability of sugars and nitrogen (Martin et al., 2002; Moore et al., 2003). Recently, the *osu1* (*oversensitive to sugar1*) mutant, deficient in a putative methyltransferase, was isolated based on enhanced anthocyanin accumulation and root inhibition under imbalanced sugar and nitrogen conditions (Gao et al., 2008). Interestingly, genes encoding diverse transporters for nitrogen and other nutrient uptake are up-regulated by sugars (Lejay et al., 1999; Lejay et al., 2003; Lejay et al., 2008). Several studies have demonstrated the genome-wide expression regulation by nitrogen (nitrate and ammonium), sugars and their various combinations (Wang et al., 2003; Palenchar et al., 2004; Price et al., 2004; Scheible et al., 2004; Gutiérrez et al., 2007). Although nitrogen and sugars each specifically regulate the expression of a subset of genes, sucrose or glucose are more potent signals, regulating the majority of the genome responses (Palenchar et al., 2004; Price et al., 2004), and nitrogen appears to play an important role in modifying sugar responses (Palenchar et al., 2004). A recent microarray study supports the idea that nitrogen and sugars are sensed through sugar- and nitrogen-responsive pathways in Arabidopsis roots, illustrating an interconnection at the level of signaling or metabolism (Gutiérrez et al., 2007).

An informative system to study the effects of nitrogen and sugars on plant growth and development is based on the formation of lateral roots since their number and position are strongly dependent on nutritional cues. High sugar concentrations repress lateral root formation, and the *lateral root initiation1* (*lin1*) mutant shows lateral root initiation even in repressing environmental conditions (high external sucrose/low external nitrate) (Malamy and Ryan, 2001). The *lin1* mutant is deficient in the high affinity nitrate transporter, NRT2. Since expression of NRT2 is induced by low exogenous nitrogen and by sucrose under low nitrogen conditions (Lejay et al., 1999), NRT2 is a negative regulator of lateral root development in the signal transduction pathway sensing environmental status (Malamy and Ryan, 2001; Little et al., 2005).

Another nutrient closely interacting with sugar signaling is inorganic phosphate (Pi). Pi is an essential component in many plant metabolites and plays an important role in energy balance and carbon assimilation. Since Pi is often limiting in soils, plants have evolved specific mechanisms to sense and adapt to Pi starvation. Interestingly, changes in internal Pi concentrations are often linked with altered source-sink distribution and carbohydrate content. Characterization of the phosphorus-deficient *pho3* mutant (mutated in the *SUC2* gene, encoding a major sucrose-proton symporter) shows that reduced levels of Pi in rosette leaves is associated with increased levels of sucrose, anthocyanins and starch (Zakhleniuk et al., 2001). In addition, knock-out of the MYB-related transcription factor PHR1, involved in the response to Pi-starvation, results in altered Pi allocation between roots and shoots, and reduced levels of starch and sugars (Nilsson et al., 2007). Microarray analyses of Pi and/or sucrose treated Arabidopsis leaf segments show that nearly 150 genes are synergistically or antagonistically regulated by both stimuli (Müller et al., 2007). Interestingly, expression of several known sucrose-regulated genes including *CHS* and *β-AMY* (β-amylase) can be modulated by Pi starvation and *vice versa*, suggesting a tight crosstalk between Pi and sucrose-regulated gene expression (Müller et al., 2005; Müller et al., 2007). Sugars are essential in regulating Pi-starvation mediated gene expression and root architecture, and it has been suggested that this response is partially mediated by HXK1 (Karthikeyan et al., 2007). However, the sugar regulation of *CHS* and *β-AMY* expression is HXK-independent (Müller et al., 2005). These studies demonstrate the complexity of crosstalk between sugar and phosphate signaling and more analyses will be necessary to reveal the molecular nature of their interactions. An overview of Arabidopsis genes mediating sugar sensitivity is summarized in Table III.

Transcription Cascades

One way for plants to respond to diverse sugar signals is to rapidly modulate gene expression. Using a variety of methods and assays, selected genes have been found to be specifically regulated by sugars (Sheen, 1990; Graham et al., 1994; Sheen, 1994; Mita et al., 1995; Koch, 1996; Rook et al., 1998; Xiao et al., 2000). Microarray technology now enables genome-wide identification of specific sets of sugar-regulated genes in Arabidopsis seedlings and adult leaves (Palenchar et al., 2004; Price et al., 2004; Bläsing et al., 2005; Li et al., 2006; Gutiérrez et al., 2007; Osuna et al., 2007). Unraveling the physiological functions of these Arabidopsis genes will bring new insight into our understanding of the sugar regulation and signaling network.

During the diurnal cycle, changes in sugar levels are accompanied by alterations in transcript levels (Gibon et al., 2004; Thimm et al., 2004; Bläsing et al., 2005). These alterations are strongly amplified in the starchless *pgm* mutant, which contains higher sugar levels than wild-type plants during the day and very low sugar levels at the end of the night, suggesting that the transcriptional changes observed during the diurnal cycle are caused by sugars (Bläsing et al., 2005). Using the *pgm* mutant, it was shown that these changes in sugar levels can even override circadian behavior (Usadel et al. 2008). Interestingly, the extensive gene expression reprogramming promoted especially by carbon depletion can be effectively blocked by the addition of sucrose or glucose (Contento et al., 2004; Gibon et al., 2004; Thimm et al., 2004; Bläsing et al., 2005; Baena-González et al., 2007). Genes involved in diverse metabolic pathways are activated or repressed by sugars. Importantly, many genes encoding transcription factors and signaling components are also highly regulated by sugars or sugar starvation, providing a molecular link of sugar signaling to multiple regulatory pathways and hormone signaling (Price et al., 2004; Li et al., 2006; Osuna et al., 2007; Baena-González et al., 2007). It is important to note that some of these microarray experiments were carried out in the presence of high nitrogen levels and may not reveal a comprehensive coverage of sugar-regulated gene expression (Martin et al., 2002; Moore et al., 2003). Future studies aim at understanding the functional relations and kinetics of sugar-regulated gene expression will faciliate the elucidation of the transcriptional cascades and signaling networks central to the control of plant growth and development.

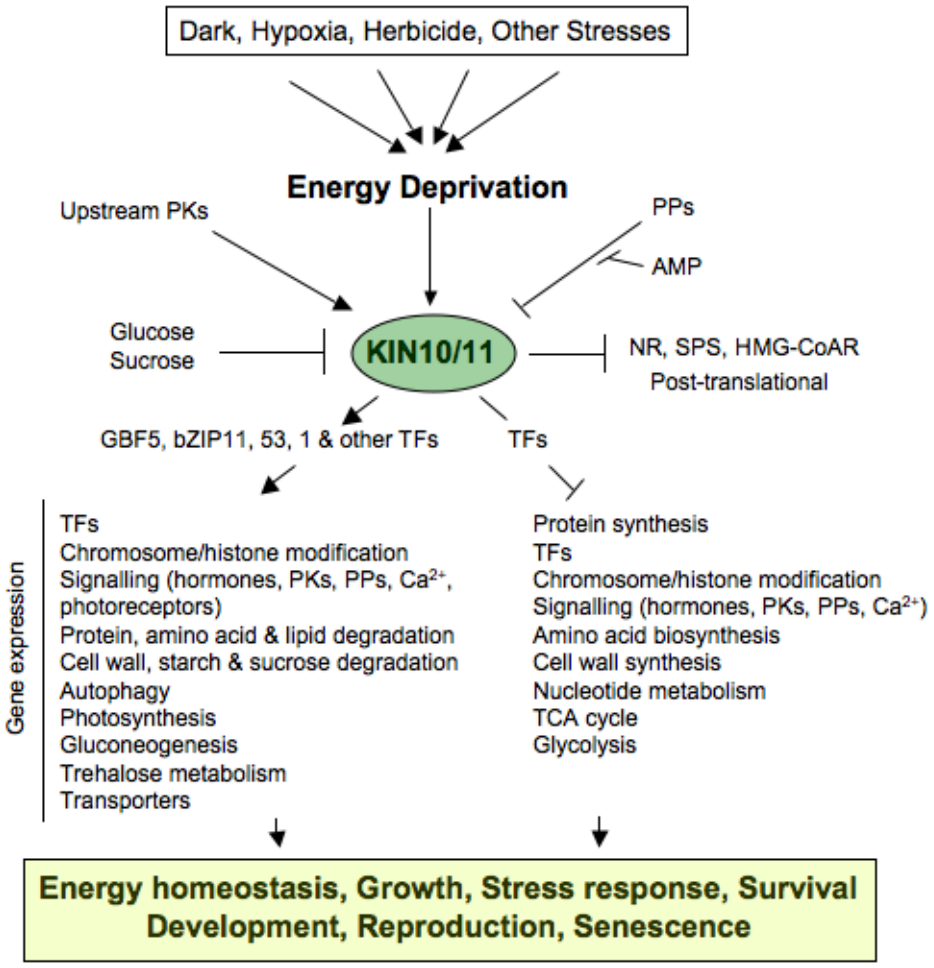

Figure 4. Overview of the role of the Arabidopsis KIN10/11 protein kinases in the response to energy depletion.

Multiple types of stress converge as an energy-deficiency signal in the cell, triggering the activation of KIN10/11. Conversely, sugars have a repressive effect. Upstream protein kinases (PKs), protein phosphatases (PPs), and additional regulatory subunits may contribute to the fine-tuning of the system. Activated KIN10/11 initiate an energy-saving program at several levels, repressing biosynthetic pathways and promoting catabolic processes and photosynthesis to increase ATP generation. This involves massive transcriptional reprogramming that affects a wide range of both plant-specific and evolutionarily conserved pathways, partly mediated by the S-class of bZIP transcription factors (TF; including bZIP1, GBF5/bZIP2, bZIP11, bZIP53). KIN10/11 also regulate key metabolic enzymes at the post-translational level to inhibit specific assimilation pathways. In addition to contributing to the maintenance of cellular energy homeostasis and tolerance to (nutrient) stress, KIN10/11 have profound effects at the whole organism level influencing growth and development, viability, reproduction and senescence. NR, nitrate reductase; SPS, sucrose phosphate synthase; HMG-CoAR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase.

Protein Kinases and Phosphatases

In all eukaryotic cell types, both protein kinases (PK) and phosphatases (PP) have established roles as central regulators of signal transduction. The first indication for the involvement of phosphatases in plant sugar signaling came from the use of PP1 and PP2A phosphatase inhibitors, mimicking sugar-induced repression of photosynthetic gene expression in maize protoplasts and *Chenopodium* cell cultures (Sheen, 1993; Ehness et al., 1997) and blocking sucrose-induced expression of genes encoding sporamin, β-amylase and the small subunit of AGP in sweet potato petioles (Takeda et al., 1994). Together with glucose- and stress-related stimuli, these PP inhibitors also activate the expression of a stress-inducible invertase and *PAL* genes, while rapidly activating a putative mitogen-activated protein kinase in *Chenopodium* cell cultures (Ehness et al., 1997). The differential effects of the PK inhibitor staurosporine on stress-and glucose-related responses suggest the involvement of different PKs in these processes (Ehness et al., 1997). Similar pharmacological approaches have also shown the involvement of distinct PPs in dark- and sugar starvation-induced *DIN* (*DARK INDUCED*) gene expression (Fujiki et al., 2000). Studies with Ca^{2+} channel blockers ($LaCl_3$), EGTA and calmodulin inhibitors have also shown the involvement of Ca^{2+} signaling in sugar induction of sporamin and β-amylase genes in sweet potato

Table III. Molecular identity of Arabidopsis genes mediating sugar sensitivity

Name	Encoded protein	Screen and conditions	Reference
Sugar insensitive			
aba1 (ABA biosynthesis 1)	ABA1	Insensitivity to 7% (390 mM) Glc	(Arenas-Huertero et al., 2000)
abf2 (ABRE binding factor 2)	ABF2 (bZIP TF)	Reduced sensitivity to 3-4% (166-222 mM) Glc	(Kim et al., 2004a)
abi3 (ABA insensitive 3)	ABI3 (B3 TF)	Increased germination rate on 1.5% (83 mM) Glc	(Yuan and Wysocka-Diller, 2006)
abi5 (ABA insensitive 5)	ABI5 (bZIP TF)	Insensitivity to 7% (390 mM) Glc	(Arenas-Huertero et al., 2000)
abi8 (ABA insensitive 8)	ABI8 (unknown)	Stunted growth rescued by addition of Glc	(Brocard-Gifford et al., 2004)
aria (arm repeat protein interacting with ABF2)	Arm repeat protein	Insensitivity to 5% (277 mM) Glc	(Kim et al., 2004b)
35S::ARR2 (Arabidopsis response regulator 2)	Cytokinin type B response regulator	Insensitivity to 6% (333 mM) Glc	(Moore et al. 2003)
axr1 (auxin resistant 1)	Subunit of RUB1 activating enzyme	Insensitivity to 6% (333 mM) Glc	(Moore et al., 2003)
axr2 (auxin resistant 2)	IAA7	Insensitivity to 6% (333 mM) Glc	(Moore et al., 2003)
35S::CKI1 (cytokinin independent 1)	Cytokinin histidine kinase 1	Insensitivity to 6% (333 mM) Glc	(Moore et al. 2003)
eto1 (ethylene overproduction 1)	Negative regulator of ACS5/ BTB/TPR adaptor of E3 ligase	Insensitivity to Glc-mediated developmental arrest	(Zhou et al., 1998)
fin219 (far-red insensitive 219)	GH3-like protein	Reduced anthocyanin accumulation and increased hypocotyl length on 1% (30 mM) Suc under far-red light conditions	(Hsieh et al., 2000)
gin1 (glucose insensitive 1)	ABA2 (short-chain dehydrogenase reductase 1)	Insensitivity to 6% (333 mM) Glc	(Zhou et al., 1998)
gin2 (glucose insensitive 2)	HXK1 (hexokinase 1)	Insensitivity to 6% (333 mM) Glc	(Moore et al., 2003)
gin4 (glucose insensitive 4)	CTR1 (MAPKKK)	Insensitivity to 6% (333 mM) Glc	(Cheng et al., 2002)
gin5 (glucose insensitive 5)	ABA3 (molybdenum cofactor sulfurase)	Insensitivity to 7% (390 mM) Glc	(Arenas-Huertero et al., 2000)
gin6 (glucose insensitive 6)	ABI4 (AP2 TF)	Insensitvity to 7.5% (416 mM) Glc	(Arenas-Huertero et al., 2000)
hup1 (hexokinase unconventional partner 1)	Vacuolar H^+ -ATPase-B1	Insensitivity to Glc-mediated developmental arrest	(Cho et al., 2006)
hup2 (hexokinase unconventional partner 2)	RPT protein 5B	Insensitivity to Glc-mediated developmental arrest	(Cho et al., 2006)
kin10 (Arabidopsis protein kinase 10)	SnRK1 catalytic α-subunit	Enhanced growth on media with 1-3% (30-88 mM) Suc	(Baena-Gonzalez et al., 2007)
lba1 (low β-amylase 1)	UPF1 RNA helicase	Reduced Suc-induced β-amylase expression	(Yoine et al., 2006)
lin1 (lateral root initiation 1)	NRT2 (nitrate transporter)	Lateral root formation on high Suc/ low nitrogen concentrations	(Little et al., 2005)
35S::MBF1 (Multiprotein bridging factor1a)	MBF1	Insensitivity to 5% (277 mM) Glc	(Kim et al., 2007)
rcd1 (radical-induced cell death 1)	WWE-domain containing protein	Insensitivity to Glc	(Ahlfors et al., 2004)
rgl2 (RGA-like 2)	DELLA protein	Increased germination rate on 1.5% (83 mM) Glc	(Yuan and Wysocka-Diller, 2006)
rgs1 (regulator of G-protein signaling 1)	RGS1	Insensitive to 6% (333 mM) Glc	(Chen and Jones, 2004)
spy (spindly)	N-acetyl glucosamine transferase	Increased germination rate on 1.5% (83 mM) Glc	(Yuan and Wysocka-Diller, 2006)
tin (homeobox domain TF)	WOX5	Normal seedling growth on 90 mM turanose	(Gonzali et al., 2005)
tir1 (transport inhibitor response 1)	F-box protein	Insensitivity to 6% (333 mM) Glc	(Moore et al., 2003)
35S::TPS1 (trehalose-6-phosphate synthase 1)	TPS1	Insensitive to 6% (333 mM) Glc	(Avonce et al., 2004)
Sugar hypersensitive			
abr1 (AP2-like ABA repressor 1)	APETALA2-Domain TF	Hypersensitivity to 4% (222 mM) Glc	(Pandey et al., 2005)
agb1 (G-protein β-subunit 1)	AGB1	Increased sensitivity to Glc	(Wang et al., 2006)
agg1/2 (G-protein γ-subunit 1/2)	AGG1/2	Increased sensitivity to Glc	(Trusov et al., 2007)
ahg1 (ABA hypersensitive germination1)	Protein phosphatase 2C	Increased Glc sensitivity	(Nishimura et al., 2007)
atg18a (autophagy 18a)	ATG18a	Hypersensitivity to carbon starvation	(Xiong et al., 2005)
cbl9 (Calcineurin B-Like 9)	Calcineurin B-like protein	Hypersensitivity on 4% (222 mM) Glc	(Pandey et al., 2004)
css1 (changed sensitivity to cellulose synthesis inhibitors 1)	At-nMat1a	Increased sensitivity to Glc	(Nakagawa and Sakurai, 2006)

(Continued)

Table III. (continued)

Name	Encoded protein	Screen and conditions	Reference
Sugar hypersensitive (continued)			
eif3h (Eukaryotic translation initiation factor 3 subunit H)	Eukaryotic translation elongation factor 3	Seedling growth arrest on 2% (58 mM) Suc	(Kim et al., 2004c)
ein2 (ethylene insensitive 2)	EIN2 (metal ion transporter)	Hypersensitivity to 4% (222 mM) Glc	(Cheng et al., 2002)
ein3 (ethylene insensitive 3)	EIN3 (TF)	Hypersensitivity to 3% (166 mM) Glc	(Yanagisawa et al., 2003)
etr1 (ethylene receptor 1)	ETR1	Hypersensitivity to 4% (222 mM) Glc	(Zhou et al., 1998)
fus6 (fusca 6)	COP11/CSN1	Hypersensitivity to 3% (166 mM) Glc	(Castle and Meinke, 1994)
ghs1 (glucose hypersensitive)	Plastid ribosomal Protein S21	Increased sensitivity to Glc	(Morita-Yamamuro et al., 2004)
glr1.1 (glutamate receptor 1.1)	Glutamate receptor 1.1	No germination on media with Suc but without nitrogen source	(Kang and Turano, 2003)
gnc (GATA, nitrate-inducible, carbon metabolism-involved)	GATA TF 24	Increased sensitivity to Glc	(Bi et al., 2005)
gpa1 (G-protein α-subunit 1)	GPA1	Increased sensitivity of dark-grown seedlings to 1% (30 mM) Suc	(Ullah et al., 2002)
hsr8 (high sugar response 8)	MUR4	Hypersensitive dark development on 0.5% (28 mM) Glc	(Li et al., 2007)
hys1 (hypersenescence1)	CPR5 (novel protein)	Increased sensitivity to Glc	(Aki et al., 2007)
keg (keep on going)	RING-ANK family member	Increased sensitivity to 4% (222 mM) Glc	(Stone et al., 2006)
mkk9 (map kinase kinase 9)	MapK kinase 9	Increased sensitivity to 4% (222 mM) Glc	(Yoo et al., 2008)
osu1 (oversensitive to sugar 1)	Putative methyltransferase	Increased anthocyanin accumulation and reduced rootlength on imbalanced C:N ratios	(Gao et al., 2008)
pp2ac-2 (protein phosphatase 2A-2)	Catalytic subunit of protein phosphatase 2A	Increased sensitivity to 3% (166 mM) Glc	(Pernas et al., 2007)
prl1 (pleiotropic regulatory locus)	PRL1 (WD40 protein)	Growth arrest on 6% (175 mM) Suc	(Nemeth et al., 1998)
rfc3 (regulator of fatty acid composition 3)	S6-like protein	Abnormal lateral root formation on 3% (88 mM) Suc	(Horiguchi et al., 2003)
rpn10 (regulatory particle non-ATPase 10)	RPN10	Hypersensitivity to 5% (146 mM) Suc	(Smalle et al., 2003)
shs1 (sugar-hypersensitive 1)	Adenylate translocator-like protein	Hypersensitivity on MS with 3% (88 mM) Suc	(Inan et al., 2007)
tag1 (triacylglycerol 1)	DGAT	Hypersensitivity to germination on Glc	(Lu and Hills, 2002)
thf1 (thylakoid formation 1)	THF1	Increased sensitivity to Glc	(Huang et al., 2006)
uns2 (unusual sugar response 2)	HLS1 (putative N-acetyltransferase)	Increased amylase activity and anthocyanin levels in sucrose supplied leaf petioles	(Ohto et al., 2006)

ABA, abscisic acid; ABRE, ABA responsive elements; ACS5, 1-aminocyclopropane-1-carboxylate synthase synthase 5; At-nMat1a, maturase-related protein; BTB, Broad complex, Tramtrack, Bric-à-bric; COP11, constitutive photomorphogenic 11; CPR5, constitutive expression of PR genes 5; CSN, COP9 signalosome; CTR1, constitutive triple response 1; DGAT, diacylglycerol acytransferase; Glc, glucose; HLS1, hookless 1; IAA, indole-3-acetic acid; MUR4, UDP-D-xylose-4-epimerase; RUB, related to ubiquitin; Suc, sucrose; snf1, sucrose non-fermenting 1; TF, transcription factor; TPR, tetratricopeptide repeat; WOX5, wuschelrelated homeobox 5

(Ohto and Nakamura, 1995). It is possible that Ca^{2+} signaling mediates protein phosphorylation (Boudsocq and Sheen, 2008) in some of the sugar signaling pathways. For example, sucrose can induce cytosolic calcium fluxes and increases expression of a plasma membrane-associated calcium-dependent protein kinase (CDPK) from tobacco (Iwata et al., 1998; Furuichi et al., 2001). The future challenges are to connect various sugar sensors and signaling pathways to the functions of specific PKs and PPs and sugar-regulated target genes.

ENERGY AND STRESS SIGNALING

Studies of sugar-regulated genes have mainly focused on establishing connections to specific sugar sensing and signal transduction pathways. However, more detailed investigation of some sugar-repressed genes reveals the interesting finding that these genes are activated in darkness, starvation or/and under diverse stress conditions (Lam et al., 1996; Fujiki et al., 2000; Contento et al., 2004; Gibon et al., 2004; Lin and Wu, 2004; Palenchar et al., 2004; Price et al., 2004; Thimm et al., 2004; Buchanan-Wollaston et al., 2005; Baena-González et al., 2007), and are likely essential for plant survival under dark-induced starvation and stress conditions (Ishizaki et al., 2006; Baena-González et al., 2007; Baena-González and Sheen, 2008; Miyashita and Good, 2008). Interestingly, sucrose enhances anoxia tolerance in Arabidopsis and partially relieves transcriptional changes under these conditions, suggesting a close relationship between more general energy-depriving stresses and sugars (Loreti et al., 2005). Importantly, the activation of these genes can be HXK1-independent (Baena-González et al., 2007) and is repressed by multiple metabolizable sugars (Lee et al., 2007). As sessile and autotrophic organisms, plants are constantly challenged by

a changing environment that compromises photosynthesis and respiration for energy supplies. Quick and adequate responses are necessary for continued metabolic activity, growth and development. The studies suggest that plant cells may sense energy depletion in a cell-autonomous manner and trigger convergent gene activation in response to diverse and seemingly unrelated stress signals independent of light (Baena-González et al., 2007; Baena-González and Sheen, 2008).

New findings now show that the evolutionarily conserved Arabidopsis protein kinases KIN10/11, catalytic subunits of the plant SnRK1 (Snf1-related protein kinase) heterotrimeric kinase complex, are master regulators of transcription networks and activate catabolism and repress anabolism under energy deprivation conditions (Baena-González et al., 2007; Baena-González and Sheen, 2008). Consistent with a role in energy depleting conditions, sugar phosphates (especially glucose-6-phosphate) have been shown to inhibit SnRK1 activity (Toroser et al., 2000). Genome-wide microarray analyses of KIN10 overexpressing cells and the characterization of *kin10/11* double mutant plants reveal that the energy sensors KIN10/11 control transcriptional responses to darkness, sugar and stress conditions (Baena-González et al., 2007; Baena-González and Sheen, 2008)(Fig. 4). In addition, KIN10/11 are important transcriptional regulators upon small changes in the carbon status during the night, suggesting high sensitivity of these components to energy status (Usadel et al., 2008). At least part of the transcriptional reprogramming under these conditions is mediated through GBF (G-box binding factors)/bZIP transcription factors (Baena-González et al., 2007; Hanson et al., 2008) (Fig. 1 and 4). In rice, the SnRK1A and SnRK1B orthologs activate the sugar-repressed α-amylase gene in germinating embryos, possibly through phosphorylation of the MYBS1 transcription factor (Lu et al., 2007). Consistently, antisense repression of SnRK1 in cultured wheat embryos represses an α-amylase gene promoter (Laurie et al., 2003).

Beside their role as global regulators of transcription networks, SnRK1 has been well known to modulate enzymes important for carbon and nitrogen metabolism through direct phosphorylation. For example, SnRK1 phosphorylates and inactivates the key enzymes 3-hydroxy-3-methylglutaryl CoA reductase, nitrate reductase and sucrose phosphate synthase *in vitro*, suggesting a pivotal role for SnRK1 in plant metabolism (Hardie et al., 1998; Sugden et al., 1999a; Sugden et al., 1999b; Polge and Thomas, 2007) (Fig. 1 and 4). Analyses of an antisense transgenic potato line suggest that SnRK1 is important for sucrose and trehalose-6-phosphate activation of AGP and sucrose induction of *SUS4* (encoding a sucrose synthase) (Purcell et al., 1998; Tiessen et al., 2003; Kolbe et al., 2005). However, unlike *KIN10/11* and another potato gene (*StubSNF1*), the specific potato SnRK1 gene (*PKIN1*) used in these transgenic line does not complement the yeast *snf1* mutant (Lovas et al., 2003), and may have novel regulation and functions distinct from those of the more conserved yeast Snf1, mammalian AMPK and Arabidopsis KIN10/11 kinases (Baena-González et al., 2007; Baena-González and Sheen, 2008).

To further study the physiological functions of *KIN10/11* in plant growth and development, transgenic and mutant plants have been characterized. KIN10 overexpression plants display delayed senescence and flowering and an altered flower architecture under long-day conditions (Baena-González et al., 2007). Silencing of both KIN10 and KIN11 genes confers dramatic growth defects, with the most silenced plants senescing before the onset of flowering (Baena-González et al., 2007). In the moss *Physcomitrella patens*, the double knockout of both SnRK1 genes results in premature senescence and altered sensitivity to auxin and cytokinin (Thelander et al., 2004). Interestingly, a Snf1-like kinase gene is expressed asymmetrically in tomato apical meristems, with higher expression in the part destined to form leaf primordia, suggesting a role for SnRK1 in linking metabolism and energy supplies with meristematic activity (Pien et al., 2001). Future studies will likely reveal more roles of KIN10/11 in plant growth and development and in antiviral defense (Shen and Hanley-Bowdoin, 2006).

In order to respond adequately to energy-mediated changes in metabolism, modulation of SnRK1 kinase activity could be important. Phosphorylation of the conserved threonine in the T-loop of the SnRK1 catalytic subunit has recently been demonstrated for Arabidopsis SnAK1 and SnAK2 (SnRK1-Activating Kinase). These two protein kinases can complement a yeast mutant deficient in Snf1 upstream kinase activity (Hey et al., 2007). Interestingly, using a similar approach, the same upstream kinases, dubbed Geminivirus Rep-interacting kinase 1 and 2 (GRIK1/2), were isolated and shown to be induced in infected leaves, suggesting a role for SnRK1 in the biotic stress response (Shen and Hanley-Bowdoin, 2006).

In addition to its conserved β, γ and the plant-specific chimeric βγ regulatory subunits (Halford et al., 2003; Baena-González et al., 2007; Polge and Thomas, 2007), several more proteins were found to interact with SnRK1, including a putative Arabidopsis dual specificity phosphatase (PTPKIS1) (Fordham-Skelton et al., 2002), novel SnRK1-interacting proteins (SnIP1 and SnIP2), the heat shock protein BHSP17 from barley endosperm (Slocombe et al., 2002; Slocombe et al., 2004), and PpSKI1 and PpSKI2 from moss (Thelander et al., 2007). In a screen for heterologous multicopy suppressors of the yeast *snf4* (Snf1 regulatory protein) deficiency, a plant casein kinase I orthologue, a novel hexose transporter (Mss1), and two Msn2/4-type zinc finger factors, AZF2 and ZAT10, involved in stress responses, have been isolated (Kleinow et al., 2000). These results suggest that a multitude of protein interactions are likely involved in the complex regulation of SnRK1 activity in plants.

TREHALOSE METABOLISM AND SIGNALING

Recently, trehalose and trehalose-6-phosphate (T6P) have emerged as novel regulators of carbon metabolism and development in plants. Synthesized from UDP-glucose and glucose-6-phosphate through the intermediate T6P, trehalose metabolism resides at the crossroads of major carbon fluxes in plants. Feeding sucrose to sucrose-starved seedlings results in a significant but transient increase in T6P levels and concomitant activation of AGP activity (Kolbe et al., 2005; Lunn et al., 2006). Moreover, direct feeding of T6P to isolated chloroplasts was reported to increase AGP activity. Disruption of the outer membrane abolishes the response, suggesting perception of T6P signals at the chloroplasts (Kolbe et al., 2005)(Fig. 1). Transgenic Arabidopsis plants overexpressing the *E. coli* trehalose-6-phosphate synthase (TPS; OtsA), the first enzyme of trehalose metabolism, exhibit better growth than wild-type seedlings on media supplemented with sugars (Schluepmann et al., 2003). Conversely, overexpression of the *E. coli* trehalose-6-phosphate phosphatase (TPP; OtsB), the sec-

ond enzyme in trehalose metabolism, causes a total arrest of seedling growth on sugar media, suggesting an important role for T6P in sugar utilization (Schluepmann et al., 2003; Paul, 2007; Paul et al., 2008). Furthermore, seedling growth is totally arrested on media with low amounts of exogenous trehalose that strongly induces *APL3* gene expression (Wingler et al., 2000). Further analyses show that expression of the starch breakdown genes, *SEX1* and β-amylase *BAM3*, is significantly reduced under these conditions, leading to high amounts of starch at the end of the night. The effects on gene expression by exogenous trehalose are mediated by the ABI4 transcription factor (Ramon et al., 2007). Interestingly, overexpression of Arabidopsis *TPS1* confers glucose and ABA insensitivity, suggesting crosstalk between T6P and sugar signaling (Avonce et al., 2004).

In the Arabidopsis genome, 21 genes with potential roles in trehalose and T6P metabolism are found. They are classified into three distinct subfamilies based on the sequence similarity with yeast *TPS1* (class I, *TPS1-4*), *TPS2* (class III, *TPPA-J*), or both (class II, *TPS5-11*) (Leyman et al., 2001; Lunn, 2007; Ramon and Rolland, 2007). From the class I genes, only *TPS1* has been studied in detail and it encodes an active TPS protein (Blazquez et al., 1998). Loss-of-function studies show that the embryo lethal *tps1* mutant arrests growth at the torpedo stage of embryo development, at the transition from cell division to cell elongation, differentiation and carbohydrate storage (Eastmond et al., 2002; Gomez et al., 2006). Conditional *TPS1* expression allows embryo maturation and further characterization of TPS1 functions in transgenic plants. Interestingly, *TPS1* knock-out plants show shorter root length with a reduction in root apical meristem size, slow growth and strongly reduced flowering, suggesting an important role for TPS1 throughout the plant's life cycle (van Dijken et al., 2004).

Enzymes of the third class of trehalose metabolism proteins are active TPPs (Vogel et al., 1998), suggesting an important role for rapid T6P breakdown under sugar-depleting conditions. Importantly, *RAMOSA3*, a gene controlling inflorescence architecture in maize, encodes such a functional TPP enzyme (Satoh-Nagasawa et al., 2006). It will be important to determine the functions of related TPP genes in Arabidopsis and other plants.

The class II *TPS* genes are more enigmatic. Although extensive expression regulation under wide-ranging conditions suggests important regulatory roles, enzymatic activity has not been demonstrated for any of these proteins (Vogel et al., 2001; Wang et al., 2003; Contento et al., 2004; Scheible et al., 2004; Schluepmann et al., 2004; Thimm et al., 2004; Brenner et al., 2005; Gibon et al., 2006; Baena-González et al., 2007; Osuna et al., 2007). Several of these proteins are phosphorylated by KIN10/11 to promote 14-3-3 protein binding (Glinski and Weckwerth, 2005; Harthill et al., 2006). Interestingly, the *tps5* mutant is thermosensitive and the *tps6* mutant exhibits altered plant architecture, epidermal pavement cell shape and trichome branching (Chary et al., 2008; Suzuki et al., 2008). These data suggest potentially important roles for trehalose metabolism in coordinating metabolic status with plant growth and development.

CONCLUSIONS AND PERSPECTIVES

Sugar sensing and signaling is mediated by a complex network comprising a multitude of interactions with metabolic and hormonal signals. Several key players involved in these processes have been identified and the complex genetic interactions are gradually being dissected and interpreted. Since many of the mutant screens are based on altered sugar sensitivity in germination and seedling growth assays, information about the spatial-temporal aspects of these interactions is still missing. Defining sugar sensing and signaling cascades for specific sugar signals and concentrations at specific time points and developmental stages will facilitate the establishment of an integrated view, linking these mechanisms with normal plant growth and development. Furthermore, the molecular and functional link between the different putative sugar sensing mechanisms is still elusive, and will require identification of the sensors, their exact location, expression and regulation, as well as an understanding of the dynamic carbon fluxes and associated signal generation during normal physiological development and in response to changing environmental stimuli. Further analyses will reveal specific sets of target genes and elucidate important protein-protein interactions and putative post-translational protein modifications in response to sugars. Plant sugar sensing and signaling is a complex field of study, but within this complexity lay great potential and opportunities for scientific exploration and discovery. Novel approaches will reveal previously unidentified components and precisely define existing mechanisms, bringing together a dynamic and comprehensive sugar-signaling network central to the modulation of plant life and processes.

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