Sugars as signaling molecules

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Recent studies indicate that, in a manner similar to classical plant hormones, sugars can act as signaling molecules that control gene expression and developmental processes in plants. Crucial evidence includes uncoupling glucose signaling from its metabolism, identification of glucose sensors, and isolation and characterization of mutants and other regulatory components in plant sugar signal transduction pathways. The emerging scenario points to the existence of a complex signaling network that interconnects transduction pathways from sugars and other hormone and nutrient signals.

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Abbreviations

HXK	hexokinase
GIN	glucose insensitive
PRL	pleiotropic regulatory locus
SNF	sucrose nonfermentation
SnRK	SNF1-related kinase

Introduction

Sugar production through photosynthesis is the most fundamental activity in plant life. The processes of sugar production, transport, consumption, and storage are dynamic and tightly linked to cellular physiology, organ identity, environmental inputs, and developmental stages. A plant's ability to monitor and respond to sugar levels could serve as a control mechanism to integrate external environmental conditions including light, other nutrients, and abiotic and biotic stresses, with intrinsic developmental programs directed by multiple plant hormones. In plants, sugars have conventionally been viewed as resources for respiration and metabolic intermediates, as well structural or storage components. The widely observed effects of sugar on gene expression and on plant growth and development have often been attributed to sugar metabolism and energy production [1–3,4•–6•,7]. Even in yeast and mammals, the prevailing wisdom still favors a regulatory role of metabolism in glucose responses [8–10]. The previous exclusion of sugars as plant signaling molecules stems from the observation that higher concentrations are needed for sugar activity than for the classically defined plant hormone effects [11,12]. Recent compelling evidence, however, supports the concept that sensing and signaling can be performed even at a millimolar range of signaling molecules using sugar binding enzymes, proteins, or transporters

[2,3,4•–6•,13–17,18•,19,20,21•,22]. Carefully designed experiments now reveal the uncoupling of sugar sensing and signaling from sugar metabolism [15–17,18•,19,20,21•,22].

Despite the anticipated complication of sugar sensing and signaling in photosynthetic plants, exciting progress has been made in the past two years. Although multiple sugar sensors/receptors are likely to exist, hexokinase (HXK) as a dual-function enzyme and glucose sensor is the first to be documented in plants [2,3,15,16,19,22]. Many signaling components — including a WD protein [23•,24•], calcium-dependent protein kinase (CDPK) [25], protein phosphatase (PP) [26,27], mitogen activated protein kinase (MAPK) [17], SNF1-related PK (SnRK) [7,9,24•,28,29•], and transcription factors [30,31•] — have been proposed to be important in plant sugar signal transduction. The isolation and characterization of sugar response mutants by using a variety of strategies have revealed surprising aspects and the large complexity of sugar regulation in plants [3,32–35,36•]. Many excellent reviews and perspectives on sugar regulated gene expression and sugar sensing and signaling have appeared in the past two years [1–3,4•–6•,7]. In this review, we focus on the interpretation of new evidence and concepts in glucose and sucrose sensing, the clarification of seemingly controversial points, and the implications of sugar mutant and transgenic plant phenotypes for the physiological role of sugars as plant hormones in plant growth and development.

Sugar signals, sensing, and effects

Although abundant publications have documented the effects of sugars in plants, the definition of sugar signals remains confusing. Sucrose is the major form of translocated sugars in plants and is the most frequently used sugar in studies of plant sugar responses in gene regulation and development [1–3,4•–6•,7]. However, in many cases the effects of sucrose could be completely substituted by hexoses, such as glucose and fructose. For instance, sucrose repression of photosynthesis genes can be mimicked by a lower concentration of hexoses [2,16,17,36•,37]. These results indicate that in many cases sucrose is not the direct signaling molecule. However, sucrose could have a signaling mission distinct from that of hexoses, and could be perceived by different types of sensors/receptors. Two such examples are: first, in the regulation of a sucrose symporter expression [38•]; and second, the post-transcriptional control of a leucine zipper transcription factor [31•]. These results suggest the existence of a specific sucrose sensor and signaling pathway.

Although numerous experiments have documented the role of hexoses in triggering sugar responses in plants, questions remain as to how hexose signals are sensed and whether the metabolism of hexoses is important for signaling. In the latter

Figure 1

Three glucose signal transduction pathways in plants. Three glucose sensing and signaling pathways are revealed on the basis of the analyses of glucose-inducible and glucose-repressible gene expression in transgenic *Arabidopsis* plants with enhanced or reduced AtHXK levels, and with a heterologous yeast HXK2. The HXK-independent pathway includes genes encoding asparagine synthase (AS1), β-amylase (β-AMY), chalcone synthase (CHS), cell-wall invertase (CIN1), glutamine synthase (GS2), and phenylalanine-ammonia lyase (PAL1). The HXK-dependent pathway controls chlorophyll a/b binding protein (CAB), nitrate reductase (NR1), oxygen evolving protein (OE33), phospholipase D (PLD), and ribulose-1,5-bisphosphate carboxylase (RBCS) genes. These genes show different glucose responses in transgenic *Arabidopsis* overexpressing plant AtHXK1 or yeast HXK2. AtHXK1 is used as a
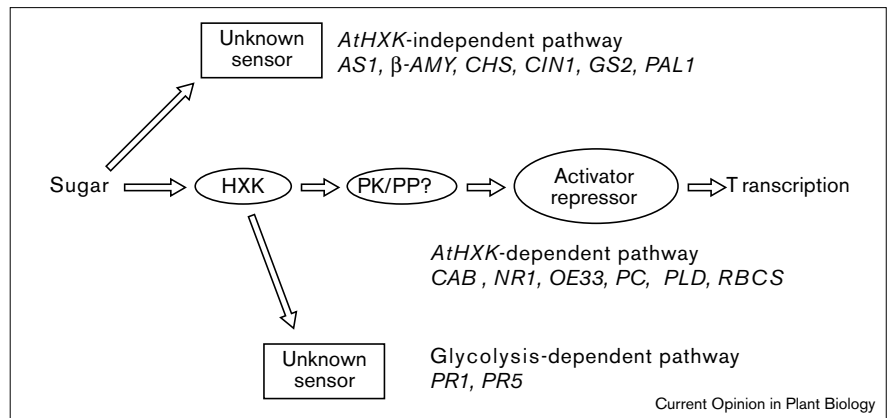
glucose sensor in this pathway that is uncoupled from glucose metabolism. The pathogenesis-related genes (*PR*) belong to the putative glycolysis-dependent pathway because enhanced *PR* gene expression is observed in transgenic *Arabidopsis* overexpressing plant AtHXK1 or yeast HXK2.

case, signaling can no longer be attributed to hexoses, but are likely to be unidentified metabolites. One approach to address the questions is to compare the effectiveness of various sugars and glucose analogues. For example, hexokinase (HXK) could be a hexose sensor because 2-deoxyglucose and mannose, the substrates of HXK, but not 6-deoxyglucose or 3-*O*-methylglucose can mimic glucose signaling in the regulation of photosynthetic and glyoxylate genes [2,15]. The use of HXK inhibitors to block glucose responses provided more supporting evidence [2,19,22]. However, the presence of unknown hexose sensors that can distinguish different glucose analogues has not been ruled out. Since 2-deoxyglucose and mannose are not effectively metabolized through glycolysis, it is suggested that glucose metabolism has a limited regulatory role [2,15,16,22]. The effectiveness of non-metabolizable 6-deoxyglucose and 3-*O*-methylglucose as sugar signals in the regulation of invertase and patatin genes supports the existence of non-HXK sugar sensors [5•,6•,17]. Recent identification of two hexose transporter-like sensors, SNF3 and RGT2, that mediate glucose regulation of glucose transporter genes in yeast has suggested that similar hexose sensors might exist in plants [5•,8]. Three unusually large glucose transporter-like proteins found in the *Arabidopsis* sequence database are potential candidates [5•]. It is likely that distinct hexose sensors are used for diverse hexose sensing and signaling pathways in plants.

The importance of HXK as a glucose sensor is further supported by the generation and characterization of transgenic *Arabidopsis* plants with altered HXK levels or with a heterologous yeast HXK that supplies a primarily catalytic function [2,16]. These studies have defined at least three distinct glucose sensing and signaling pathways: HXK-independent, HXK-dependent and metabolism-dependent, and HXK-dependent and metabolism-independent (Figure 1). Interestingly, sugar repression of photosynthesis genes and

sugar activation of pathogenesis-related genes seem to be mediated by distinct signaling pathways. Glucose regulation of some genes involved in nitrogen and carbon metabolism appears to be independent of HXK (W Xiao and J-C Jang, unpublished data).

Besides the widespread detection of sugar repression and activation of many genes with diverse functions [1–3], many vital processes — such as embryogenesis, germination, seedling development, root and leaf morphogenesis, flowering, stress responses, pathogen defense, wounding responses, and senescence — have also been shown to be influenced by sugars [2,16,23•,32,35,36•,39,40•,41,42,43•,44,45•,46]. These effects could be due to sugar signaling or metabolism or both. Exogenously applied sugars and the analyses of transgenic plants with modified invertase expression and location have linked sugar signals to photosynthesis repression, leaf senescence, altered embryo, root and shoot development, flowering transition, and pathogen defense [2,16,39,43•]. The analysis of starch mutants suggests a connection between sugars and flowering time [44]. The correlation of mitotic index and glucose distribution, revealed by a high-resolution histographical mapping method in developing bean embryos, has led to the proposal that glucose could also function as a morphogen [45]. However, the consequences of exogenous sugar applications, endogenous sugar fluctuation and distribution, and invertase manipulation are complex because the phenotypes could be due to osmotic effects, altered metabolic fluxes, as well as sugar signaling.

Molecular and biochemical mechanisms of sugar regulated gene expression

Sugar regulation of gene expression can be mediated at the transcriptional and post-transcriptional levels. Many systems,

including whole plants, intact tissues, tissue culture cells, and freshly isolated protoplasts, have been used to identify sugar responsive *cis*-DNA elements. The regulation of sugar-repressable and sugar-inducible genes is likely to be mediated by distinct mechanisms. A complicated picture emerged when six maize photosynthesis gene promoters were characterized in detail. No consensus DNA elements or negative regulatory elements were evident for the global glucose repression [27]. Thus, modulation of multiple transcription factors or chromatin might be required for this type of glucose signal transduction. Intensive investigation of the sugar repression of the rice α-amylase gene in three laboratories has revealed multiple *cis*-elements important for sugar-dependent transcription and mRNA stability [47•,48,49]. Similarly to the glucose repression of maize photosynthesis gene promoters, HXK is proposed to be the sensor mediating the sugar repression of the rice α-amylase gene [19]. *cis*-elements mediating the sugar repression of the cucumber malate synthase gene and a bean photosynthesis gene have also been characterized [50,51]. The activation of gene expression by sugars has been best studied with the promoters of genes encoding patatin, β-amylase, and vegetative storage protein [30,33,52]. Both positive and negative *cis*-elements were found. A conserved sucrose responsive element (SURE) and its cognate binding factor have also been identified [30]. A future challenge will be to connect the diverse *cis*-DNA elements on the sugar responsive promoters to the upstream sensors and signaling components through various transcription factors.

Hexokinase as a dual-function enzyme and sensor

The classical definition of HXK as a dimeric cytosolic enzyme essential for glycolysis has been the basis for controversial views on whether HXK is a glucose sensor, where intracellular glucose signals are sensed, whether the catalytic and regulatory functions of HXK are separable, and whether the ATP/AMP ratio is the actual signal. Manipulation of glucose metabolites in a simple but physiological leaf cell system showed that sugar phosphates, the changes in ATP levels, phosphate depletion, and other metabolites cannot substitute for glucose signal [2]. The inhibition of *Arabidopsis* seed germination by mannose could occur without affecting ATP or inorganic phosphate levels in seeds, and could be overcome by a HXK inhibitor [22]. These results provide evidence for the uncoupling of glucose signaling from glucose metabolism. In transgenic *Arabidopsis* plants and yeast, distinct regulatory functions in glucose repression but similar catalytic activities were exhibited by plant and yeast HXK. Therefore, glucose metabolism simply cannot account for many of the glucose responses. The function of HXK as a glucose sensor is likely to be conserved in plants, as indicated when overexpression of the *Arabidopsis* HXK1 gene promoted glucose hypersensitivity in transgenic tomato [53•] and in transformed maize leaf cells (J Sheen, unpublished data). Recent isolation of new yeast HXK2 mutants that display different enzymatic properties and ATP affinities, but similar glucose derepression, indicates that the catalytic and regulatory functions are separable [21•]. Furthermore, there is no direct correlation between the rate of sugar phosphate accumulation and the level of glucose repression in yeast cells expressing various novel HXK2 mutants [21•]. *Arabidopsis* HXK1 mutants have recently been isolated that lack the glucose repression, without corresponding changes in sugar metabolism (L Zhou and B Moore, unpublished data).

Understanding the molecular and biochemical basis of HXK function as a glucose sensor will require further investigation. As an induced-fit enzyme HXK conformation changes after binding to its substrate glucose or other hexoses, and this might be an essential mechanism of the sensing process [2,21•]. This conformational change of HXK may resemble the analogous ligand-induced conformational change of a typical receptor that allows modification of protein–protein interactions to trigger a signaling cascade. The identification of proteins that interact with HXK represents a crucial step in unraveling the mechanism of glucose sensing and signaling by HXK. The recent elucidation of how galactokinase acts as a galactose sensor provides the first vivid example of how a sugar kinase can regulate gene expression [13,14]. This signaling pathway is simple and direct involving an ATP- and galactose-dependent interaction and the regulation by galactokinase of a specific transcription repressor GAL80 and an activator GAL4 [13,14]. As hexokinase controls a broad spectrum of gene expression and developmental processes in plants, the signaling mechanism is likely to be complex and multifaceted. For example, multiple subcellular localization patterns and protein–protein interactions might allow HXK to participate in different signaling cascades in plant cells. We have observed that a significant portion of HXK is present in a large protein complex that could be important for glucose sensing and signaling (B Moore and J Sheen, unpublished data). Also, the possibility that HXK can function in the plant nucleus has not been ruled out yet since HXK protein is detected in purified nuclei of maize leaf cells (J Sheen, unpublished data). The isolation of HXK mutants that perform only signaling or only catalytic functions will be most valuable for the elucidation of the structure–function relationship of HXK as an enzyme and as a sensor. It remains unclear whether the plant AtHXK1 as a major sensor is phosphorylated as is yeast HXK2. Although the yeast S15A HXK2 mutant loses its signaling function [20], the equivalent mutation in AtHXK1 does not seem to affect glucose sensing and signaling in a transient expression assay (J Sheen, unpublished data).

Protein kinases and phosphatases in sugar signaling

The discovery and development of specific protein kinase (PK) and phosphatase (PP) activators and inhibitors in the past decade have provided valuable tools

with which to examine the involvement of protein phosphorylation/dephosphorylation in diverse signal transduction pathways [54]. It has been shown that PP1 and PP2A inhibitors can mimic glucose repression of photosynthesis genes in maize leaf cells and in photoautotrophic cultures of *Chenopodium rubrum* [17,27]. The same inhibitors also activate glucose and stress inducible invertase and phenylalanine-ammonia lyase genes in the latter system. Interestingly, glucose, PP inhibitors, and stress signals can all activate putative MAPKs that use myelin basic protein as substrate [17]. However, the differential effect of the PK inhibitor staurosporine on these signals suggests the involvement of different PKs in different transduction pathways [17]. The same PP inhibitor has also been shown to block but not activate sugar inducible β-amylase gene expression, indicating distinct phosphorylation mechanisms for glucose activation [26]. Results from experiments with inhibitors of calcium ion channels and calmodulin have been used to propose a role for calcium as a second messenger in sugar signaling. Sugar-induced increase of a plasma membrane calcium-dependent PK is an interesting observation and worth further investigation [25]. However, for the glucose repression of photosynthetic genes in maize leaf cells, no evidence for the involvement of calcium, inositolphosphate, cAMP, or cGMP could be found after treatments with a broad spectrum of reagents (J-C Jang and J Sheen, unpublished data). Thus, multiple regulatory mechanisms are used for diverse sugar responses in plants.

A plant PK with a potential function in sugar signaling is the SNF1-related PK (SnRK). At least four plant SnRKs from rye, tobacco (NPK5) and *Arabidopsis* (AKIN10 and AKIN11) can complement the yeast *snf1* mutant, which shows glucose derepression [7,9,24•,28]. This indicates that SnRK could be a regulator in plant sugar responses. As the yeast SNF1 and plant SnRK are similar to the mammalian AMP-activated PK(AMPK), it has been proposed that changes in the AMP/ATP ratio via glucose metabolism, rather than glucose itself, are important for signaling [7,9]. The direct activation of SNF1 and SnRK *in vitro by AMP* remains to be shown [9]. However, SnRK activity is required in potato for sugar activation of the sucrose synthase gene when the sugar and ATP levels are presumably high and AMP level is low [29•]. Thus, gene derepression mediated through SNF1 activation under glucose starvation in yeast cannot be directly translated to SnRK regulation of sucrose synthase gene under glucose rich condition in plants. Moreover, if the sucrose synthase gene is activated by non-metabolizable glucose analogues such as 6-deoxyglucose or 3-*O*-methylglucose in potato, as in other plant systems [6•], it will be difficult to explain the link between glucose metabolism and SnRK activation in the glucose response.

One import insight into the regulation and function of SnRK in plants is the discovery that a WD protein encoded by *Pleiotropic Regulatory Locus PRL1* interacts with AKIN10 and AKIN11 in a sugar dependent manner in yeast [23•,24•]. The *prl1* mutant exhibits pleiotropic phenotypes, including hypersensitivity to sucrose in plant growth media. *In vitro* kinase assays show that PRL1 is an inhibitor of AKIN10 and AKIN11. Although about 50% increase of AKIN kinase activity is observed in the *prl1* mutant compared with wild type, the kinase activity is stimulated similarly by 3% sucrose in both wild-type and the *prl1* mutant plants [24•]. A proposed model is that PRL1 is a negative regulator of *Arabidopsis* SNF1 homologs [24•]. From this, we would expect the sugar-activated sucrose synthase gene expression to be enhanced in the *prl1* mutant — as deduced from the analyses of transgenic potato with reduced SnRK expression [29•]. In the study of the *prl1* mutant, only the sugar-repressible sucrose synthase gene (SUS1), not the sugar-activated one was analysed [23•].

Genetics of sugar sensing and signaling

Plant sugar sensing and signaling pathways are mediated by multiple sensors and linked to plant growth and development. The underlying regulatory mechanisms are probably too complex to be completely elucidated by molecular and biochemical approaches. The power of genetics in uncovering surprising components and cross-talk will provide new and complementary means in dissecting the sugar signal transduction pathways by using *Arabidopsis thaliana* as a model system. Two general strategies have been taken to isolate sugar sensing and signaling mutants. The approach formed on the basis of sugar regulated gene repression or activation has yielded sucrose-uncoupled (*sun*), reduced sugar response (*rsr*), and low and high β–amylase (*lba*, *hba*) mutants [3,32–35]. Although these mutants were selected in the presence of sucrose, they do not define the sucrose-specific pathway as all the marker genes are also regulated by glucose [1–3,4•,6•,34–35,36•]. They might not belong to the same linear pathways — as indicated by their distinct gene expression patterns, sugar signal specificity, and morphology. There could also be altered general effects on marker gene expression that might not be directly linked to sugar levels in these mutants. For instance, the *sun1* and *sun7* mutants are different from *sun6* in displaying enhanced *PC-LUC* expression independent of sucrose and striking growth or fertility defects [32]. The reduced β–amylase gene expression in *lba2* seems to be partially due to a reduced basal level of gene expression [35]. Broader gene expression analysis could help sort out the precise deficiency in specific sugar signaling pathways. It is intriguing that *sun* mutants affect phytochrome responses [32]. The interaction between sugar and light signaling is multifaceted. For example, sugar and light have opposite effects on photosynthesis gene expression, but a synergistic effect on anthocyanin accumulation and defense gene expression [2,3,23•]. Much of the light activation of nitrate reductase gene expression could be achieved by sugar treatment in the dark [2,23•]. Similarly to light responses, the characterization of multiple sugar sensors/receptors and

Figure 2
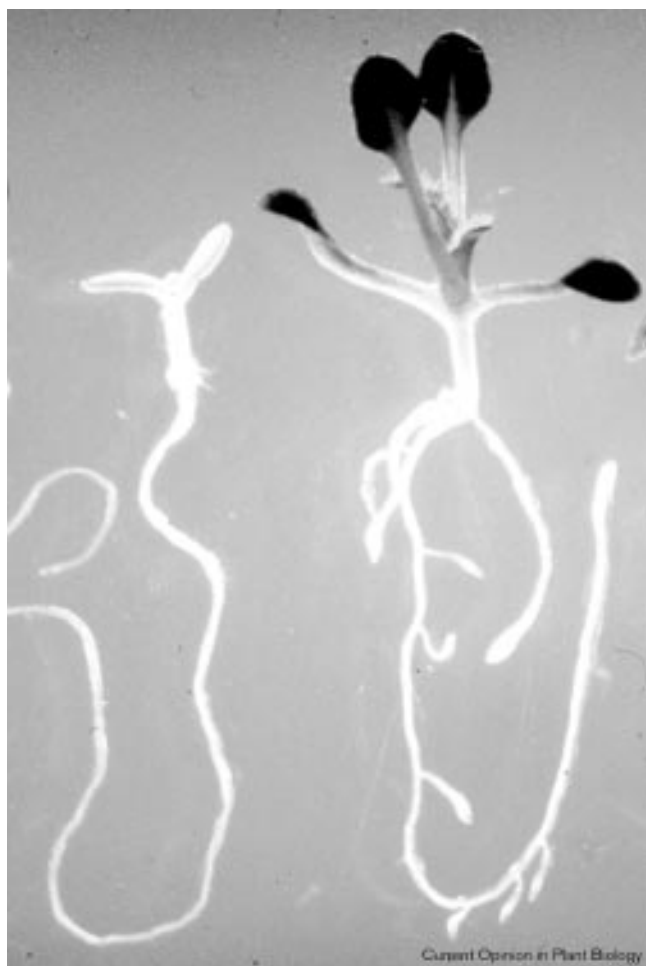
Phenotypes of the *gin1* mutant. Wild-type (left) and *gin1-1* mutant (right) plants were grown on 6% glucose medium for 10 days under constant light. The *gin1-1* mutant is insensitive to glucose repression of cotyledon greening and expansion, true leaf development, and lateral root elongation.

signaling pathways will clarify the seemingly controversial results in the future.

A second fruitful strategy for the isolation of sugar response mutants

Sugar-dependent developmental arrest at the germination or seedling stage (Figure 2) has also proven to be a useful strategy for isolating sugar response mutants. Although it is a relatively artificial condition resembling the selection of numerous plant hormone mutants, many sugar response mutants isolated by this method display interesting phenotypes in the absence of exogenous sugars. The simple selection at an early developmental stage allows an ambitious large scale genetic screen, which can be combined with the use of increasingly large pools of T-DNA mutant seeds that have become available. Mutants selected on the basis of plant development influenced by sugars include glucose insensitive (*gin*), glucose oversensitive (*glo*), carbohydrate insensitive (*cai*), sucrose insensitive (*sis*), and mannose-insensitive germination (*mig*) [3,36•]. Interestingly, the use of different sugars, selection criteria and conditions appears to select different types of mutants in terms of morphology and biochemical and molecular properties [3,36•]. However, complementation tests and mapping will be required to genetically distinguish various sugar response mutants.

The selection of the sugar response mutants by using glucose has been quite successful. The effort has so far produced *gin* mutants that show deficiencies in glucose sensing and downstream signaling and that are obviously uncoupled from sugar metabolic changes (L Zhou and J Sheen, unpublished data, [36•]). Characterization of the *gin1* mutant revealed a previously unexpected cross-talk between the glucose and ethylene signaling pathways [36•]. This discovery led to the characterization of existing ethylene overproduction (*eto*) and constitutive triple response (*ctr1*) mutants as *gin* mutants, and the ethylene insensitive mutants (*etr1* and *ein2*) as *glo* mutants [36•]. Thus, the genetic pathway originally defined as linear by the study of ethylene mutants appears to branch out and connect to the HXK-dependent glucose signaling pathway (Figure 3). The recent cloning and characterization of the *ein2* mutant also points to a link between ethylene and stress (jasmonate and oxidative) pathways [55•]. The branching and connection between the glucose and ethylene pathways are further supported by the lack of the typical ethylene 'triple response' in the dark-grown *gin1* mutant [36•] and the lack of glucose hypersensitivity in some of the *ein* mutants (L Zhou and J Sheen, unpublished data). The results from this characterization of the *gin1* mutant justify the re-examination of other existing *Arabidopsis* mutants for their responses in sugar regulated gene expression and seedling development.

Recent characterization and cloning of the new Arabidopsis *prl1* mutant provides an interesting but complicated example of sugar regulation in plants [23•]. The *prl1* mutant shows sucrose hypersensitivity in seedling growth but exhibits 'derepression' of some genes whose expression could be dependent or independent of sucrose in wild-type plants. The opposite sugar effects on gene expression and on development found in *prl1* were initially puzzling, but may be explained by its molecular identity. The *PRL1* gene encodes a WD-protein that may interact with many components involved in diverse signal transduction pathways; for example, many plant hormone responses are also altered in the *prl1* mutant [23•]. Curiously, the sugar hypersensitive phenotype of *prl1* is similar to the *fusca6* mutant that is a part of the nuclear COP9 complex [56,57]. Another plant WD-protein COP1 influences many aspects of plant development [57]. Mutation of the yeast WD-protein TUP1 results in pleiotropic phenotypes, including diminished glucose repression [58]. However, a whole genome expression analysis using yeast microarrays and RNA isolated from the *tup1* mutant shows that expression of genes affected by the *tup1* mutation is mostly (90%) distinct from those

Figure 3
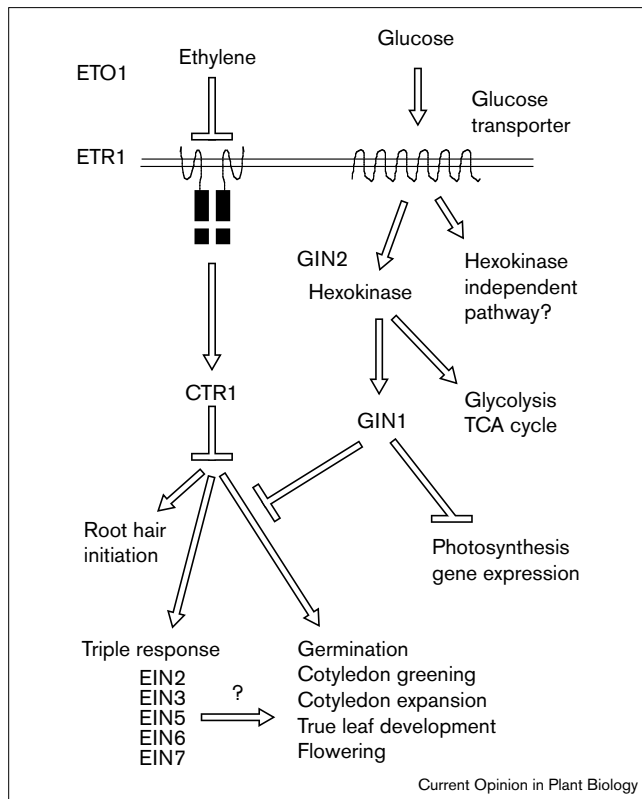
Model for glucose and ethylene signaling. The glucose and ethylene signaling pathways are antagonistic to each other. The balance between the glucose and ethylene signals determines plant growth and development. The convergent point between the two pathways is downstream of GIN1 and GIN2 (AtHXK1) in the glucose signaling pathway, and downstream of ETR1, CTR1, and EIN2 in the ethylene signaling pathway.

affected by glucose derepression [58]. Thus, PRL1 might be involved in multiple unlinked signalling pathways and cause pleiotropic phenotypes.

Glucose as a hormone and its connection to other plant hormones

The specific effects of glucose on gene expression and development are characteristic of plant hormone actions. Similar to the classic plant hormones that are also plant metabolites, the synthesis, metabolism, and transport of glucose have been well studied [1,5•]. The demonstration of HXK as a specific glucose sensor and the action of non-metabolizable glucose signals mediated through unknown sensors/receptors qualify glucose as a plant hormone with dual functions as a signaling molecule and an intermediary metabolite. The higher effective concentration of glucose relative to other hormones reflects its physiological role in allowing plants to monitor and adjust activities between sugar production and utilization.

It is not surprising that recent analyses of transgenic plants [16,39,43•,53•] and sugar response mutants [23•,32,35,36•] have uncovered intimate cross-talk between glucose and other plant hormone signaling pathways. Recent characterization of transgenic carrot plants with antisense cell wall invertase or vacuolar invertase constructs shows malformed embryos and bushy shoots that can be corrected by hexoses [43•]. These phenotypes are apparently caused by a lack of hexose signaling, which may control the balance between the plant hormones auxin and cytokinin (which are critical for embryogenesis and shoot/root development). In a recently identified *gin2* mutant, the lack of *AtHXK1* causes altered sensitivity to auxin and cytokinin (L Zhou and J Sheen, unpublished data).

The conserved F-box and leucine-rich repeats between the glucose regulated GRR1 in yeast and the auxin signaling component TIR1 in *Arabidopsis* suggest another possible connection between glucose and auxin signaling [59]. In transgenic *Arabidopsis* and tomato plants, overexpressing *AtHXK1* promotes cell death and senescence processes that are controlled by cytokinin, abscisic acid, and ethylene (W Xiao and J-C Jang, unpublished data) [2,16,53•]. In these transgenic plants, the glucose regulation of genes that are controlled by the non-traditional plant hormones, jasmonate and salicylate (that are important for plant pathogen and wounding defense), are also altered (W Xiao and J-C Jang, unpublished data). It will be interesting to determine whether the action of these hormones converge on the same DNA *cis*-element or transcription factor targets, or on upstream signaling components. The analysis of the α-amylase gene in barley embryos indicates sugar repression of a gibberellin-dependent signaling pathway [60]. Glucose repression of germination that is also controlled by abscisic acid, gibberellin, and ethylene provides another possible link between sugar and hormone signaling [22,36•]. The cross-talk between the glucose and ethylene signaling pathways has been well established from the studies of the *gin1* and various ethylene mutants [36•]. Further molecular and genetic analyses of the *gin1* and *ein* mutants might reveal the precise convergent point of the two signaling pathways. Besides interactions at the signaling level, glucose regulation of genes involved in the biosynthetic pathways of hormones might contribute to another level of connection between glucose and other plant hormones.

Sugar link to nitrogen signals

Although self-sufficient in sugar production, plant growth and development requires many nutrients from the environment. Sugar signals as an indicator of photosynthesis activity can reflect environmental and physiological status and possibly coordinate the uptake and metabolism of the major nutrient nitrogen. The regulatory relationship between sugar and nitrogen is manifested by the recent discovery that genes encoding nitrate transporters, nitrate reductase, asparagine synthase (ASN2), and glutamine synthase are activated by sugars [1,2,61,62]. The expression of a distinct asparagine synthase gene (*ASN1*) is repressed by sugars [61]. This type of gene regulation is tightly linked to the balance between sugar and nitrogen as

the sugar regulation of *ASN1* and *ASN2* can be over-ridden by asparagine, glutamate and glutamine signals [61]. Analysis of the glucose regulation of *ASN1* and *GS2* gene in transgenic *Arabidopsis* suggests that an HXK-independent pathway is involved (W Xiao and J-C Jang, unpublished data). Identification of the putative nitrogen regulator, PII-like protein, encoded by *GLB1* in Arabidopsis and castor bean indicates the existence of an evolutionarily conserved carbon and organic nitrogen sensing mechanism in plants [63•]. The expression of *GLB1* is itself regulated by sugar and amino acids. Because the *Arabidopsis* PII-like protein is localized in the chloroplast, communication between the chloroplast and nucleus is required to regulate nuclear gene transcription. Recent isolation of putative glutamate receptor genes in *Arabidopsis* raises the possibility for direct amino acid sensing through conserved sensors/receptors [64•]. Another level of interaction between sugar and nitrogen could be at the regulation of enzymatic activities. For instance, plant SnRKs may play a role in controlling carbon and nitrogen metabolism because three metabolic enzymes — HMG-CoA reductase, nitrate reductase, and sucrose phosphate synthase — have been shown to be substrates [9].

The importance of sugar and nitrogen balance in plant life is also demonstrated in maize where high nitrate signals can enhance the expression of photosynthesis genes for sugar production [27,65•]. In tobacco, nitrate has been shown to induce organic acid metabolism but to repress starch metabolism [66]. The effect of nitrate in the activation of maize photosynthesis gene expression has been proposed to be mediated through the elevation of cytokinin [65•]. Nitrate treatment in maize roots increases cytokinin accumulation more than 10-fold, and this is followed by the activation of a cytokinin-inducible response regulator gene in leaves. Thus, the mechanisms that coordinate sugar and nitrogen signals constitute an important part of a signaling network fundamental to plant growth. Mutants and transgenic plants could be used to explore the interplay between sugar and nitrate signaling.

Conclusions and perspectives

The studies of sugars as physiological signals have faced many hurdles and much skepticism due to the long-standing belief that a signaling molecule should have a single dedicated role and only act at a very low concentration in plants [11,12]. Proving that glucose is a dual-function signaling molecule and metabolite is not straightforward in plants, which produce sugars. An ingrained biochemical view of existing sugar metabolic enzymes and transporters has delayed the recent discovery of 'new' sugar sensors, such as galactokinase and glucose-transporter like proteins, after their molecular identities and genetic implication as sensors were known for a decade even in the yeast system [8,13,14,18•]. The combination of molecular, biochemical, and genetic approaches promises to unravel more detailed mechanisms underlying glucose sensing and signaling in plants.

The research progress made in the past few years has revealed a central role of glucose signal transduction in the signaling network that controls plant gene regulation and development. It is now clear that sucrose and glucose are distinct signals, and that multiple glucose sensors/receptors are mediating parallel signaling pathways to control a large number of glucose regulated genes (Figure 1). The challenges ahead include the identification of signaling components in the HXK-dependent and HXK-independent pathways by biochemical and genetic means, the identification of HXK-independent glucose sensors, the allelism and epistasis tests of different sugar response mutants, re-examination of existing hormone and development mutants for glucose responses, molecular cloning of *gin*, *sun*, *rsr*, *cai*, *lba*, *hba* and *sis* mutant loci, and the use of microarrays to gain a global and genomic view of glucose-regulated gene expression. The increasingly large seed collections mutagenized by T-DNA or transposons will serve as invaluable genetic resources for screening and cloning tagged mutants and for obtaining specific gene knockouts.

The continuous use of several plant systems for the study of sugar responses is a necessary reality. Many sugar responses have been observed in diverse plant species including the model plant *Arabidopsis thaliana*. However, *Arabidopsis* is generally considered to be less sensitive to sugars than tobacco and tomato [17,53•]. The conserved mechanisms and principles established *Arabidopsis* will be informative for similar studies carried out in other systems and crop plants. At the cellular level, multiple locations and partners will allow the same sensor or signaling molecules to conduct multiple functions. The physiological, nutritional, and developmental status of the same cell might influence responses to sugars or any other signaling molecules differently. It is also clear that sugar responses are integrated into the presence and status of other players in the signaling network. The conventional view of a linear signaling pathway is no longer realistic. The interactions between auxin and cytokinin, or between ABA and GA are well-known examples. However, thorough and creative studies that can sort out pleiotropic or specific sugar effects in mutants, will be crucial to define the physiological roles of sugar signals and to establish connections between different pathways in the plant signaling network.

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