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Update on Signal Transduction

Sugar Sensing and Sugar-Mediated Signal Transduction in Plants

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Information concerning the sugar status of plant cells is of great importance during all stages of the plant life cycle. The availability of or lack of sugars triggers many metabolic and developmental responses, and it is not surprising, therefore, that sugars profoundly affect the expression of a large number of genes (for review, see Koch, 1996; Graham, 1996). Sugar sensing occurs at the level of individual cells and the responses of such cells must be integrated at the tissue, organ, and plant level. Therefore, sugar-induced signals will interact with other sensing and signaling pathways. The mechanisms used by plant cells to sense sugars and to process this information are essentially unknown, and only recently are these questions being addressed experimentally. This lack of knowledge contrasts with the situation in yeast and bacteria, in which the molecular and physiological analysis of mutants have yielded extensive information about sugar perception (Trumbly, 1992; Ronne, 1995; Saier et al., 1995).

SUGAR SENSING IN YEAST AND ANIMALS

Yeast (*Saccharomyces cerevisiae*) serves as a model for investigating many basic biological questions about eukaryotes and is also an important paradigm for sugar sensing in plants. In yeast the availability of the preferred sugar substrate Glc signals the Glc repression phenomenon (for review, see Trumbly, 1992; Ronne, 1995; Thevelein and Hohmann, 1995). Glc repression dramatically alters yeast intermediary carbohydrate metabolism such that only Glc is being used as a carbon source, despite the presence of other readily accessible carbon sources. Glc is converted into Glu-6-P by HXK and is further metabolized via glycolysis. Genes involved in the metabolism of other carbon substrates are switched off, as are genes encoding key steps in gluconeogenic metabolism. A number of yeast mutants that are impaired in aspects of the Glc repression phenomenon have been isolated and their analysis has provided insight into the complexity of sugar sensing and signaling pathways. From these studies it was concluded that the Glc-phosphorylating enzyme HXK2 is a major Glc sensor responsible for sustained Glc repression. HXK2 activity initiates a signal transduction pathway that involves a number of different gene products (Fig. 1) and results in the repression of a large set of genes. Thus, the entry of Glc into glycolytic metabolism as mediated by HXK2 is a key step in Glc sensing.

In the repression pathway the function of two protein complexes has been elucidated. These are the GLC7 type 1 protein phosphatase complex (Tu and Carlson, 1995, and refs. therein) and the SSN6/TUP1 complex, which functions as a general repressor of transcription through modulation of chromatin structure. Binding of the SSN6/TUP1 complex to specific sites is directed by the DNA-binding protein MIG1, and in this way genes that contain MIG1-binding sites are repressed. Exactly how the HXK2, GLC7, and SSN6/TUP1/MIG1 complexes are connected is unknown. For example, in the repression pathway no substrates for the REG1/GLC7 protein phosphatase are known.

In the absence of Glc the repressed state is reversed by a different set of gene products that includes the SNF1/SNF4 protein kinase complex and the SNF2-containing chromatin modulation complex. This latter complex consists of approximately 10 proteins and reverses the Glc-induced closed chromatin conformation into an open one that can be transcribed. Like the SSN6/TUP1 complex, this SNF2 complex is a general chromatin modulator that is directed to chromosomal target sites by DNA-binding activities (transcription factors), e.g. GAL4. The SSN6/TUP1 and SNF2 complexes are probably general eukaryotic regulators of transcription. It is unknown whether the absence of Glc is sensed or whether the nonrepressed state is the default state. As for the repression pathway, the gaps in the derepression signaling pathway remain to be closed. The repressing and derepressing pathways interact. The GLC7 phosphatase complex antagonizes the function of the SNF1 protein kinase. Moreover, the SNF1 kinase can phosphorylate and thereby inactivate MIG1 (Fig. 1; Östling et al., 1996).

Several important questions remain. For example, it is unknown how the HXK-mediated conversion of Glc to Glu-6-P initiates a signaling cascade. No dominant HXK mutants have been identified in which signaling is constitutively operating in the absence of Glc and ATP. Only

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Abbreviations: dGlc, deoxyglucose; HXK, hexokinase; 3-O-mGlc, 3-*O*-methylglucose.

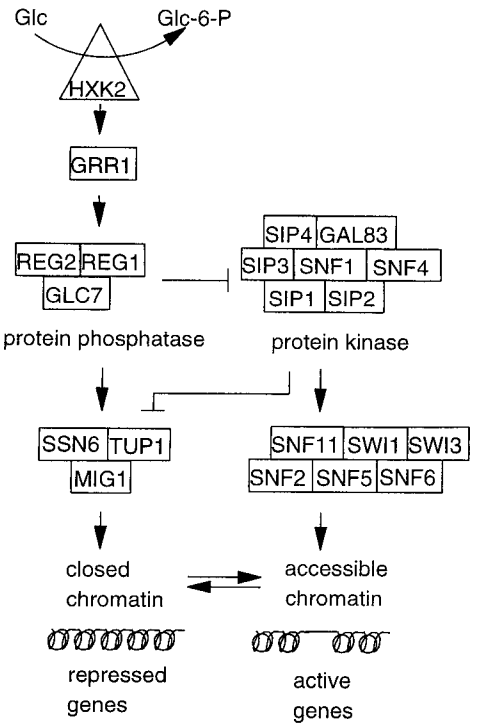

Figure 1. Overview of Glc-signaling pathways in yeast. The arrows represent gaps in the signaling cascades. Close interactions between the repression and derepression pathways exist. REG1 and REG2 proteins direct the GLC7 protein phosphatase toward the same substrates that are phosphorylated by the SNF1 protein kinase. MIG1 is a substrate of SNF1 and is inactivated by phosphorylation. More information is presented in the text and in reviews (Trumbly, 1992; Ronne, 1995). SNF1 interacts with only a subset of the proteins shown, depending on conditions.

recently has some progress been made in identifying mutants in which HXK enzymatic and signaling activities are partly separated (S. Hohmann, J.H. de Winde, J. Winderickx, and J.M. Thevelein, personal communication). Modulation of gene expression by sugars does not involve only changes in transcription; sugar sensing may affect mRNA translation and protein stability and mRNA stability as well (Ronne, 1995).

Also in mammalian systems, glucokinase (HXKIV) has been implicated as a Glc sensor (Matschinsky et al., 1993). It is required in the pancreatic β-cells for Glc-stimulated insulin release and in liver to convert excess Glc into glycogen. Point mutations in the glucokinase gene are responsible for a subset of noninsulin-dependent diabetes mellitus in humans. Targeted disruption of this gene in mice causes a severe diabetic phenotype (Grupe et al., 1995). On the contrary, β-cell-specific overexpression of yeast HXK in diabetic mice ameliorated symptoms of the disease (Epstein et al., 1992).

Whereas HXK is of major importance in yeast sugar sensing, other sensors have been identified. The yeast *SNF3* and *RGT2* genes encode Glc transporter homologs with unusually long cytosolic C-terminal domains; both SNF3 and RGT2 may act as sugar sensors (Özcan et al., 1996). Low levels of Glc are sensed by SNF3, whereas high Glc levels are sensed by RGT2. These two sugar sensors mediate the activation of different sets of Glc transporter genes and the C-terminal extension may act as a signaling domain. Dominant mutations in both genes have been identified in which the mutant sensors signal changes in gene expression. These mutants show that Glc metabolism as such is not required for signaling by these transporter homologs and that all components of the signaling pathway are present in the absence of Glc.

SUGAR SENSING IN PLANTS

Sugars control the expression of many plant genes and thereby many metabolic and developmental processes (Koch, 1996). Evidence for the existence of three different sugar-sensing systems in plants has been obtained in recent years (Fig. 2). These are (a) an HXK-sensing system similar to the one described above for yeast and animals, (b) a hexose transport-associated sensor, again similar to the situation in yeast, and (c) a Suc-specific pathway, which may involve a signaling Suc transporter. The exact mechanisms by which plants sense sugar and initiate signal transduction are still largely unknown.

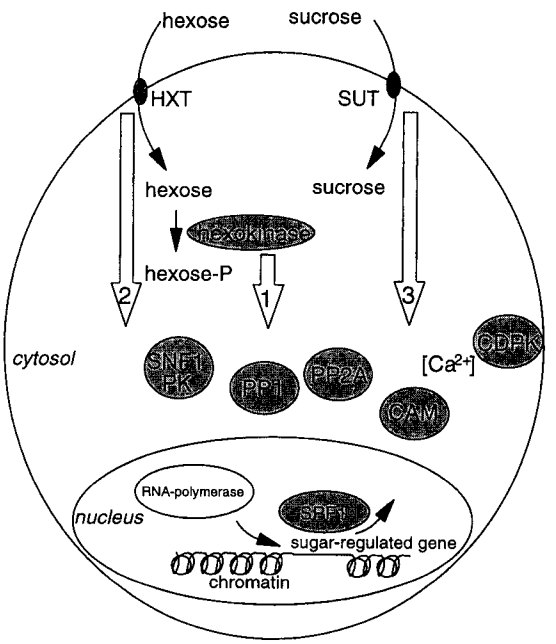

Figure 2. Sugar-sensing mechanisms discovered in plants. Hexokinase and metabolite transporters (hexose, HXT, and Suc, SUT) are proposed to function as sugar sensors that initiate signal transduction processes as indicated by the open arrows numbered 1, 2, and 3 (see text). SNF1 homologs, protein phosphatases (PP1 and PP2A), Ca^{2+} concentration, calmodulin (CAM), and a membrane-bound Ca^{2+}-dependent protein kinase (CPDK) have been implicated in signal transduction. SPF1 is a DNA-binding protein that binds to *cis*-elements in the promoters of several sugar-regulated genes. *SPF1* mRNA levels are down-regulated by sugars, suggesting that it acts as a negative regulator.

THE HXK-SENSING SYSTEM

Several lines of investigation have shown that HXK-mediated sugar sensing controls diverse processes and metabolic pathways in plants. One process is the sugar-induced feedback inhibition of photosynthesis, which has been described for many higher plants and is thought to be universal. It overrides regulation of photosynthesis by light, tissue type, and developmental stage (von Schaewen et al., 1990; Krapp et al., 1993; Sheen, 1994). The accumulation of carbohydrates in source leaves leads to inhibition of photosynthesis and a concomitant decrease in Rubisco protein, other Calvin-cycle enzymes, and chlorophyll. Repression of transcription of many photosynthesis genes is an important mechanism for sustained feedback inhibition of photosynthesis.

Decreased *RBCS* (encoding the small subunit of Rubisco) transcript levels were also observed in a *Chenopodium rubrum* photoautotrophic cell suspension when cultured in the presence of Glc (Krapp et al., 1993). Glc metabolism is essential for repression, since addition of Glc analogs, which cannot be phosphorylated, such as 6-dGlc and 3-O-mGlc, is ineffective. Jang and Sheen (1994) used a maize protoplast transient expression system to monitor the effects of a variety of sugars, Glc analogs, and metabolic intermediates on the promoter activity of genes encoding photosynthetic enzymes. They showed that Glc and other hexoses that are substrates of HXK cause repression at a low concentration (1–10 mM). The Glc analog 2-dGlc is a substrate for HXK but is not further metabolized. 2-dGlc was also an effective inhibitor of gene expression but various metabolic intermediates were ineffective, eliminating the involvement of glycolysis or other metabolic pathways. Mannoheptulose, a specific inhibitor of HXK, was able to block the repression triggered by 2-dGlc. Because the phosphorylated products did not act as repression signals, a role for HXK as a key sensor and signal transmitter of sugar repression of photosynthetic genes in higher plants was proposed. In this protoplast system, next to Glc, acetate could also inhibit expression of photosynthesis genes, suggesting that a HXK-independent sensing mechanism affects these genes as well.

Recently, more direct evidence for the involvement of HXK in the repression of photosynthesis genes was presented by Jang et al. (1997). Two HXK genes from Arabidopsis, *AtHXK1* and *AtHXK2*, were cloned and used in overexpression and antisense experiments to investigate the in vivo function of HXK in sugar sensing. When wild-type Arabidopsis seeds were germinated on a medium containing elevated levels of Glc (6%), hypocotyl elongation and greening of cotyledons were repressed. In addition, the expression of photosynthesis genes such as *RBCS* and *CAB* was reduced. Transgenic plants in which the expression of *AtHXK1* and *AtHXK2* is reduced by an antisense approach showed a markedly reduced sensitivity to Glc compared with wild-type Arabidopsis. On the other hand, HXK overexpression resulted in enhanced Glc sensitivity of the transgenic plants. In a separate experiment the yeast HXK2 gene was introduced in Arabidopsis and Glc sensitivity was tested. Such transgenic *35S*-HXK2 lines showed reduced Glc sensitivity similar to the HXK antisense lines. By competing effectively for the same substrate, Glc, the yeast enzyme bypassed the endogenous signaling HXKs, resulting in reduced Glc sensitivity (Fig. 3). Thus, yeast HXK2 is enzymatically active in plants but its signaling function is not retained.

Sugars induce the expression of many genes and Jang et al. (1997) investigated whether the HXK system is also involved in induction of gene expression. In Arabidopsis the expression of the nitrate reductase gene *NR1* is induced by sugars. Arabidopsis lines that overexpress *AtHXK* show higher Glc-induced *NR1* steady-state mRNA levels compared with wild-type plants, suggesting that HXK is important for signaling expression of sugar-inducible genes as well. However, other explanations for this observation cannot be ruled out.

HXK-mediated sugar sensing occurs not only in leaves but is important in nongreen tissues as well. In higher plants the glyoxylate cycle plays an important role in the mobilization of lipid reserves during early seedling growth. In a cucumber cell culture system the glyoxylate cycle genes malate synthase (*MS*) and isocitrate lyase (*ICL*) were shown to be repressed by the addition of Glc to the growth medium. 2-dGlc and Man, which are both phosphorylated by HXK but not further metabolized, specifically repressed *MS* and *ICL* gene expression. However, the addition of 3-O-mGlc, which cannot be phosphorylated by HXK, did not result in repression (Graham et al., 1994).

The germination of Arabidopsis seeds can be effectively inhibited by the addition of 2-dGlc or Man to the medium, whereas, again, 3-O-mGlc is ineffective (J. Pego and S. Smeekens, unpublished data). The HXK inhibitor mannoheptulose relieved the Man-induced block of germination.

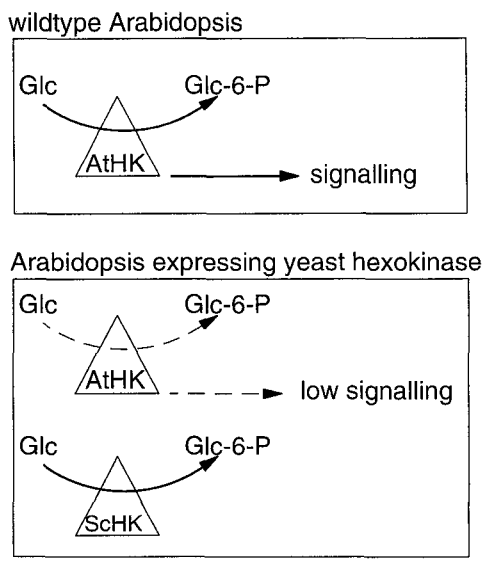

Figure 3. HXK enzymatic activity (AtHK) initiates a signal transduction process resulting in altered gene expression. In transgenic plants expressing the enzymatically active but nonsignaling yeast HXK (ScHK), the endogenous AtHK is deprived of substrate (Glc) and in this way the endogenous signaling system is bypassed, resulting in reduced sensitivity to Glc.

Arabidopsis is an oil seed and during germination the lipids are converted to Suc in a process that involves the glyoxylate cycle. Thus, HXK signaling may inhibit mobilization of lipids and, possibly, other storage compounds as well and in this way inhibit germination. These results provide evidence for an important function of sugar-induced HXK signaling in lipid metabolism and germination.

The combined evidence suggests that HXK-mediated sugar sensing and signaling are of major importance in both autotrophic and heterotrophic plant tissues and are reminiscent of the situation in yeast. Many questions remain to be answered. For example, how many different HXK genes are present in plants (Schnarrenberger, 1990); in which tissues are they expressed; what is the intracellular localization of the proteins; what is their substrate specificity; and which HXKs contribute to hexose-induced signaling and how are they controlled?

In a recent paper Herbers et al. (1996) suggested that hexose sensing occurs in association with the secretory (Golgi-ER) system. When yeast invertase is expressed in the cytosol, apoplast, or vacuole, Suc hydrolysis results in excessive monosaccharide release. Only with the apoplastic and vacuolar constructs is the hexose sensed, i.e. *CAB* genes are repressed and defense genes are induced. Cytosolic expression of yeast invertase did not induce changes in expression of these genes, even though the hexoses released are, in principle, substrates for HXK. Since apoplast- and vacuole-targeted invertases are transported through the plant endomembrane system, the authors concluded that sensing must occur in association with this system. That Suc is present in the endomembrane system and the Glc released there is sensed was shown by an experiment that involved a bacterial fructosyltransferase gene (Turk et al., 1997). Fructosyltransferase converts Suc into fructans with concomitant release of equimolar amounts of Glc. Expression of fructosyltransferase in the endomembrane system results in the accumulation of fructans and a bleached phenotype of the plants indicative of down-regulation of photosynthesis genes. These observations could also be explained by assuming that hexose influx into the cytosol is sensed but not the actual cytosolic hexose concentration. In this view the signaling hexokinase could be associated with monosaccharide transporters present in membranes lining the cytosolic compartment.

SUGAR TRANSPORTERS AND SUGAR SENSING

Evidence for the presence of monosaccharide transporter proteins with a signaling function comes from experiments with Glc analogs that are taken up by cells but not phosphorylated by HXK, such as 3-O-mGlc and 6-dGlc. In a photoautotrophic cell-suspension culture of *C. rubrum*, the addition of Glc or Suc induced the expression of genes encoding extracellular invertase and Suc synthase. This induction could be mimicked by 6-dGlc (Godt et al., 1995; Roitsch et al., 1995). The induction by 6-dGlc suggests that nonphosphorylated Glc is the signal for the sugar-induced expression of both genes. This type of sugar sensing seems to be evolutionarily conserved, since it is also reported for the unicellular green alga *Chlorella kessleri*. This alga is able to grow both autotrophically and, on Glc, in the dark. Switching *C. kessleri* cells to heterotrophic growth by adding Glc induced several genes, among which was a Glc transporter gene (*HUP1*). The Glc effect could be mimicked by 6-dGlc, and it was suggested that a Glc transporter functions as a sensor (Hilgarth et al., 1991).

The sugar- and amino acid-induced patatin class I (*B33*) promoter is also induced by the Glc analogs 6-dGlc and 3-O-mGlc in transgenic Arabidopsis plants expressing GUS under the control of the *Pat(B33)* promoter (Martin et al., 1997). This result suggests that at the intact plant level hexose transport, but not further metabolism, signals induction of gene expression.

Whereas in yeast specialized hexose transporters can function as sugar sensors (Özcan et al., 1996), there is no direct evidence for the involvement of plant signaling sugar transporters, and the above results can also be explained by assuming the presence of sugar-binding proteins with a signaling function.

SUC SENSING

Suc is an important sugar transport and storage molecule in plants and may have a signaling function as well. Several observations suggest that Suc as a molecule can be sensed by plants. Suc-specific induction of gene expression has been reported for the patatin promoter (Wenzler et al., 1989; Jefferson et al., 1990) and for the phloem-specific *rolC* promoter (Yokoyama et al., 1994). Since Suc can readily be hydrolyzed in Glc and Fru, it is difficult to establish a direct function for the Suc molecule. In these cases combinations of Glc and Fru were less effective.

Recently, an Arabidopsis Leu zipper gene, *ATB2*, was discovered that is controlled by Suc (F. Rooks and S. Smeekens, unpublished data). The expression of the *ATB2* gene is repressed posttranscriptionally at physiological Suc concentrations (50–100 mM). Other sugars and other combinations of Glc and Fru were ineffective in this repression. Arabidopsis seedlings have the capacity to synthesize Suc when Glc is added to the medium (Dijkwel et al., 1996). It is likely, therefore, that not the actual Suc concentration but the influx of Suc into the cell is being sensed. A Suc transporter protein may be involved but, here also, direct evidence is lacking.

HOW IS THE SIGNAL TRANSDUCED?

We can postulate that the activated sugar sensors initiate a signal transduction cascade resulting in a response, e.g. altered gene expression, but current knowledge about the signaling cascades is fragmentary. As mentioned before, the yeast *SNF1*-encoded protein Ser/Thr kinase plays a central role in derepression of several Glc-repressed genes. Putative *SNF1* homologs have been isolated from a number of plant species. Southern-blot analysis shows the presence of a small gene family in several species (Halford et al., 1992; Muranaka et al., 1994). Several of these homologs can restore SNF1 function in yeast *snf1* mutants, suggesting a role in carbon metabolism in plants as well, but to our

knowledge, no reports for such a function in plants have been published.

The use of inhibitors of other signaling pathways has provided some insight into potential intermediary steps in signaling and roles have been implied for protein phosphorylation and dephosphorylation, Ca^{2+}, and calmodulin. The Suc-inducible accumulation of mRNAs for sporamin and β-amylase in sweet potato depends on both the phosphorylation and continuous dephosphorylation of proteins presumably involved in expression regulation. Specific inhibitors of protein-Ser/Thr phosphatases 1 (PP1) and 2A (PP2A), as well as inhibitors of Ser/Thr protein kinases, strongly inhibited the sugar-induced accumulation of sporamin and β-amylase mRNAs (Ohto et al., 1995, and refs. therein). The use of inhibitors of calmodulin and Ca^{2+} channels showed the involvement of Ca^{2+} signaling in this system. Furthermore, results of experiments with transgenic tobacco (*Nicotiana tabacum* L.) plants that expressed a Ca^{2+}-sensitive photoprotein of jellyfish, aequorin, indicated indirectly that an increase in cytoplasmic Ca^{2+} occurs in leaf cells of tobacco during incubation with sugars. Ohto and Nakamura (1995) reported a sugar-inducible Ca^{2+}-dependent Ser/Thr protein kinase associated with the plasma membrane in leaf tissues of tobacco. It was suggested that Ca^{2+}-dependent Ser/Thr protein kinases could be involved in regulating the activity of sugar transporters in the plasma membrane.

Similar studies have also been performed with sugar-repressed genes. In cultivated rice cells the expression of α-amylase genes such as *amy3D* is repressed by sugars (Lue and Lee, 1994). In this system protein phosphatase inhibitors strongly induce *amy3D* expression and an AMP-activated protein kinase may be involved in induction.

The analysis of promoters of sugar-responsive genes has revealed regulatory sequences and *trans*-acting factors that bind to elements in the promoters. In the 5′ upstream region of the potato class I patatin gene, tuber-specific and Suc-inducible elements were found in close proximity (Liu et al., 1990). Using internal deletions, Grierson et al. (1994) were able to separate the promoter elements responsible for tissue-specific and Suc-inducible expression. The promoter element found to confer Suc-inducible expression, named SURE for Sucrose Responsive Element, showed similarity with the SP8 motifs first reported by Ishiguro and Nakamura (1994, and refs. therein). These motifs in the promoter region of the Suc-induced β-amylase and sporamin genes from sweet potato are bound by a nuclear factor called SP8BF. Also, the SURE site in the patatin promoter binds a nuclear factor with similar binding specificity to SP8BF. However, these SP8/SURE elements are not present in the 5′ upstream regions of all Suc-inducible genes, and other *cis*-acting sequences are probably responsible for Suc induction in these genes.

cDNA clones have been isolated from sweet potato petioles that bind the SP8 motif and these clones encode a new type of DNA-binding protein named SPF1. The SPF1 mRNA was detected in petioles, leaves, stems, and tuberous roots, and its transcript levels decreased when leaf-petiole cuttings were treated with Suc levels that induce accumulation of sporamin and β-amylase mRNAs, suggesting that SPF1 is a negative regulator (Ishiguro and Nakamura, 1994). A putative SPF1 homolog has also recently been isolated from cucumber (Kim et al., 1997).

SUGAR-SENSING MUTANTS

In addition to the molecular and biochemical analysis of sugar-sensing pathways, mutants can be invaluable, both to identify genes involved in sensing and signaling and to analyze the physiological function and interactions of such genes. Arabidopsis is being used for such mutant screens in several laboratories using different approaches (Table I). The plastocyanin (*PC*) gene of Arabidopsis is transiently activated, independently of light, during early seedling development (Dijkwel et al., 1996). In seedlings carrying a *PC*-promoter luciferase reporter gene (*PC-LUC*) construct, luciferase activity is similarly induced after 2 d of growth in the dark. This transient increase in *PC* mRNA and luciferase activity levels can be repressed by Suc. Mutants defective in Suc repression were identified on the basis of high luminescence levels when grown on plates with 3% Suc (Dijkwel et al., 1997). Such Suc-uncoupled (*sun*) mutants show no or reduced Suc repression of luminescence. In these mutants the accumulation of endogenous *PC*, *CAB*, and *RBCS* mRNAs was similarly insensitive to Suc repression.

The sugar sensitivity of three mutants was tested at the mature rosette stage (van Oosten et al., 1997). Whole-plant photosynthesis (gas exchange), *PC* expression, and total extractable Rubisco activity in the *sun6* mutant were unaffected by the presence of 2 mM 2-dGlc in the watering solution. These conditions strongly reduce photosynthesis, gene expression, and Rubisco activity in wild-type Arabidopsis and two other mutants tested (*sun1–2* and *sun7*). 2-dGlc is a substrate for HXK but does not enter glycolytic

Table I. *Compilation of sugar-sensing mutant classes isolated by different laboratories*

All mutants were isolated by screens at the germination or young seedling stage except *lba* and *hba*; instead, true leaves of older plants were used.

Acronym	Name	Screen	Reference
sun	Suc-uncoupled	PC-LUC, 88 mM Suc	Dijkwel et al. (1997)
gin	Glc-insensitive	Growth on 330 mM Glc	Zhou et al. (1996)
mig	Man-insensitive germination	Growth on 7.5 mM Man	J. Pego and S. Smeekens, unpublished data
rsr	Reduced sugar response	Pat(B33)-GUS, 90 mM Suc	Martin et al. (1997)
cai	Carbohydrate insensitive	Low nitrogen, 100 mM Suc	Boxall et al. (1996)
lba	Low-level β-amylase	Amylase activity, 175 mM Suc	Mita et al. (1997a)
hba	High-level β-amylase	Amylase activity, 175 mM Suc	Mita et al. (1997b)

metabolism. *sun6* germination is also insensitive to 6% Glc, further supporting the conclusion that it is affected in a HXK-dependent signaling pathway. The expression of the *PC* is controlled by phytochrome, and the presence of Suc in the medium resulted in altered PHYA responses in these mutants compared with wild-type PHYA responses (Dijkwel et al., 1997). The mutants thus provide genetic evidence for an intimate relationship between light and sugar sensing and/or signaling.

As mentioned above, Arabidopsis seedling development is arrested at high (6%) Glc levels. A number of Glc-insensitive (*gin*) mutants have been isolated in Arabidopsis in which seedling development is less sensitive to the presence of 6% Glc in the growth medium (Zhou et al., 1996). Other mutants that are insensitive to increased carbohydrate levels are the carbohydrate-insensitive (*cai*) mutants. Growth of Arabidopsis seedlings on medium containing high (100 mM) Suc but low nitrate levels results in increased intracellular sugar concentrations. Wild-type seedlings grown under these conditions accumulate high levels of anthocyanins and are low in chlorophyll. When these growth conditions have been used, a number of mutants have been isolated that do not accumulate anthocyanins and have significantly higher levels of chlorophyll (Boxall et al., 1996).

Sensing and transduction mutants were also isolated using the monosaccharide Man. As mentioned above, Man is phosphorylated by HXK to Man-6-P, which only slowly enters metabolism. HXK signaling inhibits germination and this principle was used to isolate Man-insensitive germination (*mig*) mutants from ethyl methansulfonate-mutagenized and T-DNA- and transposon-tagged seed collections (J. Pego and S. Smeekens, unpublished data). Several of the *sun* mutants also show a *mig* and/or *gin* phenotype.

The above mutants were isolated by screens based on sugar-mediated repression of gene expression or plant development by sugars. Screens for mutants in sugar induction have also yielded several mutants. The patatin class I (*B33*) promoter is induced by sugars, and signaling mutants were selected by using transgenic Arabidopsis plants harboring the *Pat(B33)-iudA* construct in a nondestructive GUS activity assay. In this way reduced sugar response (*rsr*) mutants were identified in which Suc-induced expression of patatin is perturbed. Genetic analysis suggests that one of the mutants isolated, *rsr4*, is codominant (Martin et al., 1997) and likely encodes an activator, whereas most other sugar-sensing mutants isolated are recessive and probably encode repressing activities. Also, mutants have been isolated in Arabidopsis in which expression of the gene for β-amylase exhibits an altered response to sugars (Mita et al., 1997a, 1997b). These low-level beta amylase (*lba*) mutants have reduced ability to induce β-amylase gene expression when sugars are present. Remarkably, the Arabidopsis Landsberg *erecta* (L*er*) ecotype represents a natural *lba* mutant, named *lba2*. A single recessive L*er* trait reduced the sugar responsiveness of β-amylase gene expression. In the same amylase activity screen of Mita et al. (1997a), a mutant (*hba1*) was identified in which β-amylase is constitutively expressed, independently of the presence of sugars in the medium. In both *lba* and *hba* mutants the expression of only a subset of sugar-regulated genes is affected, suggesting the existence of several potentially independent signal transduction pathways.

FUTURE PROSPECTS

The question of how plants sense sugars and how this sensing induces signal transduction and altered gene expression is being approached using a variety of molecular and biochemical techniques and is complemented by using Arabidopsis as a genetic system for identification of the genes involved. Information from these different sources still is very limited and the challenge will be to close the gaps and establish functional relations between the individual sensing and signaling steps. Equally challenging will be to unravel interactions with other signaling pathways that allow plants to integrate their responses to altered conditions. Arabidopsis mutants will be important tools in these studies since they allow the functional investigation of the genes in the plant. The coming years will yield rapid progress in the field. It is sobering to think that the "perfect" yeast system, after more than 15 years of investigations, still has many important but unanswered questions about sugar sensing and signaling.

Sugar-sensing systems control metabolic pathways and developmental decisions in plants. Most approaches to modify these processes through the manipulation of individual metabolic conversions ("limiting" steps) have met with limited success (Stitt and Sonnewald, 1995). Knowledge about the endogenous mechanisms used by plants to control metabolic pathways may allow for the predictable modification of carbohydrate production and utilization in plants.

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