Plant Sugar-Response Pathways. Part of a Complex Regulatory Web[1]

Susan I. Gibson*

Department of Biochemistry and Cell Biology, MS140, Rice University, 6100 Main Street, Houston, Texas 77005–1892

In addition to playing a central role in metabolism, soluble sugars such as Glc and Suc help regulate many developmental and physiological processes in plants (for review, see Koch, 1996; Smeekens, 1998; Sheen et al., 1999; Yu, 1999). For example, sugar levels have been postulated to play an important role in determining the time at which some plant species flower. Treatments that induce flowering can also lead to increased transport of carbohydrates from leaves to shoot apical meristems (Corbesier et al., 1998). This increased sugar transport takes place prior to the rise in metabolic activity that occurs during the transition to flowering, suggesting that sugar levels do not simply rise in response to greater metabolic demand and that sugars may be acting to signal the transition to flowering (for review, see Bernier et al., 1993). Recent findings that Arabidopsis can be induced to flower under conditions of complete darkness by supplying the aerial portions of the plant with exogenous Suc also suggest a role for sugar levels in regulating floral induction (Roldán et al., 1999). A role for sugars in seed germination is suggested by reports that exogenous sugars allow wild-type seeds to germinate in the presence of abscisic acid (ABA; Garciarrubio et al., 1997; Finkelstein and Lynch, 2000). Other developmental processes affected by soluble sugar levels include tuber formation by potatoes (Müller-Röber et al., 1992) and control of root to shoot ratios in a variety of plant species (for review, see Wilson 1988). Sugars are also thought to help control key metabolic processes such as photosynthesis (Krapp et al., 1993) and starch synthesis and breakdown (for review, see Koch, 1996). Strong evidence for the importance of sugars in controlling plant processes is also provided by reports that sugars help regulate the expression of a significant number of plant genes (for review, see Koch, 1996).

Whereas sugars have been implicated in control of many plant processes, the molecular mechanisms by which sugars act remain largely unknown. In contrast, sugar-response pathways have been relatively well characterized in the yeast *Saccharomyces cerevisiae* (for review, see Johnston, 1999). Information obtained from studies on yeast may be used to make predictions regarding plant sugar-response pathways. For example, as yeast mediate sugar responses via multiple signal transduction pathways, plants may also be expected to utilize several sugar-response pathways. In addition, plant sugar-response pathways may employ homologs of some of the components of yeast sugar-response pathways. The available evidence suggests that some of the most important factors thought to act in yeast sugar-response pathways, such as hexokinase (Hohmann et al., 1999) and SNF1 protein kinase (for review, see Hardie et al., 1998), also play important roles in plant sugar-response pathways. However, although plant and yeast sugar-response pathways are likely to share many features, plants utilize some factors not involved in yeast sugar-response pathways. For example, a calcium-dependent protein kinase acts in sugar-regulated gene expression in plants, but is not known to play a similar role in yeast (Ohto and Nakamura, 1995).

Despite the importance of soluble sugars in regulating plant development and physiology, many fundamental questions regarding plant sugar responses have barely begun to be addressed. For example, precisely which plant processes are sugar regulated remains controversial. In addition, which plant processes are affected by sugars acting in metabolism, as opposed to in signaling, has yet to be determined in most cases. In fact the identities of the molecule(s) that trigger sugar-response pathways remain in question. Although Glc or Suc may act directly as signaling molecules in some sugar-response pathways, other pathways may sense the level of a different sugar or sugar metabolite. In addition, some sugar-response pathways may sense the rate of flux through a particular metabolic pathway, rather than the absolute levels of sugars or sugar metabolites (Krapp et al., 1993). The pathways by which plants respond to sugars as signaling molecules remain to be elucidated. Characterization of these pathways is complicated by the fact that they form part of a complex regulatory web that also includes phytohormone and environmental-response pathways. Several approaches are being employed to address these issues. The remainder of this review focuses on a description of these approaches and some of the results obtained using them.

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* E-mail sig@bioc.rice.edu; fax 713–348–5154.

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USE OF SUGAR ANALOGS TO CHARACTERIZE PLANT SUGAR RESPONSES AND RESPONSE PATHWAYS

Experiments aimed at testing the effects of sugar concentration on plant developmental or physiological processes often employ treatments designed to alter endogenous sugar concentrations. However, these experiments typically do not allow a distinction to be made between the role of sugars as metabolites as opposed to as signaling molecules.

How then can the role of sugars as signaling molecules be distinguished from their role as metabolites? One possibility is to use non-metabolizable sugar analogs. In theory, demonstration that a non-metabolizable sugar analog can trigger a particular sugar response should provide a good indication that the response is the result of sugars acting as signaling molecules, rather than as metabolites. In addition, it should be possible to use different sugar analogs to obtain information about sugar-response pathways. For example, to determine whether uptake via a specific sugar-transport system is necessary to trigger a particular sugar response, the effects of sugar analogs that are, or are not, substrates for that transport system can be determined. In a similar manner, whether phosphorylation by hexokinase is an essential step in a sugar-response pathway can be assessed by comparing the effects of sugar analogs that are hexokinase substrates versus those that are not. This type of analysis has led to the hypothesis that phosphorylation of sugars by hexokinase, but not further metabolism, plays a key role in triggering sugar-regulated expression of certain genes (Graham et al., 1994; Jang and Sheen, 1994).

WHICH SUGAR ANALOGS ARE REALLY "NON-METABOLIZABLE"?

Experiments of the type described above require the availability of appropriate sugar analogs. Experiments to test the role of a particular Glc transporter in sugar responses, for example, ideally would compare the effects of a Glc analog that is transported as efficiently as Glc with the effects of a Glc analog that is not transported at all. Figure 1 presents an idealized model of the transport and metabolism of some of the most commonly used sugar analogs. Although this idealized model may be fairly accurate for some plant systems, unfortunately it is quite inaccurate for others. For example, celery suspension cultures have similar growth rates when fed Man, Suc, or mannitol, suggesting that contrary to the idealized model, Man and mannitol are efficiently metabolized by these cells (Stoop and Pharr, 1993). In *Chenopodium rubrum* cell-suspension cultures, the primary metabolic products of exogenously-supplied Man and 2-deoxy-Glc are not Man-6-P and 2-deoxy-Glc-6-P (Klein and Stitt, 1998). Also, 3-*O*-methyl-Glc is a poor substrate for some Glc-uptake systems (Komor et al., 1985). Therefore, the extent to which a particular sugar analog is transported and metabolized varies tremendously among plant species. As a result, unequivocal interpretation of sugar-analog experiments requires that transport and metabolism of the analogs be characterized in the species used in the experiments. This characterization, unfortunately, has yet to be reported for some popular model organisms, such as Arabidopsis.

DO HEXOKINASES ACT AS SENSORS?

The question of how plants sense sugar is fundamental to our understanding of plant sugar-response pathways. In yeast, hexokinases have been postulated to have dual functions in Glc sensing and phosphorylation. According to this model the conformational alteration induced in hexokinase PII during binding and/or phosphorylation of Glc may allow hexokinase PII to interact with some as yet unidentified factor(s), thereby triggering sugar responses

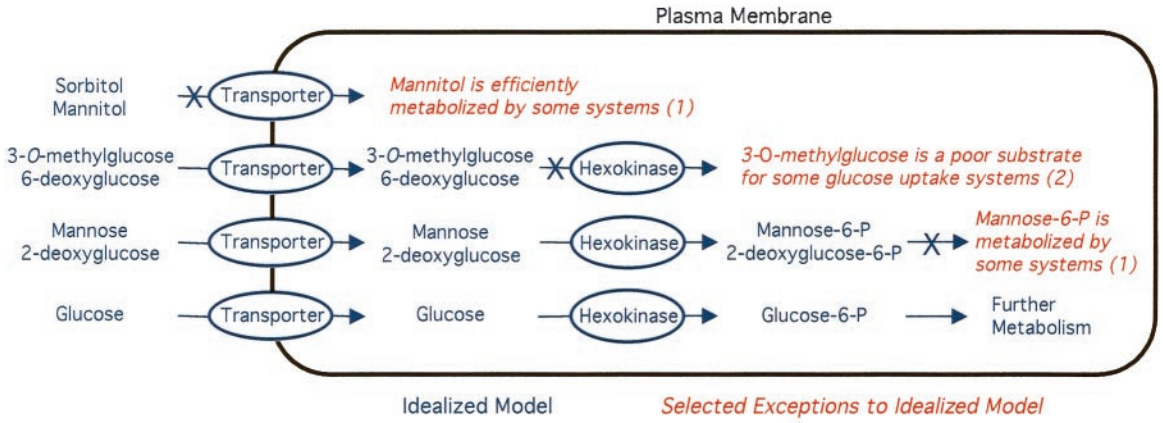

Figure 1. Idealized model of sugar-analog metabolism. Arrows indicate different steps in transport and metabolism of sugar analogs. Crosses through arrows indicate that, according to the idealized model (shown in blue), the indicated compounds do not progress through that step. Selected exceptions to the idealized model are shown in red and italicized. "1" refers to reference by Stoop and Pharr (1993). "2" refers to reference by Komor et al. (1985).

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(Entian and Fröhlich, 1984). Hexokinases have also been suggested to act as hexose sensors in plants (Graham et al., 1994; Jang and Sheen, 1994). However, models postulating that hexokinases play a role in Glc sensing that is separable from their role in Glc phosphorylation remain controversial and require further testing (Halford et al., 1999). Progress toward testing these models is provided by the recent identification of mutations that have differential effects on the hexose-sensing and phosphorylation activities of yeast hexokinase PII (Hohmann et al., 1999). In addition, transgenic Arabidopsis expressing a yeast hexokinase gene exhibit increased hexokinase catalytic activity, but are actually less sensitive to exogenous Glc (Jang et al., 1997). These findings suggest that it is possible to separate the Glc sensing and phosphorylation activities of hexokinases. Further identification and characterization of hexokinase mutants should help clarify the role of hexokinases in hexose sensing.

Although the debate over whether hexokinases act as sensors for some sugar-response pathways is likely to continue, the available evidence indicates that hexokinases are not involved in all plant sugar-response pathways. For example, expression of several genes is known to be regulated by Suc, but not by Glc, suggesting that hexokinases are not involved in sugar-regulated expression of these genes (Chiou and Bush, 1998; Rook et al., 1998; Loreti et al., 2000; Müller et al., 2000).

SNF1-RELATED PROTEIN KINASES ACT AS "METABOLIC SENSORS"

The SNF1-related protein kinases represent a large family of proteins that are conserved among animals, fungi, and plants. Many organisms encode several SNF1-related protein kinases. In mammalian systems, SNF1-related protein kinases were first identified as key components of environmental stress-response pathways. In *S. cerevisiae*, SNF1 protein kinases are activated by Glc deprivation and play a central role in response to nutritional stress. Both environmental and nutritional stresses result in increased AMP:ATP ratios, suggesting that SNF1-related protein kinases may function as "metabolic sensors" that enable organisms to regulate metabolism and gene expression in response to changes in cellular energy status. Although less is known about the role(s) of SNF1-related protein kinases in plants, they are likely to be involved in response to nutritional and/or environmental stress (for review, see Hardie et al., 1998). A role for these proteins in plant sugar-response pathways is suggested by experiments in which antisense expression of a SNF1-related protein kinase gene in potato resulted in loss of sugar-inducible expression of Suc synthase (Purcell et al., 1998). A role for plant SNF1-related protein kinases in stress responses is similarly suggested by the finding that mutations in the Arabidopsis *SOS2* gene, which encodes a protein that is similar in sequence to the yeast SNF1 protein kinase, lead to an osmo-sensitive phenotype (Liu et al., 2000). As plant SNF1-related protein kinases may function in both environmental stress and sugar responses, some apparent sugar responses may actually result from stimulation of an SNF1-mediated stress-response pathway, rather than induction of an SNF1-mediated sugar-response pathway.

Understanding how plant SNF1-related protein kinases act as metabolic sensors will require increased information regarding the regulation of these proteins and the identities of their substrates. Progress in this area has been rapid, with recent reports indicating Suc deprivation, cytokinin, and light increase transcript levels of a SNF1-related protein kinase from wheat (Ikeda et al., 1999). In addition, an SNF1-related protein kinase is up-regulated by ABA and inhibits gibberellin-induced expression of certain genes (Gómez-Cadenas et al., 1999). These findings suggest that phytohormones play an important role in some SNF1-mediated responses. Several SNF1-related protein kinase substrates have been identified. Proteins shown to be phosphorylated, and consequently inactivated, by SNF1-related protein kinases include 3-hydroxy-3-methylglutaryl coenzyme A reductase, nitrate reductase, and Suc phosphate synthase. As these enzymes play key roles in isoprenoid biosynthesis, nitrogen metabolism, and Suc synthesis, respectively, their inactivation by SNF1-related protein kinases provides a means by which these kinases may help regulate several major metabolic pathways (Sugden et al., 1999).

IDENTIFYING SUGAR-RESPONSE MUTANTS

A powerful method for characterizing plant sugar responses is to identify and analyze mutants that are defective in one or more sugar responses. Sugar-response mutants can also serve as invaluable tools for identifying components of sugar-response pathways. In recent years, several laboratories have conducted genetic screens to identify plant sugar-response mutants (Table I). These screens have used Arabidopsis as a model system and have generally employed one of two approaches. One approach has been to screen for mutants that are defective in the expression of a particular sugar-regulated gene. Mutants isolated using this approach include the *lba* and *hba* mutants, which are defective in β-amylase expression (Mita et al., 1997a, 1997b), the *rsr* mutants, which are defective in patatin expression (Martin et al., 1997), and the *sun* mutants, which are defective in plastocyanin expression (Dijkwel et al., 1997).

A second approach used to identify sugar-response mutants is to screen for altered sensitivity to the inhibitory effects of high concentrations of exogenous Glc or Suc on early seedling development. As

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Table I. *Sugar-response mutants and corresponding loci*

Mutants	Original Selection	Loci	References
rsr	Reduced sensitivity to Suc induction of patatin expression		Martin et al., 1997
lba	Reduced sensitivity to Suc induction of β-amylase expression		Mita et al., 1997b
hba	Increased sensitivity to Suc induction of β-amylase expression		Mita et al., 1997a
sun	Reduced sensitivity to Suc repression of plastocyanin expression	*sun6* Is allelic to *abi4*	Dijkwel et al., 1997; Huijser et al., 2000
sis	Reduced sensitivity to Glc or Suc-mediated inhibition of early seedling development	*sis1* Is allelic to *ctr1* *sis4* Is allelic to *aba2* *sis5* Is allelic to *aba4*	Laby et al., 2000; S. Gibson, R. Laby, and D. Kim, unpublished data
gin	Reduced sensitivity to Glc-mediated inhibition of early seedling development	*gin1* Is allelic to *aba2* *gin6* Is allelic to *abi4*	Zhou et al., 1998; Arenas-Huertero et al., 2000; J. Sheen, personal communication
prl	Increased sensitivity to sugar-mediated inhibition of early seedling development	*PRL1* Encodes a WD-40 protein	Németh et al., 1998; Bhalerao et al., 1999

shown in Figure 2, wild-type Arabidopsis seeds sown on media containing high (e.g. 0.3 M) concentrations of Suc germinate, but the majority of seedlings fail to develop green, expanded cotyledons or true leaves. High concentrations of exogenous Glc exert a similar effect. It is interesting that germinating seeds/seedlings are only sensitive to high sugar concentrations during the first approximately 48 h after the start of imbibition. This finding suggests that completion of some critical, but as yet unidentified, developmental or metabolic transition results in loss of susceptibility to high sugar concentrations (S. Gibson, R. Laby, and D. Kim, unpublished data).

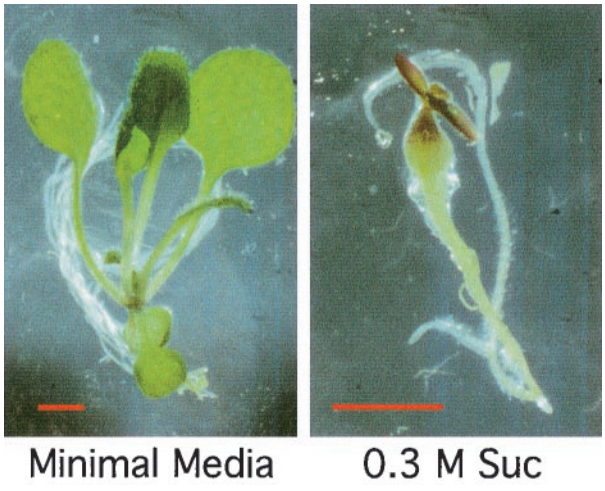

Figure 2. Effects of high concentrations of exogenous sugar on seedling development. Wild-type Arabidopsis seedlings were grown on the indicated media for 16 d prior to photographing. The red bars indicate 2 mm.

The *sugar-insensitive*, or *sis* (Laby et al., 2000) and *Glc-insensitive*, or *gin* (Zhou et al., 1998; Arenas-Huertero et al., 2000), mutants exhibit reduced sensitivity to high sugar concentrations. As high concentrations of Suc and Glc inhibit wild-type seedling development, one of the first questions that arises is whether these mutants exhibit altered sensitivity to both sugars, or just to the one used in their selection. To date, there have been no reports regarding the response of *gin* mutants to Suc. However, the *sis* mutants have altered responses to both sugars (Laby et al., 2000; S. Gibson, D. Kim, L. Hoot, and R. Laby, unpublished data). These results suggest that Suc inhibits wild-type seedling development by being metabolized to Glc, thereby triggering a hexose-response pathway. Besides being insensitive to high concentrations of Glc and Suc, at least most of the *sis* and *gin* mutants also exhibit decreased sensitivity to the inhibitory effects of exogenous Man on early seedling development (Zhou et al., 1998; Laby et al., 2000).

In contrast to the *sis* and *gin* mutants, the *pleiotropic regulatory locus 1* (*prl1*) mutant shows increased sensitivity to Glc and Suc (Németh et al., 1998). The *PRL1* gene encodes a protein with sequences characteristic of WD-40 repeat proteins (Németh et al., 1998). It is interesting that the PRL1 protein interacts with the yeast SNF1 protein, as well as with two SNF1-related proteins from Arabidopsis, in a yeast two-hybrid system (Bhalerao et al., 1999). These results suggest that PRL1 may play an important role in one or more sugar-response pathways. However, as SNF1 and related proteins may act in stress responses, as well as in sugar responses, the possibility that PRL1 pri-

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marily functions in stress responses must also be considered (Gibson and Graham, 1999).

MANY SUGAR-RESPONSE MUTANTS ARE OSMO-TOLERANT

As high concentrations of exogenous sugars were used to identify the *prl1, sis*, and *gin* mutants, an important question is whether these mutants are defective in their response to osmotic stress. When grown on concentrations of either mannitol or sorbitol that are equimolar to the Glc or Suc concentrations used in their selection (0.3–0.33 M), the *gin1* (Zhou et al., 1998), *sis1, 2, 4*, and *5* (Laby et al., 2000; S. Gibson, D. Kim, L. Hoot, and R. Laby, unpublished data) mutants appear similar to wild-type plants. However, when grown on higher concentrations of sorbitol, the *sis1, 2, 4*, and *5* mutants display osmo-tolerant phenotypes during early seedling development (Laby et al., 2000; S. Gibson, D. Kim, L. Hoot, and R. Laby, unpublished data). Testing of the *gin1* mutant at higher concentrations of sorbitol or mannitol has not been reported. The molecular basis for the osmo-tolerant phenotype of these mutants currently remains to be determined. One possibility is that mutants defective in the ability to sense and/or respond to sugar may accumulate unusually high concentrations of endogenous sugars, which could have an osmo-protectant effect.

Although most, and possibly all, mutants that are resistant to the inhibitory effects of high concentrations of exogenous sugars on early seedling development are also resistant to osmotic stress during the same developmental time period, several lines of evidence suggest that the mutants' sugar-response phenotypes are not simply a result of osmotic-stress tolerance. First, osmo-tolerant and sugar-response phenotypes are genetically separable, as some mutants, such as the *abi2-1* mutant, that are osmo-tolerant exhibit normal sugar responses (Laby et al., 2000). In addition, at least most of the sugar-response mutants characterized to date are also resistant to the inhibitory effects of concentrations of Man (e.g. 1–4 mM) that are too low to exert an osmotic stress. Man is a Glc analog that has been postulated to inhibit seed germination via a hexokinase-mediated sugar-response pathway (Pego et al., 1999). However, Man metabolism has yet to be analyzed in Arabidopsis. Therefore, the possibility remains that hexokinase-mediated inhibition of Arabidopsis seed germination by Man is the result of Man-6-P being metabolized to form a toxic product, rather than of induction of a sugar-response pathway. As a result, the possibility that some sugar-response mutants may have relatively broad defects in stress responses, rather than being specifically defective in sugar responses, cannot be ruled out at this time.

MANY SUGAR-RESPONSE MUTANTS ARE ALSO DEFECTIVE IN PHYTOHORMONE RESPONSE OR METABOLISM

Characterization of plant sugar-response mutants reveals that many of them also exhibit defects in phytohormone response or metabolism. For example, the *prl1* mutant shows increased sensitivity not only to sugars, but also to ABA, ethylene, cytokinin, and auxin (Németh et al., 1998). In addition, the *sis5* (Laby et al., 2000), *sun6* (Huijser et al., 2000), and *gin6* (Arenas-Huertero et al., 2000) mutants are allelic to the ABA-insensitive mutant *abi4*, and the *sis4* (Laby et al., 2000) and *gin1* (J. Sheen, personal communication) mutants are allelic to the ABA-biosynthesis mutant *aba2* (Table I). It is interesting that mutations in the *ABI5* gene, but not mutations in the *ABI1*, *ABI2*, or *ABI3* genes also confer a weak sugar-insensitive phenotype (Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000). These results are significant because they show that only mutations in specific *ABI* genes cause a sugar-insensitive phenotype. Only certain paclobutrazol-resistant mutants also display a sugar-insensitive phenotype. For example, the *sis2* mutant is resistant to high concentrations of sugar and to paclobutrazol, an inhibitor of gibberellin biosynthesis. In contrast, the *spy3* mutant is resistant to paclobutrazol, but not to high concentrations of sugar (D. Kim, L. Hoot, and S. Gibson, unpublished data). Mutants that over-produce ethylene or that exhibit a constitutive ethylene response also have sugar-insensitive phenotypes (Zhou et al., 1998; S. Gibson, R. Laby, and D. Kim, unpublished data).

The above results raise the question of how certain mutations might affect both sugar responses and phytohormone responses or metabolism. For example, how might mutations in the *ABI4* gene lead to ABA and sugar-insensitive phenotypes? As shown in Figure 3, several different types of mechanisms are possible. Distinguishing between these mechanisms will require more information about ABA and sugar-response pathways than is currently available. One possibility is that ABA and Glc act in the same pathway, with either acting first (Fig. 3, A and B). For example, ABA might act via a signal transduction pathway that requires ABI4, but not ABI1, 2, or 3 to induce expression of a Glc sensor. Wild-type plants would then be expected to produce relatively low levels of the Glc sensor in response to endogenous ABA. Endogenous Glc might then act via the Glc sensor to cause low level activation of a pathway that slows early seedling development. Exogenous ABA or Glc might then lead to over-stimulation of this pathway, strongly inhibiting early seedling development. Plants carrying mutations in *ABI4* would be ABA insensitive as a result of being unable to increase Glc sensor levels in response to ABA. These plants would also be Glc insensitive as a result of producing lower levels of the Glc sensor in response to endogenous ABA.

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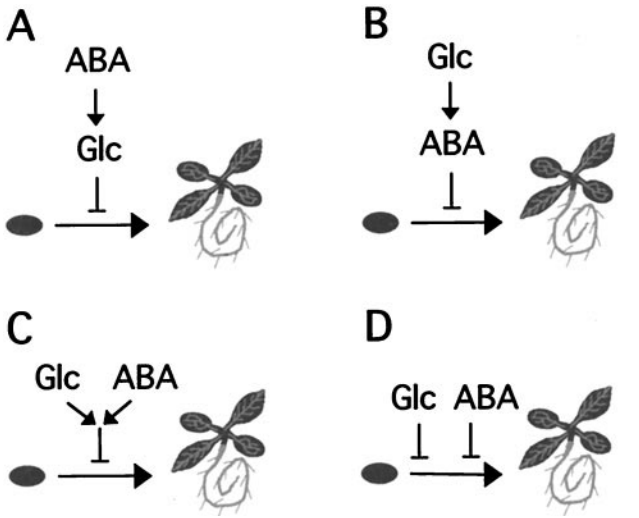

Figure 3. Models for inhibition of early seedling development by Glc and ABA. Glc and ABA may inhibit seed germination and early seedling development via one of the following mechanisms, or by a combination of two or more of them. A, Glc and ABA may act in the same pathway, with ABA acting first. B, Glc and ABA may act in the same pathway, with Glc acting first. C, Glc and ABA may act via converging pathways. D, Glc and ABA may act via completely independent pathways.

Glc and ABA, alternatively, might act via pathways that are initially independent, but then converge (Fig. 3C). For example, Glc and ABA might act via independent pathways to induce expression of ABI4, thereby activating a pathway that slows early seedling development. Mutations in ABI4 would then lead to ABA- and Glc-insensitive phenotypes. The relationship between the converging pathways might also be less direct. For example, they might have complementary effects on second messenger levels. Glc and ABA might also act via completely independent pathways (Fig. 3D). For example, ABA (acting via a pathway that requires ABI4, but not ABI1, 2 or 3) and Glc might inhibit expression of different lipase genes involved in seed storage lipid mobilization. A mutation in *ABI4* might then result in increased levels of the ABA-inhibited lipase, which could lead to a Glc-insensitive phenotype by compensating for decreased levels of the Glc-inhibited lipase in response to exogenous Glc.

The mechanism(s) by which phytohormones and sugars inhibit early seedling development are likely to be even more complex than suggested by the above models. First, these models describe responses to only a single phytohormone (ABA) and Glc, whereas several phytohormones, Glc, light, and other factors are involved in early seedling development. Second, more than one of the above models may be required to describe the mechanism(s) by which just one phytohormone and Glc affect early seedling development. In other words, ABA and Glc might each affect early seedling development via several response pathways. Some of these pathways might be involved in response to ABA and Glc, whereas others might be involved in response to only one of these factors. Precedent for this type of "combinatorial" model is provided by studies on the effects of ABA and gibberellin on seed germination. ABA and gibberellin are postulated to regulate seed germination via several pathways, some of which are responsive to both phytohormones and others of which are responsive to just one phytohormone. For example, ABA and gibberellin have antagonistic effects on Ca^{2+} levels, whereas only gibberellin acts via effects on cGMP levels (for review, see Lovegrove and Hooley, 2000).

CONCLUSIONS

Plant development, physiology, and metabolism are regulated by input from a number of signaling/response pathways. These pathways include those involved in response to phytohormones, environmental stimuli, and metabolites such as sugars and nitrogen. In recent years it has become increasingly clear that the idea that plants respond to each of these stimuli via separate, linear pathways is oversimplified. Instead, many researchers feel it may be more useful to consider plant response pathways as forming an interconnected web. A signal that affects one part of the web can then affect other parts of the web, more or less strongly and directly. As a result, determining whether a mutation affects response to a particular stimulus by altering a factor that acts directly or indirectly in a response pathway for that stimulus, or by exerting a compensatory effect on a response pathway for a different stimulus, may be difficult.

Elucidation of sugar-response pathways, as well as characterization of the relationships between these pathways and other response pathways, will require much more extensive knowledge regarding the components of these pathways than is currently available. Isolation of more genes identified by sugar-response mutations represents an important step toward this goal. The availability of these genes will then make possible, for example, experiments designed to detect direct, physical interactions between the factors encoded by these genes, as well as regulation of one gene product by another. Use of high-density cDNA microarrays and similar technologies to identify genes regulated in response to a variety of stimuli will also help provide information critical to constructing models of plant response webs.

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LITERATURE CITED

Arenas-Huertero F, Arroyo A, Zhou L, Sheen J, León P (2000) Analysis of *Arabidopsis* glucose insensitive mutants, *gin5* and *gin6*, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. Genes Dev **14:** 2085–2096

Bernier G, Havelange A, Houssa C, Petitjean A, Lejeune P (1993) Physiological signals that induce flowering. Plant Cell **5:** 1147–1155

Bhalerao RP, Salchert K, Bakó L, Ökrész L, Szabados L, Muranaka T, Machida Y, Schell J, Koncz C (1999) Regulatory interaction of PRL1 WD protein with *Arabidopsis* SNF1-like protein kinases. Proc Natl Acad Sci USA **96:** 5322–5327

Chiou T-J, Bush DR (1998) Sucrose is a signal molecule in assimilate partitioning. Proc Natl Acad Sci USA **95:** 4784–4788

Corbesier L, Lejeune P, Bernier G (1998) The role of carbohydrates in the induction of flowering in *Arabidopsis thaliana*: comparison between the wild type and a starchless mutant. Planta **206:** 131–137

Dijkwel PP, Huijser C, Weisbeek PJ, Chua N-H, Smeekens SCM (1997) Sucrose control of phytochrome A signaling in *Arabidopsis*. Plant Cell **9:** 583–595

Entian K-D, Fröhlich K-U (1984) *Saccharomyces cerevisiae* mutants provide evidence of hexokinase PII as a bifunctional enzyme with catalytic and regulatory domains for triggering carbon catabolite repression. J Bacteriol **158:** 29–35

Finkelstein RR, Lynch TJ (2000) Abscisic acid inhibition of radicle emergence but not seedling growth is suppressed by sugars. Plant Physiol **122:** 1179–1186

Garciarrubio A, Legaria JP, Covarrubias AA (1997) Abscisic acid inhibits germination of mature *Arabidopsis* seeds by limiting the availability of energy and nutrients. Planta **203:** 182–187

Gibson SI, Graham IA (1999) Another player joins the complex field of sugar-regulated gene expression in plants. Proc Natl Acad Sci USA **96:** 4746–4748

Gómez-Cadenas A, Verhey SD, Holappa LD, Shen Q, Ho THD, Walker-Simmons MK (1999) An abscisic acid-induced protein kinase, PKABA1, mediates abscisic acid-suppressed gene expression in barley aleurone layers. Proc Natl Acad Sci USA **96:** 1767–1772

Graham IA, Denby KJ, Leaver CJ (1994) Carbon catabolite repression regulates glyoxylate cycle gene expression in cucumber. Plant Cell **6:** 761–772

Halford NG, Purcell PC, Hardie DG (1999) Is hexokinase really a sugar sensor in plants? Trends Plant Sci **4:** 117–119

Hardie DG, Carling D, Carlson M (1998) The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Annu Rev Biochem **67:** 821–855

Hohmann S, Winderickx J, de Winde JH, Valckx D, Cobbaert P, Luyten K, de Meirsman C, Ramos J, Thevelein JM (1999) Novel alleles of yeast hexokinase PII with distinct effects on catalytic activity and catabolite repression of *SUC2*. Microbiology **145:** 703–714

Huijser C, Kortstee A, Pego J, Weisbeek P, Wisman E, Smeekens S (2000) The *Arabidopsis SUCROSE UNCOUPLED-6* gene is identical to *ABSCISIC ACID INSENSITIVE-4*: involvement of abscisic acid in sugar responses. Plant J **23:** 577–586

Ikeda Y, Koizumi N, Kusano T, Sano H (1999) Sucrose and cytokinin modulation of *WPK4*, a gene encoding a SNF1-related protein kinase from wheat. Plant Physiol **121:** 813–820

Jang J-C, León P, Zhou L, Sheen J (1997) Hexokinase as a sugar sensor in higher plants. Plant Cell **9:** 5–19

Jang J-C, Sheen J (1994) Sugar sensing in higher plants. Plant Cell **6:** 1665–1679

Johnston M (1999) Feasting, fasting and fermenting: glucose sensing in yeast and other cells. Trends Genet **15:** 29–33

Klein D, Stitt M (1998) Effects of 2-deoxyglucose on the expression of *rbc*S and the metabolism of *Chenopodium rubrum* cell-suspension cultures. Planta **205:** 223–234

Koch KE (1996) Carbohydrate-modulated gene expression in plants. Annu Rev Plant Physiol Plant Mol Biol **47:** 509–540

Komor E, Schobert C, Cho B-H (1985) Sugar specificity and sugar-proton interaction for the hexose-proton-symport system of *Chlorella*. Eur J Biochem **146:** 649–656

Krapp A, Hofmann B, Schäfer C, Stitt M (1993) Regulation of the expression of *rbc*S and other photosynthetic genes by carbohydrates: a mechanism for the "sink regulation" of photosynthesis? Plant J **3:** 817–828

Laby RJ, Kincaid MS, Kim D, Gibson SI (2000) The *Arabidopsis* sugar-insensitive mutants *sis4* and *sis5* are defective in abscisic acid synthesis and response. Plant J **23:** 587–596

Liu J, Ishitani M, Halfter U, Kim C-S, Zhu J-K (2000) The *Arabidopsis thaliana SOS2* gene encodes a protein kinase that is required for salt tolerance. Proc Natl Acad Sci USA **97:** 3730–3734

Loreti E, Alpi A, Perata P (2000) Glucose and disaccharide-sensing mechanisms modulate the expression of α-amylase in barley embryos. Plant Physiol **123:** 939–948

Lovegrove A, Hooley R (2000) Gibberellin and abscisic acid signalling in aleurone. Trends Plant Sci **5:** 102–110

Martin T, Hellmann H, Schmidt R, Willmitzer L, Frommer WB (1997) Identification of mutants in metabolically regulated gene expression. Plant J **11:** 53–62

Mita S, Hirano H, Nakamura K (1997a) Negative regulation in the expression of a sugar-inducible gene in *Arabidopsis thaliana*: a recessive mutation causing enhanced expression of a gene for β-amylase. Plant Physiol **114:** 575–582

Mita S, Murano N, Akaike M, Nakamura K (1997b) Mutants of *Arabidopsis thaliana* with pleiotropic effects on the expression of the gene for β-amylase and on the accumulation of anthocyanin that are inducible by sugars. Plant J **11:** 841–851

Müller J, Aeschbacher RA, Sprenger N, Boller T, Wiemken A (2000) Disaccharide-mediated regulation of sucrose:

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fructan-6-fructosyltransferase, a key enzyme of fructan synthesis in barley leaves. Plant Physiol **123:** 265–273

Müller-Röber B, Sonnewald U, Willmitzer L (1992) Inhibition of the ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage protein genes. EMBO J **11:** 1229–1238

Németh K, Salchert K, Putnoky P, Bhalerao R, Koncz-Kálmán Z, Stankovic-Stangeland B, Bakó L, Mathur J, Ökrész L, Stabel S, Geigenberger P, Stitt M, Rédei GP, Schell J, Koncz C (1998) Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in *Arabidopsis*. Genes Dev **12:** 3059–3073

Ohto M, Nakamura K (1995) Sugar-induced increase of calcium-dependent protein kinases associated with the plasma membrane in leaf tissues of tobacco. Plant Physiol **109:** 973–981

Pego JV, Weisbeek PJ, Smeekens SCM (1999) Mannose inhibits Arabidopsis germination via a hexokinase-mediated step. Plant Physiol **119:** 1017–1023

Purcell PC, Smith AM, Halford NG (1998) Antisense expression of a sucrose non-fermenting-1-related protein kinase sequence in potato results in decreased expression of sucrose synthase in tubers and loss of sucrose-inducibility of sucrose synthase transcripts in leaves. Plant J **14:** 195–202

Roldán M, Gómez-Mena C, Ruiz-García L, Salinas J, Martínez-Zapater JM (1999) Sucrose availability on the aerial part of the plant promotes morphogenesis and flowering of *Arabidopsis* in the dark. Plant J **20:** 581–590

Rook F, Gerrits N, Kortstee A, van Kampen M, Borrias M, Weisbeek P, Smeekens S (1998) Sucrose-specific signalling represses translation of the *Arabidopsis ATB2* bZIP transcription factor gene. Plant J **15:** 253–263

Sheen J, Zhou L, Jang J-C (1999) Sugars as signaling molecules. Curr Opin Plant Biol **2:** 410–418

Smeekens S (1998) Sugar regulation of gene expression in plants. Curr Opin Plant Biol **1:** 230–234

Stoop JMH, Pharr DM (1993) Effect of different carbon sources on relative growth rate, internal carbohydrates, and mannitol 1-oxidoreductase activity in celery suspension cultures. Plant Physiol **103:** 1001–1008

Sugden C, Donaghy PG, Halford NG, Hardie DG (1999) Two SNF1-related protein kinases from spinach leaf phosphorylate and inactivate 3-hydroxy-3-methylglutaryl-coenzyme A reductase, nitrate reductase, and sucrose phosphate synthase in vitro. Plant Physiol **120:** 257–274

Wilson J (1988) A review of evidence on the control of shoot:root ratio, in relation to models. Ann Bot **61:** 433–449

Yu S-M (1999) Cellular and genetic responses of plants to sugar starvation. Plant Physiol **121:** 687–693

Zhou L, Jang J-C, Jones TL, Sheen J (1998) Glucose and ethylene signal transduction crosstalk revealed by an *Arabidopsis* glucose-insensitive mutant. Proc Natl Acad Sci USA **95:** 10294–10299

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CORRECTIONS

Vol. 124: 935–939, 2000

Schopfer, C.R., and Nasrallah, J.B. Self-Incompatibility. Prospects for a Novel Putative Peptide-Signaling Molecule.

Figure 2 was erroneously printed in black and white. Figure 2 has been reprinted in color on p 2204.

Vol. 124: 1007–1017, 2000

Stotz, H.U., Pittendrigh, B.R., Kroymann, J., Weniger, K., Fritsche, J., Bauke, A., and Mitchell-Olds, T. Induced Plant Defense Responses against Chewing Insects. Ethylene Signaling Reduces Resistance of Arabidopsis against Egyptian Cotton Worm But Not Diamondback Moth.

The GenBank accession number of the β-glucosidase gene was not included when this article was first published. The GenBank accession number is AJ251301.

Vol. 124: 1511–1514, 2000

Dennison, K.L., and Spalding, E.P. Glutamate-Gated Calcium Fluxes in Arabidopsis.

Figure 1 was erroneously printed in black and white in the original publication and again in Vol. 125 on p 1151. Figure 1 has been reprinted in color on p 2205.

Vol. 124: 1532–1539, 2000

Gibson, S.I. Plant Sugar-Response Pathways. Part of a Complex Regulatory Web.

In Table I, the line "*sis5* Is allelic to *aba4*" should have appeared as "*sis5* Is allelic to *abi4*." Table I has been reprinted on p 2206.

Vol. 125: 15–19, 2001

Meyerowitz, E.M. Prehistory and History of Arabidopsis Research.

Professor Georges Bernier of the Universite de Liege (Belgium) kindly sent the following corrections for the photographs that appeared as Figures 1 and 2. In Figure 1, the last person on the right of the first row is Silvano Bonotto, not J. Bouharmont; in the third row, between A.R. Kranz and M. Jacobs, the unidentified person is J. Bouharmont. In Figure 2, in the back row, the person identified as Matigne is in fact R. Matagne. We welcome any additional information on the names of those who appear in the photographs.

Vol. 125: 329–338, 2001

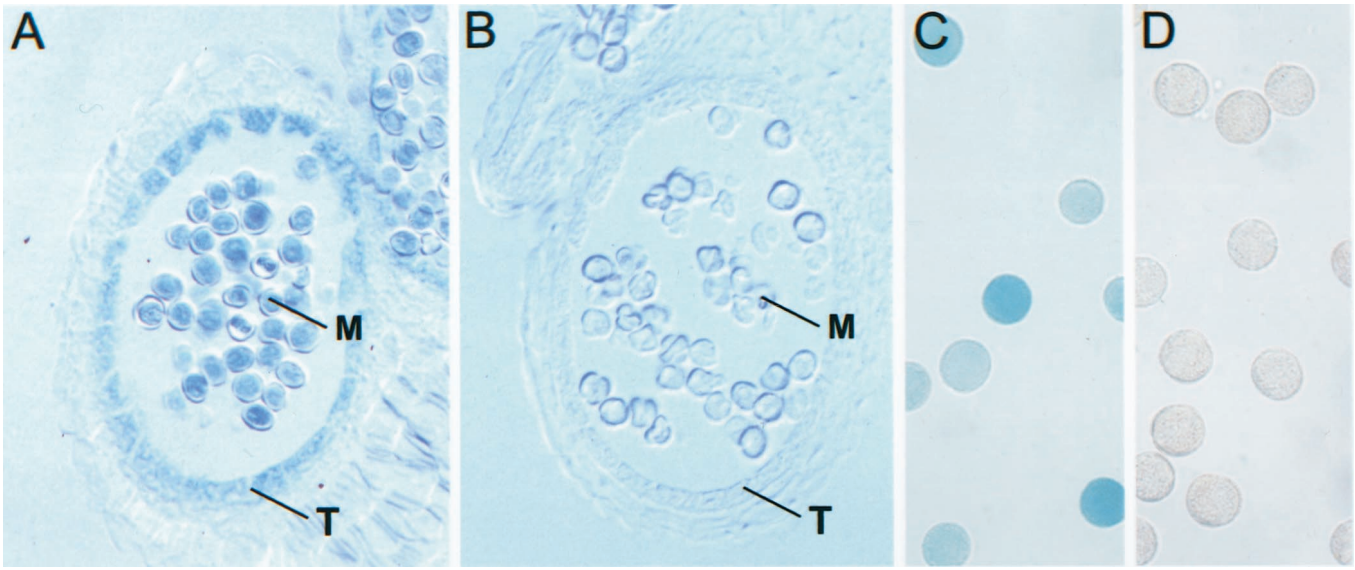
Taylor, A.R., and Assmann, S.M. Apparent Absence of a Redox Requirement for Blue Light Activation of Pump Current in Broad Bean Guard Cells.

Figures 2, 3, and 4 were not printed in the correct order. The correctly numbered figures with legends are reprinted on pp 2207–2209.

Acknowledgment

Vol. 125: No. 1, ii, 2001

We would like to acknowledge Jan Zeevaart, who supplied the photograph of the morning glory flower that appears on the cover of the January 2001 75th Anniversary Special Issue.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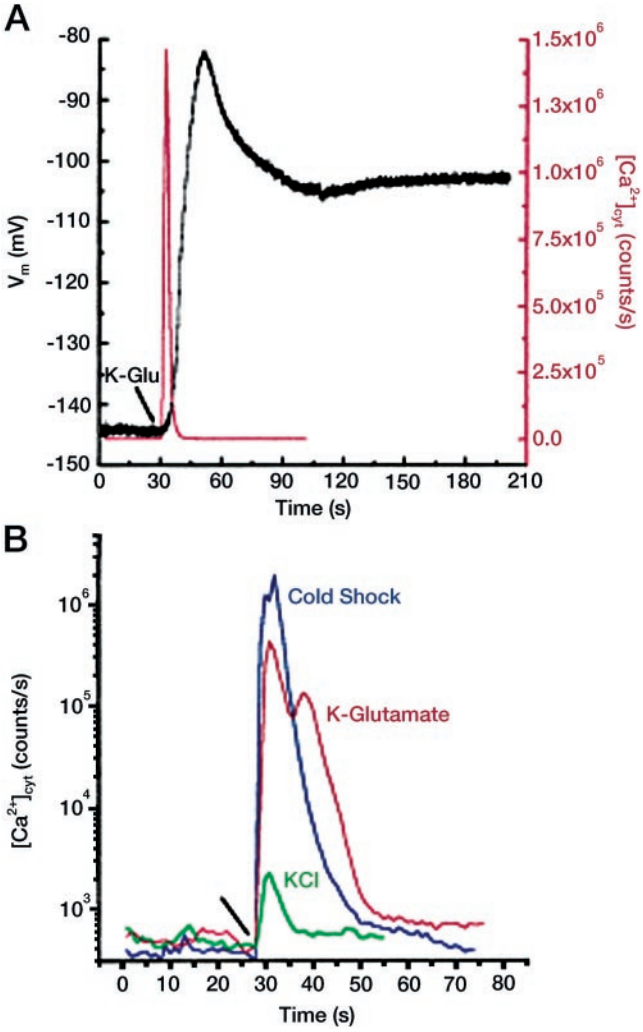
A
-80
-90
-100
-110
-120
-130
-140
-150
V_m (mV)
K-Glu
0
30
60
90
120
150
180
210
Time (s)
1.5x10^6
1.3x10^6
1.0x10^6
7.5x10^5
5.0x10^5
2.5x10^5
0.0
$[Ca^{2+}]_{cyt}$ (counts/s)
B
10^6
10^5
10^4
10^3
$[Ca^{2+}]_{cyt}$ (counts/s)
Cold Shock
K-Glutamate
KCl
0
10
20
30
40
50
60
70
80
Time (s)

Table I. *Sugar-response mutants and corresponding loci*

Mutants	Originals Selection	Loci	References
rsr	Reduced sensitivity to Suc induction of patatin expression		Martin et al., 1997
lba	Reduced sensitivity to Suc induction of β-amylase expression		Mita et al., 1997b
hba	Increased sensitivity to Suc induction of β-amylase expression		Mita et al., 1997a
sun	Reduced sensitivity to Suc repression of plastocyanin expression	*sun6* ls allelic to *abi4*	Dijkwel et al., 1997; Huijser ete al., 2000
sis	Reduced sensitivity to Glc or Suc-mediated inhibition of early seedling development	*sis1* ls allelic to *ctr1* *sis4* ls allelic to *aba2* *sis5* ls allelic to *abi4*	Laby et al., 2000; S. Gibson, R. Laby, and D. Kim, unpublished data
gin	Reduced sensitivity to Glc-mediated inhibition of early seedling development	*gin1* ls allelic to *aba2* *gin6* ls allelic to *abi4*	Zhou et al., 1998; Arenas-Huertero et al., 2000; J. Sheen, personal communication
prl	Increased sensitivity to sugar-mediated inhibition of early seedling development	*PRL1* Encodes a WD-40 protein	Németh et al., 1998; Bhalerao et al., 1999

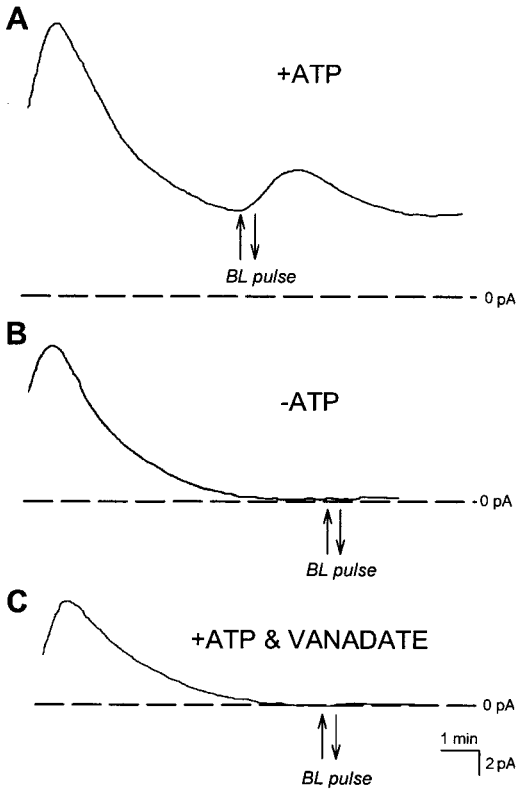

Figure 2. Steady-state- and BL-stimulated pump currents require ATP and are inhibited by vanadate. A, A typical recording with 5 mM ATP in the pipette under saturating RL. The cell responded to a 30-s pulse of BL with a typical transient increase in pump current. B, When ATP is absent from the pipette, cell currents quickly decay to 0 pA under saturating RL and are unresponsive to a pulse of BL. C, Inclusion of ATP and 20 μM vanadate in the pipette causes inhibition of pump current. All cells where pump current was inhibited by vanadate were unresponsive to BL pulses.

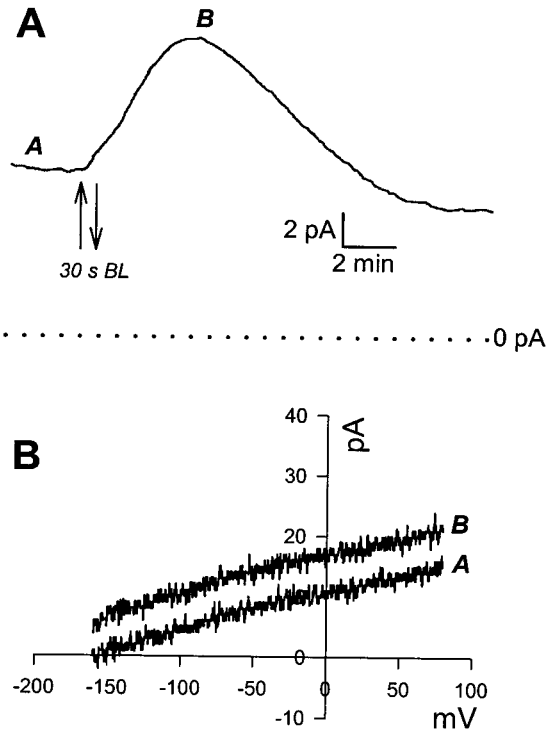

Figure 3. H^+-ATPase activation by a pulse of BL. Saturating RL background illumination was switched on before the beginning of the trace. A, Once stable baseline current is achieved a pulse of BL causes a transient increase in pump current. B, I/V ramps conducted before (*A*) and at the peak (*B*) of the response in A show the parallel shunt in pump current.

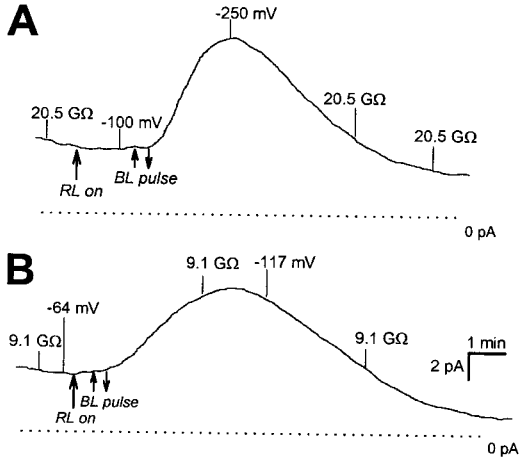

Figure 4. The effect of plasma membrane H^+-ATPase currents on membrane potential. The two traces show the pump current measured with ATP in the pipette. Membrane potential and input resistance are indicated on the traces at steady state and during BL-activated stimulation of pump current. Note the insensitivity to saturating RL illumination.