



Sugar and phytohormone response pathways: navigating a signalling network

Susan I. Gibson*

Department of Plant Biology, University of Minnesota, 250 Bio Sci Center, 1445 Gortner Avenue, St Paul, MN 55108-1095, USA

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Abstract

Many plant developmental, physiological and metabolic processes are regulated, at least in part, by nutrient availability. In particular, alterations in the availability of soluble sugars, such as glucose and sucrose, help regulate a diverse array of processes. Multiple lines of evidence indicate that many of these processes are also regulated in response to other signalling molecules, such as phytohormones. This review draws examples from a variety of plant systems, including bean, *Arabidopsis*, potato, and cereals. Five of the most interesting and best developed examples of processes regulated via 'interactions' or 'crosstalk' between sugars and phytohormones are described, including embryogenesis, seed germination, early seedling development, tuberization, and the regulation of α -amylase activity. The types of mechanisms by which different response pathways are known or postulated to interact are also described. These mechanisms include regulation of the metabolism and/or transport of a signalling molecule by a different response pathway. For example, sugars have been postulated to help regulate the synthesis, conjugation and/or transport of phytohormones, such as gibberellins and abscisic acid. Conversely, phytohormones, such as abscisic acid, gibberellins and cytokinins have been shown to help regulate sugar metabolism and/or transport. Similarly, sugars have been shown to regulate the expression of components of phytohormone-response pathways and phytohormones regulate the expression of some genes encoding possible components of sugar-response pathways. Examples of proteins and second messengers that appear to

act in multiple response pathways are also described.

Key words: Abscisic acid, amylase, embryogenesis, germination, gibberellin, glucose, review, sucrose, sugar, tuberization.

Introduction

All organisms need to be able to sense and respond to the levels of critical nutrients, such as sugars. Plants, being sessile organisms, have a particular need to be able to adapt to potential environmentally imposed limits on the availability of certain nutrients. This particular need to adapt to sugar availability is matched by a particular ability to respond to sugar levels or flux. For example, plants and certain other organisms are able to produce sugars through photosynthesis. Plants also have an unusually high potential to modify their developmental programmes in response to sugar availability, as much of plant development occurs post-embryonically, rather than embryonically as in animals. As a result, a diverse array of plant developmental, physiological and metabolic processes are known or believed to be regulated, at least in part, in response to alterations in the levels or flux of soluble sugars, such as glucose and sucrose (reviewed in Graham, 1996; Koch, 1996; Smeekens, 1998, 2000; Wobus and Weber, 1999; Yu, 1999; Gibson, 2000; Pego *et al.*, 2000; Rolland *et al.*, 2002; Rook and Bevan, 2003). In addition, further study is expected to reveal an involvement of sugars in a significant number of additional processes.

The field of plant sugar response is complicated by the fact that plants appear to respond to soluble sugar levels or flux by not one, but probably several,

* Fax: +1 612 625 1738. E-mail: gibso043@tc.umn.edu

response pathways. Experiments using glucose analogues have provided evidence for a hexokinase-dependent sugar response pathway (Graham *et al.*, 1994; Jang and Sheen, 1994). In addition, characterization of transgenic plant lines over-expressing plant or yeast hexokinases (Jang *et al.*, 1997) and of plants carrying mutations in the *HEXOKINASE1* gene (Moore *et al.*, 2003) have provided evidence that hexokinases play an important role in glucose response. However, precise elucidation of the role of hexokinases in the plant glucose response is complicated by the fact that hexokinases play critical roles in glucose metabolism. Regardless of the precise role of hexokinases in glucose response, the identification of genes that are regulated in response to sucrose but not hexoses indicates that other factors, in addition to hexokinases, are likely to function as sugar sensors (Chiou and Bush, 1998; Rook *et al.*, 1998; Barker *et al.*, 2000; Loreti *et al.*, 2000a; Müller *et al.*, 2000; Ciereszko *et al.*, 2001). The available evidence suggests that some processes may be mediated by the absolute levels of a particular sugar, such as glucose (Borisjuk *et al.*, 1998) or sucrose (Borisjuk *et al.*, 2002), whereas other processes may be mediated by flux through particular sugars (Krapp *et al.*, 1993).

Further complicating the characterization of the sugar-response pathways is the fact that these pathways 'interact' or exhibit 'crosstalk' with many other plant response pathways. For example, sugar-response pathways interact with response pathways for other key nutrients, such as nitrogen (reviewed in Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001). Sugar-response pathways also interact with environmental-response pathways, such as those involved in light-response (Ellis *et al.*, 2002; Brocard-Gifford *et al.*, 2003). Recent studies have also provided significant evidence of interactions between sugar and phytohormone response and metabolic pathways (reviewed in Gazzarrini and McCourt, 2001; Finkelstein and Gibson, 2002; Léon and Sheen, 2003).

Interactions between sugar- and phytohormone-response pathways form the focus of the remainder of this review. As the mechanisms by which different response pathways affect one another remain largely unknown, 'interaction' is used here in its loosest possible sense. Five of the best-developed examples of interactions between sugars and phytohormones in the regulation of plant developmental and other processes are described. These examples are drawn from a variety of plant systems, including bean, *Arabidopsis*, potato, and cereals. Possible molecular mechanisms by which different response pathways may interact are also discussed, as well as future avenues of research that are expected to provide additional insights into these mechanisms.

Processes affected by sugar and phytohormone-response pathways

Sugar- and abscisic acid-mediated regulation of embryogenesis

Research on a number of organisms has indicated that progress through the cell cycle is regulated, in part, in response to nutrient availability. For example, in yeast, a glucose-derived signal up-regulates expression of critical components of the cell cycle regulatory machinery that facilitates progress through Start (Newcomb *et al.*, 2003). In addition, recent evidence indicates that nutrient availability also plays a role in cell cycle control by multicellular organisms, including both animals (Stocker and Hafen, 2000) and plants (Meijer and Murray, 2000; Riou-Khamlichi *et al.*, 2000; Halford and Dickinson, 2001). It is, therefore, perhaps not surprising that the availability of important nutrients, such as sugars, helps regulate progress through critical developmental transitions as well as through the cell cycle. For example, sucrose and glucose help regulate the transition from growth by cell division to growth by cell expansion and reserve accumulation in developing plant embryos (Wobus and Weber, 1999; Borisjuk *et al.*, 2003). High-resolution imaging of glucose concentrations across the cotyledons of developing faba beans has shown that the undifferentiated, mitotically active tissue contains high levels of glucose whereas the most highly differentiated tissue contains low levels of glucose. In fact, this imaging revealed a glucose gradient that closely mirrors the developmental gradient across the cotyledons, suggesting that glucose gradients can have morphogenic functions (Borisjuk *et al.*, 1998). Interestingly, glucose and sucrose appear to play different roles during embryogenesis. Imaging of sucrose gradients across developing cotyledons during the primary storage phase of development reveals that the highest sucrose levels are found in the most actively expanding cells. High sucrose concentrations also correlated with increased starch accumulation. These findings suggest that high sucrose concentrations are important in promoting storage cell differentiation (Borisjuk *et al.*, 2002).

In addition to sugars, abscisic acid (ABA) also plays an important role during cotyledon development. The effects of ABA on mitotic activity during early cotyledon development appear to be opposite those of glucose. Whereas glucose levels are highest in undifferentiated, mitotically active cells and decrease during development, ABA levels increase during development and may promote cell cycle arrest. In contrast to the antagonistic effects of ABA and glucose during early cotyledon development, ABA and sucrose both appear to promote accumulation of storage reserves later in development (reviewed in Finkelstein *et al.*, 2002; Finkelstein and Gibson, 2002).

Sugar and phytohormone regulation of seed germination

Sugars and phytohormones also interact in the regulation of seed germination. Some sugars have been shown to inhibit seed germination when present at even very low concentrations. For example, less than 5% of wild-type *Arabidopsis* seeds sown on media containing 10 mM mannose or 2-deoxyglucose germinate. By contrast, almost 100% of wild-type *Arabidopsis* seeds sown on 10 mM 3-*O*-methylglucose or 6-deoxyglucose germinate. As mannose and 2-deoxyglucose, but not 3-*O*-methylglucose and 6-deoxyglucose, have been widely cited as substrates for hexokinases, these results have been interpreted to suggest that mannose and 2-deoxyglucose inhibit seed germination via a hexokinase-mediated step (Pego *et al.*, 1999). However, glucose analogue studies of this type, although useful, can be somewhat difficult to interpret. For example, when using glucose analogues, such as mannose, that can be phosphorylated but that undergo little subsequent metabolism, the possible effects of those analogues on ATP and phosphate levels must be considered. Although the results of some studies suggest that mannose does not inhibit seed germination via effects on ATP levels (Pego *et al.*, 1999), the results of other studies suggest that mannose can affect some processes by sequestering phosphate (Brouquisse *et al.*, 2001). In addition, metabolism of glucose analogues has only been examined in a few organisms. As even relatively closely related organisms can exhibit large metabolic differences, extrapolating results regarding metabolism of glucose analogues between different species may be problematic (reviewed in Gibson, 2000). In fact, a recent study demonstrated that 3-*O*-methylglucose is phosphorylated by hexokinase in maize (Cortés *et al.*, 2003). However, this result does not necessarily disprove the hypothesis that mannose inhibits seed germination through a hexokinase-mediated step, as the maize hexokinase was found to phosphorylate 3-*O*-methylglucose with an efficiency that was five orders of magnitude less than for glucose and mannose. Consequently, 3-*O*-methylglucose may simply be too poor a substrate for hexokinase to activate efficiently a hexokinase-mediated response pathway. In addition, further support for mannose acting via a hexokinase-mediated step is provided by results showing that mannoheptulose, an inhibitor of hexokinases, alleviates much of the negative effect of mannose on seed germination (Pego *et al.*, 1999).

ABA has long been known to inhibit seed germination (reviewed in Finkelstein *et al.*, 2002). Surprisingly, although some poorly metabolized sugar analogues also inhibit seed germination (Pego *et al.*, 1999) and glucose retards seed germination rates in *Arabidopsis* (To *et al.*, 2002; Price *et al.*, 2003), exogenous glucose allows wild-type *Arabidopsis* seeds to germinate in the presence of

otherwise inhibitory concentrations of ABA (Garcarrubio *et al.*, 1997; Finkelstein and Lynch, 2000). Although the molecular basis for this effect remains to be determined, the fact that the optimal glucose concentration for alleviating the inhibitory effects of ABA is relatively low suggests that the effect may not be entirely nutritional (Finkelstein and Lynch, 2000).

Sugar and phytohormone regulation of early seedling development

Although low (e.g. 10 mM) concentrations of some poorly metabolized glucose analogues inhibit seed germination (Pego *et al.*, 1999) and moderate to high (e.g. 30 to 300 mM) concentrations of glucose delay seed germination (To *et al.*, 2002; Price *et al.*, 2003), even high (e.g. 300 mM) concentrations of glucose and sucrose do not prevent seed germination. Within 2 weeks of the start of imbibition, 90–100% of wild-type *Arabidopsis* seeds germinate in the presence of 300 mM glucose or sucrose (Laby *et al.*, 2000). However, these concentrations of glucose and sucrose do severely inhibit very early seedling development. *Arabidopsis* seedlings grown on media containing 300 mM glucose or sucrose fail to become green or to develop expanded cotyledons or true leaves (Jang *et al.*, 1997; Németh *et al.*, 1998; Zhou *et al.*, 1998; Laby *et al.*, 2000). In addition, growth on high concentrations of glucose or sucrose inhibits chloroplast development (To *et al.*, 2003) and the breakdown of seed storage lipids (Martin *et al.*, 2002; To *et al.*, 2002). This inhibitory effect of exogenous glucose and sucrose on early seedling development has been used to isolate sugar hypersensitive (Németh *et al.*, 1998) and resistant (Zhou *et al.*, 1998; Arenas-Huertero *et al.*, 2000; Laby *et al.*, 2000; Pego *et al.*, 2000; Gibson *et al.*, 2001) mutants of *Arabidopsis*.

Characterization of sugar-hypersensitive and resistant mutants has provided evidence for interactions between sugar- and phytohormone-response pathways during very early seedling development (Table 1). For example, the *prl1* mutant of *Arabidopsis*, which is hypersensitive to the inhibitory effects of high concentrations of exogenous sucrose and glucose on early seedling development, is also hypersensitive to cytokinin, ABA, ethylene, and auxin (Németh *et al.*, 1998; Salchert *et al.*, 1998). In addition, two *Arabidopsis* mutants that are resistant to the inhibitory effects of exogenous sucrose and glucose on early seedling development, *sis1* (Gibson *et al.*, 2001) and *gin4* (Rolland *et al.*, 2002), are allelic (Table 2) to the ethylene constitutive response mutant, *ctr1* (Kieber *et al.*, 1993). Similarly, the ethylene overproducer mutant *eto1* (Guzman and Ecker, 1990) is resistant to the inhibitory effects of glucose and sucrose on early seedling development (Zhou *et al.*, 1998; Gibson *et al.*, 2001). Conversely, the ethylene-insensitive mutants *etr1-1* (Bleecker *et al.*, 1988) and *ein4* (Roman *et al.*, 1995) are hypersensitive to sucrose- and glucose-mediated inhibition of early seedling development

Table 1. Sugar-response mutants defective in phytohormone response or metabolism

Mutant	Original selection	Other phenotypes	Locus/gene product	References
<i>gin</i>	Decreased sensitivity to inhibition of early seedling development by glucose	<i>gin1</i> – defective in abscisic acid biosynthesis	<i>ABA2</i> – short-chain dehydrogenase/reductase	(Zhou <i>et al.</i> , 1998; Rook <i>et al.</i> , 2001; Cheng <i>et al.</i> , 2002)
		<i>gin2</i> – auxin-insensitive, cytokinin hypersensitive	<i>HXK1</i> – hexokinase	(Moore <i>et al.</i> , 2003)
		<i>gin4</i> – ethylene constitutive response	<i>CTR1</i> – protein kinase	(Rolland <i>et al.</i> , 2002)
		<i>gin5</i> – defective in abscisic acid biosynthesis	<i>ABA3</i> – molybdenum cofactor sulphurase	(Arenas-Huertero <i>et al.</i> , 2000; Rolland <i>et al.</i> , 2002)
		<i>gin6</i> – abscisic acid-insensitive	<i>ABI4</i> – transcription factor	(Arenas-Huertero <i>et al.</i> , 2000)
<i>isi</i>	Impaired sugar-inducible expression of ADP-glucose pyrophosphorylase subunit ApL3	<i>isi3</i> – abscisic acid-insensitive	<i>ABI4</i> – transcription factor	(Rook <i>et al.</i> , 2001)
		<i>isi4</i> – defective in abscisic acid biosynthesis	<i>ABA2</i> – short-chain dehydrogenase/reductase	(Rook <i>et al.</i> , 2001)
<i>prl</i>	Increased sensitivity to inhibition of early seedling development by sugars	<i>prl1</i> – abscisic acid, auxin, cytokinin, and ethylene-hypersensitive	<i>PRL1</i> – WD-40 protein	(Németh <i>et al.</i> , 1998; Salchert <i>et al.</i> , 1998; Bhalerao <i>et al.</i> , 1999)
<i>sis</i>	Decreased sensitivity to inhibition of early seedling development by glucose or sucrose	<i>sis1</i> – ethylene constitutive response	<i>CTR1</i> – protein kinase	(Gibson <i>et al.</i> , 2001)
		<i>sis4</i> – defective in abscisic acid biosynthesis	<i>ABA2</i> – short-chain dehydrogenase/reductase	(Laby <i>et al.</i> , 2000)
<i>sun</i>	Decreased sensitivity to sugar repression of plastocyanin expression	<i>sis5</i> – abscisic acid-insensitive	<i>ABI4</i> – transcription factor	(Laby <i>et al.</i> , 2000)
		<i>sun6</i> – abscisic acid-insensitive	<i>ABI4</i> – transcription factor	(Dijkwel <i>et al.</i> , 1997; Huijser <i>et al.</i> , 2000)

Table 2. Sugar and phytohormone response or metabolic mutants carrying mutations in the same genes

Original mutant symbol	References	Other mutant symbols	References
<i>aba2</i>	(Léon-Kloosterziel <i>et al.</i> , 1996)	<i>gin1</i>	(Zhou <i>et al.</i> , 1998; Rook <i>et al.</i> , 2001; Cheng <i>et al.</i> , 2002)
<i>aba3</i>	(Léon-Kloosterziel <i>et al.</i> , 1996)	<i>isi4</i>	(Rook <i>et al.</i> , 2001)
		<i>sis4</i>	(Laby <i>et al.</i> , 2000)
<i>abi4</i>	(Finkelstein, 1994)	<i>gin5</i>	(Arenas-Huertero <i>et al.</i> , 2000; Rolland <i>et al.</i> , 2002)
		<i>gin6</i>	(Arenas-Huertero <i>et al.</i> , 2000)
<i>ctr1</i>	(Kieber <i>et al.</i> , 1993)	<i>isi3</i>	(Rook <i>et al.</i> , 2001)
		<i>sis5</i>	(Laby <i>et al.</i> , 2000)
		<i>sun6</i>	(Dijkwel <i>et al.</i> , 1997; Huijser <i>et al.</i> , 2000)
		<i>gin4</i>	(Rolland <i>et al.</i> , 2002)
		<i>sis1</i>	(Gibson <i>et al.</i> , 2001)

(Zhou *et al.*, 1998; Gibson *et al.*, 2001). In addition, exogenous ethylene allows wild-type *Arabidopsis* to grow and develop relatively normal shoot systems on otherwise inhibitory concentrations of exogenous glucose (Zhou *et al.*, 1998) and sucrose (S Gibson, unpublished results). These results indicate that increased ethylene response or levels can result in increased sucrose and glucose resistance and that decreased ethylene response can lead to sucrose and glucose hypersensitivity during early seedling development

Evidence for interactions between sugar and ABA response pathways during early seedling development is provided by mutants identified via several different screens. The sugar-resistant mutants *sis4* (Laby *et al.*, 2000), *gin1* (Zhou *et al.*, 1998; Rook *et al.*, 2001) and *isi4* (Rook *et al.*, 2001) are allelic (Table 2) to *aba2*, an *Arabidopsis* mutant defective in ABA biosynthesis (Léon-Kloosterziel *et al.*, 1996). Similarly, the sugar-resistant mutant *gin5* (Arenas-Huertero *et al.*, 2000; Rolland *et al.*, 2002) is allelic to the ABA biosynthesis mutant *aba3*

(Léon-Kloosterziel *et al.*, 1996). In addition, the sugar-resistant mutants *sis5* (Laby *et al.*, 2000), *gin6* (Arenas-Huertero *et al.*, 2000), *isi3* (Rook *et al.*, 2001), and *sun6* (Huijser *et al.*, 2000) are allelic to *abi4*, an ABA-insensitive mutant (Finkelstein, 1994). Testing of additional ABA-insensitive mutants revealed that *abi5-1* exhibits a weak glucose-resistant phenotype whereas *abi1-1*, *abi2-1* and *abi3-1* exhibit wild-type responses to the inhibitory effects of high concentrations of exogenous glucose on early seedling development (Arenas-Huertero *et al.*, 2000; Huijser *et al.*, 2000; Laby *et al.*, 2000). More recent results indicate that some, but not all, *abi3* mutations confer resistance to the combined inhibitory effects of ABA and exogenous glucose on early seedling development (Nambara *et al.*, 2002). In the future, it will be of interest to determine whether any *abi3* mutations confer resistance to the inhibitory effects of glucose and/or sucrose alone. In addition to characterizing loss of function mutations, overexpression of *ABI3*, *ABI4* or *ABI5* has been shown to confer a glucose-hypersensitive phenotype (Brocard *et al.*, 2002; Finkelstein *et al.*, 2002). The findings that mutations in *ABI3*, *ABI4* and *ABI5*, but not *ABI1* or *ABI2*, affect sugar response in one or more of the above assays suggest that *ABI3*, *ABI4* and *ABI5* may act in a different branch of a response pathway than *ABI1* and *ABI2*. In fact, double mutant analyses suggest that *ABI3*, *ABI4* and *ABI5* act in the same signal transduction pathway (Finkelstein, 1994).

As described above, only a subset of mutations that confer ABA resistance also affect resistance to sucrose and glucose. These results indicate that the interactions between ABA and sugar-response pathways, whether direct or relatively indirect, do exhibit a significant degree of specificity. In addition, these results indicate that mutations that cause decreased ABA levels or response can lead to sucrose and glucose resistance and that increased ABA response can lead to glucose hypersensitivity during early seedling development. Thus, alterations in ABA response or metabolism appear to exert the opposite effect on sugar sensitivity during early seedling development as alterations in ethylene response or metabolism.

Role of sugars and phytohormones in tuber development

In addition to the developmental processes described above, sugars and phytohormones play an important role in tuber formation by potatoes (reviewed in Jackson, 1999; Fernie and Willmitzer, 2001). Several studies using transgenic plants have provided evidence that increasing sugar concentrations can promote tuber formation. For example, potato plants expressing an antisense ADP-glucose pyrophosphorylase construct accumulate unusually high concentrations of sugars and produce more, but smaller, tubers than wild-type plants (Müller-Röber

et al., 1992). In addition, expression of yeast invertase in the cytosol increases the number of tubers formed, but decreases their average size, whereas expression of yeast invertase in the apoplast decreases the number, but increases the average size of tubers formed (Sonnwald *et al.*, 1997). Interestingly, neither under- nor over-expression of potato hexokinases 1 or 2 has significant effects on tuber yield, suggesting that hexokinases may not play an important role in sugar-mediated regulation of tuber development (Veramendi *et al.*, 1999, 2002). In addition to the above *in vivo* experiments, *in vitro* experiments have also shown that application of exogenous sucrose promotes tuberization (Simko, 1994; Xu *et al.*, 1998) and that the effects of sucrose on tuber formation are not due to alterations in the osmotic potential of the media (Perl *et al.*, 1991). However, as high concentrations of nitrogen have been shown to inhibit tuberization (reviewed in Krauss, 1985), it is possible that exogenous sucrose promotes tuber formation by increasing the C/N ratio rather than by increasing endogenous sucrose concentrations or flux *per se*.

An additional possibility is that sucrose promotes tuberization via effects on phytohormone levels or response. Gibberellins have been shown to inhibit tuberization using both *in vivo* and *in vitro* experiments (reviewed in Vreugdenhil and Sergeeva, 1999). For example, antisense expression of a GA 20-oxidase gene (Carrera *et al.*, 2000) or overexpression of a knotted-like homeobox gene (Rosin *et al.*, 2003) caused reductions in levels of bioactive gibberellins and increased the rate of tuberization. Similarly, exogenous application of gibberellins decreased tuberization (Simko, 1994; Xu *et al.*, 1998) whereas application of the gibberellin biosynthesis inhibitor paclobutrazol increased tuberization (Simko, 1994). Interestingly, sucrose has been proposed to affect tuberization, at least in part, via effects on the levels of bioactive gibberellins. In one study, sucrose concentration was found to exhibit a negative correlation with GA(1) levels (Xu *et al.*, 1998). Another study revealed that application of exogenous sucrose caused a decrease in bioactive free gibberellin levels with a corresponding increase in levels of inactive conjugated gibberellins. Based on these findings, a model has been proposed that states that exogenous sucrose inhibits tuberization by increasing the levels of conjugated gibberellins at the expense of tuber-inhibiting free gibberellins (Simko, 1994).

Several other phytohormones have also been implicated in tuberization. For example, the application of exogenous ABA promotes tuberization, possibly by antagonizing the effects of gibberellins (Xu *et al.*, 1998). Evidence for jasmonic acid playing a role in tuberization is provided by experiments on transgenic potato plants expressing a tuber-specific lipoxygenase, which acts in jasmonic acid biosynthesis, in an antisense orientation. The transgenic

plants exhibited decreases in tuber yield, possibly via an effect on tuber enlargement (Kolomiets *et al.*, 2001). Studies on transgenic plants have also provided evidence for cytokinins exerting an effect on tuberization. Expression of the cytokinin biosynthetic gene *ipt* in transgenic tobacco caused an increase in cytokinin levels in the axillary buds and led to the formation of swollen internodes containing high levels of starch. The formation of these tuber-like structures in response to high cytokinin levels suggests that cytokinins can promote tuberization, even in plant species that do not normally form tubers (Guivarc'h *et al.*, 2002).

Sugar- and phytohormone-mediated regulation of α -amylase expression

In addition to affecting a number of developmental processes, sugars have been implicated in the regulation of a large number of genes (reviewed in Koch, 1996). A particularly well-characterized example of sugar-regulated gene expression is provided by the α -amylase gene family. α -amylases act in the breakdown of starch to generate soluble sugars, particularly during germination of starch-bearing seeds such as rice, barley and wheat. Given their biological function, it is, perhaps, not surprising that the expression of many α -amylase genes has been shown to be repressed by soluble sugars, such as glucose and sucrose, thus providing a mechanism by which starch breakdown may be regulated to provide an adequate supply of soluble sugars (Karrer *et al.*, 1991; Yu *et al.*, 1991; Huang *et al.*, 1993). In addition, expression of some α -amylase genes is induced by gibberellin or repressed by ABA (reviewed in Bethke *et al.*, 1997).

The regulation of α -amylase expression by sugars is complicated, occurring at multiple levels and via multiple response pathways that can be dependent on sugar concentration or flux. For example, α -amylase expression is regulated by sugars at both the transcriptional and post-transcriptional levels (Sheu *et al.*, 1994, 1996; Chan and Yu, 1998; Loreti *et al.*, 2000b; Toyofuku *et al.*, 2000). Alpha-amylase expression is also regulated by both glucose and disaccharide response systems. In one study, glucose was found to affect α -amylase transcript stability whereas disaccharides, including non-metabolizable disaccharides such as lactulose, palatinose and turanose, repressed

α -amylase expression without affecting transcript stability (Loreti *et al.*, 2000a). Sugar-mediated regulation of α -amylase expression can also be dependent on sugar concentration. For example, expression of α Amy3 and α Amy8 in germinating rice embryos is decreased by treatment with 100 mM glucose and further decreased by treatment with 300 mM glucose. By contrast, expression of α Amy7 in the same system was increased by treatment with 100 mM glucose, but not by treatment with 300 mM glucose (Yu *et al.*, 1996).

Findings that very high concentrations of exogenous glucose may be required for maximum effects on amylase expression raise questions regarding whether the glucose concentrations being tested are biologically relevant and whether glucose is acting primarily as an osmoticum. Results showing that glucose concentrations reach an estimated maximum of approximately 60 mM in embryos and over 500 mM in endosperm of germinating rice seeds (Yu *et al.*, 1996) suggest that experiments using glucose concentrations of several hundred mM can be biologically relevant, depending on the system being analysed. In experiments comparing equimolar concentrations of glucose and mannitol, both chemicals were found to repress α Amy8 expression in aleurone cells of germinating rice embryos, suggesting that, in this case, glucose is acting as an osmoticum (Yu *et al.*, 1996). However, the fact that expression of some α -amylase genes is repressed only by glucose analogues that are strong substrates for hexokinases indicates that sugar-regulation of these α -amylase genes is not solely due to effects on osmotic potential (Perata *et al.*, 1997; Umemura *et al.*, 1998).

Gibberellins have been shown to induce, and ABA to repress, expression of some α -amylases (reviewed in Bethke *et al.*, 1997). Interestingly, application of exogenous glucose has been shown to affect both ABA- and gibberellin-mediated control of α -amylase expression. For example, (+)-trifluoro-ABA only inhibits α -amylase expression in rice scutella and suspension culture cells in the presence of glucose (Kashem *et al.*, 1998). Glucose can affect gibberellin-mediated α -amylase expression in different ways depending on the cell/tissue type being examined. For instance, glucose represses gibberellin-mediated induction of α -amylases in barley embryos but not in the aleurone (Perata *et al.*, 1997). In these experiments, glucose did not affect gibberellin-mediated α -amylase expression by affecting gibberellin levels (Perata *et al.*, 1997). Several studies have suggested that some promoter elements may function in both glucose and gibberellin response (Lanahan *et al.*, 1992; Lu *et al.*, 1998; Morita *et al.*, 1998; Toyofuku *et al.*, 1998; Chen *et al.*, 2002). These findings suggest that one or more of the factors that bind these elements could be involved in both glucose- and gibberellin-mediated response. In fact, three MYB factors that bind the glucose and gibberellin response element TATCCA have recently been identified from rice (Lu *et al.*, 2002). All three of these proteins act in glucose-regulation of a promoter containing the TATCCA element. In addition, all three proteins co-operate with a gibberellin-regulated transcription factor to regulate the expression of an α -amylase gene in the absence of gibberellin, suggesting that the three MYB factors play roles in both glucose- and gibberellin-mediated regulation of α -amylase expression (Lu *et al.*, 2002). In at least some systems, glucose appears to repress the gibberellin response very late along the gibberellin response pathway,

after transcription of *GAMYB* (Loreti *et al.*, 2000b), which has been shown to be both necessary and sufficient for gibberellin-mediated induction of α -amylase (Zentella *et al.*, 2002). Alternatively, glucose may antagonize gibberellin action via an independent pathway in this system (Loreti *et al.*, 2000b).

Mechanisms for interactions between sugar and phytohormone response pathways

As the above examples demonstrate, sugar- and phytohormone-response pathways 'interact' or exhibit 'cross-talk' in the regulation of many processes. However, little is known about the mechanisms by which different response pathways interact. The available evidence suggests that these interactions may be quite direct in some cases and indirect in others. For example, a component of one response pathway might interact directly with components of another response pathway to form a complex. Alternatively, response pathways might interact indirectly by altering the levels of the same second messenger.

Sugars affecting phytohormone metabolism/transport

One mechanism by which sugars affect phytohormone response is by altering the levels, localization and/or transport of different phytohormones. For example, treatment with sucrose or glucose has long been postulated to inhibit gibberellin synthesis or release from the barley scutellum (Radley, 1969). The application of exogenous glucose has also been shown to lead to decreased gibberellin levels in barley (Perata *et al.*, 1997) and rice (Yu *et al.*, 1996) embryos. In addition, sucrose has been postulated to affect gibberellin response by affecting gibberellin conjugation. The application of exogenous sucrose to potatoes caused a decrease in bioactive free gibberellin levels with a corresponding increase in levels of conjugated, potentially inactive, gibberellins (Simko, 1994). Conjugated gibberellins contain glucosyl moieties that are obtained from UDP-glucose (Sembdner *et al.*, 1991). UDP-glucose, in turn, is generated primarily from sucrose via the action of sucrose synthase (ap Rees and Morrell, 1990). Based on these findings, a model has been proposed that states that exogenous sucrose may act in some cases by increasing the amount of UDP-glucose, leading to an increase in conjugated gibberellins and a decrease in bioactive free gibberellins (Simko, 1994).

Glucose has also been shown to affect ABA levels. In rice embryos, exogenous glucose has been shown to correlate with decreased levels of ABA (Toyofuku *et al.*, 2000). Interestingly, the opposite effect has been observed in *Arabidopsis*, where the application of exogenous glucose to very young seedlings was found to cause increased levels of ABA (Arenas-Huertero *et al.*, 2000; Cheng *et al.*, 2002). In addition, high levels of glucose have been shown to induce expression of some ABA

biosynthetic genes in *Arabidopsis* (Cheng *et al.*, 2002). Interestingly, exogenous glucose was recently shown to retard the rate at which endogenous ABA levels decline in germinating *Arabidopsis* seeds (Price *et al.*, 2003). These results suggest that the increased levels of ABA found in *Arabidopsis* seedlings germinating in the presence of exogenous glucose may be due more to decreased ABA breakdown than to increased ABA synthesis. In the future, it will be of interest to test the effects of other sugars, such as sucrose, on ABA levels and metabolism.

Phytohormones affecting sugar metabolism/transport

Several phytohormones have been implicated in the regulation of sugar metabolism and/or transport. Cytokinins, in particular, have long been suggested to play a role in the regulation of source/sink interactions (reviewed in Roitsch and Ehneß, 2000). In *Chenopodium rubrum*, cytokinins have been shown to induce expression of both a cell wall invertase and a hexose transporter, providing evidence for a mechanism by which cytokinins may help regulate sugar transport (Ehneß and Roitsch, 1997). ABA has also been implicated in the regulation of sugar transport and metabolism. Treatment of germinating rice seeds with ABA plus glucose results in a higher accumulation of sugars in the scutellum than treatment with glucose alone, suggesting that ABA stimulates glucose uptake from the media. In addition, treatment with the ABA analogue (+)-trifluoro-ABA plus glucose leads to increased sucrose concentrations, suggesting that ABA stimulates sucrose formation (Kashem *et al.*, 1998). ABA and gibberellins also help regulate sugar concentrations by altering α -amylase levels, thereby affecting the rate at which sugars are produced from starch (reviewed in Bethke *et al.*, 1997). In addition to exogenous phytohormones affecting sugar levels/metabolism, the characterization of ABA response mutants of *Arabidopsis* has revealed alterations in sugar (Brocard-Gifford *et al.*, 2003) and starch (Keith *et al.*, 1994; Meinke *et al.*, 1994; Brocard-Gifford *et al.*, 2003) levels. These results provide further evidence that ABA plays a role in regulating sugar metabolism.

Sugars regulating expression of components of phytohormone response pathways and vice versa

Sugars also affect phytohormone-response pathways by affecting the expression and/or activity levels of components of those pathways. For example, exogenous glucose has been shown to induce expression of *ABI3* (Cheng *et al.*, 2002) and *ABI4* (Arenas-Huertero *et al.*, 2000; Cheng *et al.*, 2002) in *Arabidopsis* seedlings, possibly affecting the sensitivity of the tissue to endogenous ABA. Exogenous glucose also increases expression of *ABI5* in *Arabidopsis* (Brocard *et al.*, 2002; Cheng *et al.*, 2002). However, stress also stimulates *ABI5* expression, suggesting that the effect of glucose on *ABI5* expression

may be relatively non-specific (Brocard *et al.*, 2002). Both glucose and sucrose have also been shown to affect expression of three MYB proteins from rice that are implicated in both gibberellin and sugar (sucrose and glucose) response (Lu *et al.*, 2002). Conversely, phytohormones have been shown to regulate the expression of putative components of sugar-response pathways. For example, ABA has been shown to increase transcript levels of a group-2 SNF1-related protein kinase from wheat, *PKABA1* (Anderberg and Walker-Simmons, 1992). Similarly, cytokinins have been shown to increase expression of the group-3 SNF1-related protein kinases *AtSR1* from *Arabidopsis* (Chikano *et al.*, 2001) and *WPK4* from wheat (Ikeda *et al.*, 1999). In yeast, SNF1 has been shown to play a role in sugar response (reviewed in Carlson, 1998) and group-1 SNF-related protein kinases have been implicated in sugar response in plants (reviewed in Halford *et al.*, 2003). Although the biological roles of group 2 and group 3 SNF1-related protein kinases in plants are less clear, findings that *WPK4* complements a yeast *snf1* mutant suggest that *WPK4* may be involved in sugar response in plants (Ikeda *et al.*, 1999).

Sharing of components between response pathways

Recent evidence suggests that some factors may act as components in more than one response pathway. A mutation in the *Arabidopsis ICX1* gene leads to increased accumulation of anthocyanins in response to both sucrose and cytokinin. These results have been interpreted to suggest that *ICX1* acts as a negative regulator of both the sucrose and cytokinin response pathways (Wade *et al.*, 2003). Previously, *Arabidopsis* expressing the hexokinase gene *HXK1* in an antisense orientation were shown to be insensitive to glucose (Jang *et al.*, 1997). More recently, *hvk1* mutants have been shown to be insensitive to auxin and hypersensitive to cytokinin, suggesting that *HXK1* could affect all three pathways (Moore *et al.*, 2003). Similarly, mutations in the *Arabidopsis ABI4* gene, which encodes a transcription factor (Finkelstein *et al.*, 1998), confer increased resistance to the inhibitory effects of ABA on seed germination (Finkelstein, 1994) and sucrose and glucose on early seedling development (Arenas-Huertero *et al.*, 2000; Huijser *et al.*, 2000; Laby *et al.*, 2000; Rook *et al.*, 2001). However, the above results do not indicate whether *ICX1*, *HXK1* and/or *ABI4* act directly or indirectly in phytohormone and sugar response. Interestingly, a maize *ABI4* homologue has recently been shown to bind elements from both ABA and sugar-regulated promoters, suggesting that *ABI4* may act directly in both pathways (Niu *et al.*, 2002). Similarly, an ASR protein from grape has been postulated to act as part of a transcription-regulating complex that functions in both sucrose and ABA response (Çakir *et al.*, 2003).

Components of the ubiquitination/proteasome pathway for protein degradation have also been suggested to act in

both phytohormone and sugar response (reviewed in Ellis *et al.*, 2002). For example, mutations in the *Arabidopsis ASK1* gene, which encodes a subunit of a SCF ubiquitin ligase, cause a decreased response to auxin (Gray *et al.*, 1999). The *ASK1* protein has also been shown to co-immunoprecipitate with an SNF1-related protein kinase from *Arabidopsis* extracts (Farrás *et al.*, 2001). As SNF1-related protein kinases have been implicated in sugar response (Halford *et al.*, 2003), these results suggest that *ASK1* may function in both auxin and sugar response. Finally, response pathways may act via common second messengers. For example, Ca^{2+} has been suggested to act in both sugar- and phytohormone-response pathways. Application of exogenous sucrose to rice cell suspension cultures inhibits Ca^{2+} uptake whereas Ca^{2+} uptake is stimulated in response to sugar depletion (Mitsui *et al.*, 1999). Similarly, a number of ABA- and gibberellin-response pathways are known to be Ca^{2+} dependent (reviewed in Lovegrove and Hooley, 2000).

Future prospects

Different response pathways can 'interact' with each other via diverse mechanisms. Currently, much of the information about interactions between different response pathways has been obtained through the characterization of complex phenomena, such as early seedling development and tuberization. Analysis of these studies is complicated by the fact that these phenomena represent not one, but rather a complex array of processes, each of which may respond to different stimuli in different ways. For example, characterization of the roles of ABA and gibberellins in seed germination has revealed that some of the processes involved in seed germination are regulated by ABA, others by gibberellin and still others by both phytohormones (reviewed in Lovegrove and Hooley, 2000). The identification of simpler processes that are regulated by one or more stimuli of interest will be very useful in characterizing interactions between response pathways. One promising method for identifying such processes is the use of DNA microarrays. DNA microarrays can be used to screen large numbers of genes for those that are regulated at the steady-state mRNA level by different stimuli. Therefore, information from DNA microarray experiments will provide more, and more precise phenotypes, for characterizing responses to different stimuli. For example, DNA microarrays can be used to identify genes that are only glucose-regulated, only ABA-regulated or both. New and existing mutants can then be screened using DNA microarrays or other methods to determine which mutations affect which subsets of genes. By comparing the subsets of genes affected by different stimuli and different mutations, it should be possible to begin developing better models regarding the relationships between these stimuli and between the factors encoded by the mutated genes.

Information from DNA microarrays will also be useful in identifying genes involved in the metabolism of, or response to, one signalling molecule that are regulated in response to a different signal.

Other approaches also offer promise for defining characteristic interactions between response pathways. For example, techniques such as yeast two-hybrid screens and co-immunoprecipitation experiments can be invaluable in identifying direct interactions between components of different response pathways. Ultimately, elucidating interactions between response pathways will require the use of a variety of approaches.

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