Sugar and hormone connections

Patricia León[1] and Jen Sheen[2]

[1]Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, Universidad Nacional Autonoma de México, Cuernavaca, Morelos 62271, Mexico
[2]Department of Genetics, Harvard Medical School, and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA

Sugars modulate many vital processes that are also controlled by hormones during plant growth and development. Characterization of sugar-signalling mutants in *Arabidopsis* has unravelled a complex signalling network that links sugar responses to two plant stress hormones – abscisic acid and ethylene – in opposite ways. Recent molecular analyses have revealed direct, extensive glucose control of abscisic acid biosynthesis and signalling genes that partially antagonizes ethylene signalling during seedling development under light. Glucose and abscisic acid promote growth at low concentrations but act synergistically to inhibit growth at high concentrations. The effects of sugar and osmotic stress on morphogenesis and gene expression are distinct. The plasticity of plant growth and development are exemplified by the complex interplay of sugar and hormone signalling.

The role of sugars as signalling molecules has been widely recognized in microorganisms and has recently emerged in animals and plants [1]. During plant growth and development, sugars modulate a range of vital processes such as seed germination, seedling development, root and leaf differentiation, floral transition, fruit ripening, embryogenesis, and senescence, as well as responses to light, stress and pathogens [2–9]. Based on the different responses that diverse genes exhibit to particular sugars or sugar-phosphorylation activities, the existence of multiple signal-transduction pathways has been proposed [6,7]. Currently, it is unclear whether these signalling pathways interconnect or whether they function in specific cell types or at specific developmental stages. To address these types of questions, it is necessary to understand in temporal and spatial detail the mechanisms by which sugar signals are transduced in each pathway and the nature of the molecules that participate in these processes.

In an effort to understand the molecular mechanisms involved in the sugar perception and signalling network, genetic strategies have been designed independently to select either sugar-insensitive or sugar-oversensitive mutants, mainly in *Arabidopsis*. The genetic screens are reminiscent of those devised for the isolation of plant hormone mutants. By using easily detectable phenotypes such as the sugar inhibition of germination or postgermination development in seedlings, mutants with altered sugar responses have been isolated and characterized [10–15]. Sugar-response mutants have also been isolated by screening transgenic seedlings carrying reporter and selection genes under the control of sugar-regulated promoters [16,17]. These distinct screening strategies yielded overlapping mutants. Surprisingly, characterization of these mutants has led to the identification of mutants and genes previously implicated in plant-hormone biosynthesis or signalling, and revealed extensive connections between the sugar and plant-hormone pathways [18]. The aim of this article is to highlight the latest findings that might provide some molecular insights into the intricate network of plant sugar signalling and its direct, extensive relationship to abscisic acid (ABA) and ethylene hormonal pathways.

Sugar-signalling mutants reveal the hormone connections

Recent genetic and molecular studies of sugar-signalling mutants in *Arabidopsis* have uncovered many unexpected links between sugar and plant-hormone signalling (Table 1). In addition to the altered sugar responses (Fig. 1), these mutants display phenotypes found in ABA or ethylene biosynthesis mutants and in ABA or ethylene signalling mutants (Fig. 2). For example, the small, dark-green leaves of the *gin1* mutant, resembling the constitutively active ethylene mutant *ctr1* (Fig. 2), prompted the application of an ethylene precursor 1-aminocyclopropane-1-carboxylate

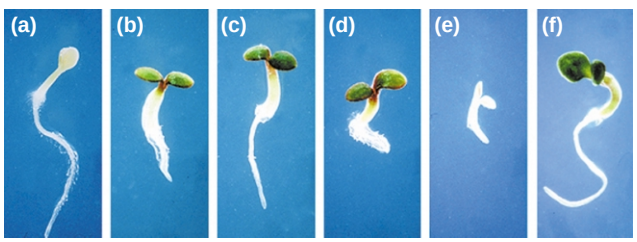

Fig. 1. *Arabidopsis* glucose-insensitive (*gin*) mutants. Seedlings were grown on 6% glucose–Murashige and Skoog (MS) medium for 4–5 days under constant light. (a) Wild type (WT). (b) The glucose-insensitive phenotype can be mimicked by treatment with the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC). (c) Abscisic acid-deficient *gin1 (aba2)* and (d) constitutive ethylene-signalling *gin4(ctr1)* mutants display a similar glucose-insensitive phenotype except for root elongation. (e) Endogenous abscisic acid is required for the glucose-oversensitive phenotype of the ethylene-insensitive mutant *ein2* because (f) the *gin1 ein2* double mutant exhibits the glucose-insensitive phenotype.

Corresponding author: Jen Sheen (sheen@molbio.mgh.harvard.edu).

 1360-1385/03/$ - see front matter doi:10.1016/S1360-1385(03)00011-6

Table 1. *Arabidopsis* mutants involved in sugar and hormone connections

Mutant	Phenotype	Gene and protein	Function	Refs
gin1	Glucose insensitive, growth retardation, wilty, seed defect	*ABA2* and SDR1	ABA biosynthesis	[12,19]
gin2	Glucose insensitive, growth retardation, delayed leaf senescence, seed defect	*GIN2* and HXK1	Glucose sensor, hexose phosphorylation	[18]
gin4	Glucose insensitive, growth retardation, constitutive triple response	CTR1	Raf-like protein kinase, negative regulator in ethylene signalling	[19]
gin5	Glucose insensitive, wilty	*ABA3/LOS5* and MCSU	Biosynthesis of the molybdenum cofactor	[13]
gin6	Glucose insensitive, ABA insensitive, osmotolerant, salt resistant	ABI4	AP2 transcription factor in ABA signalling	[13]
isi3	Glucose and mannose insensitive	ABI4	AP2 transcription factor in ABA signalling	[17]
isi4	Glucose insensitive	*ABA2* and SDR1	ABA biosynthesis	[17]
sis1	Glucose and mannose insensitive, osmotolerant, constitutive triple response	CTR1	Raf-like protein kinase, negative regulator in ethylene signalling	[20]
sis4	Sucrose and glucose insensitive, osmotolerant, wilty, paclobutrazol resistant	*ABA2* and SDR1	ABA biosynthesis	[14]
sis5	Sucrose, glucose and mannose insensitive, osmotolerant, ABA insensitive, paclobutrazol resistant	ABI4	AP2 transcription factor in ABA signalling	[14]
sun6	Sucrose, glucose, mannose and ABA insensitive	ABI4	AP2 transcription factor in ABA signalling	[16]
prl1	Sucrose, glucose, cytokinin, auxin, ABA, and ethylene hypersensitive, reduced root elongation, starch and sugar accumulation in leaves	PRL1	An α-importin WD40 protein, interacts with SnRKs	[11,57]
aba1	Glucose and sucrose insensitive	*ABA1* and ZEP1	ABA biosynthesis	[13,14,16]
abi5	Glucose insensitive	ABI5	bZIP transcription factor in ABA signalling	[13,14]
etr1	Glucose oversensitive, ethylene insensitive	ETR1	Ethylene receptor, histidine protein kinase	[12,51]
eto1	Glucose insensitive	ETO1	Ethylene biosynthesis	[12,51]
ein2	Glucose oversensitive, ethylene, jasmonate and paraquat insensitive	EIN2	Integral membrane protein, ethylene and jasmonate signalling	[19,52]
ein3	Glucose oversensitive, ethylene insensitive	EIN3	Transcription factor in ethylene signalling	[51][a]
ein6	Glucose oversensitive, ethylene insensitive	EIN6	Unknown protein	[51][a]
hys1	Glucose oversensitive, early leaf senescence, constitutive expresser of *PR* genes (*cpr*)	*HYS1/CPR5*	Unknown protein	[58]

Abbreviations: ABA, abscisic acid; bZIP, basic leucine zipper domain; CPR5, constitutive expresser of PR; HXK1, hexokinase 1; MCSU, moco sulfurase; SDR, short-chain dehydrogenase/reductase; SnRK, SNF1-related protein kinase; ZEP1, zeaxanthin epoxidase. Mutant names: *aba*, ABA deficient; *abi*, ABA insensitive; *ein*, ethylene insensitive; *eto*, ethylene overproducer; *etr*, ethylene response; *gin*, glucose insensitive; *hys*, hypersenescence; *isi*, impaired sugar induction; *prl*, pleiotropic regulatory locus; *sis*, sugar insensitive; *sun*, sucrose uncoupled. Gene names and alternative protein names are listed.
[a]W-H. Cheng and J. Sheen, unpublished.

(ACC) to wild-type plants in the presence of excess exogenous glucose. This experiment demonstrated that the glucose-dependent developmental arrest could be overcome by ethylene (Fig. 1) [12]. Although ethylene is generally known as a stress hormone that inhibits growth, this study reveals a growth-promoting role of ethylene in *Arabidopsis*. Interestingly, the *ctr1* mutant produces many more leaves than wild-type plants even in the absence of exogenous glucose (Fig. 2). It was also found that the sugar-insensitive *gin1* and *gin5* mutants had reduced-seed-dormancy and wilty phenotypes that are characteristics of ABA-deficient (*aba*) mutants [13,19]. These findings led to an awareness of the important genetic interactions between the sugar and ABA, and between the sugar and ethylene signalling pathways [12–14,16,17,19].

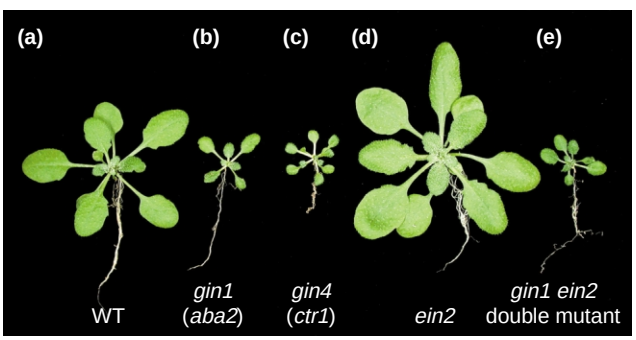

Fig. 2. Growth retardation of *gin* mutants. (a) Wild type (WT), (b) abscisic acid-deficient *gin1 (aba2)*, (c) constitutive ethylene-signalling *gin4 (ctr1)*, (d) ethylene-insensitive *ein2*, (e) *gin1 ein2* double mutant. In the absence of exogenous glucose, different *gin* mutants exhibit growth retardation. There might be a signalling network connecting endogenous glucose, abscisic acid and ethylene signals. (b) The analysis of the *gin1 (aba2)* mutant reveals the crucial role of abscisic acid as a growth-promoting hormone.

The molecular cloning of *GIN6/SUN6* as *ABI4* and *GIN1/ISI4* as *ABA2* strengthened the molecular connections between sugar and ABA [13,16,17,19]. Genetic analyses also confirmed that *gin1, sis4* and *isi4* are new *aba2* alleles, that *gin4* and *sis1* are new *ctr1* alleles, and that *gin5* is allelic to *aba3* [13,14,16,17,19,20] (A. Arroyo *et al.*, unpublished). Details of these mutant studies have recently been covered by excellent reviews [6,8,21,22]. However, with these findings, many questions have been raised about the physiological relevance and specificity of the sugar mutant screens and the deduced genetic interactions, as well as the overlap between stress and sugar signalling and the molecular bases of these complex interactions.

Glucose controls ABA biosynthesis genes

Direct biochemical analyses show that several sugar signalling mutants (*gin1, gin5*, *isi4* and *sis4*) contain lower endogenous ABA levels than wild-type plants

(Table 1) [13,14,17,19] (A. Arroyo *et al.*, unpublished). Consistent with this, the previously isolated ABA-deficient mutants *aba1, aba2* and *aba3* all display a *gin* phenotype [13,14,16]. The close relationship between ABA levels and the sugar-insensitive phenotype is further supported by the phenotypic reversion of the sugar sensitivity in these mutants by the addition of exogenous ABA at physiological concentrations (100 nM) [13,19]. It has also been shown that, in the presence of high-glucose signals, the developmentally arrested seedlings contain higher endogenous ABA levels [13,19]. In spite of the abundant genetic and phenotypic evidence, it remains unsettled whether there is a direct molecular connection between the sugar and ABA pathways [21].

Recent molecular cloning of ABA biosynthesis genes (*ABA1, ABA2, ABA3, NCED3* and *AAO3*) has revealed more insights into the role of ABA and the regulatory mechanisms of ABA accumulation during sugar responses [19,23–26]. Interestingly, it was found that the transcript levels of several ABA biosynthesis genes are raised by glucose [19]. This induction is observed with low glucose concentrations (2%) and is not further increased with higher glucose levels. A different pattern is observed with 2% and 6% mannitol as controls [19]. Unlike the negative feedback loop observed for gibberellin [27] and brassinosteroid biosynthesis [28], there could be a positive feedback loop in the control of ABA biosynthesis, because ABA by itself induces the expression of several ABA biosynthesis genes, such as *ABA1, ABA3, NCED3* and *AAO3* [19,23,24,26]. Surprisingly, this is not the case for *ABA2*, which is upregulated specifically by glucose but not by ABA [19]. By contrast, the *NCED3* gene, which is strongly activated by drought and osmotic stress in various plants, appears to be insensitive to glucose in *Arabidopsis* [19,29–31].

These results strongly suggest a direct, specific glucose modulation of ABA biosynthesis genes and ABA accumulation. This regulation, however, requires a synergistic interaction of glucose and ABA because it is abolished in the null glucose-insensitive *gin1* mutant, which displays endogenous ABA deficiency [19]. The data also indicate that the modulation of ABA levels by glucose is involved not only in the developmental arrest of seedlings but also during active plant development, arguing against the suggestion that the glucose–ABA interaction is an artificial response. The glucose-dependent developmental arrest is a complicated trait, and glucose control of ABA biosynthesis genes and ABA accumulation contribute to this phenotype. As discussed below, glucose also modulates ABA signalling genes and ethylene responses in an ABA-dependent manner.

Currently, it is unclear whether this glucose induction of ABA biosynthesis can be generalized to all cell types or to all developmental stages. The expression pattern of an *ABA2::GUS* reporter gene in transgenic plants indicates that most of the cells in young seedlings can respond to the presence of elevated glucose levels and induce the expression of this ABA biosynthesis gene [19]. Intriguingly, the *ApL3* gene (encoding an ADP–glucose pyrophosphorylase subunit that is involved in starch biosynthesis) is activated by sucrose but not ABA or mannitol in mature *Arabidopsis* leaves. Its sucrose induction depended on *ISI4/ABA2/GIN1* only when leaves were submerged in sucrose solution, not when sucrose was fed through the petiole. It was proposed that the petiole-fed leaves were subject to dehydration stress even though a wilty phenotype was not observed [17]. Because *ABA2::GUS* is highly expressed in the petiole and induced by sugar [19], an alternative explanation could be that the submerged leaves did not generate sufficient ABA through the petiole to induce *ApL3* in the *isi4* mutant. Apparently, both sucrose and ABA are required synergistically for ApL3 gene expression. In petiole-fed leaves, abundant sugar signals promoted sufficient ABA synthesis in the *isi4* mutant, which was not a null allele like *gin1* [17]. This study provides a physiologically relevant example of sugar regulation of ABA biosynthesis that is not restricted to young seedlings. It will be interesting and informative to carry out more-detailed analyses of the expression patterns of other ABA biosynthesis genes and their responses to sugars as well as their roles in sugar signalling.

Glucose controls ABA signalling genes

As shown in Table 1, the characterization of several sugar mutants (such as *gin6, isi3, sis5* and *sun6*) has revealed their allelism to the ABA-responsive mutant *abi4* [13,14,16,17,32]. The *abi4* mutant was originally selected by its ability to germinate in the presence of high levels of exogenous ABA (3 μM), which are normally inhibitory to wild-type germination and seedling development [32]. *ABI4* encodes a transcription factor of the APETALA 2 (AP2) domain family that plays a major role during seed development and germination together with two other loci, *ABI3* and *ABI5* [32,33]. The participation of *ABI4* in other aspects of vegetative growth only became clear with the finding that *abi4* alleles were isolated as sugar- and salt-resistant mutants [13,14,16,17,34]. These results are consistent with the low *ABI4* expression in vegetative tissues in addition to seeds [33] (A. Arroyo *et al.* unpublished). Interestingly, *ABI4* is activated by 6% glucose in an ABA-dependent manner but its response to ABA alone is limited [13,19] (A. Arroyo *et al.*, unpublished). The glucose-responsive regulatory DNA sequence might lie 2 kb upstream of the coding sequence, where a T-DNA insertion abolished glucose induction [13]. Thus, glucose and ABA modulate the expression of both ABA biosynthesis and an ABA signalling genes, but the control of transcription factors requires higher levels of glucose [19].

In addition to *abi4*, it was found that the *abi5* mutant also displayed a glucose-insensitive phenotype [13,14]. This phenotype appears not to be as strong as that of the *abi4* alleles and is not consistent in some studies [16]. However, overexpression of *ABI5* confers hypersensitivity to sugars [35]. The *ABI5* gene encodes a transcription factor that belongs to a large basic leucine zipper (bZIP) domain family [36]. In addition to its role during late embryogenesis, this gene plays a crucial role at the checkpoint of postgermination developmental arrest [37] and probably in specific tissues during vegetative growth [35]. Like *ABI4, ABI5* is activated by glucose in an ABA-dependent fashion [19] (A. Arroyo *et al.*, unpublished). Unlike *ABI4*, however, several members of this family, including *ABI5*, are highly inducible by ABA and

mannitol [19,35,37]. It is possible that the role of ABI5 in the glucose-induced developmental arrest partially overlaps with other bZIP factors [35]. This hypothesis is supported by the recent finding that overexpression of two other members of this family (*ABF3* and *ABF4*) resulted in glucose hypersensitivity [38]. ABA induction of other *ABI5* homologues could explain the puzzling observation that the glucose-insensitive phenotype was reverted by the addition of exogenous ABA in the ABA-insensitive *abi5* mutant [13]. It is also possible that the analysed *abi5* allele is not null and ABA increases the mutant *ABI5* expression to sufficient levels to restore a wild-type response. To dissect the participation of diverse elements and factors in sugar responses, the complex mode of regulation and direct interactions among several ABA signalling components need to be considered.

In contrast to the ABA biosynthesis deficient mutants, not all of the *abi* mutants display abnormal responses to sugars. Based on the sugar phenotype in young seedlings, *ABI1, ABI2* and *ABI3* do not seem to have a major role in glucose signalling [13,14,16]. However, *abi1* and *abi2* are dominant mutants [39]. Elucidation of the true role of ABI1 and ABI2 in sugar signalling might require more effort because of potential functional redundancy of many serine/threonine protein phosphatase 2Cs [40,41]. A recent study has shown that *ABI3* is also activated by 6% glucose in an ABA-dependent manner, albeit at a lower level than *ABI4* and *ABI5* [19]. In addition, ABI3 overproduction increases sensitivity to glucose at seedling stage [25]. The tested *abi3* mutant could be a weak allele, and a null allele should be tested to clarify the involvement of *ABI3* in sugar responses.

Distinct glucose and osmotic-stress signalling

After germination, a plant's success depends on its ability to grow autotrophically. During this transition time, plants live as heterotrophs while the photosynthetic apparatus becomes competent. Seedling establishment is such a crucial period that rapid responses to a range of factors are fundamentally important. In fact, it has been suggested that this period defines a checkpoint during which internal and external conditions are monitored [37]. The effect of excess glucose during this development stage results in an arrest of seedling growth and differentiation that seems to be mediated partly by the increase in ABA biosynthesis and by the activation of some ABA signalling genes. A concern has been raised about whether this developmental response reflects a direct sugar regulation process or an indirect osmotic effect, because ABA is well known to be involved in stress responses that allow plants to cope with environmental fluctuations [22].

Even though ABA plays a central role in sugar, salt and osmotic-stress responses [25,42], several lines of evidence indicate that the accumulation of ABA in response to sugars or to osmotic stress is mediated by distinct mechanisms. First, high glucose but not mannitol levels result in developmental arrest with no cotyledon expansion or greening [12,13,43]. Second, drought and osmotic stress, but not glucose, directly activate the *NCED3* gene, a rate-limiting step for stress-induced ABA accumulation [19,29–31]. Third, *ABA2* is activated by glucose but not by drought or osmotic stress [19]. Finally, *ABI3, ABI4* and *ABI5* expression are regulated differently by glucose and mannitol [19,35]. *ABI4* and *ABI5* transcripts are positively regulated at a low concentration by the glucose analogue 2-deoxyglucose, which acts as a sugar signal but not as an osmotic stimulus [4,6] (A. Arroyo *et al.*, unpublished). The fact that multiple mutant alleles for the same genes involved in ABA biosynthesis or ABA signalling have been isolated from sugar- and salt/osmotic-stress screens seems to reflect the important role of ABA in both types of signalling. In addition, sugar signals appear to modulate the action of other plant hormones besides ABA [12,18] (L. Zhou and J. Sheen, unpublished).

Glucose and ABA in growth promotion and inhibition

During vegetative growth, ABA has been shown to play a central role in adaptive responses to environmental cues such as drought and other stress responses [44,45]. Consequently, ABA has been considered as a stress hormone and growth inhibitor. However, recent data have revealed the importance of ABA in normal root and shoot growth [19,46], suggesting that the physiological roles of this hormone might be far more complex than those that have been conventionally defined [25]. Similar to sugars and to the plant growth hormone auxin, high and low ABA concentrations could have opposite effects on plant growth and development [7,19,46–48].

Most of the well-known ABA functions have been defined at elevated ABA levels (e.g. under stress conditions) and at the late stage of seed development. Up to 100 μM exogenous ABA is routinely used for root-inhibition or gene-regulation studies, or for mutant screens and analyses. However, the growth defects observed in *gin1* and other *aba* mutants actually suggest that endogenous ABA is required to promote plant growth and development in the absence of severe stress (Fig. 2) [19]. Similar observations are also made with the analyses of ABA-deficient mutants in tomato [46]. This important aspect of ABA function is just emerging and is not yet fully understood. Recent studies have also revealed that the growth hormone auxin can potentiate the ABA response in roots [49] and that the stress hormone ethylene can antagonize ABA signalling in seedlings [19,46,48,50]. Therefore, glucose regulation of ABA biosynthesis and signalling could impact auxin and ethylene responses.

Glucose and ethylene connections

Unexpectedly, the characterization of the *gin1* mutant uncovered a mutually antagonistic relationship between the glucose and ethylene signalling pathways [12]. This antagonistic connection is strongly supported by the discovery that the ethylene overproduction (*eto1*) and ethylene constitutive signalling (*ctr1*) mutants are glucose insensitive. Consistent with this, several ethylene-insensitive mutants (*etr1*, *ein2*, *ein3* and *ein6*) display glucose oversensitivity [12,19] (W-H. Cheng and J. Sheen, unpublished]. Further support came from the finding that two sugar-insensitive mutants, *sis1* [20] and *gin4* [19], are new alleles of *ctr1* [51]. Although *gin1* and *gin4* exhibit similar glucose-insensitive phenotypes during postgermination development (Fig. 1), only *gin4*/*ctr1* displays the

constitutive triple-response phenotype in the dark, a classical hallmark of ethylene action [51]. Recent studies have shown that overproduction of the C-terminal portion of EIN2 confers constitutive ethylene signalling with no triple response in the dark [52]. Thus, glucose signalling might affect specific ethylene pathways acting downstream of ETR1 and EIN2 [12,19,51]. Further analysis of the molecular links between glucose and ethylene signalling might distinguish branching pathways responsible for downstream responses to ethylene and elucidate interactions between ethylene and glucose, as well as other plant hormones.

The analyses of sugar signalling mutants have suggested that ethylene acts as an antagonist of the glucose response, whereas ABA promotes it. The link between these two hormones with respect to glucose has become clearer with the analysis of the double mutants *gin1 etr1* and *gin1 ein2*, which display the glucose-insensitive phenotype of the *gin1/aba2* mutant. Thus, ethylene seems to affect glucose signalling through ABA and to promote germination and seedling development. Based on the elevated ABA level found in the *ein2* mutant, it is likely that ethylene signalling partially represses the biosynthesis of ABA [19,48]. Interactions between ethylene and ABA have also been found independently [48,50]. It has been proposed that ethylene acts as a negative regulator of ABA signalling in the control of seed dormancy by decreasing the sensitivity to endogenous ABA [48]. The measurement of ABA levels and the expression of at least one ABA biosynthesis gene transcript (*ZEP1*) in 30-day-old plants suggest that ethylene decreases ABA biosynthesis [48]. Similar interactions could also occur during seed germination. However, the connection between these two stress hormones in a root-growth assay seems to be quite different. In wild-type plants, the presence of low exogenous ABA concentrations (100 nM) promotes seedling root growth, whereas the ethylene precursor ACC (0.2 μM) and a high concentration of ABA (100 μM) both inhibit root growth. In these experiments, the roots of ethylene-insensitive mutants, such as *etr1* and *ein2*, are resistant to exogenous application of ethylene and ABA [48,50]. It appears that the interactions and the mode of action of these two hormones vary depending on the hormone concentrations and the tissues analysed. These results are consistent with the new role of endogenous ABA (low concentration) in promoting root growth [19]. The high level of exogenous ABA (100 μM) probably represents a stress signal reminiscent of ethylene or the 'stress' effect of the growth-promoting hormone auxin at high exogenous levels [53]. Interestingly, the strong auxin-resistance mutant *axr2* is relatively insensitive to both ABA and ethylene signals, suggesting a possible convergence point of three hormonal signalling pathways [54].

Conclusions and perspectives

Genetic and phenotypic analyses of *Arabidopsis* sugar-signalling mutants have unravelled complex and extensive interactions between sugar and hormonal signalling pathways (Table 1, Fig. 3). Over the past few years, the simplistic idea of a linear transduction pathway in plants for regulating complex processes has begun to change. The intricate and complex webs that interrelate sugar and hormone signalling pathways are excellent examples of such complexity [22]. Multiple regulatory components originally isolated in a particular signalling pathway have now been shown to interconnect with other pathways [48,50,55]. This plasticity could facilitate central nodes of integration that are crucial for a plant's flexible responses to physical and chemical environments. Transcription factors such as ABI4 and ABI5 could act as integration nodes, receiving signals from multiple signals and generating integrated plant responses. With the increased availability of molecular probes for the genes involved in the sugar and hormone connections, more direct and specific molecular links can be established.

Although both ABA and ethylene were previously defined as stress hormones, their interaction with sugar signals is the opposite under the condition used for sugar signalling mutant screens. It was during the analysis of the *gin1* mutant that the shoot-promoting effect of ethylene was revealed in *Arabidopsis*. Even in the absence of high sugar levels, ethylene can promote hypocotyl elongation under light but inhibit the same process in the dark [52,56]. The initially puzzling interactions between ABA and ethylene in the seed-germination and root-growth assays could simply be attributed to changing responses at different concentrations of exogenously applied ABA and distinct intrinsic states of different tissues [48,50]. Interestingly, the growth-promoting effect of endogenous ABA also became clear during the characterization of the *gin1/aba2* mutant phenotypes [12,19]. Thus, the phenotypic plasticity of plant growth and

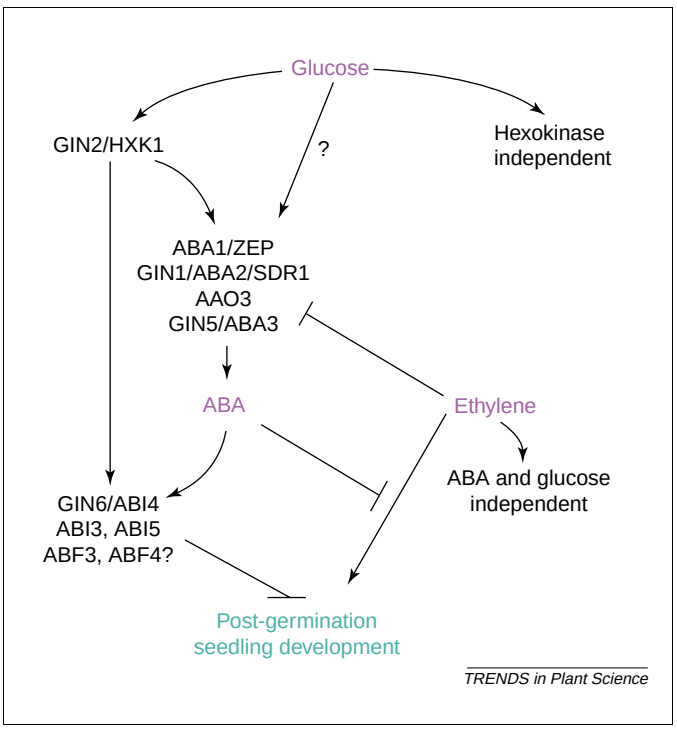

Fig. 3. Model for glucose connections to abscisic acid (ABA) and ethylene in *Arabidopsis*. The antagonistic interaction between glucose and ethylene is mediated partly through ABA biosynthesis and signalling, which might be directly modulated by glucose through hexokinase 1 (HXK1) as one of the glucose sensors.

development is the manifestation of a signalling network that integrates intrinsic and extrinsic signals, including nutrients, plant hormones and physical factors. Another layer of complexity is added when different responses are stimulated by the same signals at different concentrations. These diverse plant phenotypes reveal the remarkably broad range of growth plasticity stored in the plant genome.

Acknowledgements

We thank Filip Rolland for critical reading of the manuscript, Jyan-Chyun Jang, Wan-Hsing Cheng, Li Zhou, Brandon Moore, Stuart Reichler, Filip Rolland, Tomokazu Koshiba, Eiji Nambara and Pedro L. Rodríguez for informative discussions and sharing unpublished results. We acknowledge support from the National Science Foundation and the National Institute of Health (J.S.) and Howard Hughes Medical Institute, Consejo Nacional de Ciencia y Tecnologia 31791-N and DGAPA (P.L.).

References

1 Rolland, F. *et al.* (2001) Glucose-sensing mechanisms in eukaryotic cells. *Trends Biochem. Sci.* 26, 310–317
2 Koch, K.E. (1996) Carbohydrate modulated gene expression in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47, 509–540
3 Roitsch, T. (1999) Source-sink regulation by sugar and stress. *Curr. Opin. Plant Biol.* 2, 198–206
4 Sheen, J. *et al.* (1999) Sugars as signaling molecules. *Curr. Opin. Plant Biol.* 2, 410–418
5 Wobus, U. and Weber, H. (1999) Sugars as signal molecules in plant seed development. *Biol. Chem.* 380, 937–944
6 Smeekens, S. (2000) Sugar-induced signal transduction in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51, 49–81
7 Xiao, W. *et al.* (2000) The role of hexokinase in plant sugar signal transduction and growth and development. *Plant Mol. Biol.* 44, 451–461
8 Coruzzi, G.M. and Zhou, L. (2001) Carbon and nitrogen sensing and signaling in plants: emerging 'matrix effects'. *Curr. Opin. Plant Biol.* 4, 247–253
9 Ohto, M. *et al.* (2001) Effects of sugar on vegetative development and floral transition in *Arabidopsis*. *Plant Physiol.* 127, 252–261
10 Jang, J.C. *et al.* (1997) Hexokinase as a sugar sensor in higher plants. *Plant Cell* 9, 5–19
11 Nemeth, K. *et al.* (1998) Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in *Arabidopsis*. *Genes Dev.* 12, 3059–3073
12 Zhou, L. *et al.* (1998) Glucose and ethylene signal transduction crosstalk revealed by an *Arabidopsis* glucose-insensitive mutant. *Proc. Natl. Acad. Sci. U. S. A.* 95, 10294–10299
13 Arenas-Huertero, F. *et al.* (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
14 Laby, R.J. *et al.* (2000) The *Arabidopsis* sugar-insensitive mutants *sis4* and *sis5* are defective in abscisic acid synthesis and response. *Plant J.* 23, 587–596
15 Pego, J.V. *et al.* (2000) Photosynthesis, sugars and the regulation of gene expression. *J. Exp. Bot.* 51, 407–416
16 Huijser, C. *et al.* (2000) The *Arabidopsis SUCROSE UNCOUPLED-6* gene is identical to *ABSCISIC ACID INSENSITIVE-4*: involvement of abscisic acid in sugar responses. *Plant J.* 23, 577–585
17 Rook, F. *et al.* (2001) Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signalling. *Plant J.* 26, 421–433
18 Rolland, F. *et al.* (2002) Sugar sensing and signaling in plants. *Plant Cell* 14, S185–S205
19 Cheng, W-H. *et al.* (2002) A unique short-chain dehydrogenase/reductase in *Arabidopsis* abscisic acid biosynthesis and glucose signaling. *Plant Cell* 14, 2723–2743
20 Gibson, S.I. *et al.* (2001) The *sugar-insensitive1 (sis1)* mutant of *Arabidopsis* is allelic to *ctr1*. *Biochem. Biophys. Res. Commun.* 280, 196–203
21 Finkelstein, R.R. and Gibson, S.I. (2000) ABA and sugar interactions regulating development: cross-talk or voices in a crowd? *Curr. Opin. Plant Biol.* 5, 26–32
22 Gazzarrini, S. and McCourt, P. (2001) Genetic interactions between ABA, ethylene and sugar signaling pathways. *Curr. Opin. Plant Biol.* 4, 387–391
23 Xiong, L. *et al.* (2001) The *Arabidopsis LOS5/ABA3* locus encodes a molybdenum cofactor sulfurase and modulates cold stress- and osmotic stress-responsive gene expression. *Plant Cell* 13, 2063–2083
24 Seo, M. and Koshiba, T. (2002) The complex regulation of ABA biosynthesis in plants. *Trends Plant Sci.* 7, 41–48
25 Finkelstein, R.R. *et al.* (2002) Abscisic acid signaling in seeds and seedlings. *Plant Cell* 14, S15–S45
26 Xiong, L. *et al.* (2002) Regulation of osmotic stress-responsive gene expression by the *LOS6/ABA1* locus in *Arabidopsis*. *J. Biol. Chem.* 277, 8588–8596
27 Yamaguchi, S. and Kamiya, Y. (2000) Gibberellin biosynthesis: its regulation by endogenous and environmental signals. *Plant Cell Physiol.* 41, 251–257
28 Mathur, J. *et al.* (1998) Transcription of the *Arabidopsis CPD* gene, encoding a steroidogenic cytochrome P450, is negatively controlled by brassinosteroids. *Plant J.* 14, 593–602
29 Qin, X. and Zeevaart, J.A. (1999) The 9-*cis*-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water-stressed bean. *Proc. Natl. Acad. Sci. U. S. A.* 96, 15354–15361
30 Iuchi, S. *et al.* (2000) A stress-inducible gene for 9-*cis*-epoxycarotenoid dioxygenase involved in abscisic acid biosynthesis under water stress in drought-tolerant cowpea. *Plant Physiol.* 123, 553–562
31 Iuchi, S. *et al.* (2001) Regulation of drought tolerance by gene manipulation of 9-*cis*-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. *Plant J.* 27, 325–333
32 Finkelstein, R.R. (1994) Mutations at two new *Arabidopsis* ABA response loci are similar to the *abi3* mutations. *Plant J.* 5, 765–771
33 Finkelstein, R.R. *et al.* (1998) The *Arabidopsis* abscisic acid response locus *ABI4* encodes an APETALA 2 domain protein. *Plant Cell* 10, 1043–1054
34 Quesada, V. *et al.* (2000) Genetic analysis of salt-tolerant mutants in *Arabidopsis thaliana*. *Genetics* 154, 421–436
35 Brocard, I.M. *et al.* (2002) Regulation and role of the *Arabidopsis abscisic acid-insensitive 5* gene in abscisic acid, sugar, and stress response. *Plant Physiol.* 129, 1533–1543
36 Finkelstein, R.R. and Lynch, T.J. (2000) The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. *Plant Cell* 12, 599–609
37 Lopez-Molina, L. *et al.* (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 4782–4787
38 Kang, J. *et al.* (2002) *Arabidopsis* basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. *Plant Cell* 14, 343–357
39 Leung, J. and Giraudat, J. (1998) Abscisic acid signal transduction. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 199–222
40 Sheen, J. (1998) Mutational analysis of protein phosphatase 2C involved in abscisic signal transduction in higher plants. *Proc. Natl. Acad. Sci. U. S. A.* 95, 975–980
41 Gosti, F. *et al.* (1999) ABI1 protein phosphatase 2C is a negative regulator of abscisic acid signaling. *Plant Cell* 11, 1897–1909
42 González-Guzmán, M. *et al.* (2002) Molecular characterization of the *Arabidopsis* abscisic acid biosynthesis *ABA2* gene reveals a member of the short-chain alcohol dehydrogenase family. *Plant Cell* 14, 1833–1846
43 Martin, T. *et al.* (2002) *Arabidopsis* seedling growth, storage lipid mobilization, and photosynthetic gene expression are regulated by carbon:nitrogen availability. *Plant Physiol.* 128, 472–481
44 Zeevaart, J.A.D. and Creelman, R.A. (1988) Metabolism and physiology of abscisic acid. *Annu. Rev. Plant Physiol Plant Mol. Biol.* 39, 439–473
45 Schroeder, J.I. *et al.* (2001) Guard cell abscisic acid signalling and engineering drought hardiness in plants. *Nature* 410, 327–330
46 Sharp, R.E. *et al.* (2000) Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: evidence for an interaction with ethylene. *J. Exp. Bot.* 51, 1575–1584
47 Garciarrubio, A. *et al.* (1997) Abscisic acid inhibits germination of

mature *Arabidopsis* seeds by limiting the availability of energy and nutrients. *Planta* 203, 182–187
48 Ghassemian, M. *et al.* (2000) Regulation of abscisic acid signaling by the ethylene response pathway in *Arabidopsis*. *Plant Cell* 12, 1117–1126
49 Suzuki, M. *et al.* (2001) Maize VP1 complements *Arabidopsis abi3* and confers a novel ABA/auxin interaction in roots. *Plant J.* 28, 409–418
50 Beaudoin, N. *et al.* (2000) Interactions between abscisic acid and ethylene signaling cascades. *Plant Cell* 12, 1103–1115
51 Wang, K.L-C. *et al.* (2002) Ethylene biosynthesis and signaling networks. *Plant Cell* 14, S141–S151
52 Alonso, J.M. *et al.* (1999) EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. *Science* 284, 2148–2152
53 Evans, M.L. (1985) The action of auxin on plant cell elongation. *Crit. Rev. Plant Sci.* 2, 317–365
54 Wilson, A.K. *et al.* (1990) A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. *Mol. Gen. Genet.* 222, 377–383
55 Ephritikhine, G. *et al.* (1999) The *sax1* dwarf mutant of *Arabidopsis thaliana* shows altered sensitivity of growth responses to abscisic acid, auxin, gibberellins and ethylene and is partially rescued by exogenous brassinosteroid. *Plant J.* 18, 303–314
56 Smalle, J. *et al.* (1997) Ethylene can stimulate *Arabidopsis* hypocotyl elongation in the light. *Proc. Natl. Acad. Sci. U. S. A.* 94, 2756–2761
57 Bhalerao, R.P. *et al.* (1999) Regulatory interaction of PRL1 WD protein with *Arabidopsis* SNF1-like protein kinases. *Proc. Natl. Acad. Sci. U. S. A.* 96, 5322–5327
58 Yoshida, S. *et al.* (2002) Identification of a novel gene *HYS1/CPR5* that has a repressive role in the induction of leaf senescence and pathogen-defense responses in *Arabidopsis thaliana*. *Plant J.* 29, 427–437