Sugar sensing and signalling networks in plants

F. Rolland*† and J. Sheen*[1]

*Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA 02114, U.S.A., and †Department of Molecular Microbiology (VIB10), Flanders Interuniversity Institute for Biotechnology and Laboratory of Molecular Cell Biology, K.U. Leuven, B-3001 Leuven, Belgium

Abstract

Plant sugar signalling operates in a complex network with plant-specific hormone signalling pathways. Hexokinase was identified as an evolutionarily conserved glucose sensor that integrates light, hormone and nutrient signalling to control plant growth and development.

Introduction

Plant growth is controlled not only by developmental and environmental signals, but also by the physiological and metabolic state. As autotrophic, photosynthetic organisms, plants use sugars as metabolic messengers or signalling molecules to co-ordinate metabolic activities in source (sugar-producing) and sink (sugar-consuming or storage) tissues. It is, for example, well established that increased levels of glucose or sucrose, the end products of photosynthesis, repress photosynthetic gene expression in source leaves. This feedback mechanism overrides other levels of regulation such as light induction, ensuring a tight control of the plant's energy budget. However, from research in different fields of plant biology and recent studies on global gene expression it is becoming clear that sugar signalling not only controls photosynthetic gene expression but also affects numerous other metabolic and developmental processes during the entire plant life cycle, from seed germination to flowering and senescence (see [1] for a recent review). The apparent fundamental importance and potential for genetic modification of crop yield has recently led to an increased interest in plant sugar signalling mechanisms. To elucidate the complex plant glucose signalling networks, our laboratory has taken a combination of cellular, genetic, genomic and proteomic approaches.

Glucose signalling mediated by hexokinase

The development of a mesophyll protoplast transient expression system facilitated the exploration of metabolic gene regulation in higher plants [2]. Studies with a variety of sugars, sugar analogues and metabolic intermediates pointed to hexokinase (HXK), the first enzyme in glycolysis, as a putative sugar sensor with a distinct regulatory function [3]. Two *Arabidopsis* HXK genes, *AtHXK1* and *AtHXK2*, were cloned by complementation of the yeast *hxk1Δhxk2Δ* growth defect on fructose [4]. Consistent with a function for plant HXK in sugar sensing, transgenic *Arabidopsis* plants overexpressing sense or antisense HXK showed altered sugar responses in seedling development and gene expression [4]. While wild-type seedlings failed to develop expanded green cotyledons and arrested shoot meristem development in the presence of 6% glucose in the medium, antisense plants with reduced HXK levels were glucose insensitive. Sense AtHXK1-overexpression plants, on the other hand, were oversensitive to exogenous glucose and arrested in development by low levels [4]. Appropriate controls excluded osmotic effects.

Genetic analysis of complex interactions between glucose and hormone signalling

Using *Arabidopsis* as a genetic model and the developmental arrest assay on high exogenous glucose, we have isolated and characterized several glucose insensitive (*gin*) and glucose oversensitive (*glo*) mutants. Surprisingly, our studies of *gin* and *glo* mutants and similar screens in other laboratories have revealed extensive and intimate connections between glucose and plant hormone signalling pathways (reviewed in [5]). The insensitivity to glucose repression of cotyledon and shoot development could be phenocopied by treatment of wild-type plants with the precursor of the plant stress hormone ethylene, and by constitutive ethylene biosynthesis and signalling mutants. Ethylene-insensitive mutants, such as *ein2* (ethylene-insensitive2) and *etr1-1* (*ethylene receptor1-1*), conversely exhibited glucose hypersensitivity [6]. Epistasis analysis showed that GIN1 acted downstream of both HXK and the ETR1 ethylene receptor [6].

Subsequent molecular map-based cloning identified GIN1 as ABA2/SDR1, a short-chain dehydrogenase/reductase involved in the synthesis of the plant hormone ABA (abscisic acid) [7]. A central role for ABA in plant sugar signalling was also apparent from the analysis of the *gin5* and *gin6* mutants [8]. GIN6 is allelic to ABI4 (ABSCISIC ACID-INSENSITIVE4), a transcription factor involved in ABA signalling in seed and seedlings. However, not all *abi* mutants are glucose insensitive, suggesting that distinct ABA signalling pathways are involved in glucose and stress responses.

Key words: *Arabidopsis*, glucose, hexokinase (HXK), hormone signalling, sugar.

Abbreviations used: ABA, abscisic acid; AtHXK, *Arabidopsis thaliana* hexokinase; *ein*, ethylene insensitive; *gin*, glucose insensitive; *glo*, glucose oversensitive; HXK, hexokinase.

[1]To whom correspondence should be addressed (email sheen@molbio.mgh.harvard.edu).

GIN5 is allelic to ABA3 [5,8]. Since exogenous glucose also specifically increases both the expression of ABA signalling and biosynthesis genes and the endogenous ABA levels [7], a glucose-specific accumulation of ABA appears to be essential for HXK-mediated glucose signalling.

With the identification of *gin4* as a mutant allele of CTR1 (CONSTITUTIVE TRIPLE RESPONSE1) [7], encoding a Raf-like protein kinase and negative regulator in ethylene signalling, questions about the exact nature of the interactions between glucose and ethylene signalling resurfaced. A molecular link was finally established with the finding that protein stability of EIN3, a transcription factor and major downstream component in the ethylene-signalling cascade, is modulated by sugars [9]. EIN3 overexpressing plants were glucose insensitive, while *ein3* mutants had a *glo* phenotype. Using protoplasts expressing a luciferase reporter controlled by a synthetic promoter with multiple EIN3 binding sites, it was found that the EIN3-dependent transcription was repressed by glucose. The analysis of EIN3–GFP (green fluorescent protein) fusion proteins and immunoblot analysis of endogenous EIN3 protein levels revealed that glucose enhances the degradation of nuclear EIN3 mediated by the proteasome, while ethylene enhances its stability. Overexpression of AtHXK1 but not yeast Hxk2 increased EIN3 degradation, suggesting that this response is also dependent on AtHXK1. This was confirmed in protoplasts of the *gin2* (AtHXK1 null) mutant.

The glucose sensor AtHXK1 integrates nutrient, light and hormone signalling

The recent detailed characterization of the *gin2* mutants for the first time enabled the definition of the exact functions of this glucose sensor [10]. To study AtHXK1 functions in a physiological context (avoiding the artificial high exogenous glucose condition), plants were grown under different light conditions that altered endogenous glucose levels [10]. While wild-type and *gin2* plants looked similar under low light intensities, higher intensities resulted in significantly different phenotypes. The increase in energy supply (through photosynthesis) accelerated wild-type plant growth and development while mutant plants remained smaller with dark green leaves. This supports a role for AtHXK1 in growth promotion in addition to its growth inhibiting effect on high glucose. The delayed senescence phenotype (and reduced fertility) of the *gin2* mutants is reminiscent of the increased longevity in response to calorie restriction in other eukaryotic organisms.

The fact that the AtXHK1 null mutants retain 50% of their glucose kinase activity and have normal sugar phosphate levels suggested that the observed phenotypes were signalling effects rather than metabolic effects. Consistently, no overt correlation was found between glucose kinase activity and glucose-regulated growth, chlorophyll content and photosynthetic gene expression under different growth conditions. Conclusive evidence for the uncoupling of glucose signalling and metabolism in controlling gene expression and developmental processes came from the analysis of two specific AtHXK1 mutant alleles that lacked catalytic activity (ATP binding and phosphoryl transfer respectively) but retained the glucose binding site [10]. In a protoplast transient expression assay, these alleles sustained repression of photosynthetic promoter activity to the same extent as wild-type AtHXK1. In the *gin2* background, these mutant alleles, like wild-type AtHXK1, complemented the glucose-mediated growth phenotypes as well as glucose repression of gene expression.

The cell expansion defect and delayed senescence of the *gin2* mutants also prompted the investigation of a possible link between AtHXK1-mediated signalling and the growth hormones auxin and cytokinin [10]. Indeed, *gin2* mutants were found to be relatively insensitive to auxin (IAA, indole-3-acetic acid), with a clear defect in auxin-induced cell proliferation and root formation in hypocotyl explants. The auxin-resistant mutants *axr1*, *axr2* and *tir1* were also found to be insensitive to high glucose concentrations. Conversely, *gin2* mutant calli were hypersensitive to shoot-inducing cytokinin (2IP, 2-isopentenylaminopurine). Cytokinin treatment can also overcome the high glucose-induced developmental arrest in seedlings, independently from ethylene signalling. The glucose-insensitive phenotype of plants with a constitutive cytokinin response (e.g. overexpressing a constitutively active cytokinin receptor kinase or a key response regulator, ARR2) confirmed the antagonistic effects of glucose and cytokinins.

New investigations of the HXK-dependent signalling pathways

The genetic screens have uncovered the enormous complexity of sugar signalling networks in plants. The flexible and reversible responses to both low and high glucose signals in plant growth promotion and inhibition, respectively, depend on cell type, developmental state, multiple nutrient status and environmental conditions. The plasticity of plant developmental programmes could therefore be attributed to the versatile sugar sensing and signalling activities in the plant signal transduction networks. Interactions with ABA, ethylene, auxin and cytokinin signalling obviously complicate the elucidation of the molecular mechanisms at the core of the glucose sensing and signal transduction pathway. To gain further understanding of the molecular mechanisms of AtHXK1 actions, new approaches using genetic, proteomic and genomic tools have been taken to focus on the identification of key components directly involved in the AtHXK1-mediated sensing process. For example, *gin2* suppressor (*gis*) mutants were isolated that restored glucose sensitivity and growth phenotypes without altering glucose kinase activity (Q. Hall and J. Sheen, unpublished work). Mapping and molecular cloning of the mutated genes will possibly identify direct downstream components of the AtHXK1 signalling pathway. Interestingly, plant HXKs reside in high-molecular-mass protein complexes at multiple subcellular locations (B. Moore and J. Sheen, unpublished work; Y.-H. Cho and

J. Sheen, unpublished work). In *Arabidopsis*, for example, a completely functional glycolytic 'metabolon' is present on the outside of the mitochondrial membrane [11] and both HXK1, HXK2 and hexokinase-like (HKL) proteins appear to be associated with mitochondria (B. Moore and J. Sheen, unpublished work). In addition, HXK activities have been found to be associated with plastids (e.g. [12]). In both cases, optimal substrate availability appears to be the major advantage. Whether and how this compartmentalization is important for signalling remains unclear. Remarkably, AtHXK1 is also found in the nuclear fraction [9], suggesting that the glucose sensor might control gene expression directly as a universal glucose signalling mechanism in eukaryotic cells [13] (Y.-H. Cho and J. Sheen, unpublished work; Q. Hall and J. Sheen, unpublished work). Proteomic analysis of the various AtHXK1 protein complexes will likely reveal the direct and indirect physical interactions with regulatory or signalling target components.

To obtain an overview of the extent of transcriptional regulation by AtHXK1-dependent sugar signalling and the nature of its target genes and processes, we have carried out a comprehensive gene expression analysis of wild-type and *gin2* plants using Affymetrix GeneChip technology (F. Rolland and E. Baena-González, unpublished work). To reveal a full picture of the early transcriptional cascade, we monitored gene expression at different time points after addition of glucose to seedlings grown in liquid medium to ensure uniform responses. Clustering analysis of genes with similar regulation dynamics will prove useful in identifying regulatory *cis*-elements and their associated transcription factors. Early up- or down-regulated transcription factors are also interesting candidates for a role in mediating the downstream transcriptional cascade.

It is clear that a greater part of glucose regulation of gene expression is affected in *gin2* mutant seedlings, confirming the central role of AtHXK1 in sugar signalling. The largest group of genes affected by AtHXK1-dependent signalling is involved in photosynthesis in the broadest sense. The majority of these genes are activated by light but repressed by glucose. Thus, studying gene expression in dark-grown plants will overlook this major and important category of glucose-regulated genes. Glucose also represses genes involved in photorespiration, fatty acid synthesis and mobilization, and nitrogen metabolism, but activates genes involved in sucrose metabolism, respiration, cell wall and starch biosynthesis, which are usually associated with growth and storage. Sugar signalling also interacts with hormone, light and stress signalling by regulating the expression of diverse pathway components and transcription factors.

Conclusions

Leading a unique 'lifestyle', plants obviously display specific interactions among signalling pathways and exhibit distinct responses to environmental cues. It is therefore remarkable that many genes involved in carbon and nitrogen metabolism and storage, cell cycle and stress responses are similarly regulated by glucose in yeast, animals and plants. The evolutionarily conserved glucose sensor HXK may control the energy budget and resource utilizations through the function of different signalling complexes. It will be interesting to determine how conserved or diverse the molecular mechanisms underlying these ancient and fundamental responses really are as new sugar sensing and signalling mechanisms are uncovered in the near future.

We thank Qi Hall, Young-Hee Cho, Elena Baena-González and Brandon Moore for sharing their unpublished results. Research on sugar signalling in the Sheen laboratory is supported by the NSF and NIH grants. F.R. is supported by a return grant from the Belgian Office for Scientific, Technical and Cultural Affairs.

References

1 Rolland, F., Moore, B. and Sheen, J. (2002) Plant Cell **14**, S185–S205
2 Sheen, J. (1990) Plant Cell **2**, 1027–1038
3 Jang, J.-C. and Sheen, J. (1994) Plant Cell **6**, 1665–1679
4 Jang, J.-C., Leon, P., Zhou, L. and Sheen, J. (1997) Plant Cell **9**, 5–19
5 Leon, P. and Sheen, J. (2003) Trends Plant Sci. **8**, 110–116
6 Zhou, L., Jang, J.C., Jones, T.L. and Sheen, J. (1998) Proc. Natl. Acad. Sci. U.S.A. **95**, 10294–10299
7 Cheng, W.-H., Endo, A., Zhou, L., Penney, J., Chen, H.-C., Arroyo, A., Leon, P., Nambara, E., Asami, T., Seo, M., Koshiba, T. and Sheen, J. (2002) Plant Cell **14**, 2723–2743
8 Arenas-Huertero, F., Arroyo, A., Zhou, L., Sheen, J. and Leon, P. (2000) Genes Dev. **14**, 2085–2096
9 Yanagisawa, S., Yoo, S.-D. and Sheen, J. (2003) Nature (London) **425**, 521–525
10 Moore, B., Zhou, L., Rolland, F., Hall, Q., Cheng, W.-H., Liu, Y.X., Hwang, I., Jones, T. and Sheen, J. (2003) Science **300**, 332–336
11 Giege, P., Heazlewood, J.L., Roessner-Tunali, U., Millar, A.H., Fernie, A.R., Leaver, C.J. and Sweetlove, L.J. (2003) Plant Cell **15**, 2140–2151
12 Wiese, A., Groner, F., Sonnewald, U., Deppner, H., Lerchl, J., Hebbeker, U., Flugge, U. and Weber, A. (1999) FEBS Lett. **461**, 13–18
13 Moreno, F. and Herrero, P. (2002) FEMS Microbiol. Rev. **26**, 83–90

Received 2 September 2004