Genetic approaches to understanding sugar-response pathways

Fred Rook1 and Michael W. Bevan

Department of Cell and Developmental Biology, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

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Abstract

Plants as photoautotrophic organisms are able to produce the carbohydrates they require and have developed mechanisms to co-ordinate carbohydrate production and its metabolism. Carbohydrate-derived signals regulate the expression of genes involved in both photosynthesis and metabolism, and control carbohydrate partitioning. A number of genetic approaches have been initiated to understand sugar-response pathways in plants and identify the components involved. Screening strategies to date have been based on the effects of high sugar media on early seedling development or on changes in the enzyme activity or expression of sugar-responsive genes. These screens have established roles for plant hormones in sugar-response pathways, in particular for abscisic acid. The present emphasis on the role of plant hormones in sugar responses is due to the fact that mutants could be readily identified as belonging to these established pathways, but also results from the nature of the mutant screens in use. Progress is being made on the identification of mutants and genes that may be specific to sugar-signalling pathways. It is also expected that the modification of existing screens may target sugar-signalling pathways more directly. Genetic approaches may be especially useful in identifying components of novel signalling pathways unique to plants, and their combination with genomic and molecular approaches will guide future research.

Key words: Abscisic acid, Arabidopsis, sugar responses, sugar signalling.

Introduction

All organisms need to balance the availability and demand for carbohydrates to sustain their metabolism and support their growth and development. Plants and other photoautotrophic organisms are unique in that they are able to produce the carbohydrates they require. Photoassimilates produced in mature leaves are transported via the phloem to support the growth of developing tissues, to meet the requirements of heterotrophic tissues or to be stored in anticipation of less-favourable environmental conditions. To co-ordinate this partitioning of carbohydrates, plants require information regarding their carbohydrate status and information on where and for what purposes the available carbohydrates are needed.

The co-ordination of carbohydrate production, metabolism and partitioning is controlled at numerous levels such as the allosteric regulation of metabolic enzymes or the tissue-specific and temporal expression of the genes involved. Moreover, sugar itself functions as a signal molecule in regulating its own production and use. For instance, high sugar levels in leaves result in the feedback inhibition of photosynthetic gene expression and the induction of starch biosynthetic genes. Investigations into the mechanisms by which plants determine their carbohydrate status (‘sugar-sensing’) and regulate sugar responses are complicated by the central role of sugars in metabolism and growth and development.

A number of physiological, biochemical and molecular approaches have been used to study sugar responses in plants (for reviews see Smeekens, 2000; Rolland et al., 2002). Here the focus is on genetic approaches to sugar-response pathways and an overview is presented of the strategies that have been used to date. As the genetic model system of choice these
approaches use Arabidopsis for the isolation of sugar-response mutants.

### Sugar-response mutants

The general term ‘sugar-response mutants’ is usually preferred when referring to mutants in which sugar-responsive gene expression or sugar-induced changes in growth and development differ from those in the wild type. That a particular sugar-response mutant is affected in the actual perception or transduction (‘sugar sensing and signalling’) of a sugar signal is more difficult to establish. For instance, several mutants related to plant hormone signalling pathways are also ‘sugar-response’ mutants, but are not necessarily involved in ‘sugar signalling’. That is to say, they may not be part of the mechanism by which plant cells determine their carbohydrate status. An increasing number of strategies are being used to isolate mutants with altered sugar responses. Most are based on the effects of high sugar media on seedling development or on changes in the activity or expression of sugar-responsive genes (Table 1). The latter include screens based on transgenic lines containing fusions of sugar-responsive promoters to reporter genes or selection markers.

Genetic screens based on the observation that growth media with high sugar levels can prevent seedling establishment have been widely used (Table 1). In the glucose insensitive (gin, Zhou et al., 1998) screen, media containing 6% glucose repressed seedling greening and arrested development after germination. Mutants were isolated which were insensitive to these high glucose levels and developed normally. Similar sugar-insensitive screens based on seedling establishment used high levels of sucrose, for example, the sugar insensitive (sis; Laby et al., 2000) and sucrose insensitive growth (sig; Pego et al., 2000) mutants were selected on media containing, respectively, 300 mM and 350 mM sucrose. The same principle of seedling developmental arrest by sugar has been used to isolate mutants with enhanced sugar sensitivity. The glucose super sensitive (gss; Pego et al., 2000), sucrose super sensitive (sss; Pego et al., 2000), glucose oversensitive (glo; Rolland et al., 2002), and pleiotropic regulatory locus1 (prl1; Németh et al., 1998) mutant screens are all based on reduced seedling growth or seedling developmental arrest at lower sugar concentrations.

### Table 1. Genetic screens for sugar response mutants in Arabidopsis

<table>
<thead>
<tr>
<th>Mutant name</th>
<th>Screening approach and conditions</th>
<th>Number of mutants isolated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>cai, carbohydrate insensitive</td>
<td>Seedling development on low nitrogen (0.1 mM); and 100 mM sucrose</td>
<td>35</td>
<td>Boxall et al., 1996b Martin et al., 2002c</td>
</tr>
<tr>
<td>gin, glucose insensitive</td>
<td>Seedling establishment on 330 mM glucose</td>
<td>6</td>
<td>Zhou et al., 1998; Arenas-Huertero et al., 2000</td>
</tr>
<tr>
<td>glo, glucose oversensitive</td>
<td>Seedling developmental arrest on 220 mM glucose</td>
<td>–</td>
<td>Rolland et al., 2002b</td>
</tr>
<tr>
<td>gss, glucose super sensitive</td>
<td>Seedling developmental arrest on 56 mM glucose; Amylase activity in protein extracts of plants grown on 175 mM sucrose</td>
<td>22</td>
<td>Pego et al., 2000b</td>
</tr>
<tr>
<td>hba, high-level beta amylase</td>
<td>Increased activity of a ApL3::Luciferase construct on 34 mM sucrose media</td>
<td>&gt;10</td>
<td>Hadingham et al., 2002b</td>
</tr>
<tr>
<td>hsr, high sugar response</td>
<td>Survival of a ApL3::P450 transgenic line on 100 mM sucrose and R7402 proherbicide</td>
<td>10</td>
<td>Rook et al., 2001</td>
</tr>
<tr>
<td>isi, impaired sucrose induction</td>
<td>Amylase activity in protein extracts of plants grown on 175 mM sucrose</td>
<td>2</td>
<td>Mita et al., 1997a</td>
</tr>
<tr>
<td>lba, low-level beta amylase</td>
<td>Seed germination on 7.5 mM mannose</td>
<td>72</td>
<td>Pego et al., 2000b</td>
</tr>
<tr>
<td>mig, mannose insensitive</td>
<td>Reduced seedling growth on 175 mM sucrose</td>
<td>1</td>
<td>Németh et al., 1998</td>
</tr>
<tr>
<td>germination</td>
<td>Reduced amylase activity of plants grown under a 12 h photoperiod, in pgm1 background</td>
<td>&gt;4</td>
<td>Laby et al., 2001</td>
</tr>
<tr>
<td>rsl, reduced sucrose response</td>
<td>Reduced expression of a potato patatin promoter GUS construct in Arabidopsis on 90 mM sucrose</td>
<td>4</td>
<td>Martin et al., 1997</td>
</tr>
<tr>
<td>sig, sucrose insensitive growth</td>
<td>Seedling development on 350 mM sucrose</td>
<td>59</td>
<td>Pego et al., 2000b</td>
</tr>
<tr>
<td>sis, sugar insensitive</td>
<td>Seedling development on 300 mM sucrose</td>
<td>12</td>
<td>Laby et al., 2000</td>
</tr>
<tr>
<td>sss, sucrose super sensitive</td>
<td>Non-germination on 350 mM sucrose</td>
<td>37</td>
<td>Pego et al., 2000b</td>
</tr>
<tr>
<td>sun, sucrose uncoupled</td>
<td>Reduced repression of the developmental expression of a PC::Luciferase construct on 88 mM sucrose</td>
<td>16</td>
<td>Dijkwel et al., 1997</td>
</tr>
<tr>
<td>uns, unusual sugar response</td>
<td>Early flowering and/or low chlorophyll on 146 mM sucrose, altered sugar-responsive gene expression</td>
<td>4</td>
<td>Ohoto et al., 2000b                                                      Ohoto et al., 2001</td>
</tr>
</tbody>
</table>

- In several cases complementation groups have not been established and mutants may be allelic. Allelism between mutants isolated in different screens has usually not been determined.
- Preliminary reports of the indicated screens.
- These publications present the principle on which the screen is based.
tions. The repression of seed germination by low levels of mannose was the basis for the mannose insensitive germination (mig; Pego et al., 2000) screen, while the importance of the carbon-to-nitrogen ratio for seedling growth and photogene expression provided the basis for the carbohydrate insensitive (cai) screen (Boxall et al., 1996).

Sugar-response screens are also based on identifying mutants with changes in the expression of specific genes known to be sugar regulated. A semi-quantitative assay of total amylase activity in leaf protein extracts was used to isolate mutants with changes in β-amylase expression (Mita et al., 1997a, b; Laby et al., 2001). However, most screens based on changes in the sugar-regulated expression of specific genes, make use of transgenic lines which contain fusions of the regulatory regions to reporter genes or selection markers.

The sucrose uncoupled (sun, Dijkwel et al., 1997) mutants were identified using transgenic lines containing a luciferase reporter gene driven by the plastocyanin promoter. Like other photosynthetic genes plastocyanin expression is repressed by high sugar levels. Mutants were selected in which a developmentally controlled induction of plastocyanin was no longer repressed by sugar. While high sugar levels repress photogene expression, genes encoding storage proteins or genes involved in starch biosynthesis are induced by sugar. Patatin is the main storage protein in potato tubers and the expression of patatin class I genes is induced by sugar. This sugar-regulated expression is maintained in transgenic Arabidopsis containing a potato patatin promoter fused to the β-glucuronidase reporter gene and formed the basis for the isolation of the reduced sucrose response (rsr) mutants (Martin et al., 1997). The highly sugar inducible promoter of the ApL3 gene, coding for an ADP-glucose pyrophosphorylase large subunit, was fused to a negative selection marker and used to isolate the impaired sucrose induction (isi, Rook et al., 2001) mutants. The same promoter fused to the luciferase reporter gene was used to isolate the high sugar-response (hsr) mutants, which show enhanced ApL3 expression under low sugar conditions (M Baier, G Hemmann, F Rook, M Bevan, unpublished results).

Sugar responses and abscisic acid

The identification of abscisic acid and ethylene-related mutants in sugar-response screens demonstrates the close interaction between sugar responses and hormonal control of developmental processes. ABA in particular was found to be of major importance for the way tissues respond to sugar. Both genetic screens based on seedling developmental arrest on high sugar media and those based on transgenic plants containing reporter gene constructs have yielded a large number of mutants allelic to known ABA-related mutants such as abi4 or aba2. Constitutive ethylene biosynthesis and signalling mutants also display sugar-insensitive phenotypes on high sugar media (Zhou et al., 1998; Gibson et al., 2001). These ethylene effects may, however, be explained by reports that ethylene appears to be a negative regulator of ABA action during germination (Beaudoin et al., 2000; Ghassemian et al., 2000).

Abscisic acid insensitive4 was originally isolated because of its ability to germinate on ABA-containing media normally inhibitory for wild-type germination. The responsible gene was identified as an Arabidopsis-type transcription factor (Finkelstein et al., 1998) and has since been found in a large number of sugar-response screens (sun6, Huijser et al., 2000; sis5, Laby et al., 2000; gin6, Arenas-Huerto et al., 2000; isi3, Rook et al., 2001) but also in a salt-tolerance screen (Quesada et al., 2000). RNA gel blot analyses and transgenic lines containing ABI4 promoter-GUS fusions have established that ABI4 is predominantly expressed in developing embryos with the highest expression at seed maturation. Expression decreases after germination and is low in vegetative tissues (Söderman et al., 2000). Consequently, the clearest phenotypes are observed at the seed and seedling stages. It was initially suggested that ABI4 expression was induced by glucose (Arenas-Huerto et al., 2000), but this has since been reported not to be the case (Söderman et al., 2000). The high ABI4 expression observed in wild-type seedlings grown on 7% glucose media is most likely the result of their developmental arrest on the high glucose medium (as discussed in Rook et al., 2001).

Mutants involved in ABA biosynthesis have also been isolated in sugar-response screens, most notably alleles of aba2 (gin1, Zhou et al., 1998; sis4, Laby et al., 2000; isi4, Rook et al., 2001). We have proposed that the aba2 gene encodes a member of the short-chain dehydrogenase/reductase (SDR) gene family (Rook et al., 2001) and biochemical evidence supporting its proposed role in ABA biosynthesis as a xanthoxin oxidase has since been reported (Seo and Koshiba, 2002; Gonzalez-Guzman et al., 2002).

Opinions differ to what the precise roles are for ABA and genes like ABI4 in sugar responses. Arenas-Huerto et al. (2000) have suggested that sugar responses are directly mediated by ABA via the induction of its biosynthesis. Their model is primarily based on crossovers between transgenic lines overexpressing hexokinase, which confers sugar hypersensitivity, and glucose-insensitive mutants such as gin1/aba2. They reasoned that as the ‘double mutant’ is still glucose insensitive, ABA is epistatic to the sugar sensor hexokinase. We have argued that this interpretation presupposes that sugar-signalling pathways are not saturated by the high sugar media used and have suggested an alternative model in which ABA modulates sugar responses by regulating the responsiveness of tissues to sugar signals (Rook et al., 2001).
The idea that sugar responses depend on the ‘responsive state’ of the tissue was based on our work with the starch biosynthetic ApL3 gene. We observed that ABA itself did not induce ApL3 gene expression, but that ABA significantly enhanced the induction by sugar (Rook et al., 2001). We argued that genetic screens based on high sugar media had an osmotic component. The increase in ABA levels as a result of the high osmotic medium made the seedlings more responsive to an inhibitory sugar signal. This was supported by the observation that while high osmotic media alone did not prevent seedling establishment, the addition of a small amount of sugar resulted in seedling developmental arrest (e.g. 400 mM sorbitol combined with 28 mM glucose; Laby et al., 2000). Similarly, in the presence of very low levels of ABA (100 nM), lower sugar levels were able to induce seedling developmental arrest (Arenas-Huerto et al., 2000).

Seedling developmental arrest by high sugar media can be seen as a regression of metabolism from a state in which seed reserves are mobilized to support seedling establishment to the storage-dominated metabolic state which existed during late embryogenesis (Rook et al., 2001; Gazzarrini and McCourt, 2001). The high levels of ABI4 expression observed in developmentally arrested wild-type seedlings (Arenas-Huerto et al., 2000) are also an indication of this reversion to a ‘storage mode’. Past the initial stages of seedling establishment, transfer of seedlings to high sugar media no longer results in their developmental arrest, suggesting that metabolism has become committed to growth (Gibson et al., 2001). Lopez-Molina et al. (2001) showed that ABA and drought-stress or salt-stress induced the accumulation of the ABI5 transcription factor when they were applied within a narrow developmental time interval following germination. This correlated with the arrest of seedling growth and the maintenance of a desiccation-tolerant state. They suggested a post-germination developmental checkpoint that delays seedling growth under unfavourable environmental conditions (e.g. drought). Both these interpretations indicate that ABA and transcription factors such as ABI4 and ABI5 are most relevant during seed maturation and early seedling growth. The present emphasis on the relationship between sugar and ABA in the literature reflects the fact that most genetic screens have been based on seed germination and seedling growth.

### Sugar-signalling mutants?

As most sugar-response mutants with established gene identities to date seem to be related to plant hormone biosynthesis or signalling (e.g. aba2, abi4; Table 2), the existence of mutants specific to ‘sugar-signalling’ is an important question. Can such mutants be identified in the genetic screens presently in use, or is there a need for improved or even novel screens? Arguably, the present emphasis on the role of plant hormones in sugar responses is partly based on the fact that mutants could be readily identified as belonging to these established pathways. The available knowledge on plant hormone signalling pathways and the identity of the genes involved, has greatly facilitated their identification in sugar-response screens. Mutants and most certainly the identity of genes specifically involved in sugar signalling, may so far have been under-reported. Such mutants are less likely to have been previously isolated in unrelated screens and would require extensive analysis.

Nonetheless, present screens have been able to isolate some mutants affected in genes with proposed roles in sugar signalling (Table 2). For instance, preliminary reports have identified gin2 as a mutation in the hexokinase encoding AtHXK1 gene, a proposed sugar sensor (Rolland et al., 2002). In addition, a number of mutants with glucose insensitive or sugar insensitive (e.g. sis3; Pattison et al., 2001) phenotypes seem to be unrelated to plant hormone responses. We previously reported a screen based on transgenic Arabidopsis containing a fusion between the sugar inducible ApL3 promoter and a negative selection marker (Rook et al., 2001). Apart from alleles of abi4 (isi3) and aba2 (isi4), we have also isolated an impaired sucrose induction mutant (isi5) which is insensitive to high sugar media, but seems to have no relation to plant hormone signalling pathways. These results indicate that...
screens based on seedling developmental arrest are able to identify mutants which may be unique to sugar-response screens. We also reported on two mutants (isi1 and isi2) which seemed unique to the isi-screen as they showed wild-type sensitivity to various high sugar media (Rook et al., 2001). Preliminary results following map-based cloning of isi1 show it to be a novel gene. Its vascular expression is related to the sink-to-source transition during leaf development and suggests a role in a regulatory pathway unique to plants (F Rook, M Bevan, unpublished results). The characterization of such novel plant-specific pathways can not rely on paradigms established in other organisms.

The isi screen made use of the sugar inducible ApL3 promoter fused to a negative selection marker to isolate mutants with reduced sugar responses (Rook et al., 2001). The same promoter has also been used to select for mutants with enhanced sugar responses. Transgenic Arabidopsis containing an ApL3 promoter-luciferase construct were used in a screen for mutants in which luciferase expression was observed under low, non-inducing sugar concentrations (1% sucrose). In these high sugar-response mutants (hsr), luciferase activity and the expression of the endogenous ApL3 gene were more readily induced by sugar (M Baier, G Hemmann, F Rook, M Bevan, unpublished results). Interestingly, most of the hsr mutants analysed in detail so far seem to have no relation to hormone-signalling pathways. It can be argued that, in contrast to the isi screen, mutants with increased ABA or reduced ethylene sensitivity were to be expected. It seems, however, that the relatively low sugar conditions used (30 mM sucrose), have prevented the isolation of mutants resulting from osmotic effects of the sugar media. These hsr mutants show a wide range of phenotypes related to sugar responses and metabolism, including increased starch levels (G Hemmann, M Bevan, unpublished results). The role of the hsr mutants in sugar responses can be more precisely assessed when the responsible genes have been identified.

Restrictions to present screens
The genetic screens performed to date also place restrictions on the nature of the mutants that will be isolated. For good practical reasons, most screens are performed on seedlings and depend on or select for their survival. Seedling-based screens typically allow over 500 seedlings to be assayed in a single petri dish, while screens using mature plants are more labour-intensive and restricted by available growth space. Mutants will only be isolated in seedling-based screens if the gene involved is functional at this stage. While some mutants have sugar-response phenotypes in both seedlings and mature plants, a gene like abi4 is predominantly expressed and functional during seed development and early germination. Mature abi4 plants can not easily be distinguished from the wild type. Conversely, genes involved in sugar responses, but predominantly expressed in mature plants, will be missed in mutant screens based on seedlings. The mutant screens based on changes in β-amylase activity (Mita et al., 1997a, b; Laby et al., 2001) are rare examples of the use of mature plants.

It can also be argued that mutations with severe effects on growth and development will be missed in the existing screens that are based on seedling growth. This is demonstrated by recent work on trehalose metabolism, which is proposed to have a regulatory role in sugar responses and carbohydrate partitioning. In yeast, trehalose-6-phosphate is required to regulate the flux of sugar into glycolysis by inhibiting hexokinase. Yeast mutants deficient in TPS1, encoding trehalose-6-phosphate synthase, are unable to grow on sugars (Thevelein and Hohmann, 1995). In Arabidopsis, TPS1 is transiently up-regulated in developing embryos during the cell expansion and storage reserve accumulation phase and is required for the expression of seed maturation marker genes. Disruption of the TPS1 gene in Arabidopsis results in embryo lethality, demonstrating its importance for storage reserve accumulation and embryo maturation in plants (Eastmond et al., 2002). Although the precise role of trehalose or T-6-P in sugar responses in plants is not yet clear, this work suggests that significant components regulating sugar responses may be missed in the genetic screens used to date.

Future prospects
Genetic screens for sugar-response mutants have shown the close interaction between sugar and hormone-signalling pathways. This reflects the essential role of sugars in growth and developmental processes. An adequate supply of carbohydrates is needed both to energize metabolism and provide the ‘building blocks’ to make cellular structures and cell walls. Plant hormones may integrate metabolism with developmental programmes by directing the flow of carbon and determine its use. This leaves questions about how plants determine their ‘sugar status’ largely unanswered.

With the present knowledge about the complex interactions between sugar and plant hormone signalling pathways, the focus could be directed to mutants which show sugar-response phenotypes, but have no obvious relation to plant hormones. A large number of sugar-response mutants have been isolated, but are not yet characterized in detail (Table 1). From these a subset could be selected which are most likely to affect sugar-signalling pathways.

In addition, existing sugar-response screens can be modified to select more specifically for ‘sugar-signalling’ mutants. For instance, the isolation of suppressor and
enhancer mutations of gin2 (encoding AtHXX1) has been suggested as a useful approach to elucidate HKX action further (Rolland et al., 2002). We have observed synergistic survival of double mutants between isil and isi3 (abi4) or isi4 (aba2) on the progermination selection medium. These results suggest that performing the isi screen in an ABA-deficient or insensitive background and selecting for enhancers, would preferentially isolate mutants in ABA-unrelated pathways such as the isil pathway. Our results on the hsr mutants also suggest that novel genetic screens which avoid the use of high sugar media, are less likely to suffer from osmotic side-effects and may target sugar-signalling pathways more directly.

Progress on genes thought to regulate sugar responses may confirm their proposed role in plants. The availability of increasing numbers of T-DNA and transposon tagged lines allows genomic approaches to establish the precise roles of members of gene families encoding, for instance, for hexokinases, SNF-related kinases (Halford and Hardie, 1998) and sugar transporters with potential signalling functions (Barker et al., 2000). This kind of approach will be complementary to genetic screens as it may detect the effects of subtle phenotypes, gene redundancies or even embryo lethality as reported for TPS. Most of these effects are likely to be missed in genetic screens. As more components are being identified, molecular approaches to study protein function and interactions will be valuable ways forward.

Progress on sugar-response mutants that are more directly involved in ‘sugar signalling’ will identify components of the pathways involved. Some of these components will be similar to those found in other organisms such as yeast and animals. However, the photoautotrophic nature of plants requires plant specific pathways to regulate carbon flow from source to sink tissues. The existence of novel plant-specific mechanisms, such as suggested by the isil gene, can be expected, that will regulate this partitioning of carbohydrates and balance photosynthetic carbon production with its use. Genetic approaches are especially useful in isolating these novel components. Identifying these mechanisms will improve current understanding of plant physiology and the regulation of carbohydrate partitioning and will be of future agronomical benefit.

References


Boxall SF, Martin T, Graham IA. 1996. A new class of Arabidopsis thaliana mutant that is carbohydrate-insensitive. Poster abstracts of the 7th International Conference on Arabidopsis Research, S96.


Mita S, Hirano H, Nakamura K. 1997b. Negative regulation in


