Sugars, signalling, and plant development

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Abstract

Like all organisms, plants require energy for growth. They achieve this by absorbing light and fixing it into a usable, chemical form via photosynthesis. The resulting carbohydrate (sugar) energy is then utilized as substrates for growth, or stored as reserves. It is therefore not surprising that modulation of carbohydrate metabolism can have profound effects on plant growth, particularly cell division and expansion. However, recent studies on mutants such as stimpy or ramosa3 have also suggested that sugars can act as signalling molecules that control distinct aspects of plant development. This review will focus on these more specific roles of sugars in development, and will concentrate on two major areas: (i) cross-talk between sugar and hormonal signalling; and (ii) potential direct developmental effects of sugars. In the latter, developmental mutant phenotypes that are modulated by sugars as well as a putative role for trehalose-6-phosphate in inflorescence development are discussed. Because plant growth and development are plastic, and are greatly affected by environmental and nutritional conditions, the distinction between purely metabolic and specific developmental effects is somewhat blurred, but the focus will be on clear examples where sugar-related processes or molecules have been linked to known developmental mechanisms.

Key words: Meristem, plant development, ramosa3, stimpy; sugars, trehalose.

Introduction: translating nutrient status into plant growth and development

Carbohydrates, or sugars, are essential to the fundamental processes required for plant growth. Therefore, carbohydrate production, metabolism, and use must be carefully coordinated with photosynthetic availability, environmental cues, and timing of key developmental programmes. Sugars in general can act as signalling molecules and/or as global regulators of gene expression, for example acting like hormones and translating nutrient status to regulation of growth and the floral transition (Koch, 1996, 2004; Wobus and Weber, 1999b; Smeekens, 2000; Ohto et al., 2001; Rolland et al., 2002, 2006; Price et al., 2004; Smeekens et al., 2010). Therefore, sugar-responsive gene regulation reflects carbohydrate abundance or depletion (Koch et al., 1996; Rolland et al., 2002; Koch, 2004). The translation of nutrient status to transcriptional regulation allows the plant to modulate growth, both at the whole plant level and locally, in tissue- or cell-specific patterns, potentially to coordinate developmental programmes with available carbohydrate. In response to sugar depletion, genes involved in photosynthesis, carbohydrate remobilization and export, and nitrogen (N) metabolism tend to be up-regulated. Alternatively, sugar abundance induces typical sink organ activities such as carbohydrate import, utilization, and storage, and starch and anthocyanin biosynthesis. Comprehensive reviews on sugar sensing and signalling, carbohydrate allocation, and growth are available and should be referred to for additional information (Koch et al., 1996; Smeekens, 2000; Rolland et al., 2002, 2006; Koch, 2004; Ainsworth and Bush, 2011).

Specific growth and metabolic responses tend to be activated and/or modulated based on the nature of the sugar signal. For example, sucrose, the primary transport sugar in plants, can be sensed as a signal directly (Chiou and Bush, 1998) or, alternatively, a signal can arise via its hexose cleavage products, glucose (glc) or UDP-gluc and fructose (Rolland et al., 2002; Price et al., 2004; Li et al., 2011). A number of genes involved in sugar sensing and signalling have been identified in mutant screens for altered responses to exogenous sugars during seed germination and early seedling growth in Arabidopsis (reviewed in Smeekens, 2000;
Rolland et al., 2002, 2006; Gibson, 2005). For example, glucose insensitive (gin) mutants fail to undergo growth arrest in the presence of inhibitory levels of glc, exhibiting normal hypocotyl elongation, cotyledon greening, and expansion. Independent screens for other sugar response phenotypes revealed that certain sucrose uncoupled (sun), sugar insensitive (sis), and/or impaired sucrose induction (isi) mutations were allelic to gin loci, suggesting that these genes may function at the interface of different sugar signalling pathways (Zhou et al., 1998; Smeekens, 2000; Rolland et al., 2002; Gibson, 2005). It should be noted, however, that results from such screens could potentially be secondary responses to hextose products generated from sucrose-mediated induction of sucrose-cleaving enzymes such as extracellular invertases. Additionally, the disaccharide trehalose, and its metabolic intermediate, trehalose-6-phosphate (T6P), are implicated in the regulation of specific growth responses (Eastmond and Graham, 2003; Paul, 2008; Smeekens et al., 2010), and are discussed in relation to putative roles in development later in this review.

It is obvious that, in general, metabolism should be tightly coupled to regulatory mechanisms that control growth and development, but few examples exist of how this might occur. Sugar signals can reportedly be generated either by carbohydrate concentration and relative ratios to other metabolites, such as C:N (Coruzzi and Bush, 2001; Palenchar et al., 2004), or by flux through sugar-specific sensors and/or transporters (Lalonde et al., 1999, 2004; Williams et al., 2000; Vaughn et al., 2002; Buttnер, 2010). One possible regulatory mechanism that has come to light recently is that metabolic enzymes can also function as active members of transcriptional regulatory complexes. The best example in plants involves hexokinase (HXK), which, aside from catalysing glc phosphorylation in the first committed step of glycolysis, also acts as a hextose sensor to transduce signals based on sugar availability (Jang et al., 1997; Xiao et al., 2000; Harrington and Bush, 2003; Moore et al., 2003). Interestingly, hxl mutants lacking catalytic activity are still active in some glc responses (Harrington and Bush, 2003; Moore et al., 2003), and recently it was found that some of the cellular pool of HXK1 protein is present in the nucleus, where it associates with putative transcriptional complexes (Cho et al., 2006). Several other examples of metabolic enzymes, ‘moonlighting’ as transcriptional regulators, including Arg5,6, which function in yeast arginine biosynthesis, galactokinase, and glyceraldehyde-3-phosphate dehydrogenase, have been described in other organisms (Zenke et al., 1996; Zheng et al., 2003; Hall et al., 2004), indicating that HXK1 is not an isolated example. This remarkable cross-functionalization presumably enables cross-talk between metabolic pathways or sensors, and gene regulation. An additional sensing mechanism that has been reported involves cell surface receptors of extracellular sugar signals, such as RGS1, a negative regulator of G-protein signalling (Chen et al., 2003; Chen and Jones, 2004).

In general, hextoses tend to have greater signalling potential in promoting organ growth and cell proliferation, while sucrose is typically associated with differentiation and maturation (Borisjuk et al., 2002; Koch, 2004). Relative ratios of hextoses to sucrose are perceived and maintained by sucrose metabolic enzymes, isoforms of which can act in a spatiotemporal manner to coordinate and fine-tune growth during key phases of development (Xu et al., 1996; Koch, 2004). Sugar metabolic enzymes and transporters can thus function in establishing sugar gradients within tissues (Weschke et al., 2000, 2003). Previous work in developing legume embryos showed that differential glc concentrations along a spatial gradient correlated with increased mitotic activity (Borisjuk et al., 1998, 2003), suggesting a link between hextoses and the cell cycle. Consistent with this, sugars have been shown to control cell division through modulation of cyclinD (CycD) gene expression (Riou-Khamlichi et al., 1999, 2000; Gaudin et al., 2000).

While availability of carbohydrate substrates required for organ growth naturally has an impact on plant development, sugars can also cross-talk with known phytohormone signalling networks to modulate critical growth processes such as embryo establishment, seed germination, and seedling and tuber growth (Gazzarrini and McCourt, 2001; Rolland et al., 2002, 2006; Leon and Sheen, 2003; Gibson, 2004, 2005). There is also increasing evidence that sugars can regulate specific developmental programmes and transitions via genes that control meristem maintenance and identity (Wu et al., 2005; Satoh-Nagasawa et al., 2006). Meristems are pluripotent stem cell populations, which can either divide indefinitely or respond to positional cues that direct the developmental fate of new plant organs. Because of a strong carbohydrate requirement in dividing and differentiating cells, it makes sense that meristem maintenance, identity, and organogenesis should be carefully coordinated with nutrient status (Francis and Halford, 2006). Indeed, Pien et al. (2001) observed spatiotemporal expression of carbohydrate metabolic genes in the tomato shoot apical meristem (SAM) and developing leaf primordia. In Arabidopsis, misexpression of a specific extracellular invertase, which cleaves sucrose into glc and fructose, in the SAM caused changes in flowering time and inflorescence architecture (Heyer et al., 2004). In addition, sucrose can rescue flowering time mutant phenotypes, through control of meristem identity genes, such as LEAFY (Ohito et al., 2001). Recent work has also shown that exogenous sucrose can compensate for regulators of meristem maintenance in the shoot (Wu et al., 2005) (see later discussion) and root (Wahl et al., 2010).

Potential roles of sugars in regulating specific developmental programmes are highlighted in this review. Since hormonal cues are imperative in maintaining meristem integrity and establishing developmental fate, known examples of cross-talk between sugar and hormone signalling pathways in modulating plant growth are also discussed. In addition, how sugar–hormone connections might regulate certain developmental processes is considered. Additional important examples of sugar signalling factors that have been suggested to control plant growth, such as the TOR kinase, SNF1-related kinase, and C/S1 bZip factors, have been discussed in a recent review (Smeekens et al., 2010), and will not be discussed here.
Cross-talk between sugar and hormone signalling

Sugar-based signalling pathways cross-talk with various hormones to modulate critical aspects of plant growth. In general, plants defective in abscisic acid (ABA) and/or ethylene perception and signalling tend to display altered sugar response phenotypes. Therefore, comparable mutant screens and subsequent genetic and functional analyses have revealed extensive overlap between sugar, ABA, and ethylene signals in controlling processes such as seed germination and seedling growth, as reviewed in Gazzarrini and McCourt (2001), Gibson (2004, 2005), Leon and Sheen (2003), and Rolland et al. (2002, 2006). However, additional connections between sugars and other plant hormones, such as auxin, have emerged, and some evidence suggests potential sugar–hormone regulation of specific developmental processes. Here, findings that have established mechanisms of sugar, ABA, and ethylene cross-talk during plant growth are highlighted, and an emerging role for sugar–auxin interactions in growth and development is also discussed.

Sugar, ABA, and ethylene signalling networks converge to control plant growth

Initial evidence for sugar and ABA cross-talk came from observations that a number of mutants identified in sugar response screens were also defective in ABA metabolism or response. For example, certain ABA biosynthesis (aba) and ABA-insensitive (abi) mutants are insensitive to high glc (Arenas-Huertero et al., 2000; Brocard et al., 2002; Leon and Sheen, 2003; Dekkers et al., 2008). Extensive cross-talk between sugar and ABA signalling pathways has therefore been described for various aspects of plant growth and metabolism (Gazzarrini and McCourt, 2001; Finkelstein and Gibson, 2002; Gibson, 2004, 2005). Sugars and ABA tend to act synergistically during embryo growth, in transitioning from a phase of rapid cell division to cell enlargement and accumulation of storage reserves (Wobus and Weber, 1999a, b; Finkelstein and Gibson, 2002). For example, ABA enhances sucrose induction of starch biosynthetic genes (Rook et al., 2001). Alternatively, ABA and glc act antagonistically during seed germination and early seedling growth, where exogenous glc enables wild-type Arabidopsis seeds to germinate on otherwise inhibitory ABA concentrations (Leon and Sheen, 2003). A direct link between sugar signalling and hormone biosynthesis was revealed by characterization of ABA biosynthetic genes, \( ABA1−ABA3 \), which were also independently isolated as \( gin \) mutants (Arenas-Huertero et al., 2000; Laby et al., 2000; Rook et al., 2001). Exogenous glc can increase both expression of these ABA synthesis genes and, consequently, endogenous ABA levels (Cheng et al., 2002). A key link between sugars and ABA perception is exemplified by \( ABI4 \), which encodes an AP2 domain transcription factor and is required for normal sugar response during germination and seedling growth (Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000; Rook et al., 2001). In maize seeds, \( ABI4 \) is regulated by sugar in the developing embryo, and binds regulatory elements for both ABA and sugar (Niu et al., 2002). Interestingly, not all \( ABI \) genes are responsive to sugars, suggesting multiple ABA signalling pathways at work (Laby et al., 2000; Dekkers et al., 2008). Genome-wide analysis tools are currently being used to resolve additional players in the sugar–ABA signalling network(s) (Li et al., 2006). For example, recent work identified a splicing factor, \( SR45 \), as a negative regulator of sugar signalling during early seedling growth. \( SR45 \) is involved in the repression of glc-induced ABA accumulation, and down-regulation of genes for ABA biosynthesis and signalling (Carvalho et al., 2010).

Ethylene sensing and signalling pathways are also tightly interconnected with those for sugar and ABA (Gazzarrini and McCourt, 2001; Leon and Sheen, 2003). Mutants in ethylene perception, ethylene receptor 1 (ctr1) and ethylene insensitive 2 and 3 (ein2 and ein3), are glc hypersensitive, while constitutive triple response 1 (ctr1), a negative regulator of ethylene signalling, is glc insensitive (Zhou et al., 1998; Gibson et al., 2001; Yanagisawa et al., 2003). While basic models have been suggested for regulatory mechanisms among these pathways, hormone signals may differentially affect gene expression depending on sugar concentration, localization, or the nature of the sugar signal. Glc and ethylene signalling pathways tend to act antagonistically, partially through repression of ABA biosynthetic genes. For example, ABA levels are enhanced in the \( ein2 \) mutant, and wild-type seedlings treated with the ethylene precursor 1-aminoacyclopropane-1-carboxylic acid (ACC) phenocopy \( gin \) mutants (Ghassemian et al., 2000). Interestingly, ethylene also appears to play a role in root meristem maintenance (Ortega-Martinez et al., 2007), via a mechanism that possibly involves auxin. Evidence for the latter includes attenuation of ethylene effects in roots of certain auxin mutants (Ortega-Martinez et al., 2007), and ethylene-induced expression of auxin biosynthetic genes in the root meristem (Stepanova et al., 2008). Whether sugar signals contribute to ethylene- and auxin-based regulation in root meristem maintenance remains to be determined; however, additional evidence relates sugar and auxin signals to root development, and is discussed below.

Emerging roles for sugar–auxin cross-talk during plant growth and development

Auxin is an important plant hormone that can act as a general regulator of growth and is also implicated in pattern formation, lateral organ development, and cell expansion (reviewed by Hamant et al., 2010; Kieffer et al., 2010). Directional transport and local accumulation of auxin in spatiotemporal gradients regulate these developmental processes. Auxin biosynthesis and response pathways are also interconnected with those of other hormones, such as ethylene, brassinosteroids, and cytokinins (Kieffer et al., 2010). Evidence linking sugar and auxin signals is also emerging in various aspects of plant growth and development. Initial work on \( hxk1/gin2 \) in Arabidopsis showed that the mutants displayed
resistance to exogenous auxin, and that auxin-resistant mutants were insensitive to high glc (Moore et al., 2003). In addition, hoxk-based signalling positively and negatively interacts with auxin and cytokinin, respectively (Moore et al., 2003; Rolland et al., 2006). More recently, a novel allele of hookless1 (hls1) was shown to be resistant to both sugar and auxin responses, suggesting it may partially function in negative effects of auxin on sugar-responsive gene expression (Ohto et al., 2006). In developing maize kernels, an auxin biosynthetic gene, ZmYUCCA, was recently reported to be modulated by sugar, representing a link between sugar status and auxin signals (LeCler et al., 2010). An interesting connection between polar auxin transport and sucrose metabolism was also described during the maize stem gravitropism response. Here, auxin redistribution was necessary for gravistimulated sucrose hydrolysis in generating an asymmetric hexose gradient, which caused differential cell elongation in cells of the internodal pulvinus (Long et al., 2002).

A genome-wide expression profiling study by Mishra et al. (2009) showed substantial overlap of glc and auxin response pathways controlling root growth and development in Arabidopsis seedlings. Here, 62% of genes affected by auxin were regulated by glc, and in many instances glc and auxin acted either antagonistically or synergistically to regulate transcription. While increasing concentrations of glc induced genes for auxin biosynthesis and transport, they had differential effects on individual auxin receptors (Mishra et al., 2009). Interestingly, a number of auxin-regulated genes that did not respond to glc alone were modulated by glc in the presence of auxin. Notably, these included AUX/IAA and Lateral Organ Boundary (LOB) domain gene family members. In addition, exogenous glc significantly accentuated defects in lateral root induction, root hair elongation, and gravitropism in auxin sensing and signalling mutants, suggesting that glc may affect root architecture through auxin-based signal transduction (Mishra et al., 2009). Another study in Arabidopsis roots showed that expression of a WUSCHEL (WUS)-related homeobox gene, WOX5, which maintains localized auxin maxima in the root apical meristem, is induced by auxin and turanose, a non-metabolizable sucrose analogue (Gonzali et al., 2005).

Specific developmental pathways involving sugars

In this section the focus is on examples where sugars have been linked to specific developmental processes or pathways. How sugars may play a regulatory role in maintaining meristem function, via action of key developmental genes, and emerging views on how trehalose-based signalling may be involved in plant development, are discussed.

Shoot meristem maintenance and sugars: stippy

stippy (stip) is a mutant in WOX9, a homeobox gene related to WUS, a gene expressed in the ‘organizing centre’ of the SAM and required for maintenance of shoot stem cells. stip mutants mostly arrest soon after germination (Fig. 1A), and also have SAM defects, possibly as a result of defective WUS expression. This effect appears to be more general than the role of WUS in stem cell/organizing centre specification, and it is thought that STIP functions by maintaining cell division in root as well as shoot development. Remarkably, stip mutants can be rescued by addition of sucrose, and the mutants, once rescued, can undergo normal growth and development. Sucrose thus can compensate for loss of a gene that is typically required for normal meristem development (Fig. 1). While the exact reason for sucrose rescue is not clear, one possibility is that it acts by promoting cell division through up-regulation of CycD expression (Riou-Khamlichi et al., 2000). This could reflect a cross-talk with cytokinin signalling, which also induces CycD expression (Riou-Khamlichi et al., 1999). Additional insight into the role of cytokinins comes from the observation that they are required for activation of stip expression, linking cytokinin signalling to meristem establishment through the action of stip (Skylar et al., 2010). Recently, characterization of the plant-specific FANTASTIC FOUR (FAF) genes, which encode proteins of unknown function, uncovered a putative role for FAF2 and FAF4 in controlling shoot meristem size and maintenance of meristem function, working through WUS (Wahl et al., 2010). In contrast to STIP, the overexpression of FAF2 or FAF4 arrests shoot and root growth shortly after germination, and root growth arrest can also be rescued by addition of exogenous sucrose. Although further work is needed to understand the connection between these genes, it was proposed that STIP and FAF genes might play antagonistic roles in translating sugar signals to meristem maintenance (Wahl et al., 2010).

Trehalose signalling and development: RAMOSA3 and meristem determinacy

Trehalose is a disaccharide that is present in all kingdoms, and functions in microbes and invertebrates in carbohydrate storage, transport, signalling, and stress protection (Elbein, 1974; Crowe et al., 1992; Strom and Kaasen, 1993; Paiva and Panek, 1996). At one time, it was thought that the occurrence of trehalose in plants was limited to specialized, desiccation-tolerant species (Muller et al., 1995). However, genome
sequences revealed trehalose biosynthetic genes in all plants (Leyman et al., 2001; Muller et al., 2001; Vogel et al., 2004). In most plants, however, trehalose is present at low micromolar concentrations, suggesting a regulatory rather than a metabolic role (Paul et al., 2008).

The biosynthesis of trehalose in plants occurs in two steps (Goddijn and van Dun, 1999; Paul et al., 2008). First, the intermediate, trehalose-6-phosphate (T6P), is formed from UDP-glc and glc-6-phosphate by trehalose-6-phosphate synthases (TPSs). Next, T6P is converted to trehalose by trehalose-6-phosphate phosphatases (TPPs) (Cabib and Leloir, 1958) (Fig. 2). In Saccharomyces cerevisiae, and Escherichia coli, TPS and TPP are single-copy genes (Londesborough and Vurio, 1991; Kaasen et al., 1992, 1994; Bell et al., 1998), whereas plants encode multiple homologues of each (Leyman et al., 2001; Paul et al., 2008). Functional analysis of the plant TPS genes has concentrated on TPS1. Arabidopsis tps1 loss-of-function mutants are embryo lethal, and use of misexpression or weak alleles has shown that TPS1 function is required throughout the plant life cycle; weak alleles have slow growth and delayed flowering (Eastmond et al., 2002; van Dijken et al., 2004; Gomez et al., 2010). tps1 mutants cannot be rescued by exogenous trehalose, suggesting that the intermediate T6P is important (Eastmond et al., 2002). In itself, the observation that tps1 mutants are embryo lethal does not prove a developmental role for this gene, because many ‘housekeeping’ genes encoding basic cellular functions in plants also have embryo lethal phenotypes (Tzafrir et al., 2004), and TPS1 appears to encode the only functional TPS enzyme in Arabidopsis (Vandesteene et al., 2010). Arabidopsis also contains 10 TTP genes, with conserved phosphatase motifs typical of this class of phosphohydrolases (Thaller et al., 1998). Two of these genes were first isolated by complementation of yeast tps2 mutants (Vogel et al., 1998), suggesting they have TPP function. Little other information is available on the biological roles of the TTP genes, although two of them might be regulated by the stem cell regulator WUS in Arabidopsis, pointing to possible developmental roles (see supplementary data in Leibfried et al., 2005). Plants also encode some genes that have both TPS- and TPP-like domains, though they appear to lack either TPS or TPP enzymatic activity and might rather function as regulatory or sensor proteins (Lunn, 2007; Ramon et al., 2009). An Arabidopsis mutant in one of them, AtTPS6, was found to have interesting cell shape defects, pointing to a possible role for trehalase signalling in cell fate specification (Chary et al., 2008).

Insights into potential developmental effects of the trehalase pathway have come from heterologous expression studies. For example, plants overexpressing microbial trehalose biosynthetic genes were found to have altered carbohydrate metabolism and morphological defects, such as stunted growth and lanceolate leaves (Romero et al., 1997; Garg et al., 2002; Schluempman et al., 2003, 2004).

**Fig. 2.** The trehalose biosynthetic pathway and mutant phenotypes. (a) Trehalose is produced by two enzymatic reactions, via the intermediate trehalose-6-phosphate. Compared with wild-type Arabidopsis embryos (b), tps1 mutant embryos are embryo lethal (c) (EM = embryo; CE = cellular endosperm) (from Eastmond PJ, van Dijken AJ, Spielman M, Kerr A, Tissier AF, Dickinson HG, Jones JD, Smeeckens SC, Graham IA, 2002. Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for Arabidopsis embryo maturation. The Plant Journal, with permission of John Wiley and Sons). Wild-type maize ears (d) are unbranched, whereas ramosa3 mutant ears have abnormal long branches at their base (e, f, branches false coloured in red). The trehalase phosphatase, RAMOSA3, is expressed at the base of spikelet pair meristems in developing maize ears [black signal, in situ hybridization in (g); red signal is in situ hybridization of KNOTTED1, marking the meristem cells (Satoh Nagasawa and Jackson, unpublished)]. (h) A pathway for maize inflorescence branch determinacy, showing that RA3 and RA2 act in parallel and upstream of RA1 (based on Vollbrecht et al., 2005; Satoh-Nagasawa et al., 2006).
These phenotypes are thought to result from changes in carbon allocation between sink and source tissues (Eastmond and Graham, 2003), and provoked speculation that trehalose may be involved in sugar signalling (Goddijn and van Dun, 1999; Paul et al., 2008). One possible target of the putative trehalose signal is ADP-β-gluc pyrophosphorylase, which catalyzes the first committed step in starch biosynthesis (Wingler et al., 2000; Lunn et al., 2006). Recent evidence points to the intermediate T6P, rather than trehalose itself, as the key regulatory signal (Schluepmann et al., 2003, 2004; Lunn et al., 2006; Paul et al., 2008). T6P appears to act as a regulator of carbon metabolism, and, remarkably, as an enhancer of photosynthetic capacity (Paul et al., 2001; Eastmond and Graham, 2003). Paul et al. (2001) reasoned that T6P could be the signal that allows HXK to perceive carbon status, as in yeast (Thevelein and Hohmann, 1995); however, T6P is not an inhibitor of Arabidopsis HXKs (Eastmond et al., 2002). More recent data point to a link between T6P induction by sucrose and promotion of growth (Lunn et al., 2006), although T6P is also a potent growth inhibitor in the absence of exogenous carbon sources (Schluepmann et al., 2003, 2004). It is becoming clear that T6P levels provide an important signal that links plant metabolism with growth and development, though how this occurs is not fully understood (Paul et al., 2008). However, a recent advance identified SnRKs (Snf1-related protein kinases), which integrate energy signalling and growth in plants, as possible T6P targets (Baena-Gonzalez et al., 2007). SnRK1 activity is specifically inhibited by T6P in seedling extracts, but not in immunopurified extracts, suggesting the presence of an as yet unidentified intermediary factor (Zhang et al., 2009). Related SnRKs act as important regulators of central carbon and nitrogen metabolism, and link nutrient stress to a number of response pathways (Halford and Hey, 2009; Smekens et al., 2010). In addition, Arabidopsis lines that overexpress the bZIP11 transcription factor were identified in an elegant screen to isolate genes that suppress growth inhibition by trehalose (Delatte et al., 2011). bZIP11, which itself is both induced by sucrose at the transcriptional level and repressed by sucrose at the translational level (Ma et al., 2011), is therefore another important signalling component in this network (Delatte et al., 2011). It will thus be interesting to further establish connections among these sugar-based signalling modules.

A surprising insight into a potential role for trehalose signalling in development came from the discovery that the maize inflorescence developmental gene RAMOSA3 encodes a functional TPP enzyme (Satoh-Nagasawa et al., 2006). Inflorescence morphology is strongly impacted by auxiliary meristem initiation and growth patterns (Prusinkiewicz et al., 2007; Prenner et al., 2009). In the grasses, inflorescence development is characterized by the formation of short branches called spikelets that contain the floral structures, the florets (Irish, 1998; Bommert et al., 2005; Doust, 2007). Maize forms two distinct types of inflorescences; the terminal tassel has long basal branches and develops the male flowers, and the axillary ears, which have a prominent axis lacking long branches, and develop female flowers. In each structure, development proceeds by the initiation of spikelet pair meristems (SPMs) on the flanks of the inflorescence meristem (IM); seen in scanning electron micrographs (Fig. 2f). Each SPM develops into a short axillary branch, bearing two spikelet meristems, which in turn initiate two floral meristems. In the tassel, the IM also initiates several branch meristems, which are indeterminate, and grow out to make the long branches at the base of the tassel (Cheng et al., 1983; Bommert et al., 2005; Doust, 2007).

Inflorescence morphology in the grasses is highly variable (Bommert et al., 2005; Doust, 2007), and a large factor controlling this variability is the developmental control of auxillary meristem determinacy. In maize, this determinacy decision is controlled by the RAMOSA genes, RA1, 2, and 3. RAMOSA (RA) genes (from the Latin ramus, meaning branch) control auxillary meristem growth by imposing determinacy, and consequently ra loss-of-function mutants have more indeterminate, highly branched inflorescences (McSteen, 2006; Vollbrecht and Schmidt, 2008). RA1 encodes a putative C2H2 zinc finger transcription factor that appears to have played a key role in maize domestication and grass evolution (Vollbrecht et al., 2005). RA2 also encodes a putative transcription factor, a LOB domain protein (Bortiri et al., 2006), whereas RA3 encodes a functional TPP enzyme (Satoh-Nagasawa et al., 2006). Interestingly, genetic and molecular studies suggest that both RA2 and RA3 are required for proper RA1 expression (Vollbrecht et al., 2005; Bortiri et al., 2006; Satoh-Nagasawa et al., 2006) (Fig. 2h). All three genes are expressed in overlapping domains: RA2 is expressed in the auxillary meristem itself (Bortiri et al., 2006), whereas RA1 and RA3 expression is only at the base of these meristems, suggesting that they may control a mobile signal that regulates meristem determinacy (Vollbrecht et al., 2005; Satoh-Nagasawa et al., 2006).

RA3 is a functional TPP enzyme, since the bacterial-expressed protein has specific activity for T6P, and the protein can complement a yeast TPP mutant, suggesting a role for trehalose signalling in meristem determinacy (Satoh-Nagasawa et al., 2006). However, the fact that RA3 controls levels of RA1 expression in the genetically defined RAMOSA mutant pathway suggests that RA3 might have an alternative regulatory function. One hypothesis is that RA3, like some metabolic enzymes in yeast (Zenke et al., 1996; Zheng et al., 2003; Hall et al., 2004), and HXK in Arabidopsis (Cho et al., 2006), might play a moonlighting role in transcriptional regulation, as discussed earlier. Whatever the mechanism of RA3 action is, it is clear that this pathway is not specific to maize. A rice homologue of RA3 (Os SRA) has a similar, highly localized expression at the base of inflorescence branches (Satoh-Nagasawa et al., 2006). Some Arabidopsis TPP family members also have restricted expression patterns during inflorescence development, similar to RA3, suggesting that a similar pathway might control Arabidopsis inflorescence architecture (S. Goldsmith and DJ, unpublished).

Insights into potential regulatory mechanisms of RA3 were gained through genome-wide transcript profiles, comparing
wild-type developing ear primorida with those from loss-of-function \( \text{ra3} \) mutants (Eveland \textit{et al.}, 2010). Here, a number of genes that showed differential expression in the \( \text{ra3} \) background were associated with metabolic pathways for primary carbohydrate biosynthesis and degradation, energy production, and trehalose metabolism, suggesting global effects of sugar status associated with altered TPP activity. In addition, consistent with a putative role for \( \text{RA3} \) in transcriptional regulation, developmentally regulated transcription factors were also misexpressed in the \( \text{ra3} \) mutants. Among these were genes related to various hormone sensing and signalling pathways, such as auxin and ethylene, including nine ethylene-response factor (ERF) family members, as well as genes involved in brassinosteroid signal transduction (Eveland \textit{et al.}, 2010). Testable hypotheses that emerge from genome-wide studies such as this will enable further resolution of functional roles for \( \text{RA3} \), including potential connections to sugar signals and/or overlaps with hormone-sensing pathways, and how sugar–hormone cross-talk may contribute to the genetic control of plant development.

**Summary**

Sugars are essential, and so genetic approaches to understanding their roles in plant development are complicated. Sugar-related mutants might be embryo lethal, but this could indicate either a developmental defect or simply a critical metabolic or housekeeping function. Mutants are also likely to be pleiotropic, because they could induce general growth defects. New insights will come from identifying mutants, such as \( \text{stip} \) or \( \text{ra3} \), that have specific developmental functions, and assembling these mutants into known developmental pathways, using classical genetics and/or genomics approaches, and also from integrating genomic data from sugar signalling and developmental mutants.

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