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BOTANICAL BRIEFING

Sucrose-mediated translational control

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• *Background* Environmental factors greatly impact plant gene expression and concentrations of cellular metabolites such as sugars and amino acids. The changed metabolite concentrations affect the expression of many genes both transcriptionally and post-transcriptionally.
• *Recent Progress* Sucrose acts as a signalling molecule in the control of translation of the S1 class basic leucine zipper transcription factor (bZIP) genes. In these genes the main bZIP open reading frames (ORFs) are preceded by upstream open reading frames (uORFs). The presence of uORFs generally inhibits translation of the following ORF but can also be instrumental in specific translational control. *bZIP11*, a member of the S1 class *bZIP* genes, harbours four uORFs of which uORF2 is required for translational control in response to sucrose concentrations. This uORF encodes the Sucrose Control peptide (SC-peptide), which is evolutionarily conserved among all S1 class *bZIP* genes in different plant species. *Arabidopsis thaliana bZIP11* and related *bZIP* genes seem to be important regulators of metabolism. These proteins are targets of the Snf1-related protein kinase 1 (SnRK1) KIN10 and KIN11, which are responsive to energy deprivation as well as to various stresses. In response to energy deprivation, ribosomal biogenesis is repressed to preserve cellular function and maintenance. Other key regulators of ribosomal biogenesis such as the protein kinase Target of Rapamycin (TOR) are tightly regulated in response to stress.
• *Conclusions* Plants use translational control of gene expression to optimize growth and development in response to stress as well as to energy deprivation. This Botanical Briefing discusses the role of sucrose signalling in the translational control of bZIP11 and the regulation of ribosomal biogenesis in response to metabolic changes and stress conditions.

Key words: Sucrose signalling, *Arabidopsis thaliana*, bZIP11, translational control, ribosomal biogenesis, amino acid metabolism, TOR, SnRK1.

INTRODUCTION: SUCROSE SENSING IN PLANTS

Plants are continuously exposed to changing environmental conditions that affect metabolism and growth. The daily light–dark cycle is probably the most dramatic change in growth condition that plants normally experience. This is demonstrated by the large impact of the diurnal cycle on gene expression and concentrations of metabolites such as sugars and amino acids. During the day plants generate carbon resources by photosynthesis. During the night carbon is remobilized to support metabolism and growth. The *Arabidopsis thaliana* starchless phosphoglucomutase mutant (*pgm*) lacks the buffering effect of diurnal starch turnover. As a consequence, this mutant experiences much larger diurnal changes of sugar concentrations than the wild type. Genes responsive to changes in sugar concentrations during the diurnal cycle in the wild type were qualitatively similar but showed much higher diurnal changes in *pgm*. This indicated that diurnal gene expression is primarily responsive to sugars (Blasing *et al.*, 2005).

Glucose and sucrose function as signalling molecules in plants. Glucose sensing is the best-studied sugar signalling mechanism in plants (Xiao *et al.*, 2000; Moore *et al.*, 2003; Cho *et al.*, 2006; Huang *et al.*, 2006). Hexokinase was found to act both as a glucose sensor and as an enzyme converting glucose to glucose 6-phosphate. Hexokinase 1 (HXK1) signalling integrates nutrient and hormone signals to regulate gene expression and plant growth. Cho *et al.* (2006) showed that HXK1 forms a complex with VHA-B1and RPT5B, a vacuolar H^+-ATPase and a regulatory particle of the proteasome, respectively. They proposed a model in which nuclear HXK1 activates target gene expression. Nuclear HXK1 forms a complex with VHA-B1, RPT5B and two putative transcription factors (TFs) to mediate specific target gene transcription by binding to chromatin (Cho *et al.*, 2006). Moreover, glucose signalling activates via HXK1 the abscisic acid/ABA insensitive (ABA/ABI) signalling pathway to control gene expression (reviewed in Rolland *et al.*, 2006).

The role of sucrose as a signalling molecule has been difficult to establish because sucrose is readily converted into fructose and glucose, and vice versa. The *patatin* promoter activity was induced specifically in response to sucrose in *Solanum tuberosum* and in *Nicotiana tabacum* (Wenzler *et al.*, 1989). Likewise, the *rolC* promoter of *Agrobacterium rhizogenes* Ri plasmid was specifically sucrose-induced in *N. tabacum* phloem tissue (Yokoyama *et al.*, 1994). Sucrose also affected the transcription of the *MYB75/PAP1* transcription factor. Sucrose-induced elevations in *MYB75/PAP1* mRNA

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concentrations resulted in increased expression of anthocyanin biosynthesis genes and thereby increased anthocyanin accumulation (Teng *et al.*, 2005; Solfanelli *et al.*, 2006). Another transcription factor, the basic leucine zipper 11 (bZIP11), is translationally repressed in response to sucrose (Rook *et al.*, 1998*a*; Wiese *et al.*, 2004). In all the above examples, combined treatments of glucose and fructose were less effective than sucrose treatments, suggesting that sucrose functions as a signalling molecule. In spite of the proposed sucrose signalling function, conclusive evidence on the nature of sucrose sensors is yet to be presented. However, a signalling function for sucrose transporters such as SUC3/SUT2 remains an attractive hypothesis (Barker *et al.*, 2000; Meyer *et al.*, 2000).

SUCROSE-CONTROLLED bZIP11 TRANSLATION AND METABOLITE-CONTROLLED TRANSLATION

The translation of *A. thaliana bZIP11* mRNA is repressed in response to sucrose, whereas other sugars tested where found to be less effective. The sucrose-specific repression could not be explained by changed mRNA concentrations (Rook *et al.*, 1998*b*). Sucrose-induced repression of translation (SIRT) depends on the presence of the 5′-leader sequence upstream of the *bZIP11* main coding sequence (Rook *et al.*, 1998*a*; Wiese *et al.*, 2004). The 5′-leader of *bZIP11* mRNA harbours four upstream open reading frames (uORFs), designated uORF1–4, of which uORF2 is essential for SIRT activity. Owing to the presence of an internal AUG codon within uORF2, it can be translated both as a peptide 42 amino acids in length from uORF2a, and as a shorter peptide of 28 amino acids from uORF2b. Changing uORF2b AUG codon into a stop codon abolished SIRT, showing that the uORF2b-encoded peptide is essential for SIRT (Fig. 1). Mutations in the AUG codons of uORF1 and uORF4 did not affect SIRT. The uORF2-encoded Sucrose Control peptide (SC-peptide) was shown to be translated *in vitro* (Wiese *et al.*, 2004). Previous protein similarity searches in *A. thaliana* showed that the *bZIP11* SC-peptide sequence is conserved in four *bZIP11* orthologues: *bZIP1*, *2*, *44* and *53* (Wiese *et al.*, 2004). Together with bZIP11 these bZIP proteins form the S1 class of bZIP transcription factors (Jakoby *et al.*, 2002). Only these five S1 class bZIP genes encode this SC-peptide in the *A. thaliana* genome. Recent results showed that all five S1 class bZIP mRNA 5′-leaders mediate translational repression in response to sucrose (Weltmeier *et al.*, 2009), indicating that SC-peptides impose the same sucrose regulatory feature on the downstream ORF. Sequences similar to uORF2 in other plant species are exclusively present in paralogous bZIP genes (Fig. 2). This supports the hypothesis of an evolutionarily conserved regulatory mechanism. Interestingly, within the SC-peptide coding region, the nucleotide conservation in the first two nucleotide positions of the codons is higher than that in the third position (Fig. 2A). This indicates that the amino acid sequence and not the mRNA sequence is important for SIRT. The amino acids at the C-terminus are more prominently conserved (Fig. 2B), which suggests that this part is especially important. Mutational analysis confirmed several of these amino acids to be essential for SIRT (our unpubl. obs.).

Translation initiation starts with the assembly of the 43S pre-initiation complex (PIC), consisting of several eukaryotic initiation factors (eIFs), Met-tRNA$_i^{Met}$ and the 40S ribosomal small subunit. This 43S PIC binds the 5′-end of capped mRNA, forming the 48S PIC. This complex scans the mRNA for the first AUG initiation codon in the 5′ to 3′ direction. If an AUG is encountered and recognized, bound eIFs are released, allowing the association of a 60S ribosomal large subunit to form the 80S ribosome complex required for protein translation (reviewed in Asano and Sachs, 2007). The sequence surrounding the AUG codon greatly affects the efficiency of translation initiation (Joshi *et al.*, 1997). Scanning 48S PICs require a consensus sequence to initiate translation efficiently. If not encountered, 48S PICs have a tendency to skip such weak AUG contexts and continue scanning in the 5′ to 3′ direction. Joshi *et al.* (1997) reported that the consensus AUG context in dicots is AA/CA*AUG*GC (Fig. 2C). If we count the A in AUG as +1, the +4 and +5 positions seem to be important in plants, whereas in animals the −3 position is more important for translation initiation efficiency (Lutcke *et al.*, 1987). Interestingly, all the S1 class uORF encoding SC-peptides have relatively weak AUG contexts, whereas the main ORFs have relatively strong contexts (Fig. 2C). This suggests that scanning 48S PICs frequently skip uORF2, resulting in leaky scanning, which allows translation of the main ORF. Preliminary results on mutational analysis of the AUG context area provide support for this mechanism (our unpublished observations). Eukaryotic ribosomes generally translate one ORF per mRNA. Thus, either the uORF or the main ORF is translated. The high amino acid sequence conservation at the C-terminus of the SC-peptide suggests that most, if not all, of the peptide must be translated for SIRT. This is supported by the observation that the stop codon position is highly conserved. Possibly, at high sucrose concentrations the SC-peptide is instrumental in stalling the ribosome on the mRNA, preventing other PICs from reaching the main ORF. Slowing down translational elongation or stalling of the translational complex on the mRNA can achieve this inhibition of translation of the main ORF. It is likely that the SC-peptide actively participates in preventing main ORF translation. Similar mechanisms have been described in other organisms, e.g. the drug-dependent stalling mechanism that was reported for methyltransferase gene *ermC* often found in microbial pathogens resistant to macrolide antibiotics such as erythromycin. The main ORF of *ermC* is preceded by a uORF encoding the regulatory leader peptide (ErmCL). The drug-dependent ribosome stalling at the ErmCL coding sequence depended on the interaction of ErmCL with a 23S rRNA A2062 residue and RPL22 in the ribosome exit tunnel (Vazquez-Laslop *et al.*, 2008).

The sucrose-mediated translational control mechanism of *bZIP11* is only one example of metabolite-mediated regulation of mRNA translation. During evolution organisms developed several translational control mechanisms, enabling them to adapt to nutrient availability. For example, increased concentrations of the polyamine spermidine result in several effects on translation. The *A. thaliana ACAULIS 5* (*ACL5*) gene encodes a thermospermine synthase (Knott *et al.*, 2007). Disruption of *ACL5* resulted in a severe dwarfed phenotype (Imai *et al.*, 2006). The *SUPPRESSOR OF AUCULIS 51*

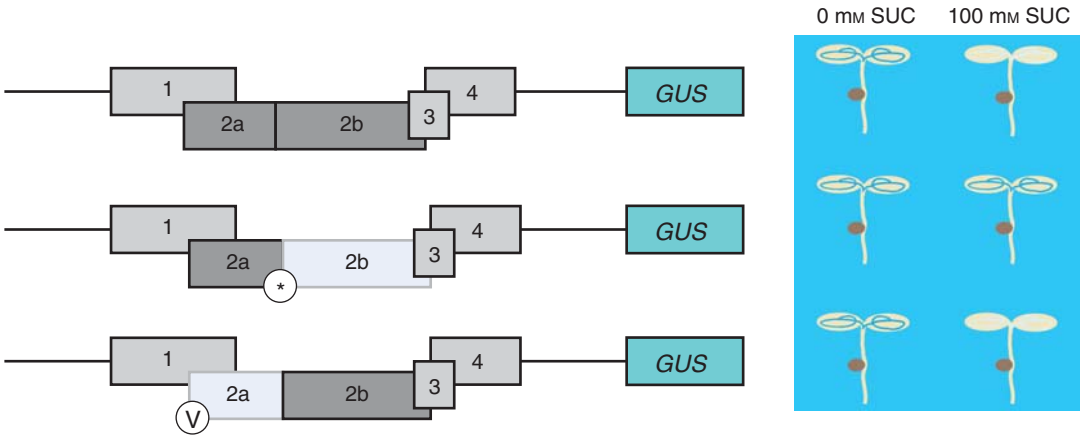


FIG. 1. *bZIP11* uORF2 is required for sucrose-induced translational repression (SIRT). Schematic representation of GUS staining patterns of seedlings grown on media supplemented with or without 100 mM sucrose (SUC). The transgenic seedlings harbour the *bZIP11* wild-type 5′-leader (top) or mutant 5′-leader sequences fused to the GUS gene (centre and bottom). AUG codons were mutated into either valine (V) or a stop-codon (*). Mutations of the uORF2a start codon showed SIRT, indicating that the truncated peptide starting from uORF2b is sufficient for SIRT activity. Adapted from Wiese *et al.* (2004).

mutant (*sac51-d*) encodes a basic helix loop helix (bHLH) TF, and suppresses the *acl5* phenotype. The *sac51-d* harbours a premature stop codon in one of the uORFs. The *sac51-d* mutation resulted in efficient translation of the main ORF and restoration of the growth phenotype. Thermospermine (a structural isomer of spermine) produced by ACL5 repressed the translation of SAC51 by affecting the translation of one of the uORFs preceding the *SAC51* main ORF. The *acl5-1* single mutant and *acl5-1 sac51-d* double mutant showed reduced SAC51-GUS expression (Imai *et al.*, 2006). These results suggested that functional ACL5 is required for the translational repression of SAC51 in response to spermidine. Recently, Imai *et al.* (2008) reported on another suppressor of *acl5*, the *sac52-d* mutant, which harbours a mutation in a gene encoding ribosomal protein L10 (RPL10). This indicated the importance of ribosomes in the thermospermine-dependent translational control of SAC51 via the uORF. Taken together, these results indicate a tight feedback regulation of polyamine biosynthesis genes. Metabolite-mediated translational control has been reported for other genes as well, e.g. *SAMDC*, *CPA1*, *arg-2* and *GCN4* in yeast (reviewed in Morris and Geballe 2000). All these genes are translationally regulated via uORFs preceding their main ORFs.

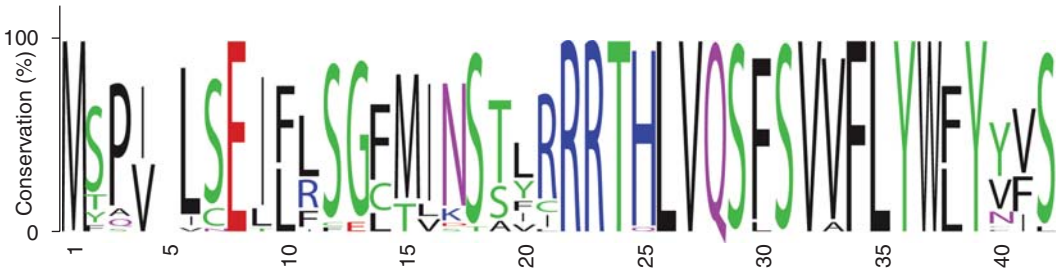
SUCROSE REGULATES AMINO ACID METABOLISM VIA TRANSLATIONAL REGULATION OF THE bZIP11 TRANSCRIPTION FACTOR

bZIP TFs have a DNA-binding domain (basic region) and a dimerization motif (leucine zipper). Plant bZIP proteins regulate diverse biological processes such as pathogen defence, seed maturation, flower development, and light and stress signalling (Jakoby *et al.*, 2002). Based on sequence similarities of the basic regions, plant *bZIP* genes are grouped into ten different groups (A to I and S) (Jakoby *et al.*, 2002). *bZIP11* is a member of the S1 class together with *bZIP1*, *2*, *44* and *53*. bZIP TFs can form both homo- and heterodimers through their zipper domain. Previously, S1 class proteins were shown to interact with C class proteins both in yeast and in plants. S1-C class heterodimers showed the highest transcriptional activation activity. bZIP11 homodimers showed a high transcriptional activity as well (Ehlert *et al.*, 2006; Weltmeier *et al.*, 2006). The ability of bZIP11 to affect growth and development was demonstrated by constitutive overexpression in *A. thaliana*. Recently, it was shown that transiently induced bZIP11 expression affects hundreds of genes, functioning in many biochemical pathways and signal transduction processes (Hanson *et al.*, 2008). Many genes affected by overexpression of bZIP11 had previously been shown to be regulated by sugars. As expected, genes induced by bZIP11 were mainly repressed by sugar treatments. Transgenic lines were created in which *bZIP11* expression was controlled by the *bZIP11* promoter but which lacked the *bZIP11* 5′ leader. In these lines *bZIP11* is no longer translationally repressed in response to sucrose owing to the lack of uORF2. These lines were used to analyse physiologically relevant target genes of bZIP11. Of the 261 genes identified as differently expressed compared with the control in a microarray experiment, 35 were tested further. Seven of these showed higher levels of expression in the transgenic lines than in the wild type when all endogenous S1 class genes were repressed by the presence of sucrose. Among these seven induced genes, two amino acid biosynthesis genes are present, *ASPARAGINE SYNTHASE1* (*ASN1*) and *PROLINE DEHYDROGENASE2* (*ProDH2*). Mutagenesis of one of the bZIP-binding G-boxes in the *ASN1* promoter resulted in abolition of induction by bZIP11 (Baena-Gonzalez *et al.*, 2007; Hanson *et al.*, 2008), which indicated a direct control of *ASN1* expression by bZIP11. *ASN1* expression was shown to be induced by several stresses such as hypoxia and darkness and repressed in the presence of sucrose or glucose (Baena-Gonzalez *et al.*, 2007; Hanson *et al.*, 2008). Overexpression of bZIP11 or orthologous bZIP genes in mesophyll protoplasts resulted in increased *ASN1* expression levels (Baena-Gonzalez *et al.*, 2007; Hanson *et al.*, 2008). Co-overexpression of KIN10 or KIN11 protein kinases strongly enhanced the transcriptional potential of the bZIP proteins to induce *ASN1* (Baena-Gonzalez *et al.*, 2007). The KIN10 and KIN11 SNF1-like kinases 1 (SnRK1 kinases) are

A

Codon position	Conservation
First	88 %
Second	94 %
Third	72 %

B



C

		uORF2a	uORF2b	main ORF
Consensus:		AMAAUGGC	AMAAUGGC	AMAAUGGC
Position:		-3-2-1+1+2+3+4+5	-3-2-1+1+2+3+4+5	-3-2-1+1+2+3+4+5
bZIP11	At	CACAUGUC	UUUAUGUU	UAAAUGGA
BG440654	Ga	UUCAUGUU	UUUAUGAU	AUAAUGGC
TA40315_3847	Gm	UUCAUGUA	UGUAUGAU	AACAUGGC
CV496133	St	UUGAUGUC	UUUAUGAU	GUCAUGGC
TA391_3848	Gs	UUCAUGUA	UGUAUGAU	ACUAUGGC
DV127068	Ee	UUCAUGUC	UUUAUGAU	UCAAUGGC
DW053302	Ls	CACAUGUC	UUUAUGAU	CUAAUGGC
TA20855_3880	Mt	UUCAUGUA	UGUAUGAU	CACAUGGC
TA26972_3635	Gh	CUCAUGUC	UUUAUGAU	UUAAUGGC
TA30608_3750	Mxd	UUGAUGAC	UGCAUGAU	UCAAUGGC
TA4489_4072	Ca	UUCAUGUC	UUUAUGAU	GUCAUGGC
TA3122_3760	Pp	UUCAUGUC	UGUAUGAU	GUCAUGGC
bZIP1	At	GAAAUGAU		AACAUGGC
bZIP2	At	CUGAUGAC		UCAAUGGC
bZIP44	At	UUCAUGUC		GAAAUGGG
bZIP53	At	UCUAUGAG		GAAAUGGG

FIG. 2. The uORF encoding the SC-peptide is highly conserved among *bZIP11* homologues. (A) Nucleotide conservation varies over the three codon positions in SC-peptide-encoding uORFs. The 43 sequences most similar to *bZIP11* uORF2 in *Arabidopsis thaliana* and other dicotyledonous species were used for the calculation. (B) Amino acid sequence logo of the SC-peptide of the 43 sequences used in (A). The highest degree of amino acid conservation is observed in the C-terminal part of the SC-peptide. (C) Alignment of the AUG context of *bZIP11* mRNA sequences similar to uORF2 identified in several different species. The importance of specific nucleotide positions is indicated by colour coding: green indicates a nucleotide corresponding to the consensus nucleotide sequence (Joshi *et al.*, 1997), and red indicates a non-corresponding nucleotide. 'M' in the consensus sequence is A or C. Note that positions +4 and +5 appear to be of greater importance in plants, whereas positions −3, −2 and −1, although well conserved, play a minor role in determining initiation efficiency (Lutcke *et al.*, 1987). Genes are indicated by gene name or accession number and are followed by abbreviations of the species name: At, *Arabidopsis thaliana* (thale cress); Ga, *Gossypium arboreum* (cotton tree); Gm, *Glycine max* (soybean); Gs, *Glycine soja* (wild soybean); St, *Solanum tuberosum* (potato); Ee, *Euphorbia esula* (leafy spurge); Ls, *Lactuca saligna* (willow leaf lettuce); Mt, *Medicago truncatula* (barrel medic); Gh, *Gossypium hirsutum* (upland cotton); Mxd, *Malus* × *domestica* (cultivated apple); Pp, *Prunus persica* (peach); Ca, *Capsicum annuum* (pepper).

activated when plants experience energy deprivation caused by hypoxia, herbicides or darkness, whereas glucose and sucrose repressed this stress effect (reviewed in Baena-Gonzalez and Sheen, 2008). The bZIP binding site in the *ASN1* promoter was shown to be required for the KIN10/11-mediated stress effect on *ASN1* expression. Plants with lowered KIN10 and KIN11 activity were unable to induce *ASN1* expression in response to stress. This demonstrated the regulatory circuit in which KIN10/11 regulated *ASN1* expression through bZIP transcription factors, probably through direct phosphorylation (Baena-Gonzalez *et al.*, 2007). ASN1 is required for conversion of aspartate (Asp) and glutamine (Gln) to asparagine (Asn) and glutamate (Glu) (Lam *et al.*, 2003). Asn is an important nitrogen transport amino acid that, compared with Gln, has a higher N/C ratio. Asn is a relatively inert amino acid that is used during the night when both organic nitrogen and organic carbon availability are limiting. Amino acid concentrations were affected in response to transient overexpression of bZIP11 (Hanson *et al.*, 2008). These results suggested a role of bZIP11 in the control of amino acid metabolism (Hanson *et al.*, 2008). Figure 3 summarizes these results and proposes a model for bZIP11-regulated reprogramming of amino acid metabolism. Further research on bZIP11-regulated genes will shed light on its broader functions in controlling metabolism.

GENERAL CONTROL OF TRANSLATIONAL RESPONSE TO NUTRIENT DEPRIVATION BY ALTERED RIBOSOME BIOGENESIS

Cells must create a balance between the need for resources to maintain cell viability and cell function, and the need for

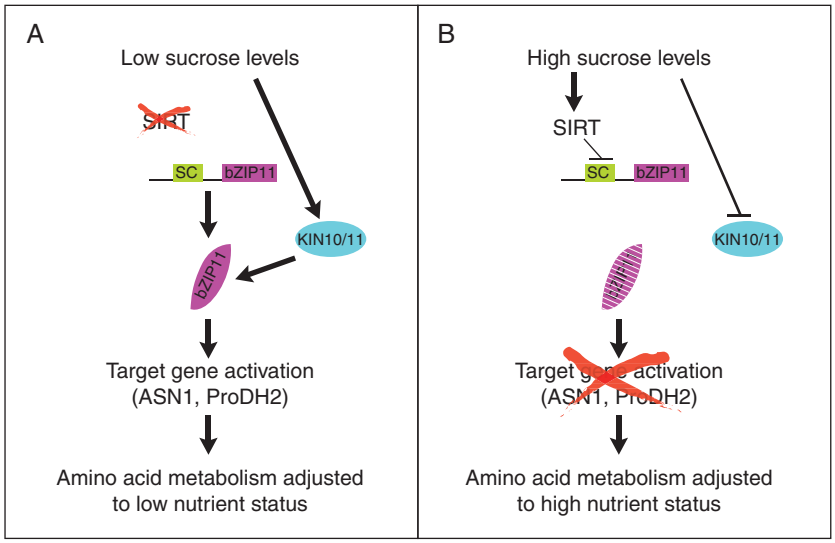


FIG. 3. Sucrose regulates amino acid metabolism reprogramming by controlling *bZIP11* translation. (A) When sucrose concentrations are low, bZIP11 is efficiently translated and the protein kinase KIN10/11 activates bZIP11, followed by activation of the expression of target genes, which results in the reprogramming of amino acid metabolism. (B) When sucrose concentrations are high, bZIP11 is translationally repressed and KIN10/11 activity is low. Thus, bZIP11 target genes will not be activated.

resources to support growth and differentiation. Cell growth and differentiation depend on continuous protein synthesis and thus require continuous ribosomal biogenesis. Ribosomal biogenesis is a tremendously energy-consuming process. In yeast it has been reported that ribosome biogenesis accounts for over 75 % of all nuclear transcription (reviewed in Warner, 1999). Ribosomal biogenesis is a demanding process for plant cells as well and must be carefully balanced with growth requirements. In response to stress such as sucrose starvation (Nicolai *et al.*, 2006) and hypoxia (Branco-Price *et al.*, 2005), ribosomal protein synthesis is repressed and subsequently general translation is put on hold. This response preserves energy and is important for cell survival. Re-establishment of ribosomal biogenesis and growth occur after the stress is relieved. Translational control in response to sucrose is thus not only regulated at the level of specific mRNAs, as for bZIP11, but may also be regulated at the general level of translational activity of the cell. So far, regulation of ribosomal biogenesis in plants has not been studied intensively. However, in mammals the protein kinase mammalian Target of Rapamycin (mTOR) serves as a key regulator promoting ribosomal biogenesis. The mTOR signalling pathway activates ribosomal biogenesis under non-stressed conditions, in which energy is not limiting. During nutrient starvation and other stress conditions mTOR is, however, inactivated through activation of AMPK, the mammalian paralogue of KIN10/11. Similarly to the situation in mammals, KIN10/11 activation in *A. thaliana* might result in the inactivation of AtTOR, the paralogue of mTOR, although this has not yet been proven experimentally. However, KIN10 overexpression greatly impacts on ribosomal protein gene expression, in agreement with a genetic interaction of KIN10 with AtTOR (reviewed in Baena-Gonzalez and Sheen, 2008). mTOR forms a complex with the raptor and mLST8 proteins, the TOR complex 1 (TORC1) (reviewed in Proud, 2007). The antibiotic rapamycin has been shown to disrupt the TORC1 complex by promoting the formation of a 12-kDa FK506-binding protein (FKBP12)–mTOR complex (Chen *et al.*, 1995). FKBP12–TOR complex formation results in mTOR inactivation. TORC1 regulates ribosomal biogenesis and protein synthesis by phosphorylating eIF4E-binding protein 1 (4E-BP1) and p70 ribosomal protein S6 kinase 1 (S6K1) (Brunn *et al.*, 1997; Burnett *et al.*, 1998). Phosphorylation inactivates 4E-BP1, resulting in the release of eukaryotic initiation factor 4E (eIF4E) from the inhibitory 4E-BP1, allowing eIF4E to form the initiation complex at the 5′-cap structure of mRNAs (Haghighat *et al.*, 1995; Mader *et al.*, 1995). mTOR signalling is also involved in the translation of a family of mRNAs that contain a polypyrimidine tract at their 5′-end [5′-terminal oligopyrimidine (5′-TOP) mRNAs]. These mRNAs encode components of the translational apparatus, e.g. ribosomal proteins.

In *A. thaliana* several TOR signalling components have been identified, but the role of AtTOR in response to nutrient availability has not been shown experimentally. Knowledge on how nutrient concentrations are perceived and result in signal transduction processes in plants is currently lacking. AtTOR plays an essential role in plants as well, given that disruption of AtTOR resulted in embryo lethality. Possibly, AtTOR regulates the integration and perception of nutrient signals in plants as well (Menand *et al.*, 2002). Silencing of AtTOR resulted in a decrease in polysome accumulation, indicating that AtTOR affects the mRNA translation process, similar to its function in mammals. Silencing of AtTOR also resulted in decreased mRNA translation of ErbB3 binding protein (EBP1), a regulator of ribosomal assembly and translation (Deprost *et al.*, 2007). This indicated that AtTOR is involved in ribosomal biogenesis. AtTOR was shown to be required for osmotic stress tolerance. (Deprost *et al.*, 2007). As in mammals, AtTOR interacted with AtRAPTOR1B, also named AtRAPTOR1, an orthologue of raptor in mammals (Mahfouz *et al.*, 2006). Similarly to AtTOR, AtRAPTOR1B was shown to be essential

for embryonic growth (Anderson *et al.*, 2005; Deprost *et al.*, 2005). AtRAPTOR1B interacted with S6K1 (Mahfouz *et al.*, 2006), which is required for the phosphorylation of Ribosomal Protein S6 (RPS6), in line with what was observed in mammals. The osmotic stress response led to the inhibition of S6K1 activity while co-expression of RAPTOR1B resulted in an increased S6K1 activity (Mahfouz *et al.*, 2006). The latter result indicated that osmotic stress affects S6K1 activity downstream of AtTOR. These findings indicated that AtTOR signalling is at least partly conserved among plants and mammals and that AtTOR signalling is likely to be involved in mediating ribosomal biogenesis and translational control, possibly in concert with KIN10/11 signalling.

CONCLUDING REMARKS AND PERSPECTIVES

Translational control of gene expression is part of the response to changing environmental cues, such as the diurnal cycle and different stresses. In accordance with this, the translationally regulated *bZIP11* was found to control amino acid metabolism. Sucrose-induced repression of *bZIP11* translation involves uORF2, which encodes the SC-peptide, which is evolutionary conserved among plant S1 class bZIPs. uORFs generally inhibit main ORF translation, but can also specifically control translation. Protein synthesis, however, is also regulated at other levels, such as ribosome biogenesis. Control of ribosomal biogenesis involves master regulators including KIN10/11 and AtTOR. Translation is thus regulated at several levels, thereby coordinating growth and metabolism.

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LITERATURE CITED

Anderson GH, Veit B, Hanson MR. 2005. The Arabidopsis *AtRaptor* genes are essential for post-embryonic plant growth. *BMC Biology* **3**: 12. doi:10.1186/1741-7007-3-12.

Asano K, Sachs MS. 2007. Translation factor control of ribosome conformation during start codon selection. *Genes & Development* **21**: 1280–1287.

Baena-Gonzalez E, Sheen J. 2008. Convergent energy and stress signaling. *Trends in Plant Science* **13**: 474–482.

Baena-Gonzalez E, Rolland F, Thevelein JM, Sheen J. 2007. A central integrator of transcription networks in plant stress and energy signalling. *Nature* **448**: 938–942.

Barker L, Kuhn C, Weise A, *et al*. 2000. SUT2, a putative sucrose sensor in sieve elements. *Plant Cell* **12**: 1153–1164.

Blasing OE, Gibon Y, Gunther M, *et al*. 2005. Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in Arabidopsis. *Plant Cell* **17**: 3257–3281.

Branco-Price C, Kawaguchi R, Ferreira RB, Bailey-Serres J. 2005. Genome-wide analysis of transcript abundance and translation in Arabidopsis seedlings subjected to oxygen deprivation. *Annals of Botany* **96**: 647–660.

Brunn GJ, Hudson CC, Sekulic A, *et al*. 1997. Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin. *Science* **277**: 99–101.

Burnett PE, Barrow RK, Cohen NA, Snyder SH, Sabatini DM. 1998. RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. *Proceedings of the National Academy of Sciences of the USA* **95**: 1432–1437.

Chen J, Zheng XF, Brown EJ, Schreiber SL. 1995. Identification of an 11-Kda Fkbp12-rapamycin-binding domain within the 289-Kda Fkbp12-rapamycin-associated protein and characterization of a critical serine residue. *Proceedings of the National Academy of Sciences of the USA* **92**: 4947–4951.

Cho YH, Yoo SD, Sheen J. 2006. Regulatory functions of nuclear hexokinase1 complex in glucose signaling. *Cell* **127**: 579–589.

Deprost D, Truong HN, Robaglia C, Meyer C. 2005. An Arabidopsis homolog of RAPTOR/KOG1 is essential for early embryo development. *Biochemical Biophysical Research Communications* **326**: 844–850.

Deprost D, Yao L, Sormani R, *et al*. 2007. The Arabidopsis TOR kinase links plant growth, yield, stress resistance and mRNA translation. *EMBO Reports* **8**: 864–870.

Ehlert A, Weltmeier F, Wang X, *et al*. 2006. Two-hybrid protein–protein interaction analysis in Arabidopsis protoplasts: establishment of a heterodimerization map of group C and group S bZIP transcription factors. *Plant Journal* **46**: 890–900.

Haghighat A, Mader S, Pause A, Sonenberg N. 1995. Repression of Cap-dependent translation by 4E-Binding Protein-1 – competition with P220 for binding to Eukaryotic Initiation Factor-4e. *EMBO Journal* **14**: 5701–5709.

Hanson J, Hanssen M, Wiese A, Hendriks MMWB, Smeekens S. 2008. The sucrose regulated transcription factor bZIP11 affects amino acid metabolism by regulating the expression of *ASPARAGINE SYNTHETASE1* and *PROLINE DEHYDROGENASE2. Plant Journal* **53**: 935–949.

Huang J, Taylor JP, Chen JG, *et al*. 2006. The plastid protein THYLAKOID FORMATION1 and the plasma membrane G-protein GPA1 interact in a novel sugar-signaling mechanism in Arabidopsis. *Plant Cell* **18**: 1226–1238.

Imai A, Hanzawa Y, Komura M, Yamamoto KT, Komeda Y, Takahashi T. 2006. The dwarf phenotype of the Arabidopsis *acl5* mutant is suppressed by a mutation in an upstream ORF of a bHLH gene. *Development* **133**: 3575–3585.

Imai A, Komura M, Kawano E, Kuwashiro Y, Takahashi T. 2008. A semi-dominant mutation in a ribosomal protein L10 gene suppresses the dwarf phenotype of the acl5 mutant in *Arabidopsis thaliana. Plant Journal* **56**: 881–890.

Jakoby M, Weisshaar B, Droge-Laser W, *et al*. 2002. bZIP transcription factors in Arabidopsis. *Trends in Plant Science* **7**: 106–111.

Joshi CP, Zhou H, Huang XQ, Chiang VL. 1997. Context sequences of translation initiation codon in plants. *Plant Molecular Biology* **35**: 993–1001.

Knott JM, Romer P, Sumper M. 2007. Putative spermine synthases from *Thalassiosira pseudonana* and *Arabidopsis thaliana* synthesize thermospermine rather than spermine. *FEBS Letters* **581**: 3081–3086.

Lam HM, Wong P, Chan HK, *et al*. 2003. Overexpression of the *ASN1* gene enhances nitrogen status in seeds of Arabidopsis. *Plant Physiology* **132**: 926–935.

Lutcke HA, Chow KC, Mickel FS, Moss KA, Kern HF, Scheele GA. 1987. Selection of AUG initiation codons differs in plants and animals. *The EMBO Journal* **6**: 43–48.

Mader S, Lee H, Pause A, Sonenberg N. 1995. The translation initiation-factor Eif-4e binds to a common motif shared by the translation factor Eif-4-gamma and the translational repressors 4E-binding proteins. *Molecular and Cellular Biology* **15**: 4990–4997.

Mahfouz MM, Kim S, Delauney AJ, Verma DPS. 2006. Arabidopsis TARGET OF RAPAMYCIN interacts with RAPTOR, which regulates the activity of S6 kinase in response to osmotic stress signals. *Plant Cell* **18**: 477–490.

Menand B, Desnos T, Nussaume L, *et al*. 2002. Expression and disruption of the Arabidopsis *TOR* (target of rapamycin) gene. *Proceedings of the National Academy of Sciences of the USA* **99**: 6422–6427.

Meyer S, Melzer M, Truernit E, *et al*. 2000. *AtSUC3*, a gene encoding a new Arabidopsis sucrose transporter, is expressed in cells adjacent to the vascular tissue and in a carpel cell layer. *Plant Journal* **24**: 869–882.

Moore B, Zhou L, Rolland F, *et al*. 2003. Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. *Science* **300**: 332–336.

Morris DR, Geballe AP. 2000. Upstream open reading frames as regulators of mRNA translation. *Molecular and Cellular Biology* **20**: 8635–8642.

Nicolai M, Roncato MA, Canoy AS, *et al*. 2006. Large-scale analysis of mRNA translation states during sucrose starvation in arabidopsis cells identifies cell proliferation and chromatin structure as targets of translational control. *Plant Physiology* **141**: 663–673.

Proud CG. 2007. Signalling to translation: how signal transduction pathways control the protein synthetic machinery. *Biochemical Journal* **403**: 217–234.

Rolland F, Baena-Gonzalez E, Sheen J. 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annual Review of Plant Biology* **57**: 675–709.

Rook F, Gerrits N, Kortstee A, *et al*. 1998*a*. Sucrose-specific signalling represses translation of the Arabidopsis *ATB2* bZIP transcription factor gene. *Plant Journal* **15**: 253–263.

Rook F, Weisbeek P, Smeekens S. 1998*b*. The light-regulated Arabidopsis bZIP transcription factor gene *ATB2* encodes a protein with an unusually long leucine zipper domain. *Plant Molecular Biology* **37**: 171–178.

Solfanelli C, Poggi A, Loreti E, Alpi A, Perata P. 2006. Sucrose-specific induction of the anthocyanin biosynthetic pathway in Arabidopsis. *Plant Physiology* **140**: 637–646.

Teng S, Keurentjes J, Bentsink L, Koornneef M, Smeekens S. 2005. Sucrose-specific induction of anthocyanin biosynthesis in Arabidopsis requires the *MYB75/PAP1* gene. *Plant Physiology* **139**: 1840–1852.

Vazquez-Laslop N, Thum C, Mankin AS. 2008. Molecular mechanism of drug-dependent ribosome stalling. *Molecular Cell* **30**: 190–202.

Warner JR. 1999. The economics of ribosome biosynthesis in yeast. *Trends in Biochemical Sciences* **24**: 437–440.

Weltmeier F, Ehlert A, Mayer CS, *et al*. 2006. Combinatorial control of Arabidopsis proline dehydrogenase transcription by specific heterodimerisation of bZIP transcription factors. *EMBO Journal* **25**: 3133–3143.

Weltmeier F, Rahmani F, Ehlert A, *et al*. 2009. Expression patterns within the Arabidopsis C/S1 bZIP transcription factor network: availability of heterodimerization partners controls gene expression during stress response and development. *Plant Molecular Biology* **69**: 107–119.

Wenzler HC, Mignery GA, Fisher LM, Park WD. 1989. Analysis of a chimeric class-I patatin-Gus gene in transgenic potato plants – high-level expression in tubers and sucrose-inducible expression in cultured leaf and stem explants. *Plant Molecular Biology* **12**: 41–50.

Wiese A, Elzinga N, Wobbes B, Smeekens S. 2004. A conserved upstream open reading frame mediates sucrose-induced repression of translation. *Plant Cell* **16**: 1717–1729.

Xiao WY, Sheen J, Jang JC. 2000. The role of hexokinase in plant sugar signal transduction and growth and development. *Plant Molecular Biology* **44**: 451–461.

Yokoyama R, Hirose T, Fujii N, Aspuria ET, Kato A, Uchimiya H. 1994. The *Rolc* promoter of *Agrobacterium rhizogenes* Ri plasmid is activated by sucrose in transgenic tobacco plants. *Molecular & General Genetics* **244**: 15–22.