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Metabolic sugar signal promotes *Arabidopsis* meristematic proliferation via G2

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ABSTRACT

Most organs in higher plants are generated postembryonically from the meristems, which harbor continuously dividing stem cells throughout a plant's life cycle. In addition to developmental regulations, mitotic activities in the meristematic tissues are modulated by nutritional cues, including carbon source availability. Here we further analyze the relationship between the sugar signal and seedling meristem establishment, taking advantage of our previous observation that exogenously supplied metabolic sugars can rescue the meristem growth arrest phenotype of the *Arabidopsis stip* mutant seedlings. Our results show that metabolic sugars reactivate the *stip* meristems by activating the expression of key cell cycle regulators, and therefore, promoting G2 to M transition in *Arabidopsis* meristematic tissues. One of the early events in this process is the transcriptional repression of *TSS*, a genetic suppressor of the *stip* mutations, by sugar signals, suggesting that *TSS* may act as an integrator of developmental and nutritional signals in regulating meristematic proliferation. We also present evidence that metabolic sugar signals are required for the activation of mitotic entry during *de novo* meristem formation from G2 arrested cells. Our observations, together with the recent findings that nutrient deprivation leads to G2 arrest of animal germline stem cells, suggest that carbohydrate availability-regulated G2 to M transition may represent a common mechanism in stem cell division regulation in multicellular organisms.

Introduction

Tissue growth driven by cell division plays a key role in the development of multicellular organisms. In higher plants, extensive cell division activities are found in the meristematic tissues postembryonically, where organ primordia originate from the stem cell clusters and the fast dividing progenitor cells. Although patterned during embryogenesis, the primary meristems enter an active growth phase immediately following germination. The *Arabidopsis* shoot apical meristem, for example, will increase its cell number by approximately 7-fold during vegetative growth in addition to producing new leaves, before reaching its mature size (Medford, 1992). Since the seeds are desiccated during maturation, all cell division activities need to be initiated *de novo* upon germination. One key regulator of this process is the hormone cytokinin, and *Arabidopsis* seedlings lacking cytokinin-sensing ability fail to develop either the shoot or the root meristem (Higuchi et al., 2004; Skylar et al., 2010).

In addition to developmental cues, cell cycle activities are modulated by nutrient availability. Simple sugars are both the main energy source and signaling molecules in plant cells (Hanson and Smeekens, 2009). It was first demonstrated in the 1960s that the division of the meristem cells relies on carbon sources (Van't Hof, 1966), and disruptions in trehalose biosynthesis and signaling pathways can often lead to severe developmental consequences (Paul et al., 2008). In the moss *Physcomitrella patens*, exogenously supplied glucose leads to prolonged juvenile growth and retarded differentiation (Lorenz et al., 2003). Genetically, the sugar signals interact with other developmental pathways. For example, *Arabidopsis gin2*-1 (*GLUCOSE-INSENSITIVE 2*) plants, which carry a mutation in *HEXOKINASE* (*HXK1*) and have reduced growth due to diminished sugar-sensing ability, show altered responses to both cytokinin and auxin hormones (Moore et al., 2003). In addition, the meristems in cytokinin receptor mutants can be partially restored by sucrose-containing medium (Higuchi et al., 2004; Skylar et al., 2010). Most strikingly, while mutations in one of the cytokinin downstream effectors, the homeobox transcription factor STIMPY (STIP; also known as WOX9) (Haecker et al., 2004; Skylar et al., 2010; Wu et al., 2005), results in the complete arrest of cell division in meristematic tissues, the application of exogenous sucrose at physiologically relevant concentrations can fully restore the arrested meristems to the wild-type size and function in both the shoot and the root (Wu et al., 2005).

Like other eukaryotic organisms, cell cycle decisions in higher plants rely on the activities of cyclin-dependent kinases (CDKs) and

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their interacting cyclins (CYCs) (Inzé and De Veylder, 2006). It has been demonstrated that sucrose, the major transported sugar in most plants, can accelerate cell division rate by activating *CYCD* expression, which promotes the G1 to S transition (Gaudin et al., 2000; Riou-Khamlichi et al., 2000). However, CYCD2 over-expression in tobacco plants results in only accelerated growth without affecting meristem size or structure (Cockcroft et al., 2000). Interestingly, both the cytokinin-sensing mutants and the *stip* mutants show increased G2-arrested cells (Higuchi et al., 2004; Wu et al., 2007). Therefore, their partial or complete rescue by sucrose raises the possibility that sugar signals may also regulate meristematic proliferation at the G2 to M transition. In this study, we examine the mechanisms by which sugar availability regulates meristematic growth in G2 arrested cells by gene expression profiling and monitoring the expression of cell cycle components in response to sugar treatment.

Materials and methods

Plant materials

Plants were grown in long days (16 h light/8 h darkness) under about 120 μE m^{-2} s^{-1} light at 22 °C. To observe seedling phenotypes, seeds were germinated on 1/2 Murashige Minimal Organics Medium (MS; Phytotechnology Lab) with 0.6% agar after 2 days of stratification at 4 °C. 44 mM of various sugars, 0.5 μg/ml 2,4-D, and 10 μM NPA were added to the media when their effects were assayed.

For callus induction in excised hypocotyls, seeds were stratified at 4 °C for 4 days on MS media containing 44 mM sucrose, and given an 8-h light treatment before germinated in the dark for 4 days at 22 °C. Hypocotyls were excised and incubated on appropriate media.

The *stip*-1 and *stip*-2 alleles were described previously (Wu et al., 2005). The CDKA1::GUS, CDKB1;1::GUS, and CYCB1;1::GUS reporters (Ferreira et al., 1994; Segers et al., 1996) were crossed to *stip*-2/+ plants, and the phenotypes were analyzed in F3. The T-DNA insertion in *TSS* (At4g28080) was obtained from GABI-KAT (Rosso et al., 2003).

Histological and morphological analysis

GUS activity staining was carried out as described (Sessions et al., 1999), using 2 mM potassium ferro and ferri cyanide, at 37 °C for 12–14 h. The GUS-stained seedlings and hypocotyls were mounted in 30% glycerol for analysis. *In situ* hybridization was performed as previously described (Skylar et al., 2010), with the modification that tissue sections were treated with 10 μg/ml proteinase K at 37 °C for 15 min pre-hybridization and 10 μg/ml RNase A at 37 °C for 30 min post-hybridization. All samples were photographed on a Zeiss Axio Imager equipped with an AxioCam HRc camera.

Gene expression analysis

Two transcriptional profiling experiments were conducted. First, roots of 1-day-old wild-type and *stip*-2 seedlings grown on MS medium were excised for comparison. Second, *stip*-2 seedlings were grown on MS medium for 1 week to allow for full growth arrest, and then treated with 1.5% sucrose. Samples were collected before and after a 3-h treatment. Total RNA was extracted using Spectrum Total Plant RNA (Sigma). The first-strand cDNA was obtained using SuperScript III First Strand Synthesis kit (Invitrogen). The microarray experiments were conducted using the Affymetrix ATH1 arrays. Two replicas were done for each sample. Raw intensity data was pre-processed and normalized using gcRMA (Wu et al., 2004), and differentially expressed genes were then identified using RankProd (Hong et al., 2006). The raw data for both experiments is deposited at ArrayExpress under accession numbers E-MEXP-2785 and E-MEXP-2784.

Real-time PCR was carried out with the SYBR-green (Molecular Probes) method on Opticon-2MJ machines. The relative changes in gene expression levels were determined using the $2^{-\Delta\Delta CT}$ method. Each sample was done in at least two biological replicates, each with technical replicates, and *eIF4A* was used for normalization.

The primers used in this study are as follows:

TSS (At4g28080): 5′-ctgaatgtaaatagcttgagaactctatt-3′ and 5′-agagacttagccacacgtatttgtt-3′;
eIF4A (At3g13920): 5′-cagcaaagaggaatcgtcccct-3′ and 5′-gcctgacactggataagggagaagt-3′.

Results

Meristematic tissue in stip seedlings enters quiescence in G2

The complete sugar rescue of the *stip* mutant meristem arrest phenotype provides a unique opportunity for studying how sugar modulates meristem growth and establishment. Our previous studies predicted that the seedling growth arrest phenotype of the *stip* mutants likely resulted from a G2 cell cycle arrest of the meristematic tissue (Wu et al., 2007, 2005). To confirm the timing of the cell cycle arrest, we compared the expression patterns of *CYCB1*;1 and *CDKB1*;1, both of which are required for the G2 to M transition (Boudolf et al., 2004; Breyne and Zabeau, 2001; Mironov et al., 1999; Porceddu et al., 2001), using existing β-glucuronidase (GUS) reporter genes (Ferreira et al., 1994; Segers et al., 1996) that had been stably integrated into the wild-type or mutant backgrounds. Two days after germination, CYCB1;1::GUS activities could be clearly detected in the wild-type shoot apex, in both the meristematic region and the first pair of true leaves (Fig. 1A). In contrast, little or no GUS activity could be found in the *stip*-2 apex (Fig. 1B). A similar absence of the CDKB1;1::GUS reporter activities was also seen in *stip*-2 shoot apex (Fig. 1D, compare to C). The same changes were the observed in the root meristematic zones (data not shown). The lack of these two key G2 to M regulators, when taken together with the relatively normal expression levels of the S phase marker histone H4 observed in *stip* mutants of similar age (Wu et al., 2005), supports the conclusion of a G2 arrest of *stip* meristematic tissues.

To further determine the differentiation state of the *stip* meristematic tissue, we examined *CDKA1* expression, which is essential for both the G1 to S and G2 to M transitions and correlates with the cellular competency to divide (Hemerly et al., 1995; Joubes et al., 2004; Porceddu et al., 2001). Although newly germinated *stip* seedlings showed lower levels of CDKA1::GUS (Segers et al., 1996) activities than the wild-type samples (Fig. 1F, compare to E), the CDKA1 promoter remained active in the shoot apex 10 days later (Fig. 1H), when these seedlings are fully arrested in growth. This result indicates that, unlike during the embryonic stage, where *stip* embryonic lethality was marked by the complete loss of CDKA1::GUS activities (Wu et al., 2007), the seedling meristematic tissues of *stip* mutants retain the memory of their undifferentiated identity. This observation is consistent with our previous finding that, although neither *CLAVATA* 3 nor *WUSCHEL* could be maintained in *stip* mutant shoot meristems, the expression of *SHOOTMERISTEMLESS* remained undisrupted even after the seedlings entered full growth arrest (Wu et al., 2005).

Metabolic sugars rescue cell cycle activities in stip mutants

Exogenous sucrose in the growth medium rescues growth-arrested *stip* seedlings by reactivating their primary meristems (Wu et al., 2005). Therefore, we concluded that sucrose must be able to reactivate cell cycle in these mutants. Since the *stip* meristematic tissues arrest growth in G2, we again examined the expression patterns of CYCB1;1 and CDKB1;1 reporters and found that they

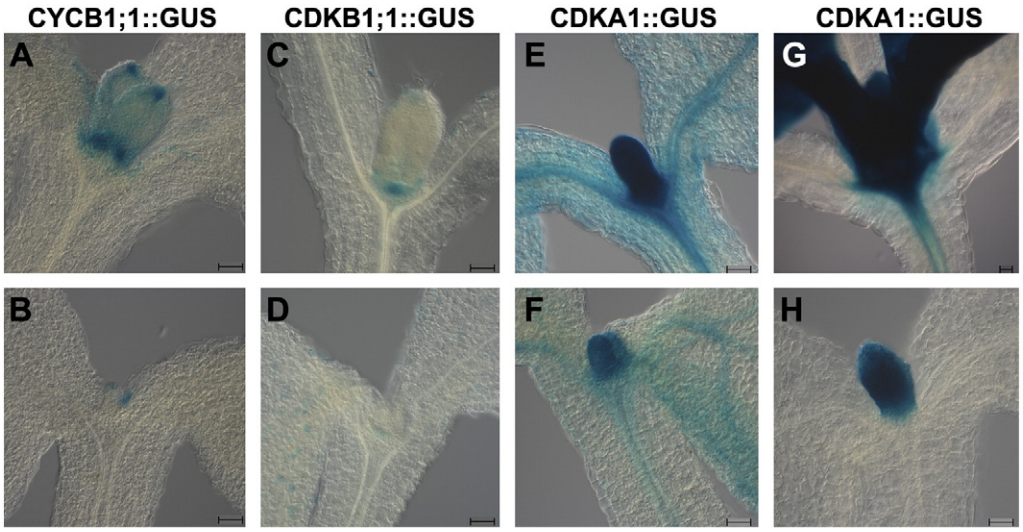

Fig. 1. The meristems in *stip* mutants arrest growth in G2. (A–F) Two days after germination, GUS reporter activities for CYCB1;1 (A and B), CDKB1;1 (C and D) and CDKA1 (E and F) can be clearly detected in the shoot apex of the wild-type seedlings (A, C, and E), while the *stip*-2 seedlings of the same age showed nearly no CYCB1;1 (B), CDKB1;1 (D) reporter activities, and slightly reduced CDKA1 reporter activities (F). (G and H) Two weeks after germination, CDKA1::GUS activities can be found in both the wild-type shoot (G) and the growth-arrested *stip*-2 shoot (H). The scale bar represents 50 μm.

behaved in the same manner in all our studies. We therefore focused on the changes in *CYCB1*;1 expression in the rest of this report. Ten days after germination, no CYCB1;1::GUS activity was detectable in *stip*-2 seedlings (Fig. 2B), which had retained the morphology of a newly germinated seedling. These fully arrested seedlings were transferred to either sugarless or sugar-containing medium, and monitored daily for any recovery of CYCB1;1::GUS activities. Three to 4 days after the transfer, the CYCB1;1 reporter activities were clearly visible in the shoot apex of the sucrose-treated *stip*-2 seedling (Fig. 2D), while no GUS activity was found in those remained on sugarless medium (Fig. 2C). Similarly, glucose and fructose were also able to reactivate *CYCB1*;1 expression in *stip*-2 seedlings. By comparison, the same concentrations of sorbitol, a non-metabolic sugar for osmotic control that does not affect the development of wild-type seedlings, was unable to rescue the cell cycle activities in *stip*-2 (Fig. 2E), suggesting that only metabolic sugars are able to induce cell cycle reentry. Consistent with this conclusion, 3-O-methylglucose (3-OMG), a transportable glucose analog that cannot be phosphorylated by hexakinases (HXK) and therefore cannot be metabolized, failed to rescue the CYCB1;1::GUS reporter activity in *stip*-2 (Fig. 2F).

Metabolic sugar promotes G2 to M transition in part by repressing TSS transcription

To understand the molecular mechanisms underlying metabolic sugar triggered cell cycle reentry from G2, we analyzed changes in gene expression profiles in response to the loss of *STIP* and the subsequent sucrose rescue, using the Affymetrix ATH1 arrays. Significant changes were detected in the expression levels of a wide range of genes, including sugar transporters and metabolic enzymes, transcription factors, protein kinases and other signaling molecules, regulators of cell wall structures, components of the photosynthetic apparatus, and other metabolic enzymes. Consistent with the 3–4 days that takes sugar to reactivate cell cycle gene expression in the growth-arrested *stip* mutants, no cell cycle machinery component was found to be differentially expressed after the 3-h sucrose treatment.

Thirty-five genes that encode transcription factors and proteins with predicted regulatory domains were selected from these candidates to test for their genetic interaction with *STIP*. Among them, *TPR-DOMAIN SUPPRESSOR OF STIMPY (TSS)*, a previously uncharacterized gene that encodes a TPR (tetratricopeptide repeat)

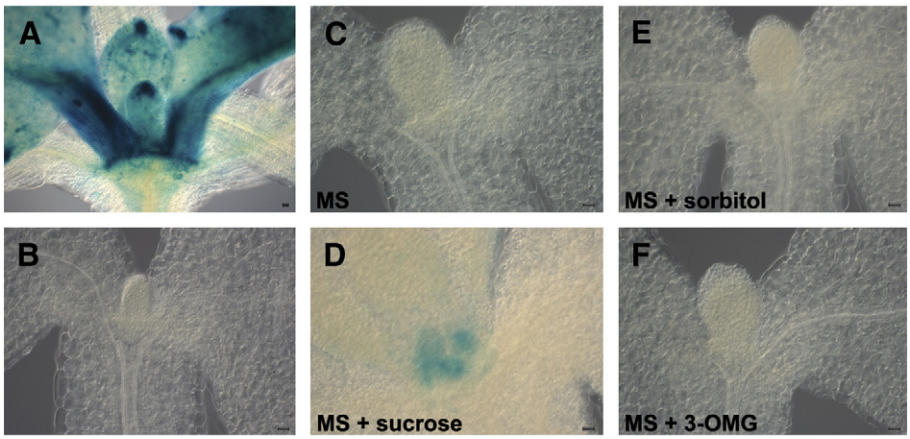

Fig. 2. Metabolic sugars reactivates *CYCB1;1* in arrested *stip* mutant meristems. (A and B) Ten days after germination, CYCB1;1::GUS reporter can be clearly seen in the wild-type shoot apex (A), but not in *stip*-2 (B). (C–F) CYCB1;1::GUS activities in the shoot apex 4 days after the growth-arrested *stip*-2 seedlings (B) were transferred to media containing different sugars. Only metabolic sugar was able to reactivate the reporter expression (D). The scale bar represents 50 μm.

domain protein, was found to be up-regulated in 1-day-old *stip*-1 roots by approximately 3-fold, before the growth arrest phenotype was morphologically apparent. Additionally, the 3-h treatment of 1.5% sucrose was able to down-regulate *TSS* in growth-arrested *stip*-2 seedlings by 2.5-fold. This sugar repression was verified in independent *stip*-1 samples using semi-quantitative RT-PCR (Fig. 3A). Perusal of a developmental expression atlas (Schmid et al., 2005) revealed that *TSS* expression is low at the shoot apex, which harbors a large percentage of meristematic tissue. Using semi-quantitative RT-PCR, we also observed a significant reduction in *TSS* transcript levels in the apical regions of both the wild-type and the *ap1 cal1* plants, which develop a large number of floral meristems (Fig. 3B). *in situ* hybridization using a *TSS* antisense probe showed that, in 5-day-old wild-type seedlings, *TSS* expression is excluded from the vegetative shoot meristem and leaf primordia, only activated in the non-epidermal tissues of the true leaves (Fig. 3C).

To test the genetic relationship between *STIP* and *TSS*, we acquired *tss*-1, an insertional allele with a T-DNA inserted in its sixth intron, which resulted in the loss of detectable *TSS* transcript using RT-PCR. When germinated on MS medium, approximately 90% ($n=212$) of the *tss*-1 seedlings developed normally, and showed no visible disruption in development during later stages. The remaining 10% *tss*-1 seedlings failed to develop after germination. In contrast, only 16% ($n=146$) of the *stip*-1 seedlings continued to develop without supplemented sugar, while the majority of seedlings arrested growth post-germination. By comparison, the *stip*-1 *tss*-1 double mutants had a seedling survival rate of 64% ($n=214$), rescuing the *stip*-1 seedling lethality by half (Fig. 3D). This partial suppression of the *stip* phenotype strongly indicates that *TSS* is partially responsible for the meristematic cell cycle arrest in *stip* mutants, and supports the hypothesis that a significant function of the metabolic sugars in promoting the G2 cell cycle reentry in *stip* seedlings is to repress *TSS* expression.

Interestingly, no significant down-regulation of *TSS* was observed in 2-day-old wild-type seedlings after an identical sucrose treatment (Fig. 3A), nor was it identified as a sucrose-responding gene in expression profiling experiments using carbon-limited wild-type seedlings (Osuna et al., 2007; Price et al., 2004). Both pieces of evidence suggest that the observed sucrose repression of *TSS* is a response specific to the rescue, i.e. the reactivation of cell cycle

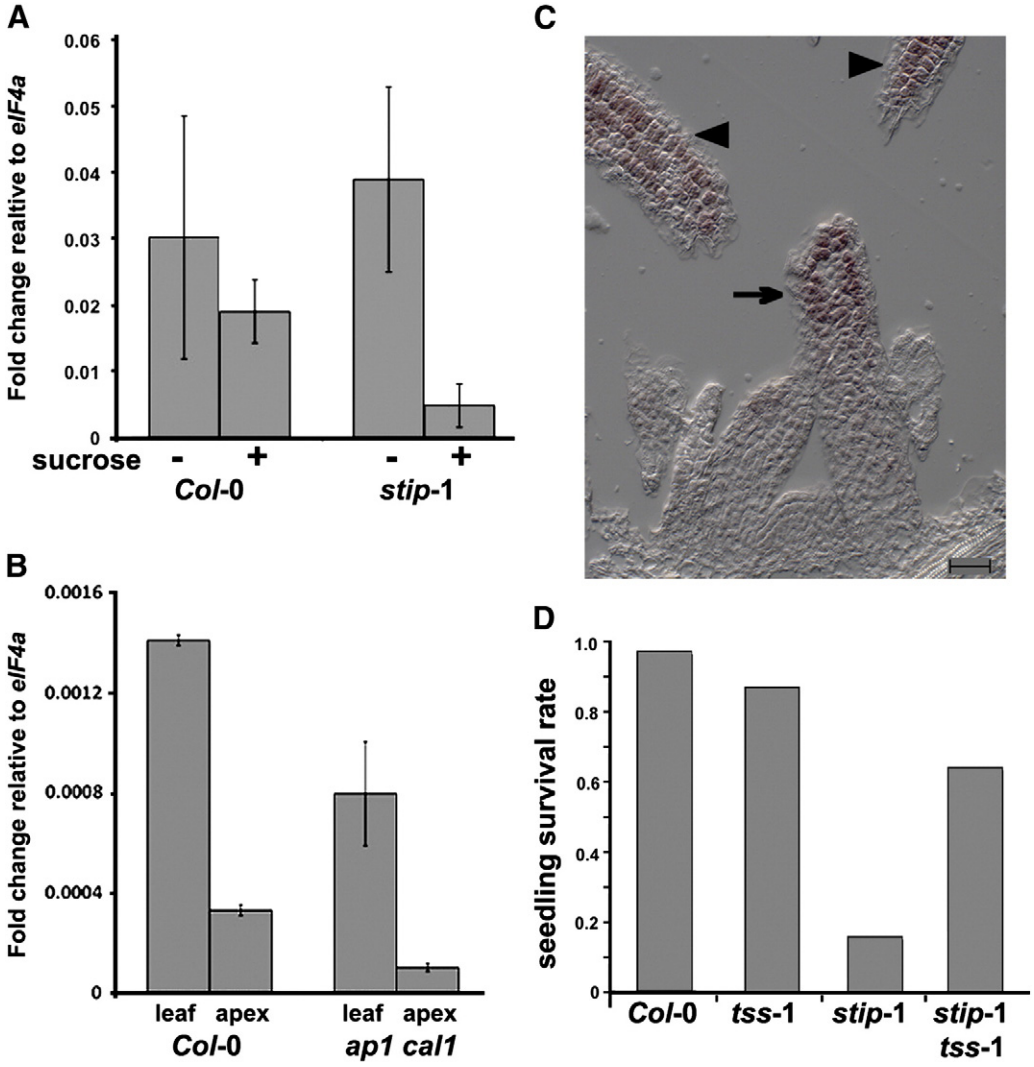

Fig. 3. The removal of *TSS* partially bypasses the sugar requirement by *stip* mutants. (A) *TSS* transcript level in response to 3 h of 1.5% sucrose treatment in 2-day-old *Col*-0 and 4-day-old *stip*-1 seedlings. There is a significant repression of *TSS* expression by sucrose in *stip* mutants. The younger *Col*-0 seedlings were used for comparison as they are morphologically similar to the *stip* mutants. (B) *TSS* transcript levels were measured by qRT-PCR in 18-day-old *Col*-0 leaves and apices, and in mature *ap1 cal*1 leaves and inflorescences. There is a significant reduction of *TSS* expression in the apical regions. (C) *In situ* hybridization of TSS in 5-day-old *Col*-0 using an antisense probe. *TSS* transcript is only detected in the first pair of true leaves (arrowheads) and the tip of the third leaf (arrow). The scale bar represents 50 μm. (D) A comparison of the seedling survival rates of wild-type, *stip*-1, *tss*-1, and *stip*-1 *tss*-1 double mutants, when grown on MS media. *tss*-1 partially rescues *stip*-1 mutants.

activities in meristematic tissues, of *stip* mutants, and the presence of *STIP* in the wild-type samples may be sufficient to repress *TSS* expression even under sugar deprivation.

Sugars reactivate cell cycle gene expression in stip meristems independent of auxin transport

Auxin is a key regulator of plant growth, and disruptions in auxin signaling can suppress the over-proliferation of the vegetative meristem caused by elevated cytokinin levels (Vidaurre et al., 2007). Since *STIP* acts partially downstream of cytokinin signaling in promoting vegetative meristem growth (Skylar et al., 2010), it is formally possible that the *stip* seedling arrest phenotype results from disruptions in auxin biosynthesis or response. However, our experimental evidence indicates that this is unlikely. Exogenous IAA or auxin analog 2,4-D failed to restore growth (Fig. 4D, compare to C and E) or to reactivate the *CYCB1*;1 reporter expression (Fig. S1C and D) in *stip* mutant seedlings. Using the DR5rev::GFP reporter (Friml et al., 2003), we detected normal auxin localization and uptake in fully arrested *stip* mutant seedlings (Fig. S1A; data not shown). In addition, 2,4-D-containing medium led to callus formation at the base of the hypocotyls in both the wild-type and *stip* mutant seedlings (Fig. S1E), suggesting that *stip* mutants have a normal auxin response, and their phenotype is not likely caused by disrupted auxin homeostasis or signaling.

Polar transport is essential for auxin signaling during plant development (Petrasek and Friml, 2009). We further examined whether auxin transport is required for the rescue of *stip* mutant seedlings by exogenous sugar. Six days after being transferred to sucrose-containing medium, the growth-arrested *stip*-2 seedlings (Fig. 4C) became fully green, and the growth of both the shoot and the root was clearly visible (Fig. 4E). In contrast, *stip*-2 seedlings transferred to sucrose medium that also contained 10 μM of the auxin transport inhibitor 1-naphthylphthalamic acid (NPA) (Rubery, 1990) showed no sign of recovery (Fig. 4G). However, sucrose was still able to reactivate the *CYCB1*;1 reporter expression in *stip* mutants in the presence of NPA, in both the shoot apex (Fig. 4F) and the root (Fig. 4H), although the GUS reporter pattern was altered by the presence of NPA. Same activation was observed for *CDKB1;*1. These results indicate that sugar signals activate the expression of key G2 to M regulators independent of auxin polar transport, and auxin signaling is most likely essential for sustaining mitotic activities in the rescued *stip* seedlings.

Sugar and auxin differentially regulate cell cycle reentry during de novo meristem formation

Our findings so far led us to the hypothesis that sugar and auxin play different roles in meristem cell cycle reentry from a G2 arrest. The presence of normal auxin distribution in *stip* mutants prevents us from testing this possibility during the sugar rescue. As such, we took advantage of the fact that although cells in hypocotyls do not normally divide post-embryonically, the xylem pericycle cells can be induced to dedifferentiate and form calli after the hypocotyl is excised and incubated on medium containing both sucrose and auxin (Atta et al., 2009). As the root xylem pericycle cells arrest the cell cycle in the G2 phase (Dewitte and Murray, 2003), it is most likely that their counterpart in the hypocotyl also exists in a G2-arrested state. We therefore tested the ability of sucrose to activate cell cycle gene expression in excised hypocotyls, with or without the help of high concentrations of exogenous auxin.

No CDKA1, CYCB1;1, or DR5 GUS reporter activity could be detected in the hypocotyls following dark germination (Fig. 5A and Fig. S2). After excision and 6 days of incubation on basic medium that also contained 1.5% sucrose, CYCB1;1::GUS activities were detected around the vascular bundle, where the xylem pericycle cells are located (Fig. 5C). This is in contrast to the hypocotyls incubated on the sugarless medium, where no reporter activity was visible (Fig. 5B). Similar activation was also observed with other metabolic sugars, but not with sorbitol or 3-OMG. In the presence of 10 μM NPA, sucrose

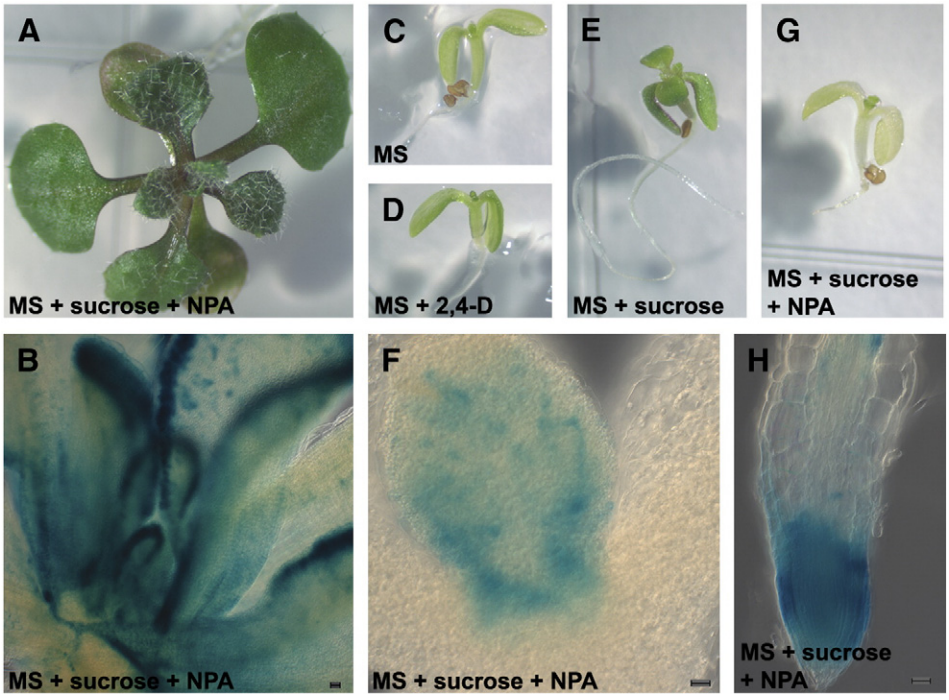

Fig. 4. Sucrose reactivates CYCB1;1 in arrested *stip*-2 meristems independent of auxin polar transport. (A and B) Ten-day-old wild-type seedlings were transferred to MS media containing both sucrose and 10 μM NPA. Four days of NPA treatment alters CYCB1;1::GUS reporter pattern (B) and leads to anthocyanin accumulation (A). (C–H) Ten-day-old fully arrested *stip*-2 seedlings were transferred to MS media with different supplement. Sucrose was able to rescue the *stip* mutants (E); MS (C), 2,4-D alone (D), or sucrose with NPA (G) failed to rescue. However, sucrose was able to reactivate CYCB1;1::GUS reporter in the presence of NPA in *stip* meristems (F,H). Note that the same CYCB1;1::GUS pattern as (H) was also found in wild-type roots treated with NPA. The scale bar represents 50 μm.

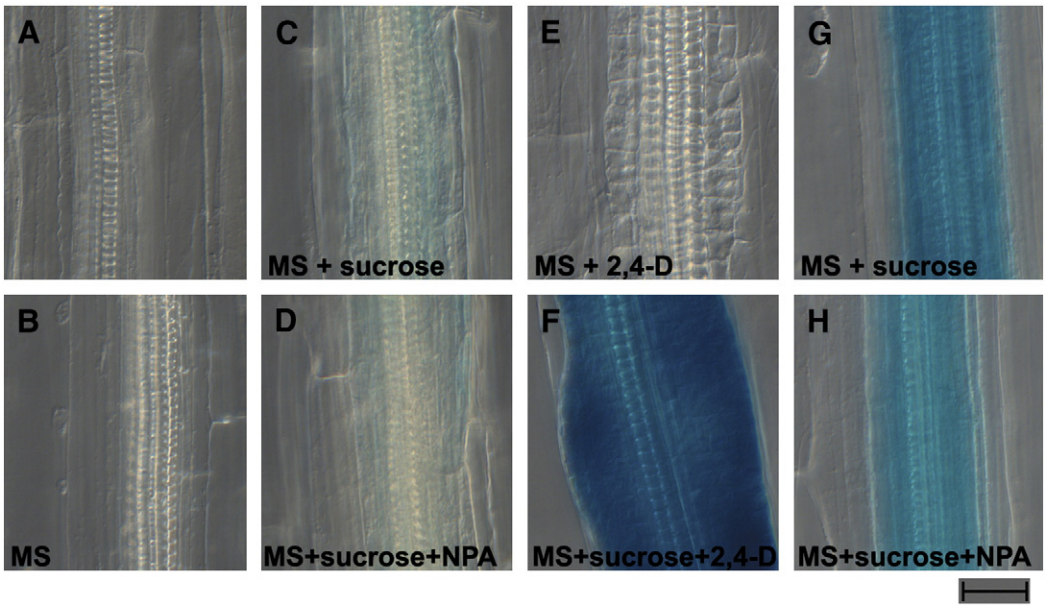

Fig. 5. Sucrose and auxin differentially regulate hypocotyl xylem pericycle cell dedifferentiation. (A–F) No CYCB1;1::GUS activity can be detected in the hypocotyls of the dark germinated wild-type seedlings (A). After excision and 6 days of incubation, the hypocotyls on MS show no CYCB1;1 activation (B); reporter activities can be seen in the samples treated with sucrose (C), and sucrose plus 10 μM NPA (D); hypocotyls treated with 2,4-D alone show limited pericycle cell division, but no detectable CYCB1;1::GUS activity; the combination of sucrose and 2,4-D induce high levels of CYB1;1::GUS activities and extensive proliferation of the pericycle cells (F). (G and H) Sucrose treatment activates CDKA1::GUS reporter in the excised hypocotyls (G), and in the presence of 10 μM NPA (H). The scale bar represents 50 μm.

was still able to activate the CYCB1;1 reporter in the same tissue, although at a reduced level (Fig. 5D). The CDKA1 and CDKB1;1 GUS reporters responded to sugar and NPA in a similar manner as CYCB1;1::GUS (Fig. 5G and H; data not shown), which is consistent with the conclusion that metabolic sugar is sufficient for the activation of key cell cycle genes in G2 arrested cells. However, these pericycle cells in the sugar-treated hypocotyls do not divide even after prolonged incubation, suggesting that sugar signal is not sufficient for triggering mitosis.

When 2,4-D was added to the incubation media, the xylem pericycle cells in the excised hypocotyls responded drastically differently depending on whether metabolic sugar was also present. With sucrose, they divided quickly and formed an enlarged cell mass around the vascular bundle, with high levels of CYCB1;1 reporter expression indicating their continued proliferation (Fig. 5F). Without sugar, limited pericycle cell divisions in response to 2,4-D could be seen within 48 h of incubation (Fig. 5E). However, no further division was ever observed even after an extended period of incubation, and we were never able to detect CYCB1;1::GUS activity in these hypocotyls (Fig. 5E). A likely explanation for this effect is that the exogenous 2,4-D was able to induce pericycle cell divisions in conjunction with the existing cellular sugar in the hypocotyls. However, without supplemental sugar, 2,4-D alone is unable to sustain either proliferation or cell cycle gene expression, supporting our hypothesis that sugar and auxin play different roles in inducing cell cycle reentry from G2.

Discussion

Metabolic sugar signals differentially regulate cell cycle progression at G1 and G2

Ample carbon supply is essential for generating new biomass during tissue growth and organ formation, which is the major role of the meristematic tissues during plant development. Simple sugars are known to stimulate plant cell cycle progression at the G1 to S transition by activating *CYCD* expression, which regulates growth rate (Cockcroft et al., 2000; Gaudin et al., 2000; Riou-Khamlichi et al., 2000). Here we demonstrated that metabolic sugars also activate the transcription of key cell cycle components for the G2 to M transition, such as *CYCB1;1* and *CDKB1;1*, in the meristematic tissues (Inzé and De Veylder, 2006). The sugar rescue of *stip* mutant seedlings suggests that this activation of the G2 to M transition by simple sugars is critical in the initiation of cell division in the primary meristems and vegetative growth following germination. However, in contrast to the D-type cyclins, which could be activated by sucrose in as little as 30 min (Riou-Khamlichi et al., 2000), the expression of these early mitotic indicators was only detected after several days of sucrose treatment, suggesting that the activation of the cell cycle components required for G2 to M transition by metabolic sugars is very much indirect.

The best understood G2 cell cycle reentry event in *Arabidopsis* is lateral root formation from the root xylem pericycle cells (Malamy and Benfey, 1997). It has been established that auxin signaling and polar transport are essential for the induction of pericycle cell division in order to initiate the lateral root primordia (Casimiro et al., 2001; Celenza et al., 1995; Vanneste et al., 2005). However, it is very difficult to evaluate the role of metabolic sugar in this process, due to the natural presence of cellular sugars during development. Here we demonstrated that metabolic sugars can activate the expression of essential mitotic regulators in pericycle cells of excised hypocotyls without auxin, while 2,4-D alone is not able to maintain cell cycle gene expression or mitotic activities, underscoring the importance of the sugar signals. This is consistent with the earlier report that the *gin2* mutants, which have lower sugar signaling and metabolism, have reduced ability to generate calli when provided with 2% sucrose and IAA (Moore et al., 2003). These observations strongly suggest that the sugar requirement during the G2 to M transition is a precondition for the auxin-driven cell division activities, and the sugar signals most likely act independent of auxin signaling (Fig. 6).

TSS acts at a checkpoint in the initiation of vegetative growth

Germination signals the beginning of vegetative development, which is marked by the initiation of active photosynthesis and tissue proliferation. In meristematic tissues, developmental cues and

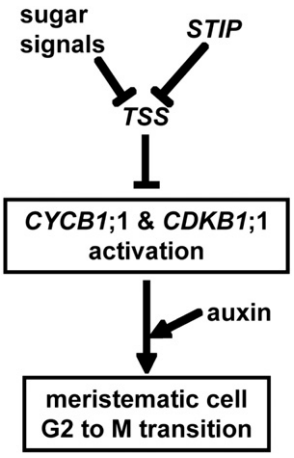

Fig. 6. A schematic representation of the genetic relationship among *STIP*, *TSS*, and sugar signaling. STIP and sugar signals initiate the G2 to M transition in meristematic tissues in part by repressing *TSS* transcription, and auxin is required for the completion of mitosis after the key cell cycle regulators are activated. Additional unidentified genes downstream of sugar and STIP signaling also play a role in the activation of mitotic entry in the meristems.

nutritional information are incorporated at the cellular level in reaching the decision of mitotic entry. Previously, we demonstrated that *STIP* mediates key developmental signals in triggering meristematic proliferation at this stage (Skylar et al., 2010; Wu et al., 2005). The removal of *TSS* is able to partially suppress *stip* seedling lethality (Fig. 3D), implying that part of the normal function of *STIP* is to repress the transcription of *TSS* to promote meristematic proliferation. Additionally, *TSS* transcription was down-regulated in the growth-arrested *stip* mutants within 3 h of applying sugar. Therefore, *TSS* transcription is repressed by both abundant carbon source and *STIP* signals, and it may act at a major checkpoint in meristematic growth by integrating these two signals in determining the timing of seedling growth.

The TSS protein shows sequence similarities to a subunit of the eukaryotic translation initiation factor 3 (eIF3) complex. The most recognizable domain in TSS is the TPR repeats, which are involved in protein-protein interactions. Therefore, it is plausible to hypothesize that TSS acts as the scaffold of a protein complex, which sequesters key factors that are required for the G2 to M transition in meristematic tissues. The meristematic tissue enters the proliferation stage after germination only when the amount of TSS is kept below a threshold by either STIP signaling or a sufficient amount of sugar. The precise role of *TSS* is currently under investigation.

Carbon source availability as a universal control mechanism for stem cell G2 to M transition

Carbohydrates are the universal cellular energy source. In unicellular systems such as budding yeast and cultured mammalian cell lines, dividing cells arrest in G1 in response to carbohydrate deprivation (Pardee, 1989). In multicelluar organisms, stem cells and progenitor cells represent the majority of dividing cells. Although most animal stem cells are found during embryonic development, where nutrients are abundant, recent studies in both *Drosophila* and *C. elegans* indicate that nutrient availability and sensing regulate the division rate of the germline stem cells (GSCs). Perceived carbon source deprivation due to disruptions in the insulin signaling pathway results in the G2 arrest of the GSCs (Drummond-Barbosa and Spradling, 2001; Fukuyama et al., 2006; Hsu and Drummond-Barbosa, 2009; Hsu et al., 2008; Ueishi et al., 2009). In higher plants such as *Arabidopsis*, the meristematic tissues consist of stem cells and other undifferentiated cells. Our results show that metabolic sugar signals activate the transcription of key cell cycle components, such as *CYCB1;1* and *CDKB1;1*, during both the recovery of the G2 quiescent meristems and *de novo* meristem formation via dedifferentiation from G2 arrested cells. These observations, taken together with the findings in the animal GSCs, suggest that carbohydrate availability-modulated G2 to M transition may represent a common mechanism in stem cell division regulation in multicellular organisms.

At the molecular level, cellular energy and nutrient levels are monitored by different branches of the systemic nutrient sensing mechanisms. Signals from these pathways are integrated by the target of rapamycin (TOR) complex, which then adjusts cell growth and proliferation accordingly. In *Drosophila* and *C. elegans*, the G2 control of GSC proliferation has been linked to known components of the systemic nutrient sensing mechanisms, including the highly conserved AMP-activated protein kinase (AMPK/SNF1) (Britton et al., 2002; Fukuyama et al., 2006; Hsu et al., 2008; LaFever and Drummond-Barbosa, 2005; Narbonne and Roy, 2006). More recently, it has been reported that TOR promotes *Drosophila* female GSC proliferation during G2, independent of insulin signaling (LaFever et al., 2010). Two SNF1-related kinases in *Arabidopsis*, KIN10 and KIN11, play similar roles in energy sensing as AMPK (Baena-Gonzalez et al., 2007; Jossier et al., 2009). A single copy of TOR has been found in *Arabidopsis*, the loss of which results in embryonic lethality (Menand et al., 2002). More interestingly, the loss of the *Arabidopsis* TOR interacting proteins RAPTOR's (regulatory associated protein of mTOR) leads to seedling growth arrest following germination (Anderson et al., 2005; Mahfouz et al., 2006), which can also be partially reversed by supplying sucrose in the growth medium (Wu, unpublished data), implying a possible link between the TOR/RAPTOR complex and early seedling development. Whether the TOR complex and the AMPK signaling pathway play a role in the G2 to M transition in meristematic tissues remains to be investigated.

Conclusions

Our results show that, in *Arabidopsis*, metabolic sugars are required for the activation of mitotic entry from G2 in meristematic tissues, likely independent of auxin signaling. In contrast to its role during the G1 to S transition, sugar signals indirectly activate the transcription of key cell cycle machinery in G2 to M transition. Through gene expression profiling, we identified the transcriptional repression of *TSS* as one of the early events in sugar-driven G2 to M transition. We propose that *TSS* negatively regulates meristematic tissue proliferation by integrating developmental signals with carbon source availability.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.ydbio.2010.12.019.

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