DELLA Signaling Mediates Stress-Induced Cell Differentiation in Arabidopsis Leaves through Modulation of Anaphase-Promoting Complex/Cyclosome Activity[1][W][OA]

Hannes Claeys[2], Aleksandra Skirycz[2], Katrien Maleux, and Dirk Inzé*

Department of Plant Systems Biology, VIB, B–9052 Ghent, Belgium (H.C., A.S., K.M., D.I.); and Department of Plant Biotechnology and Bioinformatics, Ghent University, B–9052 Ghent, Belgium (H.C., A.S., K.M., D.I.)

Drought is responsible for considerable yield losses in agriculture due to its detrimental effects on growth. Drought responses have been extensively studied, but mostly on the level of complete plants or mature tissues. However, stress responses were shown to be highly tissue and developmental stage specific, and dividing tissues have developed unique mechanisms to respond to stress. Previously, we studied the effects of osmotic stress on dividing leaf cells in Arabidopsis (*Arabidopsis thaliana*) and found that stress causes early mitotic exit, in which cells end their mitotic division and start endoreduplication earlier. In this study, we analyzed this phenomenon in more detail. Osmotic stress induces changes in gibberellin metabolism, resulting in the stabilization of DELLAs, which are responsible for mitotic exit and earlier onset of endoreduplication. Consequently, this response is absent in mutants with altered gibberellin levels or DELLA activity. Mitotic exit and onset of endoreduplication do not correlate with an up-regulation of known cell cycle inhibitors but are the result of reduced levels of DP-E2F-LIKE1/E2Fe and UV-B-INSENSITIVE4, both inhibitors of the developmental transition from mitosis to endoreduplication by modulating anaphase-promoting complex/cyclosome activity, which are down-regulated rapidly after DELLA stabilization. This work fits into an emerging view of DELLAs as regulators of cell division by regulating the transition to endoreduplication and differentiation.

Abiotic stresses, such as drought, have long been known to inhibit plant growth and thereby decrease crop productivity. This growth inhibition is an active response to stress, but little is currently known on how this is brought about (Skirycz and Inzé, 2010). Recently, it has become evident that tolerance to stress, which has been extensively studied in the past, relies on different mechanisms than growth inhibition by stress and that specific experimental setups have to be developed to investigate stress-induced growth inhibition (Skirycz et al., 2011b).

Organ growth is driven by both cell proliferation and cell expansion, in the case of Arabidopsis (*Arabidopsis thaliana*) leaf growth occurring sequentially in time. Initially, all cells in the leaf primordium are dividing, but later in development, cell division ceases from the tip to the base of the leaf, resulting in a cell cycle arrest front moving across the leaf (Donnelly et al., 1999; Kazama et al., 2010; Andriankaja et al., 2012). This transition from cell proliferation to cell expansion is accompanied by a switch from the mitotic cell cycle to endoreduplication, during which the genome is replicated, but mitosis does not occur, leading to cells with higher ploidy levels (Beemster et al., 2005). The current view is that this developmental transition from mitosis to endocycle, or mitotic exit, is triggered by a decrease in mitotic (B-type) cyclin-dependent kinase (CDK) activity (De Veylder et al., 2007; Breuer et al., 2010). This can occur through the up-regulation of cell cycle inhibitors such as Kip-Related Protein2 (KRP2; Verkest et al., 2005) and SIAMESE (SIM), which has been shown to control endoreduplication in Arabidopsis trichomes (Churchman et al., 2006) and can interact with CDKB/cyclin complexes (Van Leene et al., 2010). Another major pathway is the control of anaphase-promoting complex/cyclosome (APC/C) activity through its activating CELL CYCLE SWITCH PROTEIN52A (CCS52A) subunits, which positively regulate endoreduplication (Lammens et al., 2008) by targeting mitotic cyclins for destruction

[1] This work was supported by Ghent University (Bijzonder Onderzoeksfonds Methusalem grant no. BOF08/01M00408 and Multidisciplinary Research Partnership, Biotechnology for a Sustainable Economy grant no. 01MRB510W), the Interuniversity Attraction Poles Program (grant no. IUAP VI/33), initiated by the Belgian State, Science Policy Office, and the European Community 6th Framework Program (AGRON-OMICS grant no. LSHG–CT–2006–037704). H.C. is a predoctoral fellow of the Research Foundation-Flanders.

[2] These authors contributed equally to the article.

* Corresponding author; e-mail dirk.inze@psb.vib-ugent.be.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Dirk Inzé (dirk.inze@psb.vib-ugent.be).

[W] The online version of this article contains Web-only data.

[OA] Open Access articles can be viewed online without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.112.195032

(Boudolf et al., 2009; Kasili et al., 2010). Activation of APC/C by CCS52A proteins is counteracted by UVI4 (Hase et al., 2006; Heyman et al., 2011; Iwata et al., 2011). Upstream, the atypical E2F-like protein dimerization partner-E2F-LIKE1/E2Fe (DEL1/E2FE) represses the expression of *CCS52A* genes, thereby delaying the developmental onset of endoreduplication (Lammens et al., 2008). The regulation of *DEL1* transcription is less clear, although recently it was shown that the classical E2Fs E2Fb and E2Fc antagonistically regulate *DEL1* transcription, thereby controlling endoreduplication in the hypocotyl in response to light (Berckmans et al., 2011), whereas *UVI4* expression is also activated by E2Fa and E2Fb (Heyman et al., 2011). Endoreduplication generally correlates with cell expansion and differentiation, but its physiological role is still under debate and is likely tissue and stimulus specific (De Veylder et al., 2011). One intriguing hypothesis is that endoreduplication ensures cell fate maintenance by preventing dedifferentiation (Bramsiepe et al., 2010).

When plants are confronted with limited water availability, both cell proliferation and cell expansion are affected, leading to smaller leaves composed of fewer and smaller cells (Schuppler et al., 1998; Granier and Tardieu, 1999; Aguirrezabal et al., 2006; Skirycz et al., 2010, 2011a). Previously, we could show that when stress hits during the proliferation phase, cell division is first reversibly arrested in a posttranscriptional manner by ethylene signaling, resulting in a reduction of CDKA activity (Skirycz et al., 2011a). CDKA is the main driver of the cell cycle and is involved in both the G1-to-S and G2-to-M transitions (Inzé and De Veylder, 2006). When the stress persists, cells start the transition to cell expansion by exiting the mitotic cell cycle in favor of endoreduplication. Here, we show that this process is dependent on GA signaling.

GAs are a class of diterpenoid hormones that are involved in various processes throughout the plant life cycle, including seed germination, vegetative growth, bolting and flowering, and stress response (Achard et al., 2006; Sun, 2008). The regulation of GA levels occurs at both biosynthesis, through GA 20-oxidases (GA20OX) and GA 3-oxidases (GA3OX), and degradation, which is mainly catalyzed by GA 2-oxidases (GA2OX). All these enzymes occur in small families in Arabidopsis and have tissue-specific expression patterns (Mitchum et al., 2006; Rieu et al., 2008a, 2008b), allowing for a tight temporal and spatial control of GA levels. GA signaling occurs by binding of GA to its receptor, GA INSENSITIVE DWARF1, leading to the formation of a complex with DELLA proteins, transcriptional regulators that inhibit GA responses in the absence of GA. This results in the recognition and degradation of DELLA proteins by a Skp, Cullin, F-box complex with SLEEPY1 or SNEEZY/SLEEPY2 as the F-box components (Dill et al., 2004; Fu et al., 2004; Ariizumi et al., 2011). DELLA activity is also regulated through nonproteolytic means by phosphorylation, although its effects on DELLA activity are still under debate (Sun, 2010), and by the action of the N-acetylglucosamine transferase SPINDLY, which directly or indirectly activates DELLAs (Jacobsen and Olszewski, 1993; Silverstone et al., 2007).

DELLAs are thought to be responsible for all GA responses, which are very pleiotropic. As a result, DELLAs have the potential to induce very different transcriptomes, depending on the tissue, developmental stage, and stimulus that is studied, with only a small core involved in feedback regulation being conserved (Ogawa et al., 2003; Cao et al., 2006; Nemhauser et al., 2006; Zentella et al., 2007; Achard et al., 2008b; Hou et al., 2008; Gonzalez et al., 2010). As DELLAs have no DNA-binding domain, they function by interacting with and thereby inhibiting a wide array of other transcription factors, such as PHYTOCHROME INTERACTING FACTOR3 (PIF3), PIF4, SPATULA, JASMONATE-ZIM-DOMAIN PROTEIN1, and SCARECROW-LIKE3 (de Lucas et al., 2008; Feng et al., 2008; Hou et al., 2010; Josse et al., 2011; Zhang et al., 2011). DELLAs can also activate transcription through an association with DNA, as shown by chromatin immunoprecipitation analysis (Zentella et al., 2007), and this most likely occurs through the interaction with yet unknown DNA-binding factors. Arabidopsis contains five DELLAs (REPRESSOR OF GA1-3 [RGA], GIBBERELLIC ACID INSENSITIVE [GAI], RGA-LIKE1 [RGL1], RGL2, and RGL3) that are to some extent functionally divergent, mainly due to their expression patterns (Gallego-Bartolomé et al., 2010), with, for instance, RGA and GAI being most important for the regulation of vegetative growth (Dill et al., 2001).

DELLAs have been shown to contribute to growth inhibition by various abiotic and biotic stresses (Achard et al., 2006, 2008a; Magome et al., 2008; Navarro et al., 2008). Earlier reports also showed that various stresses can induce cell cycle inhibitors of the CDK inhibitor (ICK)/KRP or SIM family and thereby arrest the cell cycle (Wang et al., 1998; Pettkó-Szandtner et al., 2006; De Veylder et al., 2007; Peres et al., 2007). Furthermore, DELLAs appear to be able to control cell proliferation rates by regulating *KRP2*, *SIM*, *SIM-RELATED1* (*SMR1*), and *SMR2* transcription (Achard et al., 2009). However, our results suggest that although stress-induced mitotic exit of proliferating cells is fully DELLA dependent, it is not the result of elevated cell cycle inhibitor levels; rather, mitotic exit is regulated by the modulation of APC/C activity through DEL1 and UVI4.

RESULTS

DELLA Stabilization Is First Observed 24 h after Stress Onset

Studying how osmotic stress affects proliferating cells in an organ requires a specific setup, as described previously (Skirycz et al., 2011a). In short, plants are grown on nylon meshes overlaying control medium until 9 d after stratification (DAS), when all cells of the

third true leaf are still dividing. The meshes are then transferred to either control medium or medium containing 25 mM mannitol, thus causing acute, yet mild, stress to proliferating cells. For all further analyses, the third leaf is microdissected or cut from the plant at different time points after stress onset. Transcriptome analysis was performed on microdissected leaves harvested at different time points after stress onset (Skirycz et al., 2011a). In this experiment, we found changes in GA metabolism genes after 24 h, with the respective down- and up-regulation of the biosynthesis gene *GA3OX1* and the catabolism gene *GA2OX6*, which could be confirmed by quantitative PCR (Fig. 1A). Correspondingly, among genes differentially regulated in proliferating leaves 24 h after transfer to mannitol, there was an overrepresentation of genes known to be regulated by DELLAs in response to biotic stress (flg22; Navarro et al., 2008) and salt stress (Achard et al., 2008b), providing further evidence for higher DELLA activity at this time point (Fig. 1B). The timing suggested a role for GAs and DELLAs in late stress-induced mitotic exit. Therefore, we investigated the abundance of RGA by western blot on leaves of RGA::GFP-RGA plants. In accordance with the observed transcript changes, exposure of plants to osmotic stress caused a small but significant stabilization of GFP-RGA after 24 h, and this persisted after 48 h (Fig. 1C). In samples taken 3 and 12 h after transfer, no stabilization could be detected, confirming that DELLA accumulation is a relatively late event.

DELLAs Trigger Mitotic Exit

As the timing of DELLA accumulation fitted well with the timing of mitotic exit and the early onset of endoreduplication, we wanted to further explore this event and investigate whether DELLAs could be causative. To confirm that the earlier onset of endoreduplication was due to proliferating cells exiting mitosis and entering endoreduplication, we cut leaves in half at stages where the bottom half was still proliferating whereas the top half had already begun expanding (Andriankaja et al., 2012) and measured the ploidy levels of both halves. This confirmed that upon mannitol treatment, the main changes are found in the bottom half (Supplemental Fig. S1). In control leaves, high levels of 4C mitotic nuclei sharply drop as the proliferation zone collapses and then go up again after a small pause as endoreduplication starts. In mannitol-treated leaves, endoreduplication starts earlier, confirming that the changes we see in the endoreduplication index are due to proliferating cells ceasing proliferation. To investigate the effects of DELLAs on mitotic exit, we first exposed plants to paclobutrazol (PAC), a chemical that stabilizes DELLAs by inhibiting GA biosynthesis. Transfer of seedlings to PAC led to cell cycle arrest, as shown by reduced cell numbers (Fig. 2A), and triggered early differentiation, manifested by weaker and patchy CYCB1;1::DBox-GUS staining (Fig. 2B), which stains mitotic cells at the G2-to-M transition (Colón-Carmona et al., 1999), and earlier endoreduplication onset, as demonstrated by ploidy measurements (Fig. 2C). This chemical treatment was confirmed by mutant analysis. To avoid pleiotropic effects, we selected mutants with altered GA levels or DELLA activity that showed relatively limited growth phenotypes under normal conditions: *q-ga2ox*, a quintuple knockout for five GA 2-oxidases, resulting in higher GA levels (Rieu et al., 2008a); *spy-3*, a weak loss-of-function allele of the DELLA activator SPY (Jacobsen and Olszewski, 1993; Silverstone et al., 2007); the double DELLA loss-of-function mutant *rga-28 gai-2*; and *ga3ox1-3*, in which the major GA 3-oxidase responsible for GA biosynthesis in vegetative tissues is inactive, resulting in

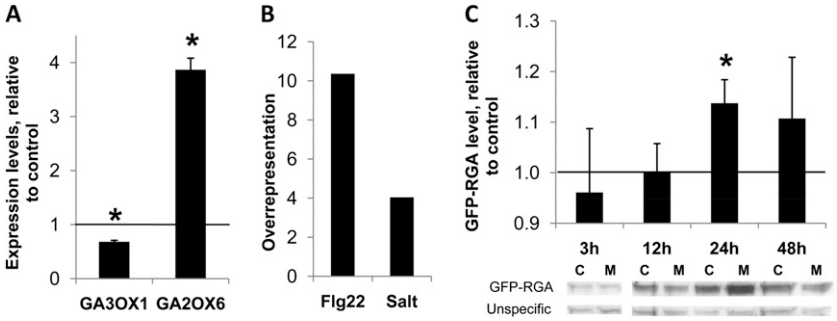

Figure 1. A, Levels of the GA biosynthesis gene *GA3OX1* and the GA catabolic gene *GA2OX6* at 24 h after transfer to mannitol. Data are expressed relative to control samples. Error bars indicate SE ($n = 3$). B, Relative overrepresentation of genes with DELLA-dependent regulation in response to flg22 (Navarro et al., 2008) or salt (Achard et al., 2008b) among genes differentially expressed in proliferating leaves after 24 h of mannitol treatment, where the expected number of genes in the overlap was set to 1. C, Levels of GFP-RGA in fully proliferating leaves determined 12, 24, and 48 h after transfer to mannitol-containing plates. Data are presented as levels of RGA-GFP from samples subjected to osmotic stress (M) relative to RGA-GFP levels in control samples (C) at each time point. Error bars indicate SE ($n = 3$). Significant differences ($P < 0.05$; two-tailed Student's *t* test) are indicated with asterisks.

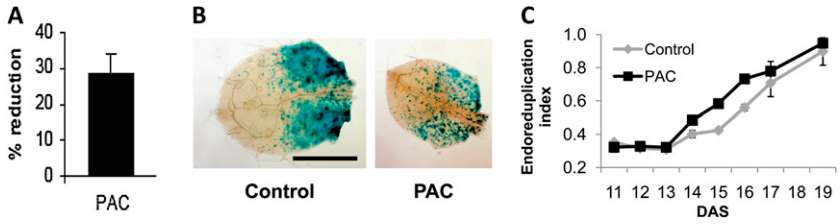

Figure 2. Effects of PAC on cell number, ploidy levels, and *CYCB1;1* expression. A, Reduction in leaf abaxial epidermal cell number 48 h after transfer to PAC-containing medium compared with transfer to control medium. B, Effects of PAC on the G2/M marker CYCB1;1 by GUS staining of CYCB1;1:DBox-GUS plants. Bar = 0.5 mm. C, Endoreduplication index over time of the third leaf from plants transferred at 9 DAS to PAC-containing or control medium. Error bars indicate SE (n = 3).

lower GA levels (Mitchum et al., 2006). *q-ga2ox*, *spy-3*, and *rga-28 gai-2* mutants showed a similar cell number reduction by mannitol compared with the wild type (Supplemental Fig. S2, A–C). Cell number reductions were quite variable between experiments, which is why care was taken to always compare with wild-type plants grown in the same experiment. Measurement of ploidy levels, however, showed that the early differentiation caused by mannitol was completely abolished in these mutants (Fig. 3, A–D). Surprisingly, although cell proliferation was more inhibited in the *ga3ox1-3* mutant (Supplemental Fig. S2D), this mutant also lacked mannitol-induced early differentiation (Fig. 3E). Importantly, none of the mutants, except for *rga-28 gai-2*, exhibited altered ploidy levels under standard conditions (Supplemental Fig. S3). Further confirmation came from 35S::*gai*-GR lines, which overexpress a nondegradable variant of the DELLA protein GAI fused to the rat glucocorticoid receptor, rendering GAI activity inducible by dexamethasone (DEX), which allows migration of the fusion protein to the nucleus, where it can be active. Although also under control conditions both the complete plants and the third leaf are smaller than in wild-type plants (Fig. 4A), the relative growth rate of the third leaf is similar during the developmental stages studied here (Fig. 4B). Upon induction with DEX, growth rates are drastically reduced (Fig. 4B) and a reduction in epidermal cell number is apparent (Fig. 4C). Importantly, early endoreduplication is observed, similar to wild-type plants treated with mannitol (Fig. 4D). This phenotype is also already apparent, albeit to a lesser extent, without DEX, either due to leakiness of the construct or a cytosolic function of GAI.

Mitotic Exit Correlates with APC/C Modulators But Not with the Expression of Cell Cycle Inhibitors

As DELLAs were previously shown to control the transcription of *KRP2*, *SIM*, *SMR1*, and *SMR2* (Achard et al., 2009), these were obvious targets to explain the mitotic exit. However, in our previous microarray analysis on mannitol-treated proliferating leaves, none of the known cell cycle inhibitors were up-regulated; some even showed down-regulation, as was the general trend among cell cycle genes 24 h after stress onset (Skirycz et al., 2011a). These microarray results were confirmed by quantitative PCR for *SIM* and *SMR1*, showing that even 72 h after stress onset, when mitotic exit had occurred, the expression remained low (Fig. 5A). Similar to mannitol treatment, in 35S::*gai*-GR leaves negative cell cycle regulators were down-regulated along with other cell cycle genes only at 12 h after DEX induction, indicative of a portion of cells going into mitotic exit (Fig. 5B). It rather seems that DELLAs influence endoreduplication onset by modulating APC/C activity, as *DEL1/E2FE* and *UVI4* were significantly

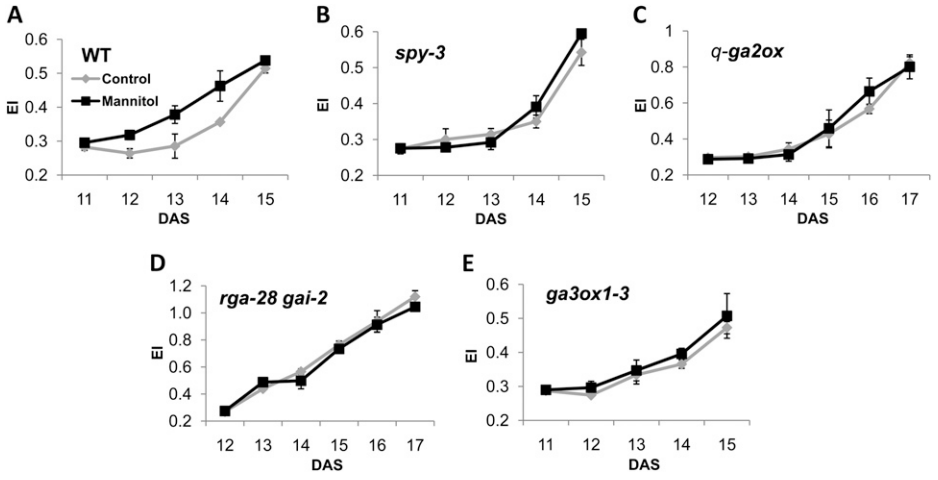

Figure 3. Mannitol-induced early onset of endoreduplication is absent in GA/DELLA mutants. Plants were transferred to mannitol-containing medium (black lines) or control medium (gray lines) at 9 DAS, and ploidy levels were measured daily. A, A representative wild type. B, *spy-3*. C, *q-ga2ox* (*ga2ox1-1 ga2ox2-1 ga2ox3-1 ga2ox4-1 ga2ox6-2*). D, *rga-28 gai-2*. E, *ga3ox1-3*. EI, Endoreduplication index. Error bars indicate SE (n = 3).

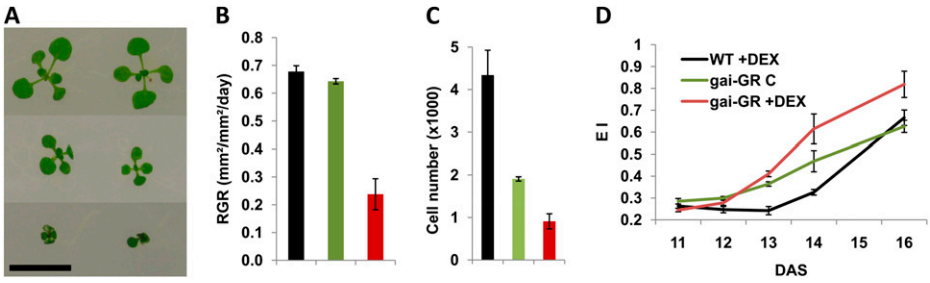

Figure 4. Analysis of 35S::*gai*-GR plants. A, Morphology of wild-type (top), noninduced (middle), and DEX-induced (bottom) *gai*-GR plants at 13 DAS (96 h after transfer to DEX). Bar = 1 cm. B, Average relative growth rate (RGR) of the third leaf between 10 and 16 DAS of wild-type (black), noninduced *gai*-GR (green), and DEX-induced *gai*-GR (red) plants. C, Cell number of the third leaf 48 h after transfer (11 DAS). Color coding is the same as in B. D, Endoreduplication index (EI). Error bars indicate SE ($n = 3$ in all panels).

down-regulated already 4 h after DEX induction, preceding the down-regulation of mitotic transcripts such as *CYCLIN B1;1* (*CYCB1;1*) and *CDKB1;1*, which occurred only 12 h after induction (Fig. 5B). The transcription dynamics of *DEL1* were next analyzed in more detail after DEX induction, showing that *DEL1* repression already started 1 h after GAI activation and reached a significant level 2 h after induction, suggesting that this is a primary target of DELLA signaling (Fig. 5C). *DEL1* and *UVI4* were also down-regulated 24 h after stress onset, and this persisted at 72 h (Fig. 5A).

Overexpression of *DEL1* Does Not Completely Abolish Early Endoreduplication on Stress

If *DEL1* down-regulation is causative of the observed early mitotic exit after mannitol treatment, then overexpression of *DEL1* is expected to counteract this. However, we still observed an early onset of endoreduplication when *DEL1*-overexpressing plants were transferred to mannitol, although this was not as pronounced as for the wild type (Fig. 6). Possibly, this is due to functional redundancy with UVI4. Overexpression of *UVI4* is most likely lethal, however (Heyman et al., 2011), so this could not be tested.

DISCUSSION

DELLAs Control Mitotic Exit Induced by Osmotic Stress

We previously demonstrated that proliferating tissues respond to stress by exiting mitosis early in favor of endoreduplication (Skirycz et al., 2011a). This limits the number of cells that form the organ and therefore contributes to the inhibition of growth by stress. Here, we showed that mild osmotic stress causes changes in GA metabolism specifically in proliferating leaf cells. What controls these changes in GA metabolism is currently unknown. In the shoot apical meristem, class I KNOTTED1-LIKE HOMEOBOX (KNOX) proteins regulate GA levels and thereby proliferation (Hay and Tsiantis, 2009), but KNOX I transcripts (SHOOT MERISTEMLESS, BREVIPEDICELLUS, KNOTTED-LIKE HOMEOBOX OF ARABIDOPSIS THALIANA2, and KNAT6) do not show changes in response to mannitol, making it unlikely that they are involved here (Skirycz et al., 2011a). Another possibility involves C-REPEAT/DRE BINDING FACTOR-related proteins, which were shown to modulate GA levels in response to cold stress (Achard et al., 2008a), but these also did not transcriptionally respond to osmotic stress (Skirycz et al., 2011a). The changes in GA metabolism then result in DELLA stabilization, which is responsible for mitotic exit. Consequently, ectopically stabilizing DELLAs, either through PAC addition or DEX induction of *gai*-GR, resulted in cell cycle inhibition and mitotic exit. However, mutants with lower DELLA activity (*spy-3*, *q-ga2ox*, and *rga-28 gai-2*) did not show any relief of cell cycle inhibition by osmotic stress. This suggests that although DELLAs are able to inhibit cell proliferation, they are not a determining factor in osmotic stress-induced inhibition of cell division, as lower DELLA activity does not release this inhibition. This is consistent with the model we previously proposed, in which ethylene arrests the cell cycle rapidly, posttranscriptionally, and reversibly (Skirycz et al., 2011a), and this mechanism is still active in these mutants. Our mutant analysis, however, confirms that the mitotic exit and early onset of endoreduplication induced by osmotic stress are regulated by DELLAs. Interestingly, both the mutants with elevated as well as those with down-regulated DELLA activity lacked the stress-induced earlier onset of cellular differentiation, indicating that this process is controlled by a fine balance of GA and DELLA activity. This may not be so uncommon in hormone effects, as, for instance, a study on the role of an ethylene-responsive factor in flg22-mediated growth inhibition revealed that both mutation and overexpression had the same effect, leading to the conclusion that it is maintained at an optimal level and that any deviation tips the fragile signaling balance (Bethke et al., 2009). Similarly, root meristem size is regulated by a fine balance of brassinosteroid signaling, and modulation of this pathway in either direction leads to short-root phenotypes

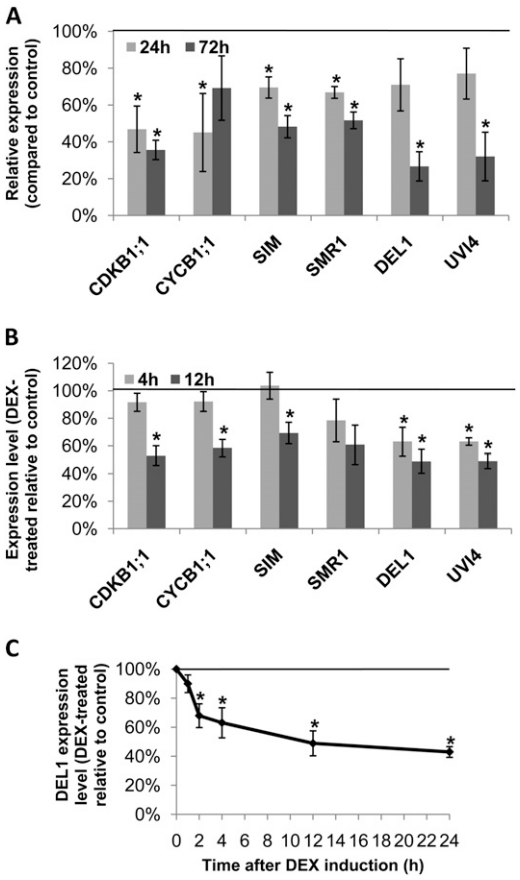

Figure 5. Expression levels of selected cell cycle genes. A, Gene expression in the third leaf of wild-type plants harvested 24 and 72 h after transfer to mannitol. B, Gene expression in the third leaf of *gai*-GR plants 4 and 12 h after transfer to DEX. C, *DEL1* expression in *gai*-GR leaves 1, 2, 4, 12, and 24 h after transfer to DEX. Data are presented as expression levels from samples subjected to mannitol/DEX relative to control samples at each time point. Error bars indicate SE ($n = 3$ in all panels). Significant differences ($P < 0.05$; two-tailed Student's t test) are indicated with asterisks.

(González-García et al., 2011). It is also interesting that perturbations of DELLA levels sufficient to disturb the stress-induced cell differentiation generally do not affect ploidy levels under normal conditions.

DELLAs Control Cell Proliferation by Inducing Differentiation

Our data fit into a network of emerging evidence that DELLAs are important for the control of differentiation and mitotic cell numbers, thereby adapting cell production rates and thus organ growth. Cell production rates are calculated by expressing the net gain of cells relative to the total number of cells in the organ and therefore integrate the fraction of cells that are dividing and their average division rate or cell cycle duration, both of which could potentially be modulated to change the cellular output of the organ. In roots, the average cell cycle duration is constant, and changes in cell production rates are mostly due to changes in the number of dividing cells and thus in the meristem size (Baskin, 2000). Correspondingly, DELLAs induce cell differentiation in the root, thereby making the root meristem smaller and thus decreasing cell production rates (Achard et al., 2009; Ubeda-Tomás et al., 2009; Moubayidin et al., 2010). Recently, the average cell cycle duration in leaves was reported to be constant throughout leaf development as well; therefore, the observed decrease in cell production rates during development is the consequence of a decrease in the fraction of proliferating epidermal cells due to the transition to cell expansion (Asl et al., 2011). Based on kinematic analysis, a role for GAs and DELLAs in the control of cell production rate during leaf development was reported in Arabidopsis (Achard et al., 2009). Our findings point to GA/DELLA-mediated control of mitotic exit in Arabidopsis leaves as well, at least under stress conditions. Furthermore, our finding that PAC and DELLA stabilization induce endoreduplication is in contrast to earlier observations based on GA addition experiments and mutant analysis showing that GA promotes endoreduplication in, for instance, pea (*Pisum sativum*; Mohamed and Bopp, 1980), Arabidopsis hypocotyls (Gendreau et al., 1999; Saibo et al., 2003), and tomato (*Solanum lycopersicum*) fruits (Serrani et al., 2007). This, again, suggests that the effects of hormones depend greatly on concentration, tissue or cell type, species, and environmental parameters.

APC/C Modulators Are Key Factors Downstream of DELLAs in Stress-Induced Mitotic Exit

Previously, DELLAs were reported to have the potential to control cell production rates in Arabidopsis by up-regulating *KRP2*, *SIM*, *SMR1*, and *SMR2*, which block the cell cycle by inhibiting CDKA activity (Achard et al., 2009). Our data rather suggest that DELLA-induced mitotic exit relies on the modulation of APC/C activity through the down-regulation

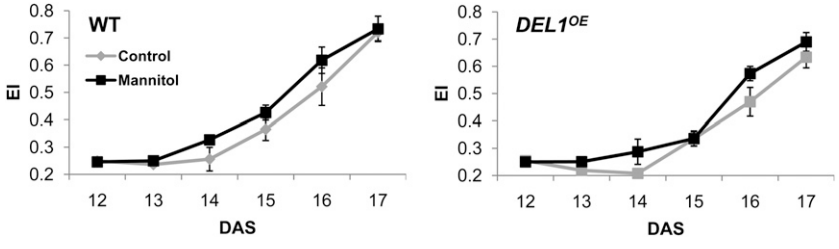

Figure 6. Ploidy levels in the third leaf of *DEL1*-overexpressing ($DEL1^{OE}$) plants compared with the wild type (WT) after transfer to mannitol. EI, Endoreduplication index.

of *DEL1* and *UVI4*. These regulators likely act together, as overexpression of *DEL1* alone was not enough to completely eliminate the early onset of endoreduplication under stress.

DELLA Stabilization Resulting in Mitotic Exit Is an Important Response to Stress

We present a model in which DELLA stabilization in proliferating cells, a relatively late event after the onset of osmotic stress, would drive the cells away from the mitotic cell cycle and into endoreduplication. As an early onset of endoreduplication is a natural response to stress, it is tempting to speculate that it contributes to stress tolerance. This is consistent with the finding that the leaf growth of *del1-1* mutants, which exhibit an early onset of endoreduplication, was found to be less sensitive to water deficit, whereas *DEL1*-overexpressing plants were more sensitive (Cookson et al., 2006). This shows that careful dissection of the responses of growing tissues to stress allows the modulation of growth inhibition, holding great promise for yield stabilization in the coming decades.

MATERIALS AND METHODS

Plant Growth

Seedlings of Arabidopsis (*Arabidopsis thaliana*; accession Columbia-0) were grown in vitro in one-half-strength Murashige and Skoog medium (Murashige and Skoog, 1962) supplemented with 1% Suc under a 16-h-day (110 μmol m^{-2} s^{-1})/8-h-night regime. Plates were overlaid with a nylon mesh (Prosep) of 20 μm pore size to avoid the growth of roots into the medium. Depending on the experiment, 32 or 64 seeds were equally distributed on a 150-mm-diameter plate. Mutant plants were grown together with their wild-type controls on the same plate.

Stress and Chemical Treatments

At 9 DAS, when the third leaf is fully proliferating, seedlings were transferred to plates containing control medium or medium supplemented with 25 mM mannitol (Sigma-Aldrich), 1 μM PAC (Sigma-Aldrich), or 5 μM DEX (Sigma-Aldrich) by gently lifting the nylon mesh with forceps. All transfers were performed 3 h into the day.

Growth Analysis

Growth analysis was performed on the third true leaf harvested at different time points after transfer. After clearing with 70% ethanol, leaves were mounted in lactic acid on microscope slides. For each experiment, eight to 12 leaves were photographed with a binocular microscope, and epidermal cells (40–300) were drawn for four representative leaves with a DMLB microscope (Leica) fitted with a drawing tubus and a differential interference contrast objective. Photographs of leaves and drawings were used to measure leaf area and cell size, respectively, using NIH ImageJ version 1.41o (http://rsb.info.nih.gov/ij/), and from these cell numbers were calculated. Relative growth rate was calculated as the slope of a linear trend line fitted to ln-transformed leaf area data.

Expression Analysis

The third leaf was harvested from plants at the indicated time points after transfer. The entire harvest was done in growth chambers and took less than 15 min. Briefly, whole seedlings were harvested in an excess of RNAlater solution (Ambion) and, after overnight storage at 4°C, dissected using a binocular microscope on a cooling plate with precision microscissors. Dissected leaves were transferred to a new tube, frozen in liquid nitrogen, and ground with 3-mm metal balls in a Retsch machine. Samples were obtained from three independent experiments and from multiple plates within one experiment. RNA was extracted with Trizol (Invitrogen), followed by clean-up with the RNeasy Mini Kit (Qiagen) including on-column DNase I (Qiagen) treatment according to the manufacturer's instructions. For cDNA synthesis, 250 to 1,000 ng of RNA was used with the iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's instructions. Primers were designed with the QuantPrime Web site (Arvidsson et al., 2008). Quantitative reverse transcription-PCR was done on a LightCycler 480 (Roche Diagnostics) on 384-well plates with LightCycler 480 SYBR Green I Master (Roche) according to the manufacturer's instructions. Melting curves were analyzed to check primer specificity. Expression values of AT1G13320, AT2G32170, and AT2G28390 were used for normalization (Czechowski et al., 2005).

Flow Cytometry

For flow cytometry analysis, four to 32 leaves were chopped with a razor blade in 200 μL of Cystain UV Precise P Nuclei Extraction buffer (Partec), followed by the addition of 800 μL of staining buffer and filtering through a 50-μm filter. Nuclei were analyzed with the Cyflow MB flow cytometer (Partec) and the corresponding FloMax software. The endoreduplication index was calculated as %4C + 2 × %8C + 3 × %16C.

Western Blot

Total soluble protein was extracted from 64 to 128 leaves by adding extraction buffer (Van Leene et al., 2007) to ground samples, followed by two freeze-thaw steps and two centrifugation steps (20,817*g*, 10 min, 4°C) whereby the supernatant was collected each time. Fifty micrograms of total soluble protein was used for protein gel-blot analysis with either primary rabbit anti-GFP antibodies (Santa Cruz; diluted 1:200) and a secondary horseradish peroxidase-conjugated donkey anti-rabbit antibody (GE Healthcare; diluted 1:10,000). Proteins were detected by enhanced chemiluminescence (Western Lightning Plus ECL; Perkin-Elmer Life Sciences). Protein amounts were quantified with NIH ImageJ version 1.41o. Cross-reacting bands were used for normalization. Control samples were arbitrarily set at 100%.

Transgenic Lines and Mutants

The 35S::*gai*-GR construct was made based on a SCR::*gai*-GR-YFP construct (Ubeda-Tomás et al., 2009), kindly donated by Malcolm Bennett (University of Nottingham), by amplifying *gai*-GR and cloning this into the Gateway-compatible pK7WG2 vector (Karimi et al., 2002), and transformed into Columbia-0 plants using *Agrobacterium tumefaciens* strain C58C1 pMP90. RGA::GFP-RGA, *rga-28*, and *rga-28 gai-2* seeds were kindly provided by Prof. Dr. Tai-ping Sun (Duke University). *q-ga2ox* mutant seeds were received from Prof. Dr. Andrew Phillips (Rothamsted Research). *spy-3* and *ga3ox1-3* mutant seeds were obtained from Nottingham Arabidopsis Stock Centre (N6268 and N6943, respectively). All transgenic lines and mutants are in the Columbia-0 background.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Endoreduplication index of bottom (proliferating) and top (expanding) halves of leaves exposed to osmotic stress.

Supplemental Figure S2. Cell number reduction at 15 DAS by mannitol in GA/DELLA mutants *q-ga2ox* (A), *spy-3* (B), *rga-28 gai-2* (C), and *ga3ox1-3* (D).

Supplemental Figure S3. Endoreduplication index of GA/DELLA mutants.

ACKNOWLEDGMENTS

We thank the Systems Biology of Yield group for the stimulating scientific atmosphere, Prof. Dr. Andrew Phillips for kindly providing *q-ga2ox* seeds, Prof. Dr. Tai-ping Sun for kindly providing RGA::GFP-RGA and *gai-2 rga-28*

seeds, Prof. Dr. Malcolm Bennett for providing us the SCR::*gai*-GR-YFP construct, and Dr. Annick Bleys for help in preparing the manuscript.

Received February 3, 2012; accepted April 24, 2012; published April 25, 2012.

LITERATURE CITED

Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. Science **311:** 91–94

Achard P, Gong F, Cheminant S, Alioua M, Hedden P, Genschik P (2008a) The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. Plant Cell **20:** 2117–2129

Achard P, Gusti A, Cheminant S, Alioua M, Dhondt S, Coppens F, Beemster GTS, Genschik P (2009) Gibberellin signaling controls cell proliferation rate in *Arabidopsis*. Curr Biol **19:** 1188–1193

Achard P, Renou J-P, Berthomé R, Harberd NP, Genschik P (2008b) Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. Curr Biol **18:** 656–660

Aguirrezabal L, Bouchier-Combaud S, Radziejwoski A, Dauzat M, Cookson SJ, Granier C (2006) Plasticity to soil water deficit in *Arabidopsis thaliana*: dissection of leaf development into underlying growth dynamic and cellular variables reveals invisible phenotypes. Plant Cell Environ **29:** 2216–2227

Andriankaja M, Dhondt S, De Bodt S, Vanhaeren H, Coppens F, De Milde L, Mühlenbock P, Skirycz A, Gonzalez N, Beemster GTS, et al (2012) Exit from proliferation during leaf development in *Arabidopsis thaliana*: a not-so-gradual process. Dev Cell **22:** 64–78

Ariizumi T, Lawrence PK, Steber CM (2011) The role of two F-box proteins, SLEEPY1 and SNEEZY, in Arabidopsis gibberellin signaling. Plant Physiol **155:** 765–775

Arvidsson S, Kwasniewski M, Riaño-Pachón DM, Mueller-Roeber B (2008) QuantPrime: a flexible tool for reliable high-throughput primer design for quantitative PCR. BMC Bioinformatics **9:** 465

Asl LK, Dhondt S, Boudolf V, Beemster GTS, Beeckman T, Inzé D, Govaerts W, De Veylder L (2011) Model-based analysis of Arabidopsis leaf epidermal cells reveals distinct division and expansion patterns for pavement and guard cells. Plant Physiol **156:** 2172–2183

Baskin TI (2000) On the constancy of cell division rate in the root meristem. Plant Mol Biol **43:** 545–554

Beemster GTS, De Veylder L, Vercruysse S, West G, Rombaut D, Van Hummelen P, Galichet A, Gruissem W, Inzé D, Vuylsteke M (2005) Genome-wide analysis of gene expression profiles associated with cell cycle transitions in growing organs of Arabidopsis. Plant Physiol **138:** 734–743

Berckmans B, Lammens T, Van Den Daele H, Magyar Z, Bögre L, De Veylder L (2011) Light-dependent regulation of *DEL1* is determined by the antagonistic action of E2Fb and E2Fc. Plant Physiol **157:** 1440–1451

Bethke G, Unthan T, Uhrig JF, Pöschl Y, Gust AA, Scheel D, Lee J (2009) Flg22 regulates the release of an ethylene response factor substrate from MAP kinase 6 in *Arabidopsis thaliana* via ethylene signaling. Proc Natl Acad Sci USA **106:** 8067–8072

Boudolf V, Lammens T, Boruc J, Van Leene J, Van Den Daele H, Maes S, Van Isterdael G, Russinova E, Kondorosi E, Witters E, et al (2009) CDKB1;1 forms a functional complex with CYCA2;3 to suppress endocycle onset. Plant Physiol **150:** 1482–1493

Bramsiepe J, Wester K, Weinl C, Roodbarkelari F, Kasili R, Larkin JC, Hülskamp M, Schnittger A (2010) Endoreplication controls cell fate maintenance. PLoS Genet **6:** e1000996

Breuer C, Ishida T, Sugimoto K (2010) Developmental control of endocycles and cell growth in plants. Curr Opin Plant Biol **13:** 654–660

Cao D, Cheng H, Wu W, Soo HM, Peng J (2006) Gibberellin mobilizes distinct DELLA-dependent transcriptomes to regulate seed germination and floral development in Arabidopsis. Plant Physiol **142:** 509–525

Churchman ML, Brown ML, Kato N, Kirik V, Hülskamp M, Inzé D, De Veylder L, Walker JD, Zheng Z, Oppenheimer DG, et al (2006) SIAMESE, a plant-specific cell cycle regulator, controls endoreplication onset in *Arabidopsis thaliana*. Plant Cell **18:** 3145–3157

Colón-Carmona A, You R, Haimovitch-Gal T, Doerner P (1999) Technical advance: spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. Plant J **20:** 503–508

Cookson SJ, Radziejwoski A, Granier C (2006) Cell and leaf size plasticity in Arabidopsis: what is the role of endoreduplication? Plant Cell Environ **29:** 1273–1283

Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible W-R (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. Plant Physiol **139:** 5–17

de Lucas M, Davière J-M, Rodríguez-Falcón M, Pontin M, Iglesias-Pedraz JM, Lorrain S, Fankhauser C, Blázquez MA, Titarenko E, Prat S (2008) A molecular framework for light and gibberellin control of cell elongation. Nature **451:** 480–484

De Veylder L, Beeckman T, Inzé D (2007) The ins and outs of the plant cell cycle. Nat Rev Mol Cell Biol **8:** 655–665

De Veylder L, Larkin JC, Schnittger A (2011) Molecular control and function of endoreplication in development and physiology. Trends Plant Sci **16:** 624–634

Dill A, Jung H-S, Sun TP (2001) The DELLA motif is essential for gibberellin-induced degradation of RGA. Proc Natl Acad Sci USA **98:** 14162–14167

Dill A, Thomas SG, Hu J, Steber CM, Sun TP (2004) The *Arabidopsis* F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell **16:** 1392–1405

Donnelly PM, Bonetta D, Tsukaya H, Dengler RE, Dengler NG (1999) Cell cycling and cell enlargement in developing leaves of *Arabidopsis*. Dev Biol **215:** 407–419

Feng S, Martinez C, Gusmaroli G, Wang Y, Zhou J, Wang F, Chen L, Yu L, Iglesias-Pedraz JM, Kircher S, et al (2008) Coordinated regulation of *Arabidopsis thaliana* development by light and gibberellins. Nature **451:** 475–479

Fu X, Richards DE, Fleck B, Xie D, Burton N, Harberd NP (2004) The *Arabidopsis* mutant sleepy1$^{gar2\text{-}1}$ protein promotes plant growth by increasing the affinity of the SCF^{SLY1} E3 ubiquitin ligase for DELLA protein substrates. Plant Cell **16:** 1406–1418

Gallego-Bartolomé J, Minguet EG, Marín JA, Prat S, Blázquez MA, Alabadí D (2010) Transcriptional diversification and functional conservation between DELLA proteins in Arabidopsis. Mol Biol Evol **27:** 1247–1256

Gendreau E, Orbovic V, Höfte H, Traas J (1999) Gibberellin and ethylene control endoreduplication levels in the *Arabidopsis thaliana* hypocotyl. Planta **209:** 513–516

Gonzalez N, De Bodt S, Sulpice R, Jikumaru Y, Chae E, Dhondt S, Van Daele T, De Milde L, Weigel D, Kamiya Y, et al (2010) Increased leaf size: different means to an end. Plant Physiol **153:** 1261–1279

González-García MP, Vilarrasa-Blasi J, Zhiponova M, Divol F, Mora-García S, Russinova E, Caño-Delgado AI (2011) Brassinosteroids control meristem size by promoting cell cycle progression in *Arabidopsis* roots. Development **138:** 849–859

Granier C, Tardieu F (1999) Water deficit and spatial pattern of leaf development: variability in responses can be simulated using a simple model of leaf development. Plant Physiol **119:** 609–620

Hase Y, Trung KH, Matsunaga T, Tanaka A (2006) A mutation in the *uvi4* gene promotes progression of endo-reduplication and confers increased tolerance towards ultraviolet B light. Plant J **46:** 317–326

Hay A, Tsiantis M (2009) A KNOX family TALE. Curr Opin Plant Biol **12:** 593–598

Heyman J, Van den Daele H, De Wit K, Boudolf V, Berckmans B, Verkest A, Kamei CLA, De Jaeger G, Koncz C, De Veylder L (2011) *Arabidopsis* ULTRAVIOLET-B-INSENSITIVE4 maintains cell division activity by temporal inhibition of the anaphase-promoting complex/cyclosome. Plant Cell **23:** 4394–4410

Hou X, Hu W-W, Shen L, Lee LYC, Tao Z, Han J-H, Yu H (2008) Global identification of DELLA target genes during Arabidopsis flower development. Plant Physiol **147:** 1126–1142

Hou X, Lee LYC, Xia K, Yan Y, Yu H (2010) DELLAs modulate jasmonate signaling via competitive binding to JAZs. Dev Cell **19:** 884–894

Inzé D, De Veylder L (2006) Cell cycle regulation in plant development. Annu Rev Genet **40:** 77–105

Iwata E, Ikeda S, Matsunaga S, Kurata M, Yoshioka Y, Criqui MC, Genschik P, Ito M (2011) GIGAS CELL1, a novel negative regulator of the anaphase-promoting complex/cyclosome, is required for proper mitotic progression and cell fate determination in *Arabidopsis*. Plant Cell **23:** 4382–4393

Jacobsen SE, Olszewski NE (1993) Mutations at the SPINDLY locus of *Arabidopsis* alter gibberellin signal transduction. Plant Cell **5:** 887–896

Josse E-M, Gan Y, Bou-Torrent J, Stewart KL, Gilday AD, Jeffree CE, Vaistij FE, Martínez-García JF, Nagy F, Graham IA, et al (2011) A DELLA in disguise: SPATULA restrains the growth of the developing *Arabidopsis* seedling. Plant Cell **23:** 1337–1351

Karimi M, Inzé D, Depicker A (2002) GATEWAY vectors for *Agrobacterium*-mediated plant transformation. Trends Plant Sci **7:** 193–195

Kasili R, Walker JD, Simmons LA, Zhou J, De Veylder L, Larkin JC (2010) SIAMESE cooperates with the CDH1-like protein CCS52A1 to establish endoreplication in *Arabidopsis thaliana* trichomes. Genetics **185:** 257–268

Kazama T, Ichihashi Y, Murata S, Tsukaya H (2010) The mechanism of cell cycle arrest front progression explained by a *KLUH/CYP78A5*-dependent mobile growth factor in developing leaves of *Arabidopsis thaliana*. Plant Cell Physiol **51:** 1046–1054

Lammens T, Boudolf V, Kheibarshekan L, Zalmas LP, Gaamouche T, Maes S, Vanstraelen M, Kondorosi E, La Thangue NB, Govaerts W, et al (2008) Atypical E2F activity restrains APC/$C^{CCS52A2}$ function obligatory for endocycle onset. Proc Natl Acad Sci USA **105:** 14721–14726

Magome H, Yamaguchi S, Hanada A, Kamiya Y, Oda K (2008) The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, *GA2ox7*, under high-salinity stress in Arabidopsis. Plant J **56:** 613–626

Mitchum MG, Yamaguchi S, Hanada A, Kuwahara A, Yoshioka Y, Kato T, Tabata S, Kamiya Y, Sun TP (2006) Distinct and overlapping roles of two gibberellin 3-oxidases in Arabidopsis development. Plant J **45:** 804–818

Mohamed Y, Bopp M (1980) Distribution of polyploidy in elongating and non-elongating shoot axis of *Pisum sativum*. Z Pflanzenphysiol **98:** 25–33

Moubayidin L, Perilli S, Dello Ioio R, Di Mambro R, Costantino P, Sabatini S (2010) The rate of cell differentiation controls the *Arabidopsis* root meristem growth phase. Curr Biol **20:** 1138–1143

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497

Navarro L, Bari R, Achard P, Lisón P, Nemri A, Harberd NP, Jones JDG (2008) DELLAs control plant immune responses by modulating the balance of jasmonic acid and salicylic acid signaling. Curr Biol **18:** 650–655

Nemhauser JL, Hong F, Chory J (2006) Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell **126:** 467–475

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S (2003) Gibberellin biosynthesis and response during *Arabidopsis* seed germination. Plant Cell **15:** 1591–1604

Peres A, Churchman ML, Hariharan S, Himanen K, Verkest A, Vandepoele K, Magyar Z, Hatzfeld Y, Van Der Schueren E, Beemster GTS, et al (2007) Novel plant-specific cyclin-dependent kinase inhibitors induced by biotic and abiotic stresses. J Biol Chem **282:** 25588–25596

Pettkó-Szandtner A, Mészáros T, Horváth GV, Bakó L, Csordás-Tóth E, Blastyák A, Zhiponova M, Miskolczi P, Dudits D (2006) Activation of an alfalfa cyclin-dependent kinase inhibitor by calmodulin-like domain protein kinase. Plant J **46:** 111–123

Rieu I, Eriksson S, Powers SJ, Gong F, Griffiths J, Woolley L, Benlloch R, Nilsson O, Thomas SG, Hedden P, et al (2008a) Genetic analysis reveals that C19-GA 2-oxidation is a major gibberellin inactivation pathway in *Arabidopsis*. Plant Cell **20:** 2420–2436

Rieu I, Ruiz-Rivero O, Fernandez-Garcia N, Griffiths J, Powers SJ, Gong F, Linhartova T, Eriksson S, Nilsson O, Thomas SG, et al (2008b) The gibberellin biosynthetic genes *AtGA20ox1* and *AtGA20ox2* act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. Plant J **53:** 488–504

Saibo NJM, Vriezen WH, Beemster GTS, Van Der Straeten D (2003) Growth and stomata development of *Arabidopsis* hypocotyls are controlled by gibberellins and modulated by ethylene and auxins. Plant J **33:** 989–1000

Schuppler U, He P-H, John PCL, Munns R (1998) Effect of water stress on cell division and Cdc2-like cell cycle kinase activity in wheat leaves. Plant Physiol **117:** 667–678

Serrani JC, Sanjuán R, Ruiz-Rivero O, Fos M, García-Martínez JL (2007) Gibberellin regulation of fruit set and growth in tomato. Plant Physiol **145:** 246–257

Silverstone AL, Tseng T-S, Swain SM, Dill A, Jeong SY, Olszewski NE, Sun TP (2007) Functional analysis of SPINDLY in gibberellin signaling in Arabidopsis. Plant Physiol **143:** 987–1000

Skirycz A, Claeys H, De Bodt S, Oikawa A, Shinoda S, Andriankaja M, Maleux K, Eloy NB, Coppens F, Yoo S-D, et al (2011a) Pause-and-stop: the effects of osmotic stress on cell proliferation during early leaf development in *Arabidopsis* and a role for ethylene signaling in cell cycle arrest. Plant Cell **23:** 1876–1888

Skirycz A, De Bodt S, Obata T, De Clercq I, Claeys H, De Rycke R, Andriankaja M, Van Aken O, Van Breusegem F, Fernie AR, et al (2010) Developmental stage specificity and the role of mitochondrial metabolism in the response of Arabidopsis leaves to prolonged mild osmotic stress. Plant Physiol **152:** 226–244

Skirycz A, Inzé D (2010) More from less: plant growth under limited water. Curr Opin Biotechnol **21:** 197–203

Skirycz A, Vandenbroucke K, Clauw P, Maleux K, De Meyer B, Dhondt S, Pucci A, Gonzalez N, Hoeberichts F, Tognetti VB, et al (2011b) Survival and growth of *Arabidopsis* plants given limited water are not equal. Nat Biotechnol **29:** 212–214

Sun TP (2008) Gibberellin metabolism, perception and signaling pathways in Arabidopsis. The Arabidopsis Book **6:** e0103, doi:10.1199/tab.0103

Sun TP (2010) Gibberellin-GID1-DELLA: a pivotal regulatory module for plant growth and development. Plant Physiol **154:** 567–570

Ubeda-Tomás S, Federici F, Casimiro I, Beemster GTS, Bhalerao R, Swarup R, Doerner P, Haseloff J, Bennett MJ (2009) Gibberellin signaling in the endodermis controls *Arabidopsis* root meristem size. Curr Biol **19:** 1194–1199

Van Leene J, Hollunder J, Eeckhout D, Persiau G, Van De Slijke E, Stals H, Van Isterdael G, Verkest A, Neirynck S, Buffel Y, et al (2010) Targeted interactomics reveals a complex core cell cycle machinery in *Arabidopsis thaliana*. Mol Syst Biol **6:** 397

Van Leene J, Stals H, Eeckhout D, Persiau G, Van De Slijke E, Van Isterdael G, De Clercq A, Bonnet E, Laukens K, Remmerie N, et al (2007) A tandem affinity purification-based technology platform to study the cell cycle interactome in *Arabidopsis thaliana*. Mol Cell Proteomics **6:** 1226–1238

Verkest A, Weinl C, Inzé D, De Veylder L, Schnittger A (2005) Switching the cell cycle: Kip-related proteins in plant cell cycle control. Plant Physiol **139:** 1099–1106

Wang H, Qi Q, Schorr P, Cutler AJ, Crosby WL, Fowke LC (1998) ICK1, a cyclin-dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and CycD3, and its expression is induced by abscisic acid. Plant J **15:** 501–510

Zentella R, Zhang Z-L, Park M, Thomas SG, Endo A, Murase K, Fleet CM, Jikumaru Y, Nambara E, Kamiya Y, et al (2007) Global analysis of DELLA direct targets in early gibberellin signaling in *Arabidopsis*. Plant Cell **19:** 3037–3057

Zhang Z-L, Ogawa M, Fleet CM, Zentella R, Hu J, Heo J-O, Lim J, Kamiya Y, Yamaguchi S, Sun TP (2011) Scarecrow-like 3 promotes gibberellin signaling by antagonizing master growth repressor DELLA in Arabidopsis. Proc Natl Acad Sci USA **108:** 2160–2165