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Report

Gibberellin Signaling Controls Cell Proliferation Rate in *Arabidopsis*

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Summary

Plant growth involves the integration of many environmental and endogenous signals that together with the intrinsic genetic program determine plant size. At the cellular level, growth rate is regulated by the combined activity of two processes: cell proliferation and expansion. Gibberellins (GA) are plant-specific hormones that play a central role in the regulation of growth and development with respect to environmental variability [1–3]. It is well established that GA promote growth through cell expansion by stimulating the destruction of growth-repressing DELLA proteins (DELLAs) [1, 4–6]; however, their effects on cell proliferation remain unknown. Kinematic analysis of leaf and root meristem growth revealed a novel function of DELLAs in restraining cell production. Moreover, by visualizing the cell cycle marker cyclinB1::β-glucuronidase in GA-signaling mutants, we show that GA modulate cell cycle activity in the root meristem via a DELLA-dependent mechanism. Accordingly, expressing gai (a nondegradable DELLA protein [4]) solely in root meristem reduced substantially the number of dividing cells. We also show that DELLAs restrain cell production by enhancing the levels of the cell cycle inhibitors *Kip-related protein 2* (*KRP2*) and *SIAMESE* (*SIM*). Therefore, DELLAs exert a general plant growth inhibitory activity by reducing both cell proliferation and expansion rates, enabling phenotypic plasticity.

Results and Discussion

GA promote important processes of plant growth through cell elongation by promoting the disappearance of nuclear DELLAs [1, 6]. Binding of GA to the GA receptors GID1 promotes interaction of GID1 with the DELLAs [7–9], subsequent polyubiquitination of the DELLAs via the E3 ubiquitin-ligase SCF^{SLY1}, and eventual destruction of DELLAs by the 26S proteasome [10–13]. Thus, mutants that stabilize DELLAs, such as the GA-deficient *ga1-3* or the F box mutant *sly1-10*, are dwarf, a phenotype that is reverted by the lack of DELLA function [1, 12, 13]. Although it is clear that DELLAs are important regulators of plant growth by restraining cell expansion, it remains unknown whether they also act on cell proliferation. To investigate this issue, we performed a kinematic analysis of leaf growth [14–16]. For this purpose, we determined the leaf blade area, the average cell area, and total cell number of abaxial epidermal cells of the first true leaf pair of *sly1-10*, *gai-t6 rga-t2 rgl1-1 rgl2-1* (also called quadruple-DELLA mutant [2]) and wild-type plants grown side by side under the same growth conditions (Figure 1; Figure S1 available online). Under these experimental conditions, leaf growth can be subdivided in three developmental phases (Figure S1). First, until 10 days after sowing, leaf growth is associated mainly with proliferation, where cell division and expansion is balanced, resulting in stable cell size [17]. In the second phase, between days 10 and ~17, the division rate decreases faster than expansion rates, causing average cell size to increase. Finally, after day 18, both cell division and expansion have stopped. Thus, final leaf size depends on rates and duration of cell proliferation and subsequent expansion [16, 17].

We found that at 10 days after sowing, *sly1-10* plants exhibited a reduction of 41% of the leaf blade area compared to wild-type plants, whereas in the quadruple-DELLA mutant, we observed an increase of 35% (Figure 1). The mutations did not affect the cell size during proliferation; therefore, the difference in leaf area was due to a difference in cell number (Figure 1B). Indeed, in comparison to wild-type leaves, average leaf cell number was decreased by 42% in *sly1-10* and increased by 38% in the quadruple-DELLA mutant, respectively. Supporting this observation, the cell division rate representing the total number of cells produced per unit of time and per meristematic cell [15] was, respectively, lower in *sly1-10* leaves and higher in quadruple-DELLA mutant leaves than in wild-type leaves at earliest time points (Figure S1E). Thus, DELLAs slow down early leaf growth by restraining the cell division rate (Figures S1B and S1E). Interestingly, we also found that the cell division rate decreased slower in *sly1-10* leaves and faster in the quadruple-DELLA mutant compared to wild-type leaves (Figure S1E). The prolonged cell division activity observed in *sly1-10* and shortened activity in quadruple-DELLA mutant leaves could be explained by a compensatory mechanism for the respective mutant's deficit or excess cell production [16].

During the second phase of leaf development, growth is mainly driven by cell expansion [17]. During this phase, DELLAs repress leaf growth through their effects on cell elongation (Figure 1). Hence, mature cells of *sly1-10* were significantly smaller than those of wild-type. However, because of the prolonged division, mature *sly1-10* leaves contained 30% more cells than wild-type leaves, indicating that DELLAs modify the balance between cell division and expansion and that reduced epidermal cell size is partially compensated by an increase in the cell number. It is noteworthy that we found similar kinetics of the appearance of stomatal complexes in wild-type, *sly1-10*, and quadruple-DELLA mutant leaves, indicating that guard cell differentiation proceeded normally (Figure S1F). Taken together, these data show that accumulation of DELLAs restrains leaf growth by a dual mechanism, first

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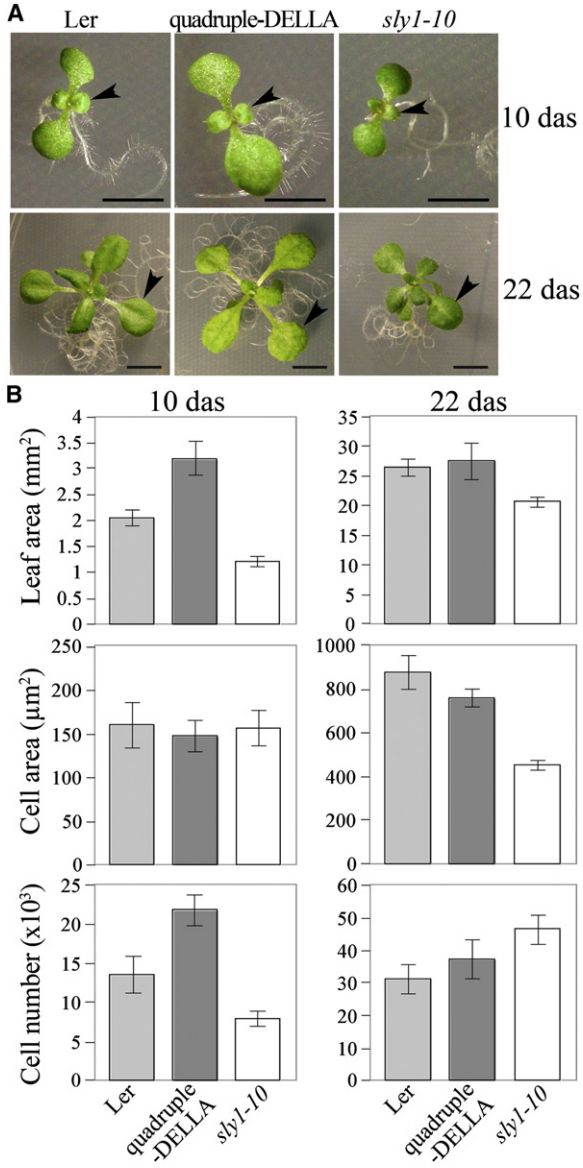

Figure 1. DELLAs Restrain Both Cell Proliferation and Expansion Rates in Leaves

(A) Representative wild-type (Ler), *gai-t6 rga-t2 rgl1-1 rgl2-1* (quadruple-DELLA), and *sly1-10* mutant plants 10 days and 22 days after sowing (das). Arrowheads indicate a leaf of the first true leaf pair on which the kinematic analysis was performed (Figure S1). Scale bars represent 5 mm.

(B) Leaf blade area, average cell area, and total cell number of abaxial epidermal cells of the first true leaf pair of wild-type (Ler; light gray), quadruple-DELLA (dark gray), and *sly1-10* (white) mutant 10 days and 22 days after sowing (das). Data represent average ± SE.

by altering cell division rate during the proliferation phase of leaf development, then by altering cell expansion rate during the expansion phase.

We next determined whether DELLAs constitute a mechanism generally controlling cell production rates. Plant growth is maintained and regulated through the activity of apical meristems, where a balance between cell production and differentiation needs to be properly coordinated. For example, root growth occurring from the distal end as a result of both cell division and elongation depends on the continuous function of the root meristem [18]. Root meristem originates from a group of undifferentiated stem cells, which generate cells that divide several times before elongating and differentiating [18, 19]. The root meristem organization is completed when the balance between cell division and cell differentiation rates is established, resulting in the formation of a meristem of stable size [20].

Previous experiments identified a key role for DELLAs in controlling root growth [21]. To specifically investigate whether DELLAs play a critical role in the control of root meristem activity, we followed the size of the root apical meristem of wild-type, GA-deficient *ga1-3* mutant, and *ga1-3* quadruple-DELLA mutant seedlings throughout their development (Figure 2). Root meristem size was expressed as the length of the meristematic zone and the number of cortex cells in a file extending from the quiescent center (QC; a small group of organizing cells defining together with the stem cells the stem cell niche [18]) to the first elongated cell exhibiting vacuolization [20]. We found that accumulation of DELLAs repressed root meristem size. Although the growth of wild-type and *ga1-3* quadruple-DELLA mutant root meristem was indistinguishable throughout their development, the size increase of *ga1-3* mutant root meristem was severely retarded (Figures 2B and 2C). Thus, the root meristem size of 5 days postimbibed (dpi) *ga1-3* mutant was significantly reduced compared to wild-type, and the absence of DELLA function in *ga1-3* quadruple-DELLA mutant suppressed the growth restraint conferred by *ga1-3* (Figures 2A–2C). We also found that the growth of the GA-insensitive *gai* root meristem (a mutant that is relatively resistant to the effects of GA [4]) was also significantly retarded in comparison to wild-type and quadruple-DELLA mutant (Figure S2). Altogether, these results (which are consistent with those reported in the accompanying manuscript by Ubeda-Tomás et al. [22] in this issue of *Current Biology*) suggest that DELLAs affect root meristem activity by acting on the rate of meristematic cell division.

To further substantiate the role of DELLAs in controlling root cell division, we introgressed a *pCYCB1;1:Dbox-GUS* construct into *ga1-3* and *gai-t6 rga-24* (lacking both GAI and RGA) mutants. The cyclin B1-GUS reporter allows us to visualize cells at the G2-M phase of the cell cycle and thus to monitor mitotic activity in the root meristem [23]. We found that the number of dividing cells was reduced in *ga1-3* compared to wild-type or *gai-t6 rga-24* mutant roots (Figures 3A and 3B). In the presence of paclobutrazol (PAC), an inhibitor of GA biosynthesis [1], the number of dividing cells was reduced in wild-type root meristem to a similar level than *ga1-3*, but was unchanged in *gai-t6 rga-24* root meristem. In contrast, GA treatment increased the number of dividing cells in *ga1-3* root meristem (Figures 3A and 3B). The kinetic of response to GA was within 1 hr time (Figure S3). Furthermore, expressing gai solely in root meristem (in RCH1-driven *gai-GFP* expression line; *RCH1* promoter is active only in the root mersitem [20]) reduced dramatically the number of dividing cells (Figures 3C–3E). Overall, these results demonstrate that GA signaling controls cell division activity in root meristem, thereby contributing in the regulation of root meristem growth [19].

The DELLA-mediated decrease in root meristematic cell division rate could be the consequence of reduced stem cell niche activity [18–20]. In root, the stem cell niche is formed by the QC, which acts as a stem cell organizer, surrounded by the stem cells [18]. QC specification and stem cell activity were shown to depend on the combinatorial action of a set of transcription factors, PLETHORA1 (PLT1), SCARECROW

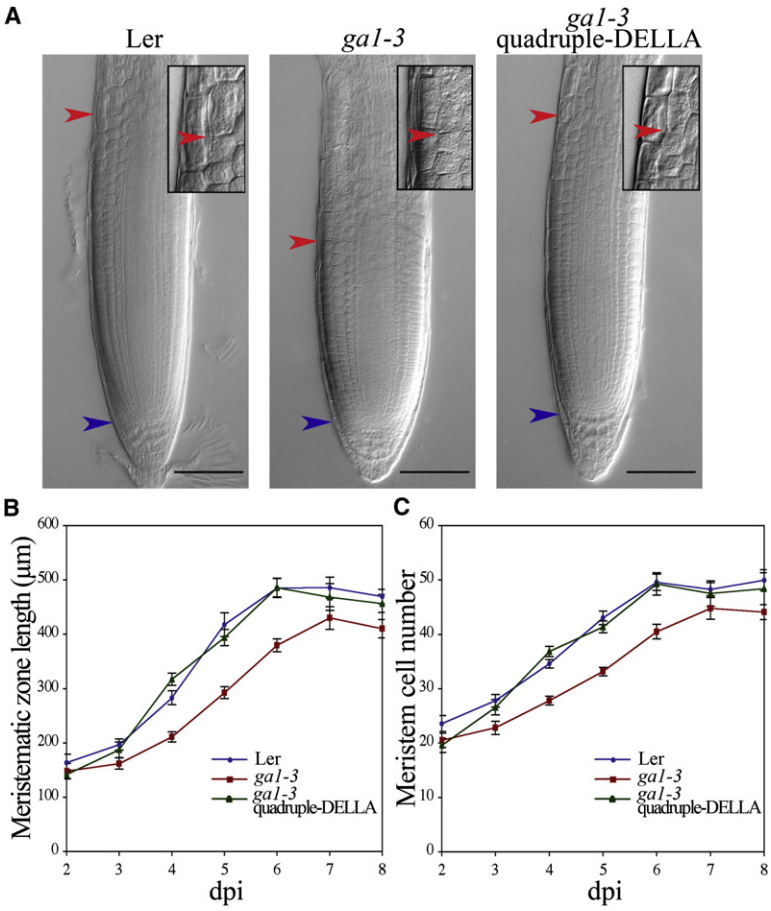

Figure 2. DELLAs Slow Root Meristem Growth

(A) Representative wild-type (Ler), *ga1-3*, and *ga1-3* quadruple-DELLA mutant root meristems at 5 days postimbibition (dpi). The insert shows a close-up of elongating cells exiting from the meristem at the transition zone. The QC and the transition zone between cortex meristematic and differentiated cells are indicated by blue and red arrowheads, respectively. Scale bars represent 100 μm.

(B and C) Means (n > 15, ± SE) root meristem length (B) and root meristem cell number (C) of wild-type (Ler; blue), *ga1-3* (red), and *ga1-3* quadruple-DELLA mutant (green) seedlings from 2 to 8 dpi. For monitoring root meristem growth, the length of the meristematic zone and the number of cortex meristematic cells enclosed between the blue and red arrowheads in (A) were determined.

(SCR), and SHORT-ROOT (SHR) [24, 25]. Therefore, we investigated the effects of GA and PAC treatments on the expression of the QC-expressed promoter trap (QC-46), the expression of SCR and SHR proteins (*pSCR:SCR-GFP* and *pSHR:SHR-GFP*), and the *PLT1* promoter activity (*pPLT1:CFP*) in the root [20] (Figure S4). Both treatments had no effect on either the localization or the expression levels of each reporter lines. Thus, these results suggest that GA signaling controls root meristematic cell division rate without interfering with stem cell niche specification or activity. These results are consistent with the lack of effect of ectopic gai expression in initial cells on root meristem size reported in the accompanying manuscript by Ubeda-Tomás et al. [22].

Finally, it is noteworthy that we found by a similar approach that DELLAs also restrain cell division activity in the shoot meristematic zone and early developing leaves (Figure S5), supporting the kinematic data presented in Figure 1, and thereby suggesting that the GA signaling provides a general mechanism to control plant cell proliferation.

To gain insight into the molecular basis of that regulatory mechanism, we next determined the expression levels of key core cell cycle regulators in 7-day-old wild-type, *ga1-3*, and *ga1-3* quadruple-DELLA mutant seedlings. Eukaryotic cell division is driven by the consecutive action of cyclin/cyclin-dependent kinase (CYC/CDK) complexes, whose activity controls two major cell cycle phase transitions, the G1/S and G2/M boundaries [26]. Interestingly, among the positive and negative cell cycle regulators tested, we found that the transcript levels of CDK inhibitors (CKIs) were increased in *ga1-3* mutant compared to wild-type seedlings (Figure 4A; Figure S6). CKIs negatively regulate the cell cycle by binding to and inhibiting CYC/CDK complexes [26]. Plants contain both conserved and plant-specific classes of CKIs, known as Kip-related proteins (KRP) and SIAMESE (SIM), respectively [14, 27]. Here we found that transcript levels of *KRP2* and 3 members of the SIM family (*SIM, SMR1*, and *SMR2*) were increased by a factor of ~2 in *ga1-3* mutant compared to wild-type seedlings (Figure 4A). Moreover, GA treatment or lack of DELLA function (in *ga1-3* quadruple-DELLA mutant) suppressed the CKI increased transcript accumulation conferred by *ga1-3*. Thus, DELLAs promote the expression of *KRP2* and members of the *SIM* family whereas GA repress their expression by overcoming DELLA-mediated promotion. Accordingly, we show that KRP2 protein accumulates to higher levels in *ga1-3* seedlings compared to wild-type or *ga1-3* quadruple-DELLA mutant seedlings (Figure 4B). Similar results were obtained on dissected root tips material, suggesting that the DELLA-dependent control of *KRP2* and *SIM* genes expression occurs in root meristematic cells (Figure S7).

Plant CKIs inhibit cell cycle progression by interacting with D-type CYC and A-type CDK subunits [26]. Overexpression of D-type CYC increases mitotic activity and suppresses the mutant phenotype conferred by ectopic expression of CKIs [26, 28, 29]. If the DELLA-mediated growth restraint phenotype exhibited during the proliferation phase is the consequence of CKI accumulation, we speculated that overexpression of *CYCD3;1* could revert, at least in part, this phenotype. Hence, whereas *ga1-3* plants exhibited a reduction of 57% of the leaf blade area compared to wild-type plants because of a deficit in cell number, overexpression of *CYCD3;1* rescued the growth of *ga1-3* plants (Figure 4C; Figure S8). As a consequence, at 10 days after sowing, leaves of *CYCD3;1* OE and *CYCD3;1* OE *ga1-3* plants are indistinguishable. Altogether, these results indicate that GA signaling, by modulating the expression levels of *KRP2* and members of *SIM* gene family, controls the cell proliferation rate.

Although it has long been known that GA promote growth through cell expansion by stimulating the destruction of growth-repressing DELLA proteins [1], their effects on plant growth through the regulation of cell production is novel. We established that GA control both cell proliferation and expansion rates, processes that both rely on the destruction of DELLAs. Thus, throughout plant development, DELLAs restrain

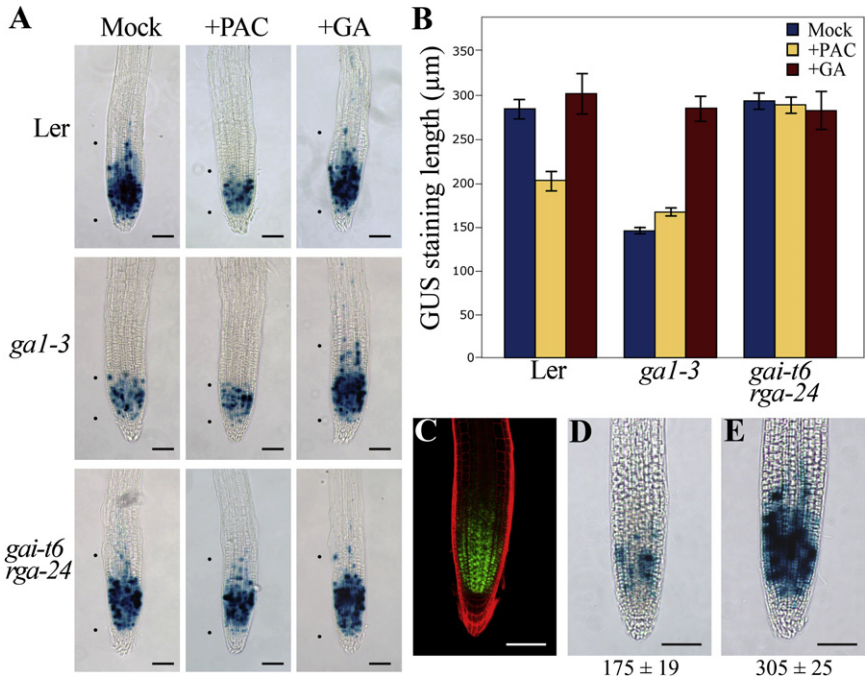

Figure 3. DELLAs Restrain Cell Division in Root Meristem

(A) Effects of gibberellin (GA) and paclobutrazol (PAC) treatments on Dbox CYCB1;1-GUS cell-division marker in wild-type (Ler), *ga1-3*, and *gai-t6 rga-24* mutant roots. Photographs show representative 8-day-old root seedlings grown for 4 days on 2 μM GA or 1 μM PAC. Black dots indicate the distance between the first and last dividing cells in the longitudinal axis of the root meristem as shown by β-glucuronidase staining. Scale bars represent 80 μm.

(B) Mean (n > 20, ± SE) length of the Dbox CYCB1;1-GUS staining in the longitudinal axis of the root meristem.

(C–E) Effect of RCH1-driven *gai* expression on cell division.

(C) Expression of *gai-GFP* in *pRCH1:gai-GFP*, *pCYCB1;1:Dbox-GUS* root meristem.

(D) Dbox CYCB1;1-GUS activity in *pRCH1:gai-GFP*, *pCYCB1;1:Dbox-GUS* root meristem.

(E) Dbox CYCB1;1-GUS activity in *pCYCB1;1:Dbox-GUS* root meristem. Numbers indicate average length (n > 20, ± SE) of Dbox CYCB1;1-GUS staining, as in (B). Scale bars represent 100 μm.

growth of organs such as leaves and primary root, by first decreasing the rate of division of proliferating cells, then by altering the rate of elongation of differentiated cells. Moreover, we showed that DELLAs restrain cell cycle activity by enhancing the accumulation of cell cycle inhibitors, particularly members of the plant-specific *SIM* gene family.

Interestingly, it has recently been shown that expression of the *SIM* gene family responds to diverse biotic and abiotic stress treatments [30]. It was thus suggested that this class of CKIs couples the cell cycle to (adverse) environmental conditions [30], in contrast to KRPs that respond to intrinsic developmental signals [14]. Moreover, recent advances revealed that DELLA restraint provides a general mechanism permitting flexible and appropriate growth in response to changes in natural environment [2, 3]. Overall, these observations suggest that DELLA-mediated control of *SIM* expression provides such a mechanism, enabling plants to modulate their growth (by modulating their cell proliferation rate) according to surrounding environments.

Experimental Procedures

Plant Lines

GA biosynthesis and signaling mutant seeds used in this study were derived from Landsberg *erecta* (Ler) ecotype. *Arabidopsis* mutants *sly1-10*, *ga1-3*, *gai*, *gai-t6 rga-24*, quadruple-DELLA (*gai-t6 rga-t2 rgl1-1 rgl2-1*), *ga1-3* quadruple-DELLA; and *Arabidopsis* transgenic lines *pCYCB1;1:Dbox-GUS*, QC-46, *pPLT1:CFP*, *pSCR:SCR-GFP*, *pSHR:SHR-GFP* and lines were as described previously [2, 13, 20, 21, 23–25]. The *CYCD3;1* OE line (*35S:CYCD3;1*) drives a constitutive expression of CYCD3;1 leading to an increase in cell proliferation [28]. *ga1-3 pCYCB1;1:Dbox-GUS*, *gai-t6 rga-24 pCYCB1;1:Dbox-GUS* and *ga1-3 CYCD3;1* OE lines were isolated from F3 progeny of the appropriate crosses. Growth analysis, genomic PCR, and GUS staining were used to confirm the *ga1-3* or *gai-t6 rga-24* genotypes [2] and the presence of *CYCD3;1* OE or *pCYCB1;1:Dbox-GUS* transgene, respectively.

Kinematic Analysis of Leaf Growth

Seeds were plated on MS-agar growth medium [14] and placed at 4°C for 5 days to synchronize germination. Plates were then placed horizontally in a growth chamber (22°C; 16 hr photoperiod), and only seeds germinating at the same time (same day) were selected for the analysis. Kinematic analysis was performed essentially as described earlier [14] by measuring whole leaf blade area and drawing 30–50 cells from abaxial epidermis of the first leaf pair harvested daily on 4 to 7 plants for each genotype from days 5 to 24 after sowing. The drawings were scanned and analyzed by an automated image analysis routine that measures total area of the drawn cells and counts number of pavement and guard cells and performs the mathematics to calculate average leaf area, average cell area, number of cells per leaf, relative rates of cell expansion (RLER), cell division rates, and Stomatal Index in function of time.

Root Meristem Size Analyses

Seeds were plated on half concentrated MS-agar growth medium and placed at 4°C for 5 days to synchronize germination. Plates were then placed vertically in a growth chamber (22°C; 16 hr photoperiod). Meristem size was expressed as the number of cells in cortex files from the QC to the first elongated cell exhibiting vacuolization [20]. Measurements were performed every day by microscopy (AxioImager Z1, Zeiss) from days 2 to 8 after imbibition on at least 15 roots for each genotype. Root meristem length was measured with Image J software (http://rsb.info.nih.gov).

Ectopic Expression of *gai*

RCH1 (AT5g48940) promoter (a 847 bp fragment [20]) and *gai-GFP* cDNA were obtained by PCR amplification from the *pRCH1:AtCKX1* construct (provided by S. Sabatini) and the *35S:gai-GFP* construct (provided by N. Harberd), respectively. The *pRCH1:gai-GFP* construct was engineered by mobilizing *pRCH1* in plasmid pDONR221-P4P1r (Invitrogen) and *gai-GFP* in plasmid pDONR207-P1P2 (Invitrogen) into the destination vector pH7m24GW,3 (Plant Genetic, psb-vib, Gent, Belgium) via a gateway cloning approach (Invitrogen). The *pRCH1:gai-GFP* construct was introduced into *Agrobacterium tumefaciens* GV3101 by electroporation. *pCYCB1;1:Dbox-GUS Arabidopsis* plants were transformed by floral dip.

Detection of Dbox CYCB1;1-GUS Activity

Seeds were plated on MS-agar growth medium. After 5 days of stratification at 4°C, plates were placed vertically in a growth chamber (22°C; 16 hr photoperiod) for 4 days. Subsequently, seedlings were transferred to MS-agar plates containing 2 μM GA or 1 μM PAC (paclobutrazol, an inhibitor of GA-biosynthesis) for further 4 days. Histochemical detection of GUS activity was carried out with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc) as a substrate. Seedlings were placed in 90% acetone on ice for 15 min and then in X-Gluc buffer solution (500 μg/ml X-Gluc, 50 mM $NaPO_4$ [pH 7], 10 mM EDTA, 0.01% Triton X-100, 2 mM $K_3F_3(CN)_3$) under vacuum (600 mm Hg) for 10 min, and then placed at 37°C overnight. For observation,

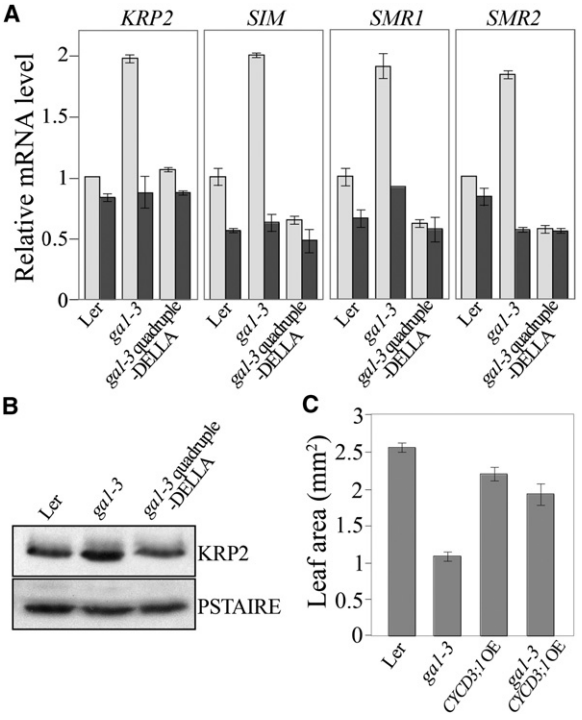

Figure 4. DELLAs Affect the Expression Levels of Cyclin-Dependent Kinase Inhibitors

(A) Relative levels of *KRP2*, *SIM, SMR1*, and *SMR2* gene transcripts (determined by quantitative RT-PCR) in 7-day-old wild-type (Ler), *ga1-3*, and *ga1-3* quadruple-DELLA mutant seedlings that had been treated by 2 μM GA (dark gray) and controls (mock, light gray). Data are means ± SE. Similar results were obtained in two independent experiments.

(B) Immunodetection of KRP2 (with antibody to KRP2) in 7-day-old wild-type (Ler), *ga1-3*, and *ga1-3* quadruple-DELLA mutant seedlings.

(C) Mean leaf blade area of the first true leaf pair of wild-type (Ler), *ga1-3*, *CYCD3;1* OE and *ga1-3 CYCD3;1* OE plants 10 days after sowing. Data represent average ± SE.

stained roots were transferred to small Petri dishes containing 0.24 N HCl in 20% methanol and incubated at 57°C for 15 min. This solution was replaced with 7% NaOH, 7% hydroxylamine-HCl in 60% ethanol for 15 min at room temperature. Roots were then rehydrated for 5 min each in 40%, 20%, and 10% ethanol and infiltrated for 15 min in 5% ethanol 25% glycerol. Roots were mounted in 50% glycerol on glass microscope slides. In Figure S3, 5-day-old *ga1-3 pCYCB1;1:Dbox-GUS* seedlings grown on MS-agar medium (22°C; 16 hr photoperiod) were transferred to MS liquid medium for 2 days then treated by 10 μM GA for the time as indicated before the detection of GUS activity. In Figure S5, 6-day-old seedlings grown on horizontal MS-agar plates (22°C; 16 hr photoperiod) were transferred to MS-agar plates containing 2 μM GA or 1 μM PAC for further 4 days before the detection of the GUS activity.

Observation of Stem Cell Niche Reporter Lines

QC-46, *pSCR:SCR-GFP*, and *pSHR:SHR-GFP* lines were grown on MS-agar plates for 5 days in a growth chamber (22°C; 16 hr photoperiod), then transferred to MS liquid medium for 2 days before to be treated by 2 μM GA or 1 μM PAC for 24 hr. 5-day-old *pPLT1:CFP* were transferred to MS liquid medium containing 2 μM GA or 1 μM PAC for 48 hr. Confocal microscopy images were obtained with a Zeiss LSM510 inverted confocal laser microscope with 20× or 40× objectives. All images were obtained with the same modifications and intensity parameters.

Quantitative RT-PCR Analyses

Total RNA was extracted with Trizol reagent (Molecular Research Center) from 7-day-old whole seedlings (or dissected root tips in Figure S7) treated by 2 μM GA and controls. 2 μg of total RNA was treated first with 2 units of DNase I (Promega) and then reverse transcribed in a total volume of 40 μl with 2 μM oligo(dT)$_{20}$, 0.5 mM deoxynucleotide triphosphate, 5 mM DTT, and 200 units of Superscript III reverse transcriptase (Invitrogen). RT-PCR was performed with gene-specific primers in a total volume of 15 μl SYBR Green Master mix (Roche) on a Lightcycler LC480 apparatus (Roche) according to manufacturer's instructions. The *GAPDH* and At4g26410 (unknown function) genes were used as internal controls. The relative expression level of each gene in mutants and in GA-treated seedlings was compared with that in wild-type seedlings with GenEx Pro 4.3.5. software (MultiD Analyses) after normalization with the *GAPDH* cDNA level and average over three replicates. qRT-PCR analyses were performed on two biological repeats.

Immunoblot Analyses

7-day-old seedlings were ground in 2× SDS-PAGE buffer followed by boiling for 5 min. After centrifugation, the protein extracts were fractionated on a 10% SDS-PAGE gel and blotted onto membrane. Immunoblots were performed with a 1000-fold dilution of KRP2 antibodies [31] and a 5000-fold dilution of horseradish peroxidase-conjugated IgG (Molecular Probes). Signals were detected by film (within linear range of detection) with the enhanced chemiluminescence protein gel blot analysis system (Amersham Biosciences). The blot was subsequently stripped with 0.2 N glycine (pH 2.5) and reprobed with cdc2 (PSTAIRE) antibody (Santa Cruz Biotechnology) for loading control. The immunoblot assay was repeated three times.

Primer List

Gene-specific primer sequences are available in Supplemental Data.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and eight figures and can be found with this article online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01194-4.

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