Current Biology 19, 1194–1199, July 28, 2009 ©2009 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2009.06.023

Report

Gibberellin Signaling in the Endodermis Controls *Arabidopsis* Root Meristem Size

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Summary

Plant growth is driven by cell proliferation and elongation [1]. The hormone gibberellin (GA) regulates *Arabidopsis* root growth [2–5] by controlling cell elongation [6], but it is currently unknown whether GA also controls root cell proliferation. Here we show that GA biosynthetic mutants are unable to increase their cell production rate and meristem size after germination. GA signals the degradation of the DELLA growth repressor proteins [7–12] GAI and RGA, promoting root cell production. Targeting the expression of gai (a non-GA-degradable mutant form of GAI) in the root meristem disrupts cell proliferation. Moreover, expressing gai in dividing endodermal cells was sufficient to block root meristem enlargement. We report a novel function for GA regulating cell proliferation where this signal acts by removing DELLA in a subset of, rather than all, meristem cells. We suggest that the GA-regulated rate of expansion of dividing endodermal cells dictates the equivalent rate in other root tissues. Cells must double in size prior to dividing but cannot do so independently, because they are physically restrained by adjacent tissues with which they share cell walls. Our study highlights the importance of probing regulatory mechanisms linking molecular- and cellular-scale processes with tissue and organ growth responses.

Results and Discussion

A germinating seedling must obtain anchorage, water, and nutrients after emerging from its seed coat. Vigorous root growth is essential if the seedling is to rapidly secure these resources. Root length is determined by the number of dividing cells and their final cell size [1]. Root cells first undergo repeated rounds of division in the root proximal meristem and then subsequently experience rapid cell expansion in the elongation-differentiation zone (EDZ; Figure 1A). In order to maximize root growth after germination, seedlings could increase either root cell production rate or final cell size or both.

In the model plant *Arabidopsis thaliana*, root growth rate rapidly increases after seed germination (Figure 1B; Figure S1A available online). We initially investigated the cellular basis for the increased root growth, measuring mature cell length, cell production in the meristem, and root meristem size. Mature cell size was essentially constant, whereas the rate of root cell production increased proportional to the root growth rate; wild-type roots exhibit a doubling in cell production rate over a 4 day period after germination (Figure 1C; Figures S1D and S1F). Increased cell production can be due to an increasing number of cells in the meristem because extensive variations in cell division rates are relatively rare [13]. Indeed, our measurements indicate that root meristem size doubles during the first days after germination (DAG), then plateaus by 4–6 DAG, depending on the *Arabidopsis* accession (Figures S1D–S1F). Similarly, cortical cell numbers in the root meristem reached a constant number of between 40 and 60 cells, depending on the *Arabidopsis* accession (Figures S1E and S1G). Hence, the acceleration in *Arabidopsis* root growth is correlated with increasing root meristem size and numbers of meristematic cells (Figure 1B; Figure S1).

The plant hormone gibberellin (GA) represents an important regulator of *Arabidopsis* root growth [2–5]. GA has recently been reported to regulate root cell elongation [6]. However, it is currently unclear whether GA also controls root cell production. To investigate this possibility, we used pharmacological and genetic approaches to test the role of GA in regulation of root meristem size. Reduction of endogenous GA levels by treating wild-type seedlings with Paclobutrazol (PAC, an inhibitor of GA biosynthesis [14]) results in a reduced root growth rate (Figure S1A). Detailed microscopy measurements revealed that PAC treatment caused a reduction in root meristem size (Figures S1D–S1G) and mature cell length (Figures S1B and S1C). Subsequently, roots of GA biosynthetic mutants *ga1-3* [15] and *ga3ox1/ga3ox2* [16] were analyzed. The *ga1-3* mutant failed to increase cell production rate, essentially remaining static (Figure 1C). Similarly, a reduction in cell production rate was also detected in *ga3ox1/ga3ox2* mutant (Figure S1K). Both mutants exhibited a smaller root meristem size compared to wild-type (Figures 1B–1D; Figure S1H). However, GA3 treatment was able to fully rescue root meristem size in the *ga1-3*, *ga3ox1/ga3ox2* mutants and PAC-treated seedlings (Figure 1B; Figures S1D–S1H). Conversely, removal of 7- to 8-day-old *ga1-3* seedlings from GA3-supplemented media resulted in a reduction in root meristem size (Figure S1L). Hence, GA levels appear to be required to promote and maintain the increase in root growth rate through control of root meristem size.

To investigate the relationship between gibberellins and root cell division, we monitored how changes in GA levels affect the expression of the mitotic cyclin CycB1;1 (marks G2/M-phase

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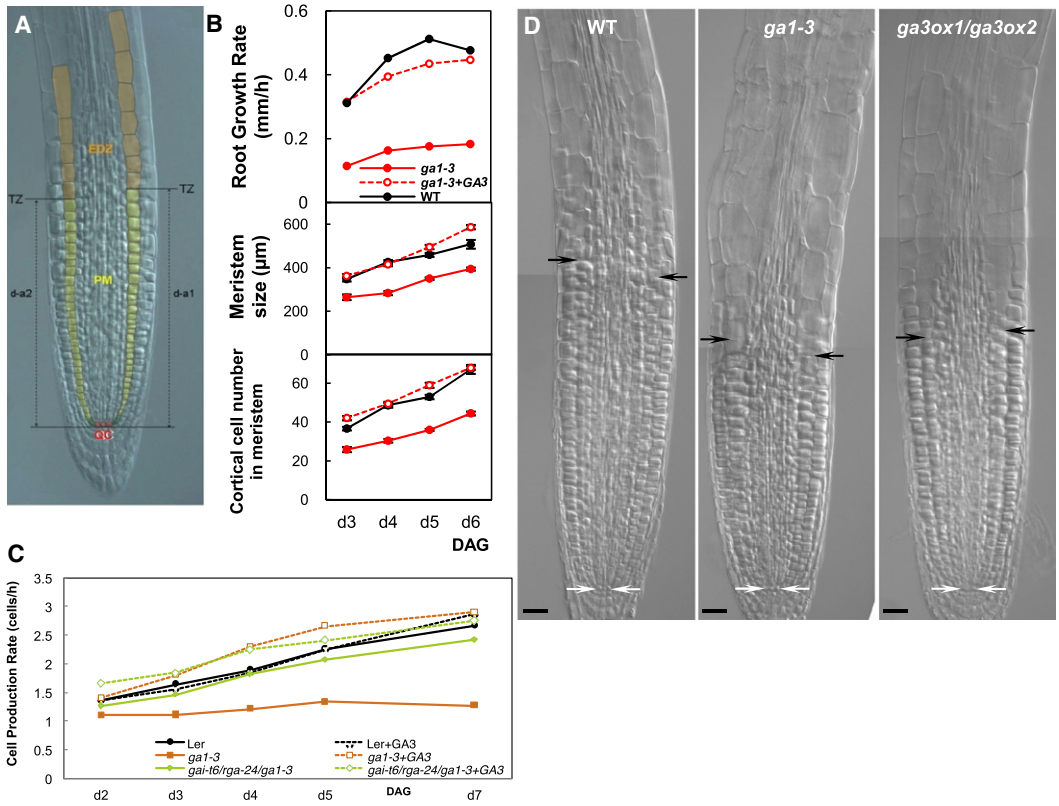

Figure 1. GA Regulates Root Meristem Size and Cell Production Rate

(A) Bright-field microscopy image of the *Arabidopsis* root meristem. Two cortical cell files within the proximal meristem (PM) are marked in yellow, and the same files within the elongation-differentiation zone (EDZ) are marked in orange. The distance (indicated as d-a1 and d-a2) between the transition zone (TZ) and the quiescent center (QC, marked in red) correspond to the meristem size (µm).

(B) The GA-deficient mutant *ga1-3* (Col-0 background) showed a reduction in its root growth rate (mm/h), meristem size (µm), and cortical cell number in meristem from day 3 to day 6 after germination (DAG). GA treatment (GA3 1 µM) restored the mutant phenotype. Error bars represent SE (n > 15).

(C) The GA-deficient mutant *ga1-3* (Ler background) showed a reduction in cell production rate (root growth rate/mature cell length) that could be recovered by GA treatment (GA3 1 µM) and by mutating the main root DELLA proteins in the triple mutant *gai-t6/rga-24/ga1-3* (Ler background). Wild-type (Ler background) roots were used as control (n > 20).

(D) Root meristem images (bright-field microscopy) of GA-deficient mutants *ga1-3* and *ga3ox1/ga3ox2* (both in Col-0 background) showing a reduction in meristem size with respect to control (wild-type, Col-0 background) of four DAG seedlings. White and black arrowheads indicate QC and TZ position, respectively. Scale bar represents 25 µm. Statistical significance of differences observed are displayed in Tables S1A and S1B.

of the cell cycle [17]), employing transcriptional and translational fusions CycB1;1::GUS [18] and CycB1;1::GFP, respectively. PAC treatment significantly decreased mitotic cell number that could be restored to a wild-type level by simultaneously treating with PAC and GA3 (Figures 2A–2I; Figures S2A–S2D). We also quantified mitotic events in roots of GA biosynthetic mutant's *ga1-3* and *ga3ox1/ga3ox2*, employing the newly formed cell wall marker KNOLLE [19]. Immunolocalization of KNOLLE revealed a reduced number of cell plates (representing cell division events) in both *ga1-3* and *ga3ox1/ga3ox2* mutants compared with wild-type roots (Figures 2J–2M). Our observations (which are consistent with those reported in the accompanying manuscript by Achard et al. [20] in this issue of *Current Biology*) suggest that GA regulates root meristem size by promoting mitotic activity.

GA acts by destabilizing DELLA proteins like RGA and GAI that function as growth repressors during *Arabidopsis* seedling development [7–12]. This is achieved by GA first binding the GA receptor GID1 [21–23], which then interacts with DELLA proteins [3–5, 21–24], resulting in their targeted degradation via the SCF/proteosome machinery [8, 25, 26]. To investigate whether GA promoted root cell division in a DELLA-dependent manner, cell production rate studies were performed on the GA-deficient mutant *ga1-3* (*Ler* background), the triple mutant *gai-t6/rga-24/ga1-3* (*Ler*), and the wild-type (*Ler*) control [2]. We observed that the reduced cell production rate in the GA-deficient *ga1-3* mutant could be restored to a wild-type level by mutating the two main DELLA proteins in the *Arabidopsis* root (RGA and GAI) in the triple mutant combination *gai-t6/rga-24/ga1-3* (Figure 1C). Similar results were obtained in the case of the triple GA receptor mutant *gid1a, b, c* in which its reduced meristem size could be rescued in the quadruple *rga-24/ gid1a, b, c* mutant (data not shown). Hence GA controls root cell proliferation in a DELLA-dependent manner.

Root cell proliferation is first initiated within a population of stem cells surrounding the quiescent center (QC) at the root apex [27] (Figure 1A). Daughter cells that remain in contact with the QC maintain an indeterminate root stem cell identity [28]. In contrast, daughter cells that lose contact with the QC will acquire a determinate cell identity and undergo a variable number of mitotic divisions in the proximal meristem prior to entering the elongation zone and ultimately differentiating

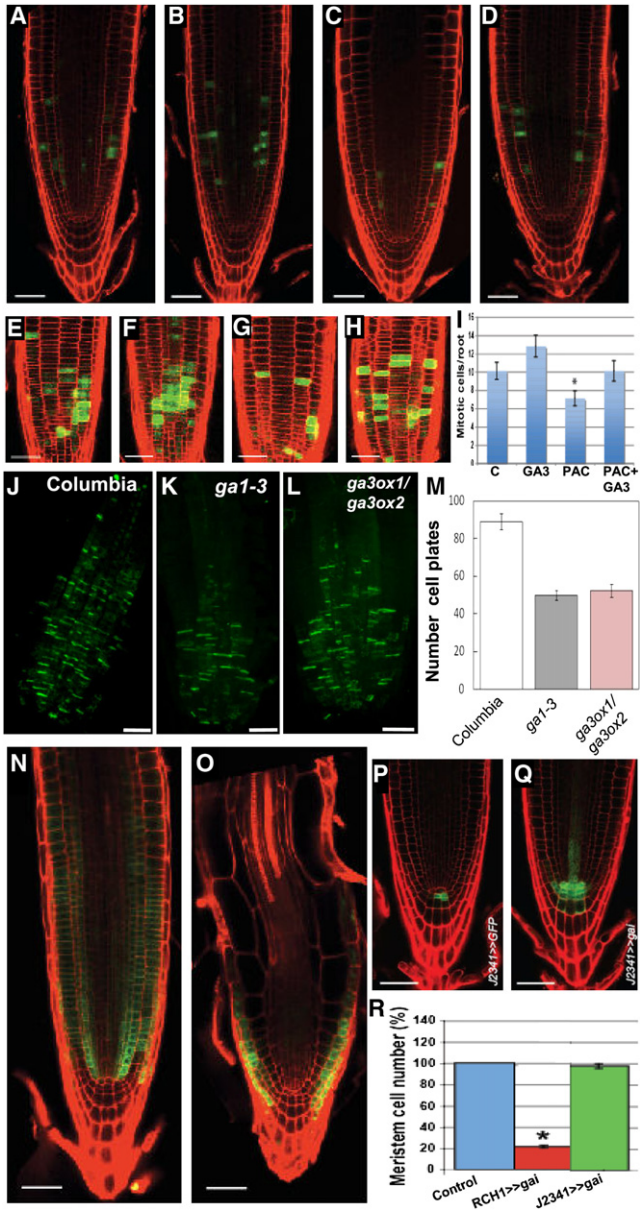

Figure 2. GA Is Required to Maintain Cell Division in the Proximal Meristem

(A–D) Confocal image of radial optical sections of root meristem of transgenic line CycB1;1::GFP.

(E–H) Tangential optical sections of cortical cells from z-series image stacks used for mitotic cell quantification. All plants were germinated in control medium, transferred to the different treatments at 4 DAG, and kept for 48 hr before imaging.

(A and E) Control roots at 6 DAG.

(B and F) Roots treated with 10 μM GA3.

(C and G) Roots treated with 10 μM paclobutrazol (PAC, GA biosynthesis inhibitor).

(D and H) Roots treated concurrently with PAC and GA3.

(I) Frequency of mitotic cells of roots analyzed in (A)–(H) (n = 20). 6-day-old plants were imaged after 48 hr of treatment with (from left to right) control solution, GA3 10 μM, PAC 10 μM, and the combination of PAC and GA3. In order to measure the frequency of cell division within a region of active proliferation, 38 cortex cells from the 2nd to the 20th position from QC in two adjacent files of cortex cells were scored in batches of 20 roots for CycB1;1::GFP expression. Propidium iodide was used as a red counterstain. Asterisk, PAC-treated roots showed a significant reduction of number of mitotic cells (7.1 ± 0.80 [SEM] versus 10.1 ± 0.94 [SEM] in control; Student's t test $p < 0.05$; n = 20).

(J–L) Maximal projections of image stacks taken through the whole root via confocal microscopy of 4- to 5-day-old Columbia, *ga1-3*, and *ga3ox1/ga3ox2* mutant seedlings. Green fluorescent marks correspond to cell plates formed in dividing cells. Seedlings were fixed and immunolocalization experiments performed with anti-Knolle primary antibody and Oregon green-coupled anti-Rabbit secondary antibody (Invitrogen).

(M) The maximal projections of image stacks were used to manually count the green fluorescent marks, corresponding to cell plates formed in dividing cells. Statistical significance of differences observed are displayed in Table S1C.

(N and O) RCH1-driven expression of gai in PM reduces the number of meristematic cells.

(N) 7-day-old control root [F1 progeny from RCH1 (Utr) × wild-type (Col-0)].

(O) 7-day-old RCH1 > > gai root [F1 progeny from RCH1 (Utr) × UAS::gai (Col-0)].

(P and Q) J2341-driven expression of gai in the QC does not significantly affect the number of meristematic cells.

(P) 6-day-old control root [F1 progeny from J2341 (C24) × wild-type (Col-0)].

(Q) 6-day-old J2341 > > gai root [F1 progeny from J2341 (C24) × UAS::gai (Col-0)].

(R) Normalized effect of GA response disruption in meristematic cells (RCH1) and stem cell niche (J2341). Asterisk indicates statistical significance in RCH1 > > gai (Student's t test $p < 4.5048E-44$; n = 25).

Scale bars represent 40 μm.

[1]. To determine which population of meristematic cells GA acted upon to control root cell proliferation, we disrupted the GA response in either QC or proximal meristematic cells by expressing a GA-insensitive mutant form of the DELLA protein GAI (termed gai) in these tissues [6]. Expressing *gai* in all dividing root cells (RCH1 > > gai) resulted in a dramatic reduction in meristem cell number (Figures 2N, 2O, and 2R), whereas targeting *gai* expression to just the QC and columella stem cells (J2341 > > gai) did not affect root meristem cell number compared to the control (Figures 2P–2R). This result is consistent with the lack of an effect of PAC or GA3 treatments on several stem cell markers reported in the accompanying manuscript by Achard et al. [20]. Hence, GA appears to control root meristem size by regulating the proliferation of proximal meristem cells rather than affecting QC function.

The *Arabidopsis* proximal root meristem is composed of lateral root cap, epidermis, cortex, endodermis, and stele tissues (Figure 1A). GA may control the rate of cell division in a specific tissue or in all proximal meristem tissues simultaneously. To address this question, we disrupted the GA response either in individual or combinations of root tissues by targeting *gai* expression employing a selection of tissue or zone-specific GAL4 driver lines [6]. We observed that expressing *gai* in only a subset of root tissues caused a significant reduction in root growth rate [6] (Figure 3A). These included expressing *gai* in the elongation zone (EDZ; J0631 > > gai), cortex/endodermis (J0571 > > gai), and in the endodermis (Q2500 > > gai) tissues (Figure 3A; Figure S3). In contrast, expressing the wild-type form *GAI* did not affect root growth in any of the cases as described in our previous work [6].

We next studied the effect of targeted *gai* expression on meristem size in detail for each of these lines. Anatomical measurements revealed that the increase in root meristem length and cell number after germination in the wild-type control was blocked in J0571 > > gai and Q2500 > > gai seedlings (Figures 3B and 3C). Hence, GA appears to cause a doubling in root meristem size by targeting DELLA degradation in a subset of root meristematic cells in the endodermis. Targeted *gai* expression in the endodermis has recently been reported to also affect cell elongation and cell morphology in the EDZ [6] (Figure 3F). To rule out the possibility that these

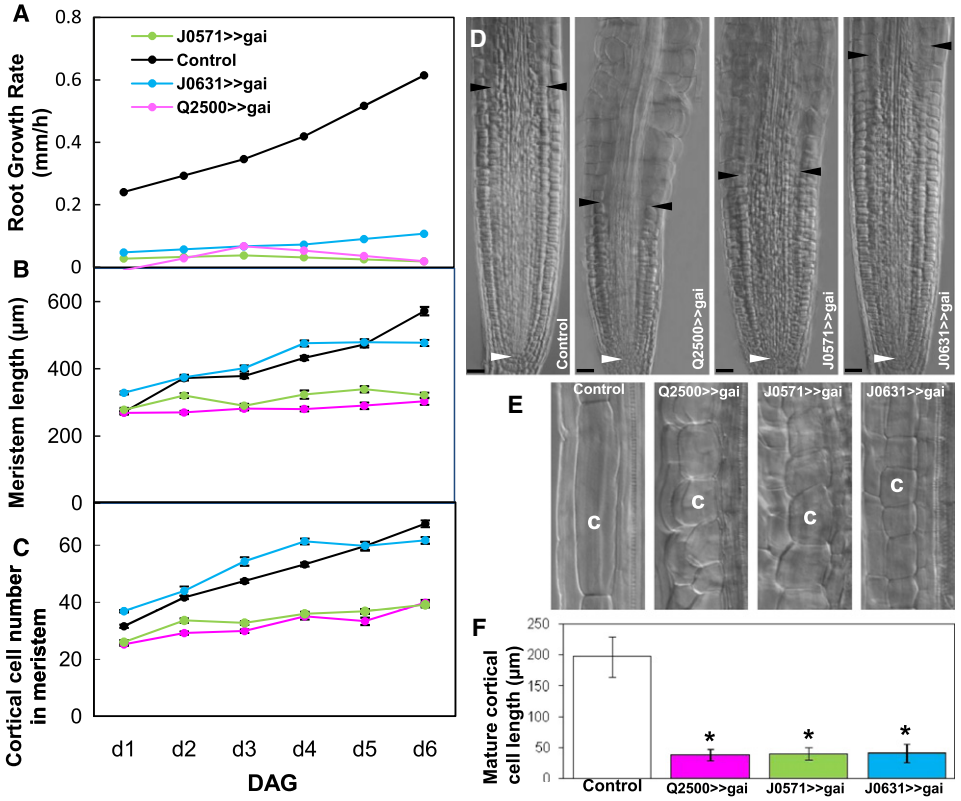

Figure 3. GA Regulates Endodermal Cell Division Controlling *Arabidopsis* Root Meristem Size

(A–D) Blocking GA response in endodermis (Q2500 > > gai) and endodermis/cortex (J0571 > > gai) reduces root elongation rate (A) and meristem size (B and C) by blocking the increase in the number of root meristematic cells. This effect was not observed when blocking GA response in all elongating tissues (J0631 > > gai).

(A–C) Graphs representing time course experiment with roots from 1 to 6 days after germination (DAG). Root meristem size was measured as distance (μm) and as cortical cell number between the transition zone (TZ) and the quiescent center (QC). Error bars represent SE (n > 100 per line).

(D) Primary root meristems of seedlings 4 DAG. TZ and QC positions are indicated by black and white arrowheads, respectively. Scale bars represent 25 μm.

(E and F) Blocking GA response in roots reduces cell elongation.

(E) Representative image of mature cortical cells showing a reduced length when blocked GA response.

(F) Measurements of cortical cell length (μm) within the mature zone of primary roots in 6 DAG seedlings. The zone selected to take measurements was located between the hypocotyl and the differentiation zone of the secondary cell wall of xylem cells. Asterisk, statistically significant differences for values compared with wild-type as determined by Student's t test ($p < 0.05$). Statistical significance of differences observed are displayed in Tables S1D–S1F.

morphological changes in the EDZ are responsible for the decrease in meristem size, we targeted *gai* expression in EDZ (but not meristem) tissues with the J0631 driver line (Figures S3A and S3B). In this case, root growth was severely reduced but no reduction in root meristem size was detected when blocked GA responses in the EDZ (J0631 > > gai, Figures 3B and 3D). Hence, the morphological changes in the EDZ described for J0571 > > gai and Q2500 > > gai roots [6] (Figures 3D–3F; Figures S3C and S3D) were not responsible for the reduction in root meristem size in these lines (Figures 3B–3D). To confirm that gai acted cell autonomously, we also expressed a YFP-tagged gai protein (termed gai-YFP) in the different root meristematic tissues, revealing the spatial specificity of this approach (Figure S4). Therefore, our targeted *gai* expression results indicate that GA controls root meristem size by promoting endodermal cell proliferation in the proximal meristem.

In summary, this study and the accompanying paper by Achard et al. [20] provide compelling new evidence that GA regulates *Arabidopsis* root growth by promoting cell proliferation. By increasing the number of root meristematic cells, a greater number of cells are produced, causing root growth to accelerate. The ability to rapidly increase root growth is particularly important for newly germinated seedlings if they are to rapidly secure vital resources such as anchorage, water, and nutrients after emerging from their seed coat. In this work we showed how GA is needed during root development after germination to attain and maintain root meristem size, by regulating cell proliferation through DELLA protein degradation. Molecular details about DELLA regulation of the cell cycle machinery are discussed in the accompanying manuscript by Achard et al. [20]. We report how GA controls root meristem size by targeting DELLA degradation in a subset of (rather than all) dividing cells. The critical question this observation poses is how dividing cells in the endodermis regulate the proliferation of meristematic cells in adjacent tissues? Cells must double in size prior to dividing but cannot do so independently, because they are physically restrained by adjacent tissues with which they share cell walls (Figure 4). Thus, the rate of expansion of dividing endodermal cells may dictate the equivalent rate in other root tissues. We have previously demonstrated that DELLA-regulated endodermal cell expansion is rate limiting for other elongation zone tissues [6]. Hence, GA appears to promote root growth by increasing endodermal

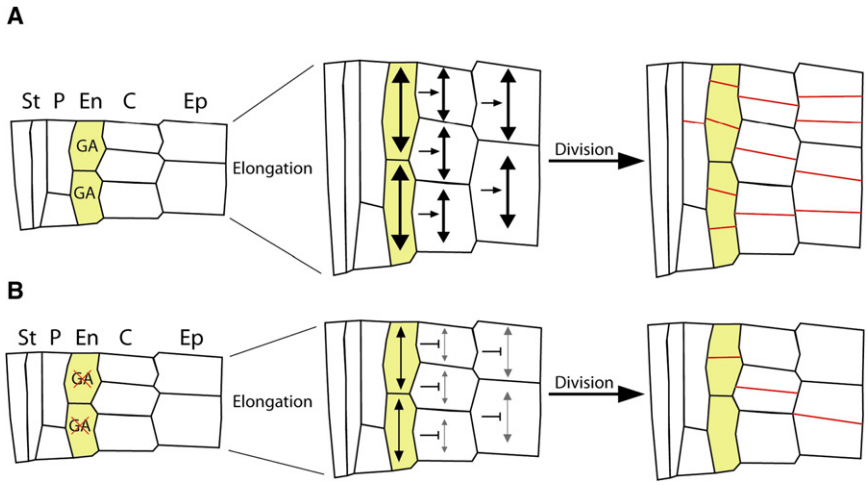

Figure 4. Model for GA Regulating Root Meristem Size

Endodermal cells (yellow) in the meristem must double in size prior division and this elongation is GA regulated [6], enabling adjacent tissues to elongate and therefore divide. This increase in number of divisions will produce a bigger meristem (A). In the case of reduced GA levels or reduced GA response in the endodermis, cells will elongate less and therefore adjacent tissues will reduce their elongation, reducing the number of division events, resulting in smaller meristems (B). Black arrows represent cell elongation processes in the meristem, black bar heads represent inhibition of cell elongation, and red lines represent cell plates corresponding to division events. The different root tissues represented in the figure are stele (St), pericycle (P), endodermis (En), cortex (C), and epidermis (Ep).

cell expansion in both meristematic and elongation zones, thereby indirectly controlling the rates of division and expansion of other root tissues (Figure 4) and root meristem size. Although we provide new evidence that GA regulates *Arabidopsis* root cell proliferation, the same signal is inhibitory to shoot apical meristem activity [29, 30]. This marked difference in GA action is likely to reflect distinct differences in (1) the organization of the tissues and (2) composition of the regulatory networks that operate in the root and shoot apical meristem to control cell proliferation [26, 29, 30].

Experimental Procedures

Plant Material and Treatments

Arabidopsis thaliana GA mutants with different backgrounds were used as indicated in the text including *ga1-3* in both Columbia-0 (Col-0) and Landsberg *erecta* (Ler) background, *gai-t6/rga-24/ga1-3* (Ler), *ga3ox1/ga3ox2* (Col-0). Transgenic line CycB1;1::GUS was described previously [18] and CycB1;1:: GFP line was constructed by replacing a BamHI-SacI fragment excised from pCDG [17], which removed the *uid*A gene, and replacing it with a BamHI-SacI fragment corresponding to mGFP5. Seeds were surface sterilized and plated on half (0.5) MS (Murashige Skoog) and agarose 1% (w/v) solidified medium. GA3 and Paclobutrazol (PAC, an inhibitor of GA biosynthesis [14]) treatments (1 μM) were applied to the media as required. 10 μM dosages were used for results presented in Figure 2. In the case of PAC treatments, seeds germinated in 0.5 MS and transferred to PAC treatment after germination (when the root penetrated the endosperm, 1.5–2 days after being transfer to growth room). Seedlings grew vertically in a growth chamber with constant conditions (24°C, 150 μmol/m^2/s), permitting roots to grow along the surface of the agarose.

Targeted Misexpression Approach

To map the site of action of GAs in the *Arabidopsis* root meristem, we analyzed the F1 progenies from crossing the line UAS::gai (in SCL3::GUS, Col-0 background) with several driver lines (Q2393, Q2500, J0631, J0951, and J0571; http://www.plantsci.cam.ac.uk/Haseloff/construction/catalogFrame.html) in C24 background as described previously [6]. The correspondent F1 progeny from crossing UAS::gai with C24 was used as a control for these lines. The line UAS::gai-YFP-9.4 (Col-0 background) was used to cross with the different driver lines in localizing gai-YFP (Figure S4).

Root Growth

To assess root growth, the root length of vertically grown seedlings was measured from root tip to hypocotyl base with ImageJ 1.32j freely available (http://rsb.info.nih.gov/ij/). Two-tail t tests were performed with Microsoft Excel software. We used a five point equation to calculate root elongation rates from the root length data [31]. Cell division rate within the proximal meristem zone (PM, Figure 1A) and cellular elongation through the elongation-differentiation zone (EDZ, Figure 1A) determines the final length of primary roots.

Root Meristem Size Analysis

Root meristem size was expressed as (1) the distance between the quiescent center (QC) and the transition zone (TZ; indicating the position of the first elongating cortical cell), represented as d-a1 and d-a2 in Figure 1A; (2) the number of cortical cells [32] in a file extending from the QC to the TZ. To calculate the root meristem size, measurements were performed every day on clarified roots. In the case of the Figure S1L, measurements were performed in confocal images of roots stained with propidium iodide (Sigma, St. Louis, MO).

Image Analysis

GUS histochemical staining was performed by immersing roots in a solution (50 mM sodium phosphate, 1 mM EDTA, 0.5 mM potassium ferrocyanide, 0.5 mM potassium ferricyanide, 0.5% Triton X-100, 0.5% dimethylformamide, 1 mM X-Glc) for 2 hr at 37°C followed by root clarification [33]. Subsequent imaging with Nomarski optics on a Nikon optiphot-2 microscope and a Leica DFC300 camera performed. Confocal analysis was performed as described previously [6] except for Figure 2, where images were obtained with a Leica TCS-SP confocal microscope (Leica, Milton Keynes, UK). In this case, roots were stained with 10 μg/ml propidium iodide (Sigma) for 15 s, rinsed, and mounted in water. EGFP was excited with the 488 nm line of an argon laser and propidium iodide was excited with the 514 nm line. Fluorescence emission was collected between 505 and 530 nm for EGFP, and 606 and 635 nm for propidium iodide. The number of mitotic cells was quantified by manually counting the GFP-positive cells.

Immunolocalization

4- to 5-day-old seedlings were fixed, immunolocalization experiments were performed as described previously [34] with anti-Knolle primary antibody (1:4000 dilution) and Oregon green-coupled anti-Rabbit secondary antibody (Invitrogen) (1:200 dilution), and visualization was by confocal microscopy. Maximal projections of image stacks taken 1 μm apart through the whole root were used to manually count the green fluorescent marks, corresponding to cell plates formed in dividing cells, represented in Figures 2J–2M.

Supplemental Data

Supplemental Data include four figures and one table and can be found with this article online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01296-2.

Acknowledgments

We thank S.G. Thomas (Rothamsted Research, UK) for *ga3ox1/ga3ox2, ga1-3, gai-t6/rga-24/ga1-3, gid1abc*, and *rga-24/gid1abc* mutants, B. Scheres for the *RCH1::GAL4* line, the Nottingham Arabidopsis Stock Centre (NASC) for providing selected GAL4 enhancer trap lines used in this study, and Benjamin Peret for assistance in drawing Figure 4. We acknowledge the support of the Biotechnology and Biological Sciences Research Council (S.U.-T. and M.J.B.); BBSRC/EPSRC CISB programme funding (S.U.-T. and M.J.B.); Belgian Scientific policy (BELSPO contract BARN to G.T.S.B.

and M.J.B.); Ayuda de movilidad GRU0790 to I.C.; and Bill and Melinda Gates Foundation (Gates Cambridge Scholarship to F.F.).

Received: March 6, 2009
Revised: June 2, 2009
Accepted: June 3, 2009
Published online: July 2, 2009

References

1. Beemster, G.T.S., and Baskin, T.I. (1998). Analysis of cell division and elongation underlying the developmental acceleration of root growth in *Arabidopsis thaliana*. Plant Physiol. *116*, 1515–1526.
2. Fu, X., and Harberd, N.P. (2003). Auxin promotes *Arabidopsis* root growth by modulating gibberellin response. Nature *421*, 740–743.
3. Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z.L., Powers, S.J., Gong, F., Phillips, A.L., Hedden, P., Sun, T.P., et al. (2006). Genetic characterization and functional analysis of the GID1 gibberellin receptors in *Arabidopsis*. Plant Cell *18*, 3399–3414.
4. Willige, B.C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E.M., Maier, A., and Schwechheimer, C. (2007). The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of *Arabidopsis*. Plant Cell *19*, 1209–1220.
5. Ueguchi-Tanaka, M., Nakajima, M., Katoh, E., Ohmiya, H., Asano, K., Saji, S., Hongyu, X., Ashikari, M., Kitano, H., Yamaguchi, I., et al. (2007). Molecular interactions of a soluble gibberellin receptor, GID1, with a rice DELLA protein, SLR1, and gibberellin. Plant Cell *19*, 2140–2155.
6. Ubeda-Tomás, S., Swarup, R., Coates, J., Swarup, K., Laplaze, L., Beemster, G.T., Hedden, P., Bhalerao, R., and Bennett, M.J. (2008). Root growth in *Arabidopsis* requires gibberellin/DELLA signalling in the endodermis. Nat. Cell Biol. *10*, 625–628.
7. Peng, J., Richards, D.E., Hartley, N.M., Murphy, G.P., Devos, K.M., Flintham, J.E., Beales, J., Fish, L.J., Worland, A.J., Pelica, F., et al. (1999). 'Green revolution' genes encode mutant gibberellin response modulators. Nature *400*, 256–261.
8. Silverstone, A.L., Jung, H.S., Dill, A., Kawaide, H., Kamiya, Y., and Sun, T.P. (2001). Repressing a repressor: Gibberellin-induced rapid reduction of the RGA protein in *Arabidopsis*. Plant Cell *13*, 1555–1566.
9. Peng, J., Carol, P., Richards, D.E., King, K.E., Cowling, R.J., Murphy, G.P., and Harberd, N.P. (1997). The *Arabidopsis* GAI gene defines a signaling pathway that negatively regulates gibberellin responses. Genes Dev. *11*, 3194–3205.
10. King, K.E., Moritz, T., and Harberd, N.P. (2001). Gibberellins are not required for normal stem growth in *Arabidopsis thaliana* in the absence of GAI and RGA. Genetics *159*, 767–776.
11. Dill, A., and Sun, T.P. (2001). Synergistic derepression of gibberellin signalling by removing RGA and GAI function in *Arabidopsis thaliana*. Genetics *159*, 777–785.
12. Cheng, H., Qin, L., Lee, S., Fu, X., Richards, D.E., Cao, D., Luo, D., Harberd, N.P., and Peng, J. (2004). Gibberellin regulates *Arabidopsis* floral development via suppression of DELLA protein function. Development *131*, 1055–1064.
13. Baskin, T.I. (2000). On the constancy of cell division rate in the root meristem. Plant Mol. Biol. *43*, 545–554.
14. Olszewski, N., Sun, T.P., and Gubler, F. (2002). Gibberellin signaling: Biosynthesis, catabolism, and response pathways. Plant Cell Suppl. *14*, S61–S80.
15. Sun, T.P., and Kamiya, Y. (1994). The *Arabidopsis* GA1 locus encodes the cyclase ent-kaurene synthetase A of gibberellin biosynthesis. Plant Cell *6*, 1509–1518.
16. Mitchum, M.G., Yamaguchi, S., Hanada, A., Kuwahara, A., Yoshioka, Y., Kato, T., Tabata, S., Kamiya, Y., and Sun, T.P. (2006). Distinct and overlapping roles of two gibberellin 3-oxidases in *Arabidopsis* development. Plant J. *45*, 804–818.
17. Ferreira, P.C.G., Hemerly, A.S., de Almeida-Engler, J., Van Montagu, M., Engler, G., and Inzé, D. (1994). Developmental expression of the *Arabidopsis* cyclin gene cyc1At. Plant Cell *6*, 1763–1774.
18. Colon-Carmona, A., You, R., Haimovitch-Gal, T., and Doerner, P. (1999). Technical advance: spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. Plant J. *20*, 503–508.
19. Lauber, M.H., Waizenegger, I., Steinmann, T., Schwarz, H., Mayer, U., Hwang, I., Lukowitz, W., and Jurgens, G. (1997). The *Arabidopsis* KNOLLE protein is a cytokinesis-specific syntaxin. J. Cell Biol. *139*, 1485–1493.
20. Achard, P., Gusti, A., Cheminant, S., Alioua, M., Dhondt, S., Coppens, F., Beemster, G.T.S., and Genschik, P. (2009). Gibberellin signaling controls cell proliferation rate in *Arabidopsis*. Curr. Biol. *19*, this issue, 1188–1193.
21. Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., Chow, T.Y., Hsing, Y.I., Kitano, H., Yamaguchi, I., et al. (2005). GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature *437*, 693–698.
22. Nakajima, M., Shimada, A., Takashi, Y., Kim, Y.C., Park, S.H., Ueguchi-Tanaka, M., Suzuki, H., Katoh, E., Iuchi, S., Kobayashi, M., et al. (2006). Identification and characterization of *Arabidopsis* gibberellin receptors. Plant J. *46*, 880–889.
23. Shimada, A., Ueguchi-Tanaka, M., Nakatsu, T., Nakajima, M., Naoe, Y., Ohmiya, H., Kato, H., and Matsuoka, M. (2008). Structural basis for gibberellin recognition by its receptor GID1. Nature *456*, 520–523.
24. Murase, K., Hirano, Y., Sun, T.P., and Hakoshima, T. (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature *456*, 459–463.
25. Fu, X., Richards, D.E., Ait-Ali, T., Hynes, L.W., Ougham, H., Peng, J., and Harberd, N.P. (2002). Gibberellin-mediated proteasome-dependent degradation of the barley DELLA protein SLN1 repressor. Plant Cell *14*, 3191–3200.
26. Dill, A., Thomas, S.G., Hu, J., Steber, C.M., and Sun, T.P. (2004). The *Arabidopsis* F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell *16*, 1392–1405.
27. Scheres, B. (2007). Stem-cell niches: Nursery rhymes across kingdoms. Nat. Rev. Mol. Cell Biol. *8*, 345–354.
28. van den Berg, C., Willemsen, V., Hendriks, G., Weisbeek, P., and Scheres, B. (1997). Short-range control of cell differentiation in the *Arabidopsis* root meristem. Nature *390*, 287–289.
29. Hay, A., Kaur, H., Phillips, A., Hedden, P., Hake, S., and Tsiantis, M. (2002). The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body parts. Curr. Biol. *12*, 1557–1565.
30. Jasinski, S., Piazza, P., Craft, J., Hay, A., Wooley, L., Rieu, I., Phillips, A., Hedden, P., and Tsiantis, M. (2005). KNOX1 action in *Arabidopsis* is mediated by coordinate regulation of cytokinin and gibberellin activities. Curr. Biol. *15*, 1560–1565.
31. Erickson, R.O. (1976). Modeling of plant growth. Annu. Rev. Plant Physiol. *27*, 407–434.
32. Dello Ioio, R., Linhares, F.S., Scacchi, E., Casamitjana-Martinez, E., Heidstra, R., Costantino, P., and Sabatini, S. (2007). Cytokinins determine *Arabidopsis* root-meristem size by controlling cell differentiation. Curr. Biol. *17*, 678–682.
33. Malamy, J.E., and Benfey, P.N. (1997). Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. Development *124*, 33–44.
34. Swarup, R., Kramer, E.M., Perry, P., Knox, K., Leyser, O.H.M., Haseloff, J., Beemster, G.T.S., Bhalerao, R., and Bennett, M.J. (2005). Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. Nat. Cell Biol. *7*, 1057–1065.