Funneling of gibberellin signaling by the GRAS transcription regulator SCARECROW-LIKE 3 in the *Arabidopsis* root

Jung-Ok Heo[a], Kwang Suk Chang[a], In A Kim[a], Mi-Hyun Lee[a], Shin Ae Lee[a], Sang-Kee Song[b], Myeong Min Lee[b], and Jun Lim[a,1]

[a]Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Korea; and [b]Department of Biology, Yonsei University, Seoul 120-749, Korea

Edited by Mark Estelle, University of California at San Diego, La Jolla, CA, and approved December 16, 2010 (received for review August 18, 2010)

During plant development, because no cell movement takes place, control of the timing and extent of cell division and coordination of the direction and extent of cell expansion are particularly important for growth and development. The plant hormone gibberellins (GAs) play key roles in the control of these developmental processes. However, little is known about the molecular components that integrate the generic GA signaling into a specific cell/tissue to coordinate cell division and cell expansion. Here we report that SCARECROW-LIKE 3 (SCL3), a GRAS protein, acts as a positive regulator to integrate and maintain a functional GA pathway by attenuating the DELLA repressors in the root endodermis. The tissue-specific maintenance of GA signaling in the root endodermis plays distinct roles along the longitudinal root axis. While in the elongation/differentiation zone (EDZ), the endodermis-confined GA pathway by SCL3 controls primarily coordination of root cell elongation; in the meristem zone (MZ) SCL3 in conjunction with the SHORT-ROOT/SCARECROW (SHR/SCR) pathway controls GA-modulated ground tissue maturation. Our findings highlight the regulatory network of the GRAS transcription regulators (SCL3, DELLAs, and SHR/SCR) in the root endodermis, shedding light on how GA homeostasis is achieved and how the maintenance of GA signaling controls developmental processes in roots.

formative division | hormonal regulation | middle cortex formation | root development

Because no cell movement takes place during plant development, control of cell division (formative and proliferative) and coordination of cell expansion are crucial in growth and development. The plant hormone gibberellins (GAs) play key roles in the control of these developmental processes (1–6). With molecular, genetic, and biochemical approaches, molecular components of GA signaling have been well characterized (7, 8). The soluble GA receptors GIBBERELLIN INSENSITIVE DWARF 1 (GID1) interact with DELLA proteins (DELLAs), major negative regulators of GA signaling, in a GA-dependent manner (9–13). Bioactive GAs promote the GID1–DELLA interaction and, in turn, lead to rapid degradation of the DELLA repressors via the ubiquitin/26S proteasome pathway (11–14). DELLAs accumulate when bioactive GA levels are low, whereas degradation of DELLAs is accelerated when bioactive GA levels are elevated. Thus, bioactive GAs act as "inhibitors of inhibitors" (7), promoting degradation of the DELLA repressors and thus GA-mediated growth responses (14). DELLAs belong to the GRAS transcription regulator family (9, 10, 15, 16), and in *Arabidopsis thaliana* there are five DELLAs (GAI, RGA, RGL1, RGL2, and RGL3), which have overlapping but distinct roles in plant growth and development (7–10, 17). Although the GA signaling pathway has become increasingly well characterized, still little is known about its integration into a specific cell/tissue to regulate developmental processes in the plant life cycle.

The SCARECROW (SCR) and SHORT-ROOT (SHR) transcription regulators, which also belong to the GRAS family, control specification of stem cell niche (18) and ground tissue formation in the *Arabidopsis* root (19, 20). Mutations in *SCR* and *SHR* cause defects in the formative periclinal (parallel to the growth axis) division that generates cortex and endodermis (19, 20). Recent work demonstrated that the SHR/SCR pathway, in conjunction with the GA pathway, controls the timing and extent of additional formative periclinal division for endodermis and additional cortex (termed middle cortex; MC) at later stages (2, 4, 21). These findings imply the involvement of additional regulatory components to integrate the GA signaling pathway in the root endodermis, because neither SHR nor SCR is subject to regulation by bioactive GAs or GA signaling per se. Recently, it was also shown that GA signaling controls cell proliferation in the root meristem (5, 6) and that the endodermis-specific disruption of GA signaling results in uncoordinated cell expansion in the root (3). However, the molecular components that integrate the generic GA signaling into the root endodermis to coordinate cell division and cell expansion are largely unknown.

In this study, we show that the GRAS transcription regulator SCARECROW-LIKE 3 (SCL3) serves as a tissue-specific integrator of the GA pathway in the *Arabidopsis* root endodermis. Our genetic and physiological results indicate that SCL3, acting downstream of RGA, is likely a positive regulator in GA signaling. Furthermore, our findings reveal that the spatial integration of the GA pathway by SCL3 plays distinct roles along the longitudinal root axis. In the elongation/differentiation zone (EDZ), SCL3, acting as an attenuator of GAI and RGA, controls coordination of root cell elongation. In the meristem zone (MZ), the maintenance of a functional GA signaling by SCL3–DELLA interaction, in conjunction with the SHR/SCR pathway, modulates the timing and extent of the formative division for ground tissue maturation.

Results and Discussion

SCL3 Acts as an Integrator of the GA/DELLA and SHR/SCR Pathways. Recent microarray analysis revealed that among GA/DELLA-regulated genes, RGA was associated with the promoter of *SCL3*, which also belongs to the GRAS family, like DELLAs (22). In addition, the SHR and SCR transcription regulators, key in root radial patterning (19, 20), form a heterodimer that regulates *SCL3* transcription being associated with its promoter (23, 24). Thus, we started with the premise that SCL3 acts downstream of both the GA and SHR/SCR pathways, serving as a convergent point.

Author contributions: J.-O.H., K.S.C., I.A.K., M.-H.L., S.A.L., and J.L. designed research; J.-O.H., K.S.C., I.A.K., M.-H.L., and S.A.L. performed research; S.-K.S. and M.M.L. contributed new reagents/analytic tools; J.-O.H., K.S.C., I.A.K., M.-H.L., S.A.L., and J.L. analyzed data; and J.-O.H., K.S.C., M.-H.L., S.A.L., and J.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. E-mail: jlim@konkuk.ac.kr.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1012215108/-/DCSupplemental.

In the *Arabidopsis* root (Fig. 1*A*), mRNA in situ hybridization analysis showed that *SCL3* transcripts localized primarily to the endodermis (Fig. 1*B*) (16). Additionally, a transcriptional fusion between the *SCL3* promoter and β-glucuronidase (GUS) (*pSCL3::GUS*) basically recapitulated the mRNA in situ hybridization pattern (Fig. 1 *C* and *D*). Furthermore, a translational fusion to green fluorescent protein (GFP) (*pSCL3::SCL3-GFP*) conferred nuclear localization in the cells of the quiescent center (QC), cortex/endodermis initial (CEI), and endodermis (Fig. 1 *A* and *E*), suggesting that SCL3 acts as a transcription regulator.

Next, we analyzed expression of *SCL3* with the *pSCL3::GUS* transcriptional fusion and reverse transcription-based quantitative PCR (qRT-PCR). In the presence of exogenous bioactive GA (GA_3), *SCL3* expression was reduced substantially (Fig. 1 *F* and *G* and Fig. S1). By contrast, its expression was up-regulated by the GA biosynthesis inhibitor paclobutrazol (PAC) (Fig. 1 *H* and *I* and Fig. S1). Consequently, in the GA-deficient *ga1-3* mutant (25), *SCL3* expression level was higher than that in wild type (WT), and it was modulated by bioactive GA levels: decreased by GA_3 and increased by PAC (Fig. S1). Our data are in agreement with the previous findings that *SCL3* expression is modulated by bioactive GA levels (22). We further investigated the *SCL3* transcript levels in loss-of-function mutants of DELLAs: *gai* (SALK_082622), *rga* (SALK_089146), and *gai rga* and *della* (*gai rga rgl1 rgl2 rgl3*). As reported previously (22), *SCL3* expression is regulated, at least in part, through accumulation of DELLAs that is modulated by bioactive GA contents.

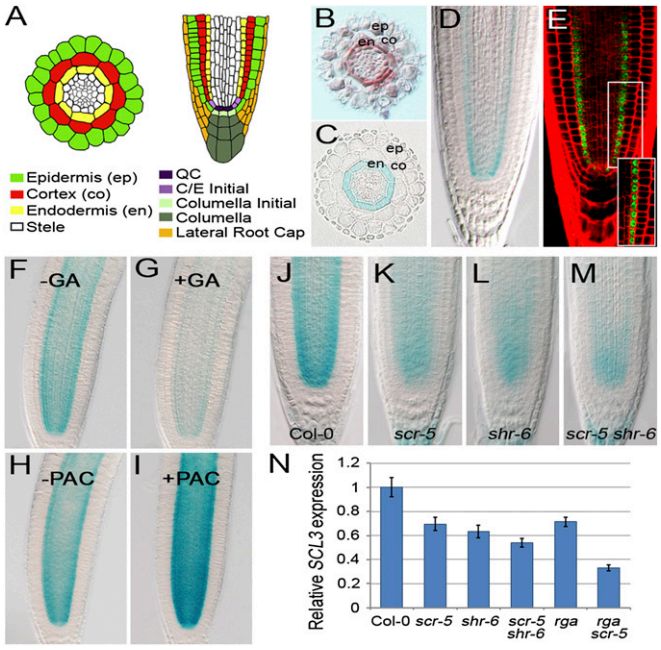

Fig. 1. Transcriptional control of *SCL3* in the root by the GA/DELLA and SHR/SCR pathways. (*A*) Schematic of the *Arabidopsis* root. (*B–E*) Localization of *SCL3* mRNA and its protein. Expression of *SCL3* is detected primarily in the endodermis by in situ hybridization (*B*) and a transcriptional fusion (*pSCL3::GUS*) (*C* and *D*). Blue staining is observed in a manner similar to the *SCL3* in situ hybridization pattern. (*E*) Translational fusion (*pSCR::SCL3-GFP*) in the root. GFP signals are observed in the nuclei of the QC and the endodermis lineage. (*F* and *G*) *pSCL3::GUS* in WT in the absence (*F*) or presence (*G*) of 10 μM of exogenous GA_3 for 6 h. (*H* and *I*) *pSCL3::GUS* in WT in the absence (*H*) or presence (*I*) of 10 μM of PAC for 6 h. Expression of *SCL3* is modulated by bioactive GA contents. (*J–M*) Expression of *pSCL3::GUS* in Col-0 (*J*), *scr-5* (*K*), *shr-6* (*L*), and *scr-5 shr-6* (*M*). (*N*) qRT-PCR of *SCL3* transcripts in Col-0, *scr*, *shr*, *scr shr*, *rga*, and *rga scr* roots. The *SCL3* transcript level is substantially reduced in *rga scr* double mutants. The *SCL3* mRNA level in Col-0 is arbitrarily set to 1. Error bars indicate SD from three biological replicates.

It is noteworthy that mRNA and protein localization of *SCL3* in the QC, CEI, and endodermis exactly overlaps with the spatial domains of *SCR* mRNA and protein as well as SHR protein, implying a molecular interplay between SCL3 and the SHR/SCR module at the cellular levels. We therefore examined the expression pattern of *SCL3* in *scr-5*, *shr-6*, and *scr-5 shr-6* mutants. In *scr-5*, the expression level of *SCL3* was reduced to ~70%, and in *shr-6* its expression was about 60%, compared with that in WT roots (Fig. 1 *J–L* and *N*). We also found a reduction of the *SCL3* expression level in *scr-5 shr-6* (~50% of the WT level) (Fig. 1 *M* and *N*), further verifying that *SCL3* transcription in the root is controlled by the SHR/SCR pathway.

Intriguingly, we observed only modest reduction of *SCL3* expression even in the presence of exogenous GA application or with mutations in both *SCR* and *SHR*. Thus, we reasoned that *SCL3* transcription is modulated by both the GA/DELLA and SHR/SCR pathways. To test this, we generated *rga scr* double mutants that harbor loss-of-function mutations in the two direct upstream genes and investigated the *SCL3* expression. Indeed, the level of *SCL3* mRNA was markedly reduced to only 30% of WT roots (Fig. 1*N*). Our findings indicate that SCL3 likely plays as a cell/tissue-specific integrator, acting downstream of both the GA/DELLA and SHR/SCR pathways.

SCL3 Acts as a Positive Regulator in the GA Response Pathway. Because *SCL3* expression is actively modulated by bioactive GAs through DELLAs, we initially postulated that SCL3 likely acts as another negative regulator in GA signaling. To test this, we first used a genetic approach by introducing either loss or gain of SCL3 function in backgrounds with defective GA biosynthesis and GA response. Interestingly, the *scl3 ga1-3* double mutant exhibited a shorter root phenotype compared with *ga1-3* in our root growth assay (Fig. S2 *A* and *C*). In contrast, root growth of the *SCL3* overexpressors (*35S::SCL3*) in *ga1-3* was indistinguishable from that of *ga1-3* (Fig. S2 *A* and *C*). In the presence of exogenous GA_3, the phenotype of *scl3 ga1-3* was restored, even though both *ga1-3* and *scl3 ga1-3* roots were slightly shorter than WT roots (Fig. S2 *B* and *C*). Furthermore, root growth of *scl3* was more sensitive to PAC treatment, whereas *35S::SCL3* seedlings conferred resistance to PAC (Fig. S2 *D* and *E*). Additionally, we observed that both *rga* and *scl3 rga* were indistinguishable in root growth in the presence of PAC (Fig. S2 *D* and *E*), indicating that *RGA* is epistatic to *SCL3*. Taken together, our observations indicate that SCL3 is likely a positive regulator in GA signaling. In accordance with our findings, Zhang et al. (26) in this issue of PNAS describe the molecular details on the role of SCL3 as an activator in the GA response pathway.

To maintain GA homeostasis, transcription of GA metabolism genes is subject to feedback regulation in which DELLA repressors play key roles (27, 28). Like DELLAs, SCL3 also belongs to the GRAS transcription regulator family (15, 16, 29). Thus, to investigate the involvement of SCL3 in the maintenance of GA homeostasis, we analyzed expression profiles of the GA metabolism genes with qRT-PCR in the absence or presence of exogenous GA or PAC, as well as in *ga1-3* and *scl3 ga1-3*. Of the GA metabolism genes examined, transcript levels of *GA20ox1*, *GA20ox2*, and *GA20ox3* were notably up-regulated in *scl3* under GA-deficient conditions (in the presence of PAC or *ga1-3*) (Fig. S2 *F* and *G*). Our findings are in accordance with the results by Zhang et al. (26) in this issue of PNAS that the transcription of *GA20ox1*, *GA20ox2*, and particularly *GA20ox3* is subject to regulation by the SCL3 transcription regulator to maintain GA homeostasis.

Endodermis-Confined GA Signaling by SCL3 Coordinates Root Cell Elongation in the EDZ. Recent reports demonstrated that GA controls root meristem size by promoting proliferative cell divisions (5, 6) and that GA response in the root endodermis also

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coordinates cell elongation (3). Endodermis-specific *SCL3* expression and root growth inhibition of *scl3* in GA-deficiency raised the question as to whether SCL3 plays a role in promoting cell division, cell elongation, or both. To address this question, we analyzed GA-mediated root growth of WT and *scl3* seedlings in the absence or presence of *ga1-3* or PAC. As described previously (5, 6), we measured the number and length of ground cells from the QC as parameters for root meristem size (Fig. S3*A*). Under standard conditions, root meristem sizes of both WT and *scl3* seedlings were indistinguishable (Fig. S3 *B* and *C*). We also monitored the CYCB1::GUS mitotic marker that was shown to correlate with division potential in the root meristem (5, 6). In the presence of PAC, the number of dividing cells was reduced in *scl3* to a level similar to that of WT roots (Fig. S3 *D–F*). In addition, the root meristem size of *scl3 ga1-3* double mutants was indistinguishable from that of *ga1-3* (Fig. S3 *G* and *H*), suggesting that GA-mediated cell proliferation in the root meristem is likely independent of SCL3 function. Thus, we investigated the role of SCL3 in cell elongation, because rapid elongation of cells that exit from the meristem drives postembryonic root growth (30). In the presence of PAC or *ga1-3*, root cell elongation in *scl3* appeared to be reduced when compared with WT, whereas *35S::SCL3* roots were PAC resistant (Fig. S4 *A* and *B*). We also found that *scl3 rga* exhibited a PAC-resistant phenotype in cell elongation indistinguishable from *rga* (Fig. S4*A*), further corroborating that SCL3 acts downstream of RGA. Notably, root cell elongation of *scl3*, *35S::SCL3*, *rga*, and *scl3 rga* correlated well with the EDZ length of the individuals (Fig. S4 *C* and *D*), suggesting that the sum of each cell's elongation contributes primarily to the EDZ length, and consequently to the whole root length.

Recently, the endodermis-specific disruption of a functional GA response by expressing the nondegradable gai under the *SCR* promoter (*pSCR::gai-GR-YFP*) was shown to affect root cell elongation and cell morphology in the EDZ (3). Thus, we introgressed the *pSCR::gai-GR-YFP* construct into WT, *scl3*, and *35S::SCL3* plants, respectively. In the absence of dexamethasone (−DEX), we found no difference in root cell elongation in WT, *scl3*, and *35S::SCL3* seedlings (Fig. 2 *A*, *C*, *E*, and *G*). When induced with DEX (+DEX), root growth of *pSCR::gai-GR-YFP* seedlings in the WT background was reduced (Fig. 2*B*), and cells in the EDZ showed inhibition of elongation (Fig. 2 *D* and *F*). Notably, cell elongation of *pSCR::gai-GR-YFP* seedlings in *scl3* was more severely inhibited (Fig. 2 *D* and *F*). Under prolonged DEX treatment (+DEX for 3 d), the surface of *pSCR::gai-GR-YFP* roots in *scl3* appeared bulged because the direction of cortex cell elongation was shifted perpendicularly compared with the untreated roots (Fig. S5 *A* and *B*). As a result, the average length of individual cells in *scl3* was more significantly reduced than in the WT background (Fig. 2*H* and Fig. S5 *B* and *C*). We also observed similar results in WT and *scl3* roots harboring the *pSCR::rga-GR-YFP* construct (Fig. S5 *D–F*). Interestingly, *35S::SCL3* seedlings with *pSCR::gai-GR-YFP* suppressed the inhibition of cell elongation when induced with DEX (Fig. 2 *B*, *D*, *F*, and *H*). These observations indicate that SCL3 attenuates the effects of nondegradable gai and rga in the root endodermis. The results from ref. 26 in this issue of PNAS lend support for the notion that SCL3 acts as an attenuator of DELLAs. Thus, the maintenance of GA signaling by SCL3–DELLA interaction in the endodermis controls coordination of cell elongation for root growth.

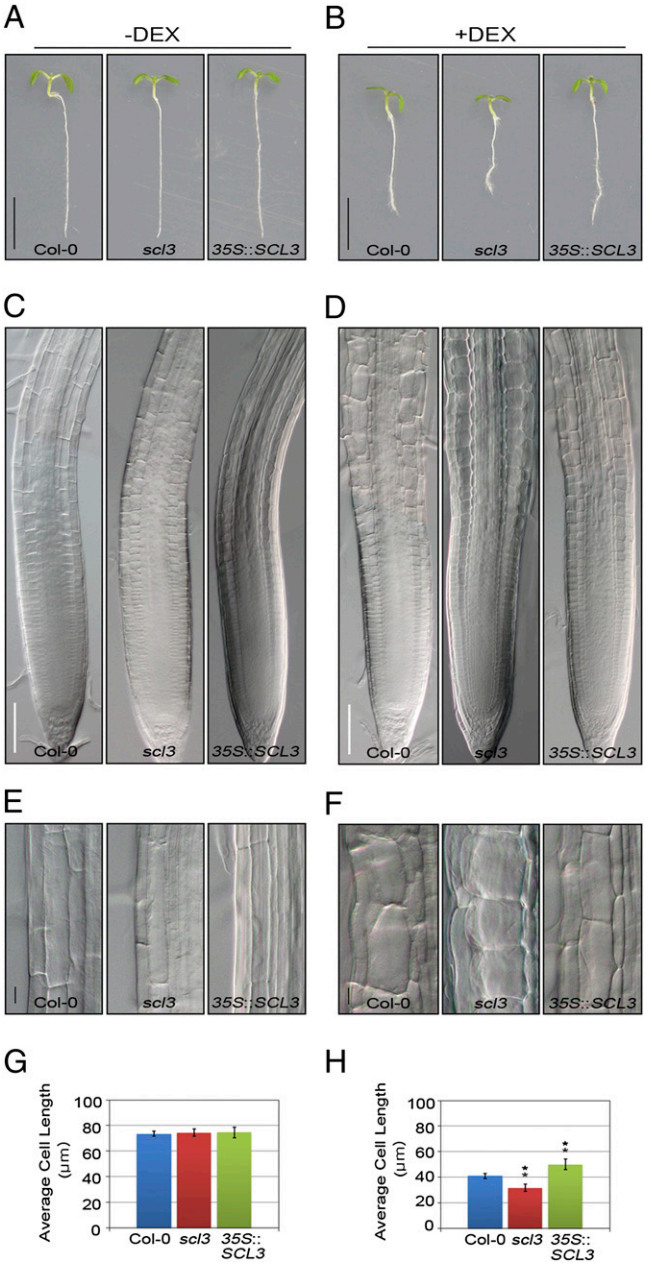

Fig. 2. Regulation of GA-mediated root cell elongation by SCL3–DELLA interaction in the EDZ. The *pSCR::gai-GR-YFP* seedlings in Col-0, *scl3*, and *35S::SCL3* were grown in MS agar plates for 4 d, transferred to MS agar plates supplemented with or without 10 μM of DEX, and incubated for another 3 d (*A* and *B*) or for 12 h (*C–H*). In the absence of DEX (−DEX), neither inhibition of root growth nor cell elongation was observed (*A*, *C*, *E*, and *G*). In the presence of 10 μM of DEX (+DEX) (*B*, *D*, *F*, and *H*), *pSCR::gai-GR-YFP* seedlings in Col-0 showed inhibition of root growth and cell elongation. Root growth and cell elongation of *pSCR::gai-GR-YFP* seedlings in *scl3* were more severely inhibited. Notably, overexpression of *SCL3* (*pSCR::gai-GR-YFP* in *35::SCL3*) suppressed the inhibition of root growth and cell elongation. As a result, the average cell length is reduced significantly in *scl3*, whereas increased in *35S::SCL3* ($n > 20$) (*H*). (Scale bars, 5 mm in *A* and *B*; 30 μm in *C* and *D*; and 10 μm in *E* and *F*, respectively.) Statistical significance of differences was determined by Student's *t* test (error bars: SE, $^{**}P < 0.01$).

SCL3 and Bioactive GA Levels Modulate the Timing and Extent of the Formative Division for Ground Tissue Maturation. The asymmetric cell division that generates cortex and endodermis is established during embryogenesis and perpetuated in postembryonic development (31). At later stages, *Arabidopsis* seedlings undergo the additional formative division giving rise to endodermis and another cortex (MC) for ground tissue maturation (21). Recent work demonstrated that the GA pathway, in conjunction with the SHR/SCR module, controls the timing and extent of MC formation (2, 4). Our results that SCL3 serves as an integrator of the GA/DELLA and SHR/SCR pathways in the endodermis

raised the possibility that SCL3 plays a role in the regulation of MC formation. To test this possibility, we focused on the timing and extent of the formative division in WT, *scl3*, and *35S::SCL3* roots. In WT roots, occurrence of the formative division was first observed at 6 d postgermination (dpg), and half of the seedlings in a given population showed MC formation at 12 dpg under our experimental conditions (Fig. S6*A*). As roots became mature, *scl3* seedlings had already undergone the periclinal division (Fig. 3 *A* and *B*). For instance, only 12% of WT roots exhibited the formative ground tissue division, whereas 42% of *scl3* seedlings had already formed MC at 7 dpg (Fig. 3*E* and Fig. S6*A*). By contrast, *35S::SCL3* roots at 7 dpg exhibited a delay in occurrence of the formative division compared with WT roots (2 vs. 12%) (Fig. 3*E* and Fig. S6*A*). Interestingly, a decreased frequency of MC formation conferred by *35S::SCL3* had diminished from 9 dpg onward, implying that SCL3 is likely subject to its own regulation, as demonstrated by Zhang et al. (26) in this issue of PNAS. Our data indicate that SCL3 modulates the timing and frequency of the formative ground tissue division for MC formation.

Previously, it was shown that GA deficiency (either by PAC or *ga1-3*) caused premature MC formation, and conversely exogenous bioactive GA application delayed MC formation, indicating that bioactive GA levels regulate ground tissue maturation (2, 4). Thus, we next investigated the mutual relation between SCL3 and bioactive GA levels in the control of the timing and extent of the formative division. In the presence of PAC, WT roots showed precocious MC formation compared with the untreated WT roots at 7 dpg (24 vs. 12%) (Fig. 3*E* and Fig. S6 *B* and *C*). In the same GA-deficient condition, *scl3* roots also showed an increase in frequency of MC formation compared with the untreated *scl3* roots (64 vs. 42%) (Fig. 3 *B*, *C*, and *E*). Similarly, we observed an increased frequency of MC formation in *scl3 ga1-3* than in *ga1-3* roots (85 vs. 29% at 6 dpg) (Fig. 3 *F*, *G*, and *I*). These observations indicate that *scl3* and GA deficiency additively or synergistically cause an increased frequency of precocious MC formation. In contrast, *35S::SCL3* roots in GA-deficient conditions (by PAC or *ga1-3*) showed a resistant phenotype in the MC formation compared with WT and *scl3* (Fig. 3 *H* and *I*). In the presence of exogenous GA_3, only 18% of WT roots exhibited MC formation at 12 dpg compared with the untreated WT (50%) (Fig. 3*J* and Fig. S6 *D* and *E*). Similarly, when applied with exogenous GA_3, *scl3* roots showed a decreased frequency of MC formation at 12 dpg compared with the untreated *scl3* (30 vs. 62%) (Fig. 3 *D* and *J*). In addition, application of exogenous GA_3 further reduced the frequency of MC formation in *35S::SCL3* roots compared with the untreated *35S::SCL3* (18 vs. 34%) (Fig. 3*J*). Taken together, our results indicate that SCL3 and bioactive GA levels additively or synergistically regulate the timing and extent of the formative division for ground tissue maturation.

Maintenance of a Functional GA Signaling by SCL3–DELLA Interaction Regulates the MC Formation. Next, we interrogated genetically the mutual relation between SCL3 and GA response in MC formation. To this end, we generated *gai rga* double mutants (loss-of-

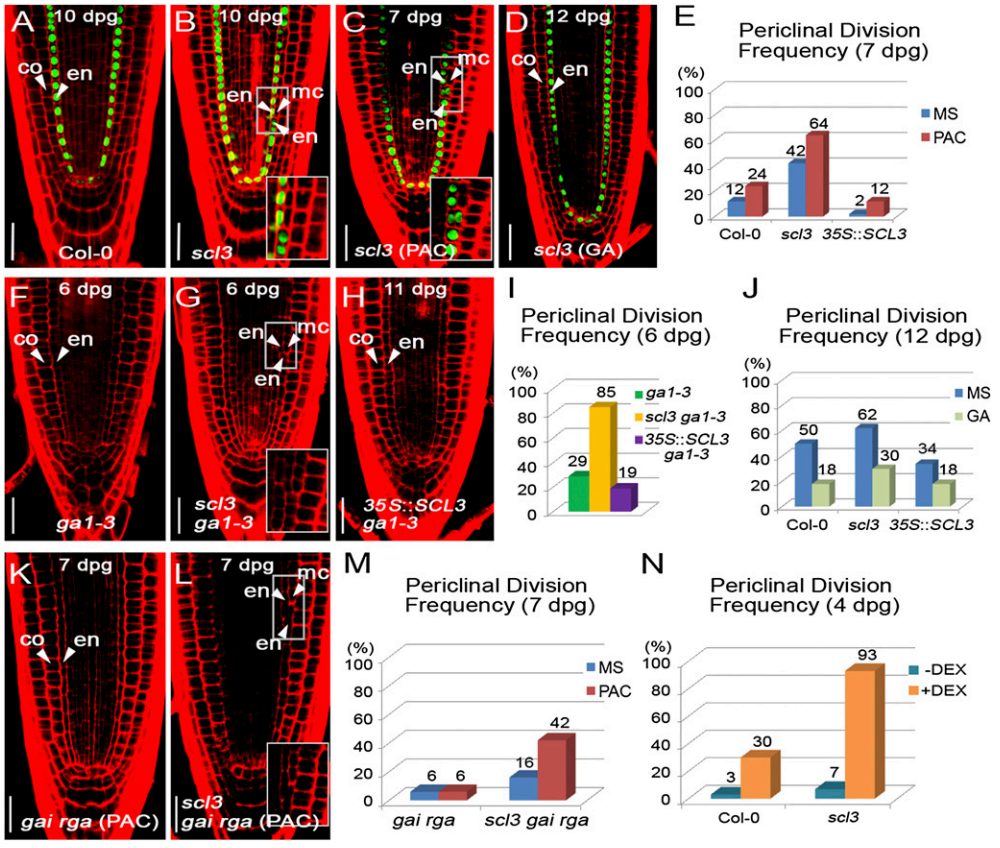

Fig. 3. Control of the formative ground tissue division in the MZ by the interaction of SCL3 and GA/DELLA pathways. MC formation in Col-0 (*A*) and *scl3* roots (*B*) at 10 dpg. The *scl3* root exhibited the additional formative periclinal division giving rise to endodermis (en) and middle cortex (mc). Arrowheads indicate endodermis (en), middle cortex (mc), and cortex (co), respectively. The nascent MC layer loses the endodermis identity marked by *pSCR::GFP-SCR* (*Insets* in *B* and *C*). Occurrence of the formative division in *scl3* in the presence of 1 μM of PAC (*C*) and 10 μM of GA_3 (*D*). The treatment with PAC increased, whereas exogenous GA_3 decreased the MC formation. (*E*) Quantitative evaluation of MC formation confirms that GA deficiency causes an increased frequency of MC formation in Col-0, *scl3*, and *35::SCL3*. In addition, loss and gain of SCL3 function also have opposing effects on the MC formation: increase in *scl3* and decrease in *35::SCL3*. (*F–I*) Occurrence of MC formation in *ga1-3*, *scl3 ga1-3*, and *35::SCL3* in *ga1-3*. Similarly, the frequency of the formative division was also modulated by loss and gain of SCL3 function. (*J*) Quantitative evaluation of MC formation confirms that exogenous bioactive GA, conversely, decreases the frequency in Col-0, *scl3*, and *35::SCL3*. (*K–M*) MC formation in *gai rga* and *scl3 gai rga* in the absence or presence of 1 μM of PAC. As predicted, *gai rga* roots were extremely resistant to PAC, whereas *scl3 gai rga* triple mutants showed far more increased occurrence of the formative division in the presence of PAC. (*N*) In the absence or presence of DEX, the *pSCR::gai-GR-YFP* seedlings in Col-0 and *scl3* were grown in MS agar plates for 4 d, transferred to MS agar plates supplemented with or without 10 μM of DEX, and incubated for 12 h. In the presence of DEX (+DEX), *pSCR::gai-GR-YFP* seedlings in *scl3* showed a dramatically increased frequency of MC formation compared with *pSCR::gai-GR-YFP* seedlings in Col-0. (Scale bar, 30 μm.)

function mutations in two major DELLAs) and *scl3 gai rga* triple mutants. Under standard conditions, *gai rga* roots showed a decreased frequency of MC formation compared with *scl3 gai rga* roots (6 vs. 16%) (Fig. 3*M*). In the presence of PAC, *gai rga* roots, as predicted, were extremely PAC resistant. Surprisingly, the difference in the frequency of MC formation between *gai rga* and *scl3 gai rga* became clearly evident at 7 dpg (6 vs. 42%) in the presence of PAC (Fig. 3 *K–M*). Our data indicate that a PAC-sensitive phenotype of *scl3 gai rga* in MC formation is likely conferred by loss of SCL3 function. The results from ref. 26 in this issue of PNAS also support the notion that SCL3 regulates its own expression to maintain a functional GA signaling. Next, we examined the MC formation in roots with defects in GA response by targeting the nondegradable rga in the endodermis under the *SCR* promoter (*pSCR::rga-GR-YFP*). In the presence of DEX, *pSCR::rga-GR-YFP* in WT showed an ~10-fold increase in the occurrence of the MC formation compared with the untreated seedlings (Fig. 3*N*). Notably, *pSCR::rga-GR-YFP* in *scl3* seedlings dramatically increased the frequency of the MC formation in the given population (Fig. 3*N*), further corroborating that SCL3 attenuates the endodermis-specific disruption of GA signaling conferred by the GA-insensitive rga function.

SCL3 and the SHR/SCR Module Regulate the MC Formation. At later stages, the radial patterning mutant *scr* also occasionally undergoes the additional formative division, resulting in the sporadic production of two ground tissue layers: an inner layer with fused characteristics of endodermis and cortex (termed mutant layer; mu) and an outer layer with cortex characteristics (Fig. 4 *A* and *B*) (2, 4). Thus, we genetically interrogated the mutual relation between SCL3 and the SHR/SCR module in the control of the formative division. We generated the double mutant combinations of *scl3 shr* and *scl3 scr*, respectively, and examined the MC formation. In *scl3 shr* double mutants, we observed no additional formative division regardless of PAC or GA_3, resulting in the single ground tissue layer (Fig. S7 *A–H*). These observations are in accordance with the previous results that SHR is required for the formative ground tissue division (2). Interestingly, *scl3 scr* roots showed much earlier occurrence of the formative division than in *scr*; at 4 dpg, 55% of *scl3 scr* had already generated two ground tissue layers (Fig. 4 *C–E* and Fig. S7*I*). By contrast, *35S::SCL3* in *scr* delayed the MC formation compared with *scr* roots (8 vs. 32% at 5 dpg) (Fig. 4*F* and Fig. S7*I*). In addition, we found no effects of *35S::SCL3* on the delay in the MC formation from 6 dpg onward (Fig. S7*I*), further corroborating that SCL3 regulates its own expression. Our findings indicate that *scl3* and *scr* additively or synergistically cause precocious MC formation.

Interplay of the SCL3, SHR/SCR, and GA/DELLA Modules Regulates the MC Formation. The results of our genetic experiments between *scl3* and mutants defective in the GA pathway and in the SHR/SCR module indicate that the two pathways converge on SCL3 to control the formative ground tissue division. To interrogate the interplay of the GA/DELLA and SHR/SCR pathways, we examined the MC formation in *scr*, *scl3 scr*, and *35S::SCL3* in *scr* in the presence of PAC or GA_3. Interestingly, all of the seedling combinations including *scr* single mutants were sensitive to PAC (10 nM), resulting in premature MC formation (Fig. 4*G*). In contrast, *scr*, *scl3 scr*, and *35S::SCL3* in *scr* showed inhibition of MC formation in the presence of GA_3 (Fig. 4*H*). These findings suggest that bioactive GA levels in combination with *scl3* and *scr* have a more profound effect on the MC formation. We further genetically analyzed the MC formation in *rga scr* (loss-of-function mutations in the two direct upstream genes of *SCL3*) in the absence or presence of SCL3 function. In the presence of PAC, the frequency of MC formation in both *rga scr* and *scl3 rga scr* increased substantially (Fig. 4*I*), indicating that a PAC-resistant phenotype of *rga* disappeared in the *scr* background. Notably, *scl3 rga scr* triple mutants exhibited a dramatically increased MC formation even in the absence of PAC compared with *rga scr* (96 vs. 18%), resulting in a lack of control in the MC formation (Fig. 4*I*). Our findings suggest that SCL3–DELLA interaction, in conjunction with the SHR/SCR module, plays an important role in the GA-mediated coordination of MC formation for ground tissue maturation.

Conclusions

Bioactive GAs promote growth by regulating cell division and cell elongation (1–8). Although the molecular components of GA signaling have become available and increasingly well characterized, still little is known about the spatial integration of the GA pathway to control these developmental processes in the plant life cycle. Here, we provide compelling evidence that SCL3 is a tissue-specific integrator of the GA pathway. Acting as a positive regulator by attenuating the DELLA repressors, SCL3 maintains GA homeostasis. Along the longitudinal root axis, the maintenance of GA signaling by SCL3–DELLA interaction in the endodermis plays distinct roles: coordination of cell elongation in the EDZ (Fig. S8*A*) and of MC formation in the MZ (Fig. S8*B*). In the EDZ, the loss of SCL3 function aggravates

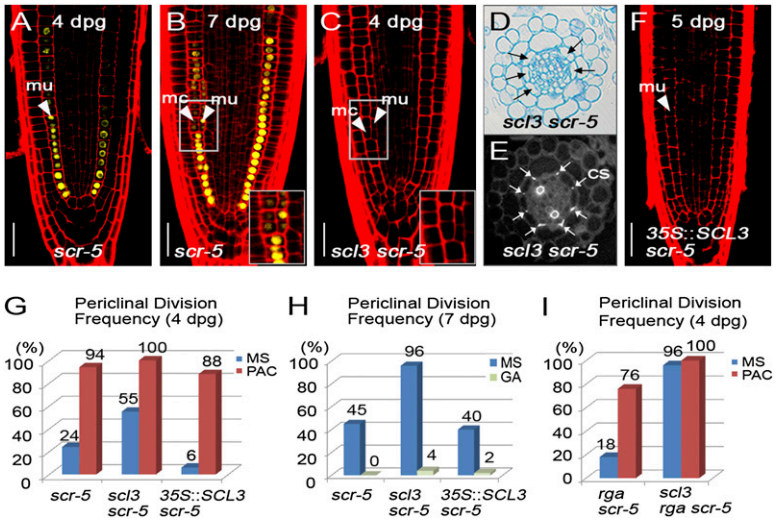

Fig. 4. Control of MC formation by the interplay of the SCL3, SHR/SCR, and GA/DELLA pathways. (*A* and *B*) MC formation in *scl3* roots marked by *pCO2::H_2B-YFP*. At 7 dpg, *scl3* roots showed the additional formative periclinal division giving rise to two ground tissue layers. Arrowheads indicate mutant layer (mu) and middle cortex (mc), respectively. (*C–E*) Occurrence of the formative division in *scl3 scr*. The *scl3 scr* double mutants exhibited uncoordinated formative divisions, which generate an inner layer with the Casparian stripe (CS) and an additional cortex layer. Black arrows indicate the additional formative division (*D*), and white arrows indicate the Casparian stripe (CS) (*E*). (*F*) *35::SCL3* in *scr* reduced the occurrence of the formative division (at 5 dpg). Quantitative evaluation of MC formation in *scr*, *scl3 scr*, and *35::SCL3* in *scr* in the absence or presence of PAC (*G*) or GA_3 (*H*). Bioactive GA levels additively or synergistically have influence on MC formation with opposing effects: increase by PAC and decrease by GA_3. (*I*) Occurrence of MC formation in *rga scr* and *scl3 rga scr* in the absence or presence of 1 μM of PAC. The *scl3 rga scr* roots showed a far more increased frequency of the formative division regardless of PAC. (Scale bar, 30 μm.)

uncoordinated cell expansion caused by endodermis-specific disruption of GA signaling, whereas the gain of SCL3 function restores cell elongation. Intriguingly, the SCL3–DELLA interaction for the maintenance of the GA pathway also employs the SHR/SCR module in the MZ to control MC formation. In the MZ, *scl3* roots show an increase of MC formation, whereas roots of *35S::SCL3* delay the timing and extent of the ground tissue division. Moreover, bioactive GA levels in combination with the SHR/SCR module additively or synergistically act in the control of MC formation: promotion by GA deficiency and inhibition by exogenous bioactive GA. Notably, *scl3 rga scr* triple mutants exhibited a lack of control in the formative division even under normal conditions, resulting in premature MC formation. Thus, our results reveal the network of the GRAS transcription regulators (SCL3, DELLAs, and SHR/SCR) in the endodermis of the MZ for the coordination of MC formation. This study and Zhang et al. (26) in this issue of PNAS shed light on how GA homeostasis is achieved and how the maintenance of GA signaling controls developmental processes in roots. Together with recent work in the shoot, in which the brassinosteroid signaling pathway in the epidermis modulates shoot growth (32), our study provides a molecular framework for the cell/tissue-specific integration of hormone signaling pathways in plant growth and development.

Materials and Methods

Plant Material and Growth Conditions. All plants were of *A. thaliana* Columbia (Col-0) background except for *della* quintuple mutant in Landsberg *erecta* (*Ler*). Origins of mutant and transgenic lines are described in *SI Materials and Methods*. Seeds were sterilized and incubated as previously described (29, 33). Primers used for genotyping are listed in Table S1.

Treatment and Root Assay. Root growth analysis was performed as described previously (6). For detailed description on the analysis of root meristem size and periclinal division, see *SI Materials and Methods*.

Molecular Cloning and Transgenic Plants. To generate pSCL3::GUS, pSCL3::SCL3-GFP, and 35S::SCL3, the Gateway recombination cloning technology (Invitrogen) was used as described previously (29, 33). Primers used for plasmid construction are listed in Table S2. For detailed description on plasmid construction and transgenic plant production, see *SI Materials and Methods*.

Expression Analysis. Total RNA extraction and reverse transcription-associated quantitative RT-PCR (qRT-PCR) were performed as described previously (29, 33). Primers used for qRT-PCR are listed in Table S3. mRNA in situ hybridization was performed as described previously (19, 20). (See *SI Materials and Methods* for detailed procedures.)

Histology and Microscopy. GUS histochemical staining was performed and visualized as described previously (29, 33). Confocal laser scanning microscopy was performed as described previously (29). (See *SI Materials and Methods* for detailed description.)

ACKNOWLEDGMENTS. We thank Philip Benfey, Malcom Bennett, John Celenza, Renze Heidstra, Ben Scheres, Tai-ping Sun, Susana Ubeda-Tomás, the *Arabidopsis* Biological Resource Center, and the Nottigham *Arabidopsis* Stock Center for materials. We are grateful to Tai-ping Sun for sharing unpublished results and critical reading of the manuscript. We also thank Philip Benfey for critical reading and suggestions. This work was supported by National Research Foundation Grant 2009-0079304/R0602494 (to M.M.L.) and Grant NRF2009-0077753 (to J.L.) and by the Konkuk University (J.L.).

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