

DELLA protein functions as a transcriptional activator through the DNA binding of the INDETERMINATE DOMAIN family proteins

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DELLA protein is a key negative regulator of gibberellin (GA) signaling. Although how DELLA regulates downstream gene expression remains unclear, DELLA has been proposed to function as a transcriptional activator. However, because DELLA lacks a DNA-binding domain, intermediate protein(s) mediating the DELLA/DNA interaction are believed to be necessary for activating DELLA target genes. Here, using yeast hybrid screenings, we identified five members of INDETERMINATE DOMAIN (IDD) protein family which bind physically to both DELLA and the promoter sequence of the GA-positive regulator *SCARECROW-LIKE 3* (*SCL3*), which previously was characterized as a DELLA direct target gene. Transient assays using *Arabidopsis* protoplasts demonstrated that a luciferase reporter controlled by the *SCL3* promoter was additively transactivated by REPRESSOR of *ga1-3* (RGA) and IDDs. Phenotypic analysis of transgenic plants expressing AtIDD3 (one of the 16 IDDs in the *Arabidopsis* genome) fused with the plant-specific repression domain (SRDX) supported the possibility that AtIDD3 is positively involved in GA signaling. In addition, we found that *SCL3* protein also interacts with IDDs, resulting in the suppression of its target gene expression. In this context, DELLA and *SCL3* interact competitively with IDD proteins to regulate downstream gene expression. These results suggest that the coregulators DELLA and *SCL3*, using IDDs as transcriptional scaffolds for DNA binding, antagonistically regulate the expression of their downstream targets to control the GA signaling pathway.

transcription factor | gibberellin feedback regulation | coactivator/corepressor exchange regulation system

Gibberellins (GAs) are diterpene phytohormones that regulate many cellular and developmental events such as cell elongation, leaf expansion, flowering, pollen maturation, and the transition from vegetative growth to flowering (1–4). Several protein factors involved in GA signaling have been identified. Among these, DELLA protein is a key player in the regulation of GA responses. DELLA proteins are characterized by a DELLA/TVHYNP motif at the N terminus and a GRAS domain [named after its first three members: GA INSENSITIVE (GAI), REPRESSOR of *ga1-3* (RGA), and SCARECROW (SCR)] at the C terminus, placing DELLAs within the GRAS family of transcriptional regulators. GRAS-domain transcription factors have diverse functions in growth and development. Recent intensive studies revealed how GA is perceived by the GA receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1) and how the perceived GA signal is transmitted to DELLA. By binding to active GAs, GID1 acquires the ability to interact with DELLA, allowing further interaction with an F box protein, SLEEPY1/GID2. DELLA is polyubiquitinated by E3 ubiquitin-ligase SCF^{SLY1/GID2} and finally is degraded through the 26S proteasome. However, how

DELLA regulates downstream gene expression in GA signaling has remained unclear.

In *Arabidopsis*, five DELLA genes have been identified; GAI, RGA, and three RGA-LIKE proteins (RGL1, RGL2, and RGL3) (1–4). Rice and barley each have a single DELLA protein, SLENDER1 (SLR1) and Slender1 (Sln1), respectively (1–4). Previous studies have predicted dual functions for DELLAs in regulating downstream genes and allowing GA to regulate various developmental processes (3). One possible mode of function is that DELLA interacts with other transcription factors to inhibit their DNA-binding and transcriptional activities. For example, *Arabidopsis* DELLA interacts with PHYTOCHROME INTERACTING FACTOR 3 and 4 (PIF3 and PIF4) and blocks their DNA-binding abilities to their target gene promoters, resulting in short hypocotyls in light-grown plants (5, 6). Likewise, DELLA is reported to interfere with several components of hormonal and developmental signaling pathways through protein–protein interaction (3, 4).

On the other hand, Zentella et al. (7) and Gallego-Bartolomé et al. (8) have used transcriptome analyses in transgenic plants expressing gain-of-function versions of DELLA to identify DELLA target genes. More recent work has demonstrated that DELLA

Significance

Gibberellin (GA)-dependent degradation of DELLA protein, a key negative regulator, is essential for GA action. However, it is unclear how DELLA regulates downstream gene expression. Although possessing strong transactivation activity, DELLA lacks a DNA-binding domain. Therefore, a model has been proposed in which DELLA acts as a transcriptional coactivator with another transcription factor or factors containing a DNA-binding domain. Here, we show that some members of the INDETERMINATE DOMAIN (IDD) protein family are such intermediate proteins. The DELLA/IDD complex up-regulates the expression of the GA-positive regulator *SCARECROW-LIKE 3* (*SCL3*). Meanwhile, *SCL3* protein also interacts with IDD proteins to suppress its own expression. We propose that a coregulator exchange system between DELLA (as coactivator) and *SCL3* (as corepressor) regulates the expression of *SCL3*.

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interacts with the core subunit of chromatin remodeling factor SWI/SNF to increase the transcription of DELLA target genes (9). Furthermore, Hirano et al. (10) have demonstrated that the N-terminal DELLA/TVHYNP motif of the rice DELLA, SLR1, possesses transactivation activity. The transactivation activity of mutated SLR1s observed in yeast corresponds well to the severity of dwarfism in rice plants overexpressing mutated SLR1, indicating that SLR1 suppresses plant growth through its transactivation activity. Thus, DELLA also functions as a transactivator *in planta*. Because DELLA is thought not to possess a DNA-binding domain (DBD), one or more other transcription factors may serve as an intermediate protein between DELLA and DNA to up-regulate their downstream genes. However, such intermediate proteins have not yet been identified.

To identify intermediate proteins mediating DELLA/DNA interaction, we conducted yeast two-hybrid (Y2H) screening using RGA, an *Arabidopsis* DELLA, as bait against a transcription factor library. We also conducted yeast one-hybrid (Y1H) screening using the promoter of a GA-positive regulator, *SCARECROW-LIKE 3* (*SCL3*), which is a putative direct target of RGA (7, 11). From these screenings we identified five transcription factors belonging to the C2H2 zinc finger family that interact with both the RGA protein and *SCL3* promoter. Interestingly, all the candidates belong to a single subfamily, the INDETERMINATE DOMAIN (IDD) family. Subsequent experiments confirmed that RGA uses these IDD proteins as transcriptional scaffolds to up-regulate *SCL3* expression for GA signaling regulation. Furthermore, we found that the *SCL3* protein interacts competitively with IDD against RGA and interferes with transactivation by the RGA/IDD complex. In conclusion, we propose a coactivator/corepressor exchange regulation system in which DELLA and *SCL3* are used as a transcriptional coactivator and a corepressor, respectively, and IDDs are used as transcriptional scaffolds to regulate the expression of *SCL3* and other genes in GA signaling.

Results

Members of the IDD Family Interact with both DELLA and the Promoter Region of *SCL3*. One possible reason for the failure to identify DELLA/DNA-mediating factors to date may be that DELLA has strong self-transactivation activity in the N-terminal DELLA/TVHYNP motif, complicating screening for its interacting proteins by a Y2H approach. However, we previously identified the GRAS domain, which is separated from the transactivation domain in the N-terminal region of DELLA, as the region through which DELLA interacts with other proteins for DNA binding (10). Therefore, a Y2H screen using the GRAS domain lacking self-transactivating activity is likely to return fewer false-positive clones but retain the ability to identify genuine partners of DELLA that are important for DNA interaction and transactivation of DELLA targets. Thus, we used a truncated version of RGA (RGA-GRAS) lacking the N-terminal region (amino acids 186–587) as bait construct for Y2H screening. Another considerable problem is that the available cDNA libraries often do not include the transcription factors of interest because of their low expression levels. To overcome this drawback, we used a cDNA library composed only of transcription factors, which covered ~75% of all *Arabidopsis* transcription factors (12). By this approach, using Y2H and Y1H screenings, we identified five clones encoding proteins belonging to the C2H2 type zinc finger superfamily.

Interestingly, all five C2H2 type zinc finger superfamily proteins identified by the Y2H and Y1H screens also belong to the IDD subfamily. The *Arabidopsis* genome encodes 16 IDD proteins, collectively designated AtIDDs (13). The proteins of this subfamily have a characteristic domain, the IDD, which includes traditional and irregular zinc finger motifs at the N terminus that possess DNA-binding ability, as well as two other conserved

motifs at the C terminus (Fig. S1) (13, 14). In Y2H screens, the identified IDD proteins AtIDD3 (AT1G03840), AtIDD4 (AT2G02080), AtIDD5 (AT2G02070), AtIDD9 (AT3G45260), and AtIDD10 (AT5G03150) interacted with RGA-GRAS (Fig. 1A). Furthermore, we confirmed that AtIDD3 interacts with intact RGA as well as with other DELLA members (Fig. S2). These IDD proteins also bound to the promoter region of *SCL3* (Fig. 1B). *SCL3*, which also is a GRAS family member, is thought to act as a tissue-specific integrator of the GA pathway in the root endodermis by antagonizing DELLA, and its expression is positively regulated by RGA through indirect interaction between RGA and its promoter sequence (11, 15). The interaction of these IDD proteins with RGA *in vivo* was confirmed by bimolecular fluorescence complementation (BiFC) assays (Fig. 1C). A strong fluorescence signal was observed in the

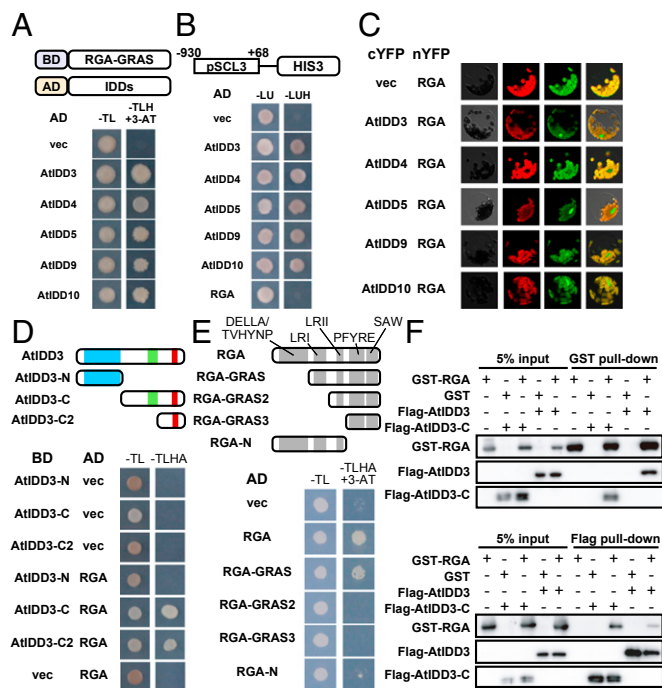


Fig. 1. Some members of the IDD protein family interact with RGA protein and the promoter sequence of *SCL3*. (A) Interactions between RGA-GRAS and IDDs in a Y2H assay. Schematic representations of the construct, BD-RGA-GRAS, and AD-IDDs are shown in the upper panel. AD, GAL4-activating domain; BD, GAL4-binding domain; -TL, synthetic complete medium lacking Trp and Leu; and His; -TLH, synthetic complete medium lacking Trp, Leu, and His; +3-AT, 3-aminotriazole, which is a competitive inhibitor of HIS3 enzyme. The -TLH +3-AT medium contained 10 mM 3-AT. (B) Interaction between IDDs and the promoter of *SCL3* (pSCL3) in a Y1H assay. Schematic representations of the construction, pSCL3::HIS3 is shown in the upper panel in which the numbers correspond to the distance from the transcription start site. -LU, synthetic complete medium lacking Leu and Ura; -LUH, synthetic complete medium lacking Leu, Ura, and His. (C) BiFC analyses testing the interaction between IDDs and RGA in *Arabidopsis* mesophyll protoplasts. Individual and merged images of YFP (green) and chlorophyll autofluorescence (red) as well as differential interference contrast images of protoplasts are shown. (D) Domain analyses of AtIDD3 in Y2H assays. The upper panel indicates AtIDD3 and its derivatives showing the conserved domain/motifs by color: N-terminal ID domain (light blue box) and two C-terminal motifs, MSATALLQKAA (light green box) and TR/LDFLG (red box). -TLHA, synthetic complete medium lacking Trp, Leu, His, and adenine. (E) Domain analyses of RGA in Y2H assays. The upper panel indicates RGA and its derivatives. The selection medium contained 50 mM 3-AT. (F) In vitro pull-down assays assessing physical interaction between RGA and AtIDD3. AtIDD3-C indicates the truncated AtIDD3, as used in D. GST-RGA fusion protein or GST (Upper) and Flag-AtIDD3 or Flag-AtIDD3-C (Lower) were used as bait.

nucleus of *Arabidopsis* mesophyll protoplasts that were cotransfected with RGA-YFP^N and IDD-YFP^C but not in protoplasts transfected with the vector control.

Next, we analyzed a domain of AtIDD3 necessary for interaction with RGA (Fig. 1D). AtIDD3 containing only the N-terminal region, including the DBD (AtIDD3-N, amino acids 1–229) did not interact with DELLA, whereas AtIDD3 variants containing only C-terminal regions (AtIDD3-C, amino acids 230–506, and AtIDD3-C2, amino acids 401–506) were able to interact with DELLA. These results suggest that IDD proteins can interact simultaneously with DNA and DELLA, unlike other reported DELLA-interaction partners whose interaction with DELLA abolishes their DNA-binding activities (3–6).

We also performed domain analysis of RGA for interaction with AtIDD3 (Fig. 1E). The GRAS domain of RGA can be subdivided into five distinct motifs: leucine-rich region I (LRI), VHID, leucine-rich region II (LRII), PFYRE, and SAW (16). The entire RGA and RGA-GRAS containing these five motifs interacted with AtIDD3, whereas RGA-GRAS2, which lacks only the LRI domain, could not. RGA-N, which contains the DELLA/TVHYNP, LRI, and LRRII domains, also was unable to interact with AtIDD3. These results indicate that the LRI domain of RGA is essential but not sufficient for the interaction between RGA and AtIDD3 and that at least one other C-terminal region is necessary. In vitro pull-down assays also confirmed the interaction between GST-tagged full-length RGA and Flag-tagged AtIDD3 regardless of the DBD of AtIDD3 (Fig. 1F). Taken together, these results strongly suggest that the AtIDD3, -4, -5, -9, and -10 members of the IDD family are able to mediate the regulatory function of RGA for the expression of *SCL3*.

The DELLA/IDD Complex Promotes the Expression of *SCL3*. A transient reporter assay was used to study the transcriptional activity of the DELLA/IDD complex, in which a firefly luciferase (fLUC) reporter under the control of the 1-kb 5' upstream promoter region of *SCL3*, with or without effector plasmids (enhanced 35S::AtIDD3, -4, -5, -9, -10 and RGA), was introduced into *Arabidopsis* protoplasts (Fig. 2A). When protoplasts were transfected with the fLUC reporter alone, only trace fLUC activity was detected (Fig. 2B), whereas cotransfection of the reporter and IDDs (–RGA), resulted in a significantly increased fLUC activity. Cotransfection of the reporter and both IDD and RGA plasmids (+RGA) again was able to produce substantially higher fLUC activity, which was dependent on the IDD protein variant used. These results demonstrate that IDDs function as intermediate proteins for the transcriptional activation of *SCL3* by RGA, whereas IDDs alone possess only a small transcriptional activation activity. To localize target sites of the DELLA/IDD complex in the *SCL3* promoter, we conducted a transient reporter assay using a series of truncated *SCL3* promoters together with an AtIDD3 protein fused with a transcriptional activation domain from viral protein 16 (VP16) replacing RGA (IDD3-VP) (Fig. 2C). High fLUC activity was observed when the promoter was –183 bp (or more) upstream of the transcription start site, but promoters less than –100 bp upstream of the transcription start site displayed no fLUC activity, indicating that the region from –183 to –100 bp upstream from the transcription site is essential for transcriptional activation of *SCL3* by AtIDD3. In addition, because VP16 can substitute for RGA, RGA likely acts as a transcriptional activator rather than by strengthening the binding between IDD and DNA. We performed the same experiment using AtIDD3 and RGA (instead of IDD3-VP) as effectors (Fig. 2D) and confirmed that the region from –183 to –100 is essential for the transcription of *SCL3* by the DELLA/IDD complex.

To determine which DNA sequences within the *SCL3* promoter are targeted by the DELLA/IDD complex, we searched for sequences homologous to the consensus target motifs of IDD

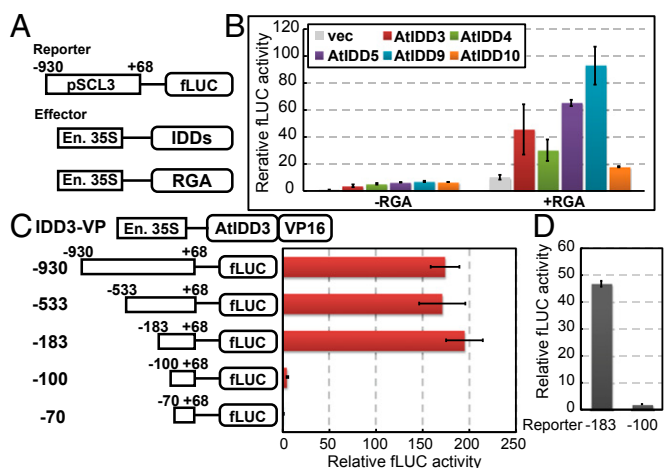


Fig. 2. The effects of IDDs and RGA on the expression of *SCL3*. (A and B) A transient reporter assay was used to examine the transactivation effects of IDDs and RGA on the expression of *SCL3* in protoplasts from *Arabidopsis* T87 suspension-cultured cells. (A) Schematic representations of the effector and reporter constructs. (B) Bars marked –RGA represent experiments in which the reporter, IDD effector plasmid, and empty RGA effector plasmids were cotransfected. Bars marked +RGA correspond to experiments in which each of the IDDs, RGA, and reporter plasmids were cotransfected. As a negative control, an empty effector vector (vec) was used in place of the IDD plasmids. The relative activity caused by vector control (far left bar) was set as 1. Results represent the means of three experiments; error bars represent SD. En. 35S, enhanced 35S promoter whose details are described in *SI Materials and Methods*. (C) Transient reporter assay in cultured cell protoplast to determine where AtIDD3 exerts its effect on the *SCL3* promoter. Schematic representations of the effector construct, IDD3-VP16, and *SCL3* promoter deletion are shown on the left. (D) Transient reporter assays using AtIDD3, RGA, and the –183 and –100 *SCL3* promoter constructs. The AtIDD3 and RGA constructs were cotransfected as effectors. The relative activity caused by vector control was set as 1.

homologs *ZmID1*, *AtIDD8*, and *OsIDD10* (14, 17, 18) and found one candidate carrying AGACAA from –111 to –106 in the *SCL3* promoter (Fig. S3). The physical interaction between AtIDD3 and the biotinylated DNA fragments containing the region from –123 to –94 was confirmed by an EMSA, whereas the same DNA fragment in which the target sequence (AGACAA) is replaced by CTCAGG lost interacting activity (Fig. S3). These data indicate that RGA positively regulates the expression of *SCL3* through interaction with AtIDD3, which binds to specific DNA sequences containing AGACAA as a core motif.

***atidd10jkd-4* and AtIDD3-SRDX Plants Induced Characteristic Phenotypes Related to GA.** Welch et al. (19) reported that AtIDD3 (MAGPIE; MGP) and AtIDD10 (JACKDAW; JKD) are involved in the root development, which also is controlled by DELLA and *SCL3* (15). Therefore, we compared the root phenotype of *atidd10jkd-4* (SALK_054242C) with WT and GA-deficient plants. We observed an unusually high frequency of periclinal cell division in the root endodermis of *atidd10* plants, which also was observed in the WT plants under GA-deficient conditions (Fig. 3A and B and Fig. S4) and in GA-deficient and *scl3* mutants, as previously reported (15). However, we were unable to observe additional *atidd10* phenotypes typical of GA-related defects or any such phenotypes in plants with down-regulated AtIDD3 expression as previously reported (17, 19), perhaps because of the partially redundant activities of IDDs. Therefore, we generated transgenic plants overexpressing AtIDD3 (IDD3-ox) and plants in which the IDD function was suppressed by the introduction of AtIDD3 fused with the plant-specific repression domain (IDD3-SRDX) (20). IDD3-SRDX plants should be

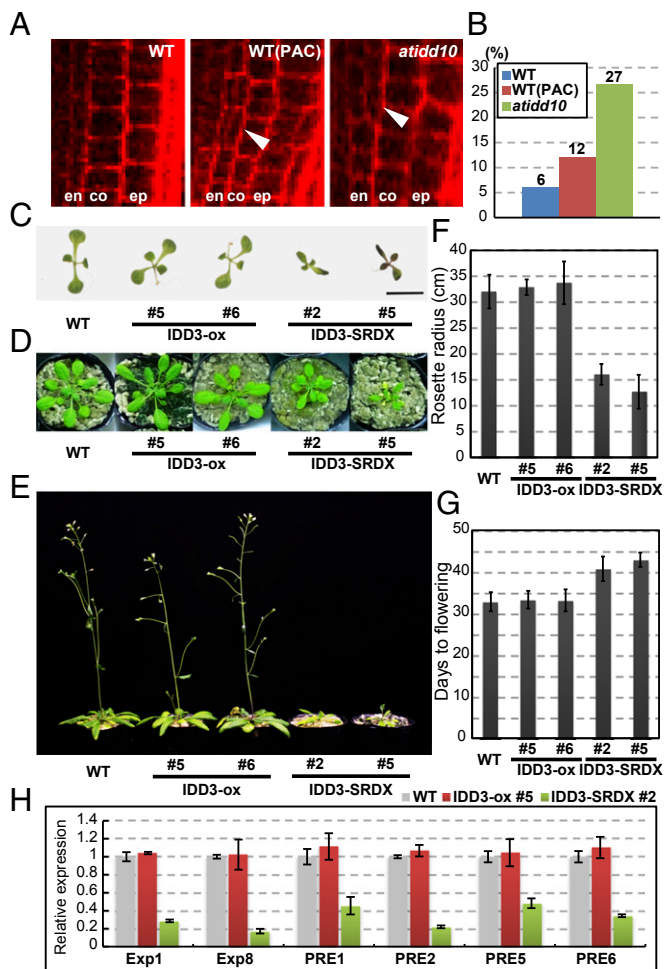


Fig. 3. The mutation of AtIDD10 and the expression of AtIDD3 fused with SRDX reduce GA signaling. (A) Higher-magnification view of periclinal cell division in the endodermis. Whole images are shown in Fig. S4. Arrowheads indicate periclinal cell division in the endodermis. en, endodermis; ep, epidermis; co, cortex; WT(PAC), WT plants treated with the GA biosynthesis inhibitor paclobutrazol. (B) The frequency of periclinal cell division in the endodermis at 4 d after germination. (C and D) Leaf phenotype in 1-wk-old (C) and 4-wk-old (D) plants. AtIDD3 in the IDD3-ox plants and AtIDD3 fused with SRDX in the IDD3-SRDX plants were both driven by the 35S promoter. (E) Late flowering of IDD3-SRDX in 6-wk-old plants. (F and G) Quantification of data in D and E. Results represent the means of more than three samples; error bars indicate SD. (H) Relative transcript levels in the leaves of 1-wk-old seedlings. The housekeeping gene *glyceraldehyde-3-phosphate dehydrogenase C subunit* (GAPC), whose expression is not responsive to GA, was used to normalize different samples. Biological triplicate samples were averaged. Error bars indicate SD of the means.

phenocopies of mutants with defects in which multiple IDD3s (including AtIDD3, -4, -5, -9, and -10) share the common target sequence; therefore it is considered useful for investigating the physiological functions of the redundant transcription factors (20). IDD3-ox plants were rarely different from WT; however, the IDD3-SRDX plants had smaller leaves, shorter roots, and delayed flowering as compared with WT plants (Fig. 3 C–G and Fig. S5A), mimicking the previously reported phenotypes of GA-signaling and -deficient mutants (1–4, 21, 22). Furthermore, cell numbers in the first true leaves were reduced in both IDD3-SRDX plants and paclobutrazol-treated WT plants as compared with untreated WT plants (Fig. S5B), a finding that is consistent with GA promoting cell proliferation in young leaves (23). Taken together, these results suggest that IDD3s are related to plant GA

signaling. IDD3-SRDX also exhibited an abnormal leaf phenotype at 1 wk but not at 4 wk after germination; this phenotype did not seem to be related to GA signaling (Fig. 3 C and D). To confirm that these GA-related phenotypes in IDD3-SRDX were caused by repression of GA signaling, we analyzed the gene expressions of the *Expansin1* and -8, and *paclobutrazol resistance factor* (*PRE*) 1, 2, 5, and 6, which previously were reported to be up-regulated by GA (24, 25). Quantitative RT-PCR (qRT-PCR) analyses showed that the expression of the GA-regulated genes was reduced significantly in IDD3-SRDX plants but was not obviously changed in IDD3-ox plants (Fig. 3H). The *SCL3* expression pattern was similar in WT, IDD3-ox, and IDD3-SRDX plants (Fig. S6).

Given these results, we conclude that IDD proteins regulate the GA-signaling pathway by serving as an intermediate protein between DELLA and the region of the promoters of downstream genes containing the sequence AGACAA.

RGA and SCL3 Antagonize Each Other Through the Competition to Interact with IDD Proteins. Previous studies demonstrated that SCL3 protein functions antagonistically against RGA as a GA-positive regulator (11). Additionally, DELLA and SCL3 are both members of the GRAS family, although SCL3 lacks the DELLA domain, and DELLA interacts with IDDs via their GRAS domain, possibly suggesting that SCL3 interacts with its target DNA in a manner similar to that of the DELLA protein (11). This circumstantial evidence led us to speculate that SCL3 may interfere with the interaction between DELLA and IDDs by interacting directly with IDDs. Indeed, a physical interaction between SCL3 and IDD proteins was confirmed by Y2H (Fig. 4A) and BiFC assays (Fig. S7). Moreover, Y2H results have shown that the interaction of AtIDD3 with SCL3 occurs through their C-terminal domains, as is the case for DELLA (Fig. S8). We next performed a yeast three-hybrid (Y3H) assay to examine the competitive relationship between the RGA/IDD and SCL3/IDD interactions (Fig. 4B). The activity of a reporter gene *HIS3*, which indicates the interaction between SCL3 and AtIDD3, apparently was reduced in the presence of RGA. An *in vitro* pull-down assay demonstrated that an increasing abundance of RGA causes a decrease in the SCL3/AtIDD3 complex (Fig. 4C). These results indicated a competitive relationship between RGA and SCL3 for interaction with AtIDD3 rather than the formation of a triple RGA/SCL3/AtIDD3 complex. Such a competitive relationship also was demonstrated *in vivo*, in which protoplast *luc* activity promoted by the RGA/AtIDD3 complex was diminished by SCL3 expression (Fig. 4D). Taken together, these results suggest the SCL3 corepressor also is able to interact with IDDs, thus competing with the coactivator DELLA; this competition may result in a feedback loop mechanism for transcriptional activity of DELLA and SCL3 (Fig. 4E and Discussion).

Discussion

It has been suggested that GA-regulated growth is governed by the GA-dependent de-repression of a growth-repressive factor, DELLA, and that GA-dependent degradation of DELLA is a core mechanism in the GA-signaling pathway (1–4). In this context, Davière and Achard (3) proposed two possible functions of DELLA. The first is that DELLA functions to block the transcriptional activity of transcription factors by physical interaction, as for the PIF proteins. The other possibility is that DELLA functions as a transcriptional coactivator through interaction with another transcription factor(s) for DNA binding. We conducted Y1/2H screening from a library covering almost all *Arabidopsis* transcription factors to identify proteins that contain a DBD and that interact with both DELLA and the promoter sequence of *SCL3*, one of the known target genes of DELLA, both properties being consistent with a role as a DELLA transcriptional coactivator (7, 11). We identified five such proteins,

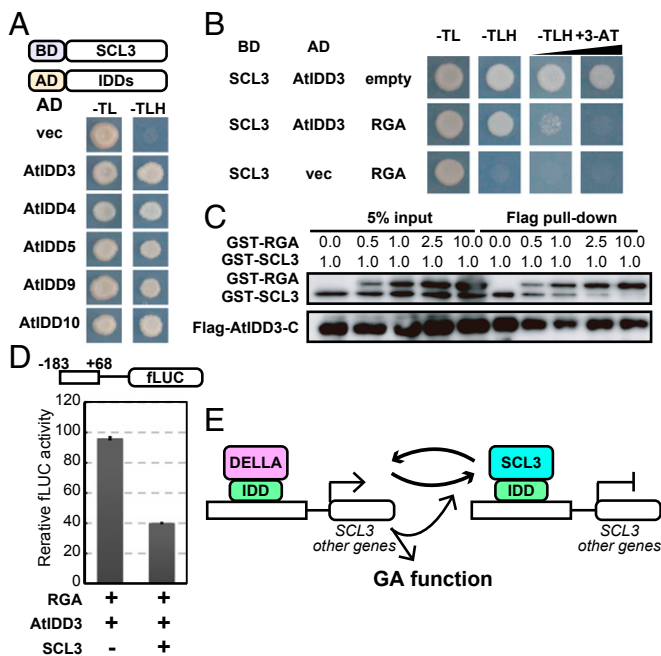


Fig. 4. SCL3 competes with DELLA and prevents its transactivation activity. (A) Interactions between IDEs and SCL3 in the Y2H assay. Schematic representations of the construct, BD-SCL3, and AD-IDDs are shown in the upper panel. (B) Y3H assay to determine the physical relationship between RGA, SCL3, and AtIDD3. The two lines on the right indicate yeasts on selection medium containing 5 and 10 mM 3-AT, respectively. (C) In vitro pull-down assays for determining the physical association of SCL3 and AtIDD3 and competition between RGA and SCL3 for AtIDD3. Flag-AtIDD3-C was used as bait. Numbers indicate the concentrations (in mM) of each protein in the reaction solution. (D) Transient reporter assay for identifying the effect of SCL3 on the transcriptional regulation of the DELLA/IDD complex. The -183 construct shown in Fig. 2C was used as the reporter. Results represent the means of three experiments; error bars indicate SD. The relative activity caused by vector control was set as 1. (E) A model for GA feedback regulation by the interaction among DELLA, SCL3, and IDD.

all of which were members of the IDD family (Fig. 1A and B). Further analyses confirmed that DELLA acts as a transcriptional coactivator through the IDD proteins (Fig. 2B–D and Fig. S3).

The IDD family is highly conserved protein family in angiosperms (13). Because the first IDD family gene identified (*ZmID1*) was isolated as a causal gene for a late-flowering mutant of maize (26), the function of this family has been discussed in the context of flowering time regulation. For example, a rice *ZmID1* homolog, *OsID1*, promotes flowering, as does *AtIDD8* (NUTCRAKER; NUC) (17, 27–29). Later, a functional role in gravitropism was reported for *AtIDD15* (SHOOT GRAVITROPISM 5; SGR5) and *OsID14* (LOOSE PLANT ARCHITECTURE1; LPA1) (30–32). To date, only one report has described physical interactions of *AtIDD1* (ENHYDROUS; ENY) with each of the five DELLA members (33). Feurtado et al. (33) also demonstrated that *AtIDD1* represses the expression of GA biosynthesis and signaling genes, including *SCL3*, and discussed the possibility that *AtIDD1* may disrupt the interaction between DELLA and other proteins, including PIFs. This proposed function of *AtIDD1* is different from our description that DELLA and IDEs cooperate to up-regulate their downstream genes. It is noteworthy that *AtIDD1* possesses a putative repression domain, whereas *AtIDD3*, -4, -5, -9, and -10 do not (34), suggesting that there are functional and physical differences between *AtIDD1* and the other IDD family members. In addition, although the library we used contains *AtIDD1* (12), we failed to isolate it in our Y2H screening; this result suggests that the binding affinity of *AtIDD1*

for RGA is likely to be lower than that of the other IDEs. Here, we propose the novel function of IDEs as intermediate proteins between DELLA and target gene promoters, acting to enhance gene expression. That at least five of the 16 *Arabidopsis* IDEs have been demonstrated to have such a function suggests this is a principal function of the IDD family proteins. However, because some of *IDD3-SRDX* phenotypes [e.g., the abnormal leaf phenotype at 1 wk after germination (Fig. 3C)] were not the same as those of GA-deficient mutants, IDEs also may work in other signaling pathways, in addition to GA signaling.

GA deficiency and *AtIDD10* knockout mutation increased the frequency of periclinal cell division in the root endodermis (Fig. 3A and B and Fig. S4) (15, 35). Recent work has demonstrated that *SCL3* also is involved in this division in the context of GA signaling (15). The IDD family members are expressed in the endodermal cell, where RGA and *SCL3* are expressed also (19, 36–39). Such overlapping expression of IDEs, RGA, and *SCL3* strongly supports our model in which DELLA, *SCL3*, and IDD proteins cooperate to control GA signaling in the endodermis by regulating downstream gene expression. Consistent with previous studies (11, 15), we considered the relationship of the three factors in this study: DELLA promotes the periclinal cell division in the root endodermis by repressing GA signaling; on the contrary, *SCL3*, a positive regulator of GA signaling, suppresses the periclinal cell division; and lack of *AtIDD10* causes a defect in DELLA-mediated feedback, thus resulting in increased cell division.

We found that RGA up-regulates the expression of *SCL3* by collaborating with IDEs (Fig. 2B–D and Fig. S3). Like DELLA, *SCL3* is a putative transcription factor of the GRAS family, but, unlike DELLA, *SCL3* acts as a positive regulator of GA signaling (11, 40). A previous study also has shown that *SCL3* interferes with the function of DELLA to repress transcription of GA biosynthesis genes as well as its own (*SCL3*) transcription (11). In this study, we identified an interaction between *SCL3* and IDEs (Fig. 4A and Fig. S7). We also found that RGA and *SCL3* interact competitively with IDEs to regulate the expression of *SCL3* (Fig. 4B–D). According to these results, we propose a novel feedback-loop system to regulate GA signaling (Fig. 4E). DELLA activates the expression of the downstream genes, including *SCL3*, by IDD-mediated interaction with their promoters. The subsequent increase in the abundance of *SCL3* protein results in an increase of the *SCL3*/IDD complex, decreasing the formation of the DELLA/IDD complex and consequent suppression of *SCL3* expression (Fig. 4E). This DELLA-*SCL3*-mediated feedback loop explains homeostatic regulation of protein levels of the downstream genes, including the positive regulator *SCL3*, resulting in homeostatic GA signaling. Therefore, the transcription activity of *AtIDD3* may depend on which coregulator it binds to. This model can explain the observations that the *IDD3-ox* plants were rarely different from WT (Fig. 3C–E). In fact, although the increase in IDD protein does not determine the level of *SCL3*, the balance between DELLA and *SCL3* protein level does. On the other hand, *IDD3-SRDX* plants exhibited a GA-deficient phenotype (Fig. 3C–E), which can be explained by the *IDD3-SRDX* fusion protein functioning as a repressor of the downstream gene expression to negatively regulate GA signaling regardless of the presence of coregulators.

To our knowledge, such a coactivator/corepressor exchange regulation system (Fig. 4E) has not been described previously for any plant signaling pathway. However, similar regulation systems have been well studied in animals (41). For example, estrogen nuclear receptor can act as a transcriptional activator or repressor by interacting with coactivators such as histone acetyltransferase p300 and a mediator recruiting RNA polymerase complex or with corepressors such as HDAC and NCoR (42). A similar system also has been observed in the regulation of cortisol biosynthesis (43) and the function of Liver X receptor, one of the nuclear oxysterol receptors (44). Rosenfeld et al. (41)

reported that this regulation system plays important roles in integrating signal-dependent programs of transcriptional responses at the molecular level in animal systems. Our findings demonstrated that the coactivator/corepressor exchange system should explain, at least in part, the intricate regulation of GA signaling in plants. Moreover, such a system may be involved in transcriptional regulation of other signaling pathway.

Materials and Methods

Yeast One-Hybrid/Two-Hybrid Screening. Yeast one-hybrid/two-hybrid (Y1/2H) screening was performed as previously described (12) with yeast strains AH109 harboring BD-RGA-GRAS and YM4271 harboring pSCL3::HIS3. Selection medium lacking histidine but containing 5 mM 3-amino-1,2,4-triazole (3-AT; Wako) or 10 mM 3-AT were used for Y1H and Y2H screening, respectively.

Transient Reporter Assays. Plasmids were extracted using the NucleoBond Xtra Midi Plus according to the manufacturer's instruction (Takara). Transient reporter assays using protoplasts prepared from *Arabidopsis* T87 suspension-cultured cells were performed and calculated as previously described (45). The fLUC activity was normalized according to the

humanized renilla luciferase activity in each assay, and the relative ratio was determined by comparing this ratio with that obtained with the empty vector. The mean relative ratios were calculated from three or four independent experiments.

Primers used in this study are listed in Table S1.

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