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Report

Step-by-Step Acquisition of the Gibberellin-DELLA Growth-Regulatory Mechanism during Land-Plant Evolution

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Summary

Angiosperms (flowering plants) evolved relatively recently and are substantially diverged from early land plants (bryophytes, lycophytes, and others [1]). The phytohormone gibberellin (GA) adaptively regulates angiosperm growth via the GA-DELLA signaling mechanism [2–7]. GA binds to GA receptors (GID1s), thus stimulating interactions between GID1s and the growth-repressing DELLAs [8–12]. Subsequent 26*S* proteasome-mediated destruction of the DELLAs promotes growth [13–17]. Here we outline the evolution of the GA-DELLA mechanism. We show that the interaction between GID1 and DELLA components from *Selaginella kraussiana* (a lycophyte) is GA stimulated. In contrast, GID1-like (GLP1) and DELLA components from *Physcomitrella patens* (a bryophyte) do not interact, suggesting that GA-stimulated GID1-DELLA interactions arose in the land-plant lineage after the bryophyte divergence (~430 million years ago [1]). We further show that a DELLA-deficient *P. patens* mutant strain lacks the derepressed growth characteristic of DELLA-deficient angiosperms, and that both *S. kraussiana* and *P. patens* lack detectable growth responses to GA. These observations indicate that early land-plant DELLAs do not repress growth in situ. However, *S. kraussiana* and *P. patens* DELLAs function as growth-repressors when expressed in the angiosperm *Arabidopsis thaliana*. We conclude that the GA-DELLA growth-regulatory mechanism arose during land-plant evolution and via independent stepwise recruitment of GA-stimulated GID1-DELLA interaction and DELLA growth-repression functions.

Results and Discussion

S. kraussiana and *P. patens* Possess Candidate GA-DELLA Signaling Components yet Lack Detectable Growth Responses to GA

DELLAs are a subset of the GRAS (GAI, RGA, and SCARECROW) family of candidate transcription factors [18]. We identified genes encoding DELLAs SkDELLA (from *S. kraussiana*) and PpDELLAa and PpDELLAb (from *P. patens*) (see Experimental Procedures). Phylogenetic analysis revealed that SkDELLA, SmDELLA (from the related *Selaginella moellendorffii*), PpDELLAa, and PpDELLAb are included within a monophyletic land-plant DELLA group (Figure S1A in the Supplemental Data available online). Angiosperm DELLAs contain in their N termini two highly conserved domains (I and II) that are necessary for GID1-DELLA interactions [10, 11, 19–21] (Figure 1A). SkDELLA contains divergent but conserved domains I and II (as does SmDELLA; Figure 1A; see Tables S1 and S2). PpDELLAa and PpDELLAb are more widely divergent (as is SpDELLA from the bryophyte *Sphagnum palustre*), with only small sections of domains I and II matching the overall consensus for these regions (Figure 1A; Tables S1 and S2). We also identified genes encoding *S. kraussiana* and *P. patens* gibberellin (GA) receptors (GID1s) (and related proteins) SkGID1, SmGID1, PpGLP1, and PpGLP2 (Figure S1B; see Experimental Procedures). Although SkGID1 and SmGID1 are clearly included in and basal to the clade that distinguishes gymnosperm and angiosperm GID1s, PpGLP1 and PpGLP2 are more substantially diverged (Figure S1B).

The GA-DELLA mechanism regulates growth and development throughout the angiosperm life cycle [22] but was not previously known to operate in basal ("ancestral") land plants. We found that exogenous GA_3 (which promotes angiosperm and gymnosperm growth [23]) did not detectably promote the growth of *S. kraussiana* (sporophyte; data not shown) or *P. patens* (gametophyte; Figure 1B). Furthermore, although the GA-biosynthesis inhibitor paclobutrazol (PAC [24]) inhibited the growth of *P. patens*, exogenous GA_3 did not reverse this effect (Figure 1B). Similarly, GA_3 did not reverse the inhibitory effect of PAC on the growth of *S. kraussiana* (data not shown).

Substantial DELLA deficiency confers derepressed growth, relatively rapid life-cycle progression, and resistance to the growth-inhibitory effects of PAC, salt stress, and the phytohormone abscisic acid (ABA) on *A. thaliana* [5, 25]. In contrast, we found that a DELLA-deficient *P. patens* strain (*Ppdellaa Ppdellab*) did not exhibit derepressed growth and did not display accelerated life-cycle progression (data not shown) or increased resistance to PAC, ABA-, or salt-induced growth inhibition (Figure 1B). Thus PpDELLAs do not repress the growth of *P. patens*, suggesting that DELLAs do not restrain bryophyte growth.

In addition to regulating the growth of angiosperms, GA regulates the levels of various angiosperm gene transcripts, such as those encoding GID1 GA receptors (e.g., [10]). We found that SkGID1-encoding *SkGID1* transcript levels were reduced in *S. kraussiana* plants treated with GA_3 (Figure 1C). Thus, GA_3 does not promote the growth of *S. kraussiana* or *P. patens*, and the lack of PpDELLAs does not derepress growth of *P. patens*. However, although *P. patens* growth may be responsive to GA forms other than GA_3, *S. kraussiana* *SkGID1* transcript levels are GA_3 responsive. This suggests that lycophytes exhibit a limited range of (non-growth) GA_3 responses.

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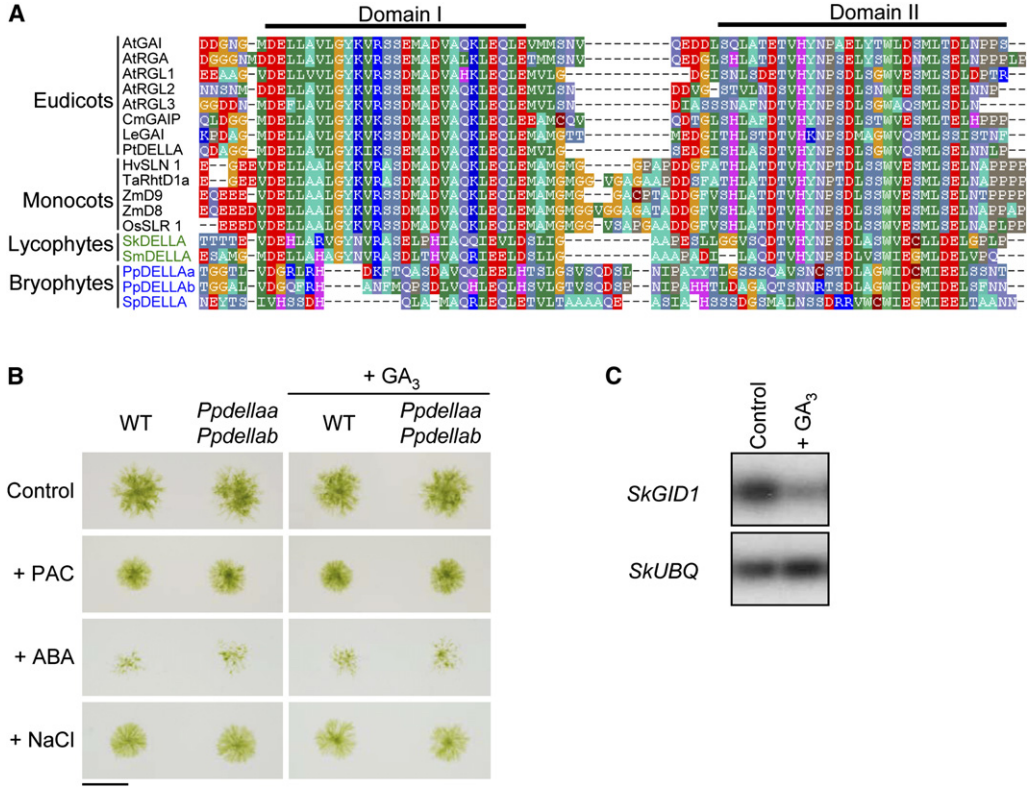

Figure 1. Initial Characterization of GA-DELLA Components and GA-Responses of the Basal Land Plants *S. kraussiana* and *P. patens*

(A) Alignment of N-terminal amino acid sequences (including domains I and II [19]) from angiosperm, lycophyte, and bryophyte DELLAs. Although domains I and II of SkDELLA and SmDELLA are clearly related to those of angiosperms, the more widely divergent N-termini of bryophyte DELLAs (PpDELLAa, PpDELLAb, and SpDELLA) only have recognizable domain I and II sequences toward the C-terminal end of each domain. The alignment was generated with BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Database sequence accession numbers are in Table S1, and the color coding of amino acid properties is in Table S2.

(B) Comparison of growth of *P. patens* (WT and *Ppdellaa Ppdellab*) in the presence or absence of 10 μM GA_3 in response to PAC (20 μM), ABA (5 μM), or NaCl (75 mM). PAC, ABA and NaCl are equally inhibitory to the growth of the WT and *Ppdellaa Ppdellab*, and exogenous GA_3 does not overcome this inhibition. For details of construction of the *Ppdellaa Ppdellab* mutant strain, see the Supplemental Experimental Procedures and Figure S2. Scale bars represent 5 mm.

(C) Comparison (via semiquantitative RT-PCR) of *SkGID1* transcript levels in *S. kraussiana* plants (and controls) treated with 100 μM GA_3. Ubiquitin-encoding transcripts (*SkUBQ*) provide loading control. The result is representative of three biological replicates.

GID1-DELLA Interactions Probably Arose Subsequent to the Divergence of the Bryophytes from the Land-Plant Lineage

We next investigated interactions between *S. kraussiana* and *P. patens* GID1s or GLPs and DELLAs. First studying within-species interactions, we found that *S. kraussiana* SkGID1 and SkDELLA components interact with one another and that this interaction, like the *A. thaliana* AtGID1c-AtRGA interaction [9, 10], was stimulated by GA_3 (Figure 2A). In contrast, the *P. patens* PpGLP1 and PpDELLAa components did not detectably interact with one another in the presence or absence of GA_3 (Figure 2A). Thus *A. thaliana* and *S. kraussiana* exhibit GA_3-stimulated GID1-DELLA interactions, whereas *P. patens* does not. The lack of a GA_3-stimulated PpGLP1-PpDELLAa interaction explains the absence of detectable GA_3 responses in *P. patens* (Figure 1B), whereas the GA_3-stimulated SkGID1-SkDELLA interaction presumably explains the transcript-level GA_3 response exhibited by *S. kraussiana* (Figure 1C).

GID1-DELLA interactions require dual affinities: GID1 affinity for DELLA, and DELLA affinity for GID1. Our studies of between-species GID1-DELLA interactions revealed the evolution of these two separate affinities. We found that PpGLP1 interacts strongly with SkDELLA (Figure 2A). Thus *P. patens* PpGLP1 interacts with a lycophyte DELLA but not with the bryophyte PpDELLAa of *P. patens* itself. We also found that neither SkGID1 nor AtGID1c interact with PpDELLAa (Figure 2A). Thus the affinity of the bryophyte GLP1 for DELLAs was preexistent and presumably preserved during subsequent evolution of the GID1 clade (i.e., retained in lycophyte and angiosperm GID1s). Conversely, the DELLAs evolved affinity for the GID1s sometime between the bryophyte and lycophyte divergences (as probably reflected in the differences between PpDELLAa and SkDELLA domains I and II; Figure 1A).

We also compared the GA enhancibility of between-species GID1-DELLA interactions and found that interactions involving AtGID1c or SkGID1 (AtGID1c-AtRGA, AtGID1c-SkDELLA, and SkGID1-SkDELLA) were GA_3 potentiated, whereas the interaction involving PpGLP1 and SkDELLA was not (Figure 2A). Although supported by only a single interaction (PpGLP1 with SkDELLA),

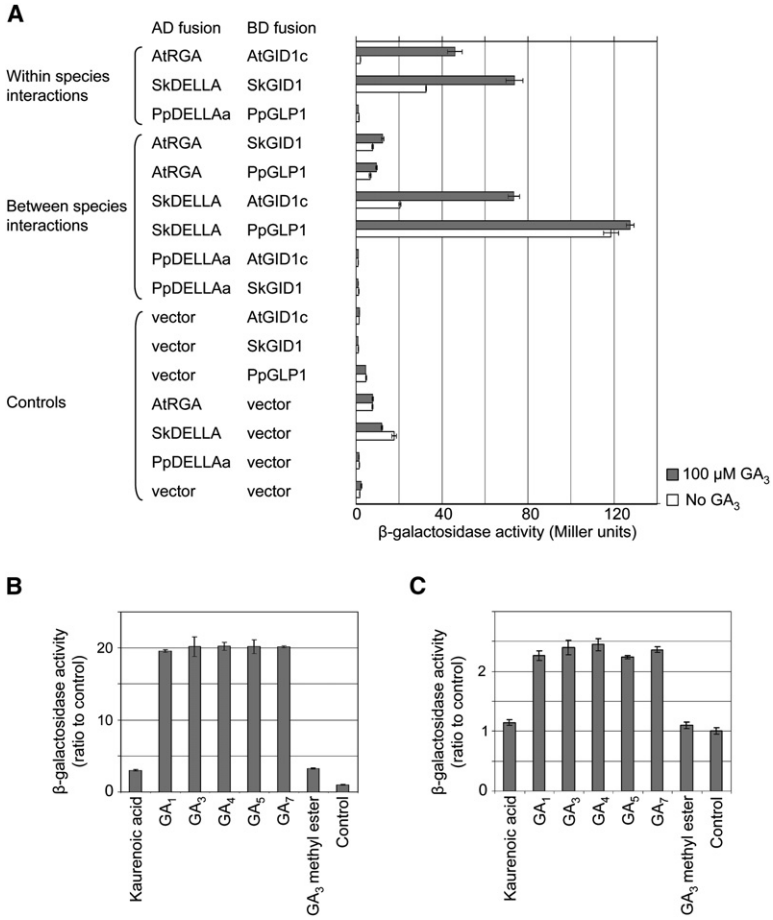

Figure 2. Detection of Lycophyte and Angiosperm GID1-DELLA Interactions

(A) Quantitation of within- and between-species GID1-DELLA interactions in the presence or absence of GA_3 (versus vector controls) by yeast two-hybrid assay. Results are expressed as means ± the standard deviation (SD) (n = 3). The experiment was conducted twice. Experiments with PpDELLAb (data not shown) gave results similar to those obtained with PpDELLAa.

(B and C) Effects of different (active and inactive) GA forms on the strength of GID-DELLA interactions. GA_1, GA_3, GA_4, GA_5, and GA_7 all promote angiosperm growth, whereas kaurenoic acid and GA_3 methyl ester lack biological activity per se. Results (ratio to control) are expressed as means ± SD (n = 3) for (B) AtGID1c-RGA and (C) SkGID1-SkDELLA. The experiment was conducted twice.

our observations suggest that the capacity for the GA potentiation of the newly arisen GID1-DELLA interaction also arose sometime between the bryophyte and lycophyte divergences.

SkDELLA interacts with almost equal strength with both SkGID1 and AtGID1c (Figure 2A). Conversely, AtRGA and SkGID1 do not detectably interact (Figure 2A). Perhaps AtRGA has evolved a tight specificity for its natural partner (AtGID1c). Furthermore, GA_3 potentiated the AtGID1c-AtRGA interaction more effectively than it did the SkGID1-SkDELLA interaction, suggesting that the GID1-DELLA interaction has become more susceptible to GA potentiation during the course of evolution.

Relative bioactivity of GA forms is usually defined by (angiosperm) bioassays [26]. We found that SkGID1-SkDELLA and AtGID1c-AtRGA interactions were potentiated (or not) by the same GAs (Figures 2B and 2C). This suggests that there has been little change in the GID1 ligand-specificity since the appearance of the lycophytes.

Basal Land-Plant DELLAs Can Restrain Angiosperm Growth

Angiosperm DELLAs restrain plant growth, whereas GA releases angiosperms from DELLA-mediated growth restraint [17]. We next found that basal land-plant DELLAs restrain growth when expressed in angiosperms. First, we observed fluorescence due to green fluorescent protein (GFP)-tagged DELLAs (GFP-PpDELLAa, GFP-SkDELLA, and GFP-AtRGA) in root-cell nuclei of *A. thaliana* seedlings [27] (Figure 3A; expression driven by the *AtRGA* promoter). GA_3 treatment resulted in a rapid loss of GFP-AtRGA fluorescence [27, 28], a clearly detectable but less-rapid loss of GFP-SkDELLA fluorescence, and no detectable loss of GFP-PpDELLAa fluorescence (Figure 3A; data not shown). These results, consistent with those in Figure 2A, indicate that GA_3 stimulates the AtGID1-dependent destruction of GFP-AtRGA and GFP-SkDELLA in *A. thaliana*, but not that of GFP-PpDELLAa.

The GA-deficient *A. thaliana ga1-3* mutant displays DELLA-dependent dwarfism. The lack of both AtGAI and AtRGA (e.g., in a *gai-t6 rga-24 ga1-3* mutant line) substantially suppresses the *ga1-3* phenotype, conferring a tall rather than a dwarf phenotype, whereas the lack of AtGAI alone has relatively minor effects [29, 30]. As expected [27], we found that transgenic expression of GFP-AtRGA substantially restored the dwarfism characteristic of *ga1-3* to *gai-t6 rga-24 ga1-3* (Figure 3B). GFP-PpDELLAa or GFP-SkDELLA also conferred dwarfism (resembling that exhibited by *ga1-3*) when expressed in *gai-t6 rga-24 ga1-3* (Figure 3B). Furthermore, in accordance with our previous observations (Figures 2A and 3A), exogenous GA_3 overcame the effects of GFP-AtRGA and GFP-SkDELLA on *gai-t6 rga-24 ga1-3*, but not those of GFP-PpDELLAa (data not shown).

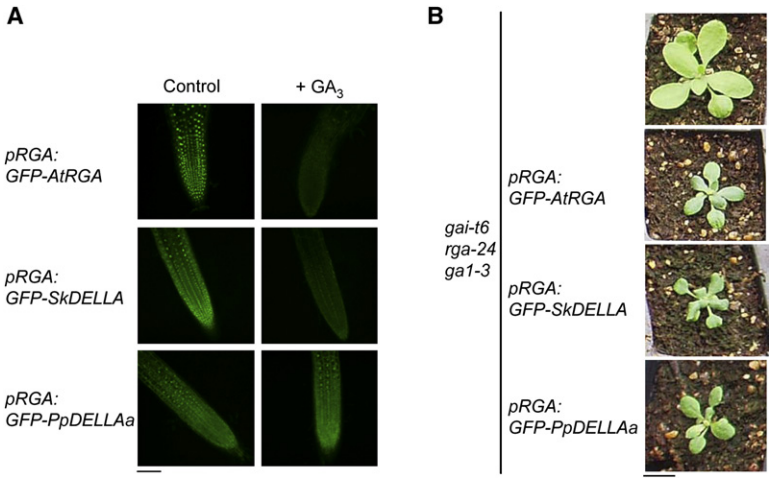

Figure 3. Basal Land-Plant DELLAs Repress Growth of *A. thaliana*

(A) GFP-fluorescence due to GFP-AtRGA, GFP-SkDELLA, and GFP-PpDELLAa is seen in control *A. thaliana gai-t6 rga-24 ga1-3* root-cell nuclei. Treatment with GA_3 (10 μM; 4 hr) causes a reduction in GFP-AtRGA and GFP-SkDELLA but not in GFP-PpDELLAa. Scale bars represent 0.1 mm.

(B) Expression of GFP-AtRGA, GFP-SkDELLA and GFP-PpDELLAa restores the dwarfism conferred by *ga1-3* and suppressed by the lack of GAI and RGA in *gai-t6 rga-24 ga1-3*. Three-week-old plants are shown. The scale bar represents 1 cm.

Thus PpDELLAa and SkDELLA both restrain the growth of *A. thaliana*, although neither restrains growth in its species of origin. This suggests that DELLA-mediated growth restraint evolved subsequent to the lycophyte divergence and prior to that of the gymnosperms (whose growth is GA stimulated [23]). Furthermore, growth restraint appears to be a property of the transgenic recipients rather than of the DELLAs themselves. DELLAs are candidate transcriptional regulators [18], suggesting that the growth-restraint function arose because downstream growth-controlling genes became DELLA responsive via evolutionary change in *cis*-regulatory regions [31].

Conclusions

Plants are sessile organisms and are susceptible to environmental fluctuation. The angiosperm GA-DELLA mechanism permits the adaptively significant, environmentally responsive regulation of life-cycle progression at the seed-dormancy and -germination [2, 4, 32], flowering [3, 7], and floral-development [25, 33, 34] stages. Furthermore, the GA-DELLA mechanism integrates the effects of numerous growth-regulatory signals, including the phytohormones ABA [32], auxin [28], and ethylene [7, 35]; environmental stress [5]; and light [6]. However, it was not previously clear how the GA-DELLA mechanism arose during plant evolution.

Land plants are a monophyletic group thought to have evolved from an aquatic ancestor [1]. Our work suggests that the GA-DELLA mechanism arose after the colonization of the land (Figure 4). In addition, we can position various distinct stages in the evolution of the GA-DELLA mechanism with respect to some major events in land-plant evolution. Initially, the DELLAs (like PpDELLAa) lacked the ability to interact with GID1s (although GID1 affinity for DELLAs was pre-existent). Next (step 1; Figure 4), the DELLAs evolved an affinity for GID1s, and GID1-DELLA interactions became possible (as seen in SkDELLA). In step 2 (possibly achieved in parallel with Step 1; Figure 4) GID1-DELLA interactions became susceptible to potentiation by bioactive GA. In step 3, plants evolved the capacity for growth restraint in response to the DELLAs. Steps 1 and 2 occurred between the bryophyte and lycophyte divergences (~430–400 million years ago [MYA] [1]; Figure 4). Step 3 occurred later, subsequent to the divergence of the lycophytes and prior to that of the gymnosperms (~300 MYA [36]; Figure 4). The increased capacity of GA to potentiate angiosperm versus that of lycophyte GID1-DELLA interactions suggests that GA-potentiation was

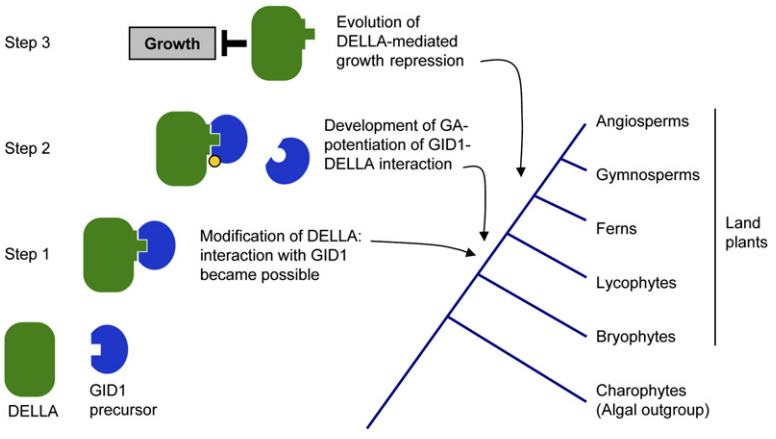

Figure 4. Stepwise Evolution of the Land-Plant GA-DELLA Growth-Regulatory Mechanism

Timing of the stages of evolution of the GA-GID1-DELLA mechanism is shown in relation to major events in land-plant evolution. Steps 1 and 2 took place after the bryophyte divergence (~430 MYA) but before the lycophyte divergence (~400 MYA). Step 3 occurred before the gymnosperm divergence (~300 MYA). Arrows relate each step to a simplified land-plant phylogeny, which also includes the charophytic algae (representative of the likely aquatic ancestor of the land plants [1]). Note that bryophytes and gymnosperms as represented here comprise several lineages and might not be monophyletic. Also, it is not currently known whether the evolution of DELLA-mediated growth repression occurred before or after the fern divergence.

refined during evolution, and that this process occurred in parallel with the rise in prominence of the GA-DELLA system as a mechanism for the adaptive regulation of growth and development.

There is considerable current interest in the relative evolutionary contributions of changes in the structure and function of transcriptional regulators versus changes in the *cis*-regulatory regions of genes responding to those regulators [31]. Although the evolution of GA-stimulated GID1-DELLA interactions occurred via changes in protein structure, the evolution of the DELLA growth-restraint function appears to have been more a function of changes in the transcriptional response of growth-controlling genes to DELLAs. Thus both types of evolutionary change have contributed to the step-wise acquisition of the growth-regulatory GA-DELLA mechanism seen in present-day angiosperms.

Experimental Procedures

Plant Material and Growth Conditions

Physcomitrella patens (Gransden2004 strain) and *Selaginella kraussiana* were kindly provided by Yasuko Kamisugi (University of Leeds, UK) and Jane Langdale (University of Oxford, UK), respectively. The *Arabidopsis gai-t6 rga-24 ga1-3* line was as previously described [29]. Moss cultures were grown under sterile conditions on BCD medium [37] at 25°C in a 16-hr-light/8-hr-dark cycle with or without supplements for 15 days after the spot inoculation of filamentous tissue (1 mm diameter). *Selaginella* and *Arabidopsis* were grown at 21°C in a 16-hr-light/8-hr-dark cycle.

Gene Identification and Isolation

PpDELLAa, *PpDELLAb*, *PpGLP1*, *PpGLP2*, *SmDELLA*, and *SmGID1* were obtained through basic logical alignment search tool (BLAST) search from the Phycobase (http://moss.nibb.ac.jp/) and *Selaginella* Genomics (http://selaginella.genomics.purdue.edu/) databases. See also the *Physcomitrella* genome website (http://genome.jgi-psf.org/Phypa1_1/Phypa1_1.home.html) for annotations and ID numbers for *PpGLP1* (118478) and *PpGLP2* (121825). For *SkDELLA*, *SkGID1*, and *SpDELLA*, gene fragments were amplified from genomic DNA by degenerate polymerase chain reaction (PCR), and the remaining coding sequences were obtained by the rapid amplification of cDNA ends or by thermal-asymmetric-interlaced PCR (see Table S4 for primer sequences).

Yeast Two-Hybrid Analysis

Expression constructs were made with plasmids pB42AD and pLexA and introduced to the yeast strain EGY48 + pSH18.2. β-galactosidase assays were performed in the presence or absence of 100 μM GA_3, following the instructions in the Yeast Protocols Handbook (PT3024-1) (http://www.clontech.com/). The expression of fusion proteins was confirmed by immunoblot analysis with LEXA (Invitrogen) and HA antibodies (Roche) (data not shown).

Arabidopsis Transformation

A 3146 bp upstream sequence of AtRGA (pRGA) and a 1976 bp downstream sequence of AtRGA (tRGA) were amplified from genomic DNA of *Arabidopsis* Landsberg *erecta* (see Table S4 for primer sequences). N-terminal fusions of GFP-DELLA flanked by pRGA and tRGA were cloned into pGreenII0229 (http://www.pgreen.ac.uk/). The constructs were introduced into the *gai-t6 rga-24 ga1-3* mutant as described in [38].

Confocal Detection of GFP-DELLA

Seeds were sterilized and germinated on germination medium (GM) plates [29]. Seven-day-old seedlings were treated with water or 10 μM GA_3 for 4 hr and observed with a Leica confocal laser microscope (Wetzlar, Germany) with 20× objectives at a 488 nm excitation wavelength. All images were obtained with the same modifications and intensity parameters.

RT-PCR

RNA was extracted from 30 mg of bifurcating tips of GA_3- or water-treated *S. kraussiana* with TRIzol LS Reagent (Invitrogen) according to the manufacturer's instructions. cDNA was generated with Superscript II reverse transcriptase (Invitrogen), and PCR was performed with 20 cycles for the *SkGID1* fragment and 15 cycles for the *SkUBQ* fragment (see Table S4 for primer information). PCR products were detected by gel-blot analysis with the radiolabeled expected fragment as probe.

Supplemental Data

Experimental Procedures, two figures, and four tables are available at http://www.current-biology.com/cgi/content/full/17/14/1225/DC1/.

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Accession Numbers

The SkGID1, SkDELLA, PpDELLAa, PpDELLAb, and SpDELLA sequences reported in this paper have been deposited in the National Center for Biotechnology Information (NCBI) GenBank with the accession numbers EF646469, EF646471, EF646472, EF646473, and EF646470, respectively.