The DELLA Domain of GA INSENSITIVE Mediates the Interaction with the GA INSENSITIVE DWARF1A Gibberellin Receptor of Arabidopsis

Björn C. Willige, Soumya Ghosh, Carola Nill, Melina Zourelidou, Esther M.N. Dohmann, Andreas Maier, and Claus Schwechheimer

Department of Developmental Genetics, Center for Plant Molecular Biology, Tübingen University, 72076 Tübingen, Germany

Gibberellic acid (GA) promotes seed germination, elongation growth, and flowering time in plants. GA responses are repressed by DELLA proteins, which contain an N-terminal DELLA domain essential for GA-dependent proteasomal degradation of DELLA repressors. Mutations of or within the DELLA domain of DELLA repressors have been described for species including Arabidopsis thaliana, wheat (Triticum aestivum), maize (Zea mays), and barley (Hordeum vulgare), and we show that these mutations confer GA insensitivity when introduced into the Arabidopsis GA INSENSITIVE (GAI) DELLA repressor. We also demonstrate that Arabidopsis mutants lacking the three GA INSENSITIVE DWARF1 (GID1) GA receptor genes are GA-insensitive with respect to GA-promoted growth responses, GA-promoted DELLA repressor degradation, and GA-regulated gene expression. Our genetic interaction studies indicate that GAI and its close homolog REPRESSOR OF GA1-3 are the major growth repressors in a GA receptor mutant background. We further demonstrate that the GA insensitivity of the GAI DELLA domain mutants is explained in all cases by the inability of the mutant proteins to interact with the GID1A GA receptor. Since we found that the GAI DELLA domain alone can mediate GA-dependent GID1A interactions, we propose that the DELLA domain functions as a receiver domain for activated GA receptors.

INTRODUCTION

The phytohormone gibberellic acid (GA) promotes important processes of plant growth and development, such as seed germination, elongation growth, and flowering time (Richards et al., 2001). The GA signaling pathway is controlled by the DELLA repressors, which are characterized by their N-terminal DELLA domain (Pysh et al., 1999). The Arabidopsis thaliana genome encodes five highly homologous DELLA protein repressors, including GA INSENSITIVE (GAI) and REPRESSOR OF GA1-3 (RGA) (Peng et al., 1997; Silverstone et al., 1998; Richards et al., 2001). While GAI and RGA have overlapping functions as repressors of elongation growth, RGA-LIKE1 (RGL1) and RGL2 play a predominant role in controlling germination and floral development, respectively (Dill and Sun, 2001; King et al., 2001; Lee et al., 2002; Cheng et al., 2004; Tyler et al., 2004). The DELLA repressors are inactivated in response to GA by ubiquitin proteasome–dependent protein degradation (Silverstone et al., 2001; Fu et al., 2002; McGinnis et al., 2003; Sasaki et al., 2003). A 17-amino acid deletion in the conserved DELLA domain, which is the mutation present in the dominant Arabidopsis gai-1 mutant, renders mutant gai and rga proteins insensitive to GA induced proteolysis, and plants expressing these mutant DELLA repressors are GA-insensitive, dark-green, late-flowering dwarfs (Peng and Harberd, 1997; Dill and Sun, 2001; Silverstone et al., 2001; Fleck and Harberd, 2002; Itoh et al., 2002; Dill et al., 2004). Interestingly, mutations in and of the DELLA domain were also identified in dwarfing alleles of the DELLA repressors Reduced height1 (Rht1) from wheat (Triticum aestivum), dwarf8 (d8) from maize (Zea mays), and Slender1 (Sln1) from barley (Hordeum vulgare), and these mutations were hypothesized to be the molecular cause for the GA insensitivity of the respective alleles (Gale and Marshall, 1976; Peng et al., 1999b; Chandler et al., 2002). In line with this hypothesis, it was demonstrated in the case of the barley Sln1D allele that the SLN1 protein produced by this mutant is partially impaired in GA-dependent SLN1 degradation (Gubler et al., 2002). Conversely, the understanding of the molecular mechanism underlying the dwarfing phenotypes of the wheat Rht1 alleles is not understood but of particular importance since their use in breeding permitted to generate the lodging-resistant high-yield wheat varieties of the so-called green revolution (Gale and Marshall, 1973, 1976; Peng et al., 1999b).

In Arabidopsis, the GA-dependent degradation of GAI and RGA is promoted by the F-box protein SLEEPY1 (SLY1), which functions as the degradation substrate receptor subunit of the E3 ubiquitin ligase SCF^{SLY1}. sly1 mutants fail to degrade GAI and RGA, and the sly1 mutant phenotype is suppressed by gai and rga loss-of-function alleles. SLY1 interacts in the yeast two-hybrid system with GAI and RGA, and the gai–1 gain-of-function phenotype is suppressed by sly1 gain-of-function alleles with increased affinity for the DELLA repressors (Peng et al., 1999a; Dill et al., 2004; Fu et al., 2004; Tyler et al., 2004). The SLY1 protein contains the DELLA (DWARF1, GA–INSENSITIVE) domain, which is recognized by the GAI DELLA domain, suggesting that the DELLA domain may be the receptor for GA-regulated gene expression.
DELLA protein interaction also occurs when the DELLA domain is deleted. Thus, the possibility that the DELLA domain serves as an interaction domain for SLY1 has been excluded.

The identification of the GA INSENSITIVE DWARF1 (GID1) proteins as soluble GA receptors in rice (Oryza sativa) and Arabidopsis was a major breakthrough in the understanding of GA signaling (Ueguchi-Tanaka et al., 2005; Nakajima et al., 2006). In rice and Arabidopsis, the analysis of GID1 proteins revealed that these GA receptors interact in a GA-dependent manner with the DELLA proteins from the respective species (Ueguchi-Tanaka et al., 2005; Nakajima et al., 2006). A recent study shows that loss of the three Arabidopsis GID1 receptors results in GA insensitivity and that the N-terminal DELLA and VHYNP domains of the DELLA protein RGA are required for GID1 interactions in Arabidopsis (Griffiths et al., 2006).

As introduced above, several DELLA domain mutations have been described that result in GA-insensitive growth in different plant species. In most cases, the consequences of these mutations on DELLA protein behavior had not been tested at the molecular level, and how these mutations affect GA signaling remained to be addressed. In this article, we characterize plants expressing gai variants with DELLA domain mutations that had previously been identified in DELLA repressors from maize, wheat, and barley. In all cases examined, these mutations result in GA-insensitive plant growth and a stabilization of the mutant gai proteins. Consistent with a recently published report, we also found that all three Arabidopsis GID1 genes participate in GA responses, and we extend this analysis by showing that the growth repression of the GA receptor mutants is largely caused by GAI and RGA. Finally, we show that the GAI DELLA domain is required and sufficient for interactions with the Arabidopsis GA receptor protein GID1A. We therefore conclude that the DELLA domain serves as a receiver domain for activated GID1 GA receptors.

RESULTS

DELLA Domain Mutations Impair GA-Promoted Protein Degradation and Plant Growth

The dominant GA-insensitive Arabidopsis gai-1 mutant expresses a gai mutant protein with a 17–amino acid deletion of the highly conserved N-terminal DELLA domain (Peng et al., 1997). Following the identification of the DELLA domain deletion in Arabidopsis gai-1, DELLA domain mutations were also identified in dwarfing alleles of DELLA repressor genes from several crop species, including wheat, maize, and barley (Gale and Marshall, 1976; Peng et al., 1999b; Chandler et al., 2002). Based on the apparent importance of the DELLA domain for GA-induced DELLA protein degradation, it was hypothesized (but with the exception of the barley protein never shown) that these mutations lead to a stabilization of the respective DELLA proteins (Dill et al., 2001; Gubler et al., 2002). Interestingly, there is no obvious correlation between the extent of growth repression conferred by the different DELLA mutations and their expected severity. For example, while a 5–amino acid deletion in the DELLA domain seems to be responsible for extreme dwarfism in D8-1 mutant maize, a predicted large truncation of the protein’s N terminus, including the DELLA domain and the neighboring VHYNP domain, suppresses plant growth only moderately in maize D8-Mp1 mutants (see Supplemental Figure 1A online) (Harberd and Freeling, 1989; Winkler and Freeling, 1994; Peng et al., 1999b).

This gives rise to the hypotheses that either the nature of the DELLA domain mutations or the specific genetic backgrounds determine the severity conferred by these mutations.

We wanted to study the effect of the different DELLA domain mutations in a homogenous genetic background. To this end, we generated transgenic Arabidopsis plants that contain genomic fragments for the expression of wild-type Arabidopsis GAI or GAI variants carrying DELLA domain mutations reported for the dwarfing alleles from Arabidopsis gai-1, maize D8-1 and D8-Mp1, wheat Rht-B1b and Rht-D1b, and barley Sln1D (Figures 1A and 1B) (Peng et al., 1997, 1999b; Chandler et al., 2002; Gubler et al., 2002). For each construct, at least 10 transgenic lines were generated, and eight lines were analyzed at the biochemical and physiological level. While endogenous wild-type GAI and wild-type GAI expressed from the GAI:GAI transgene is efficiently degraded in response to GA, we found that the mutant gai proteins are fully stabilized in lines expressing variants with a partial or full deletion of the DELLA domain (GAI:gai and gai-1, GAI:D8-1, GAI:Rht, and GAI:D8-Mp) (Figure 1C). In agreement with the previously reported observation that the Sln1D protein from barley is still partially GA sensitive, GAI:Sln1D plants, producing a GAI variant with a single amino acid substitution in the DELLA domain, express a partially stabilized gai mutant protein (Figure 1C) (Gubler et al., 2002).

In all cases, the degree of GAI stabilization correlates well with the level of growth suppression in the transgenic lines. All transgenic plants expressing fully stabilized GAI variants are GA-insensitive, dark-green, late-flowering dwarfs that are phenotypically indistinguishable from the Arabidopsis gai-1 mutant (Figure 1D; see Supplemental Figures 1B and 3 online). Again in agreement with the previously reported observation that the Sln1D allele from barley is still partially GA sensitive, we observed a partial reduction of elongation growth and a delay in the onset of flowering in GAI:Sln1D lines that was significantly less severe than that observed in lines expressing fully stabilized mutant gai proteins (Figure 1D).

When we examined the consequences of GAI stabilization on RGA protein accumulation, we noticed that RGA protein levels are strongly reduced in all lines expressing stabilized GAI variants (Figure 1C). Since GA-insensitive mutants, such as gai-1, were reported to contain increased levels of GA (Peng and Harberd, 1997), we reasoned that increased GA-promoted RGA degradation may be responsible for this effect. Indeed, when we treated plants expressing stabilized GAI variants with the GA biosynthesis inhibitor paclobutrazol (PAC), RGA protein levels increased, suggesting that GA levels regulate DELLA repressor abundance, at least in part, by promoting their degradation (see Supplemental Figure 2B online). In summary, we conclude that partial or full deletions of the DELLA domain, as previously reported for dwarfing alleles of several crop species, cause (when introduced into Arabidopsis GAI) GA insensitivity with respect to GA-promoted protein degradation and GA-promoted plant growth. Hence, the differences in the severity of dwarfing mutations, such as the D8-1 and D8-Mp mutations from maize, may be attributable to differences in the genetic background of these alleles.
Figure 1. Physiologically Relevant DELLA Domain Mutations Stabilize GAI.

(A) Clustal alignment of the N-terminal 140-amino acid residues of Arabidopsis GAI and gai mutant variants that were designed based on the mutations identified in Arabidopsis gai-1, maize D8-1 and D8-MP, wheat Rht-B1b/D1b (Rht), and barley Slr1D alleles (see Supplemental Figure 1A online for a detailed alignment).

(B) Schematic representation of the GAI-derived transgenes carrying the mutations shown in (A). The mutations in the GAI coding sequence, but not the flanking 5' - and 3' -GAI genomic regions, are drawn to scale.

(C) Immunoblots with specific anti-GAI and anti-RGA antibodies (see Supplemental Figure 2 online) using 45 μg of protein extract from GA-treated inflorescences of wild-type, gai-1 mutant, and transgenic plants. A cross-reacting band serves as a loading control.

(D) Wild-type and transgenic 7.5-week-old (left panel) and 8.5-week-old (right panel) Arabidopsis plants.
The Three Arabidopsis GID1 Genes Participate in GA Responses

The biological role of the three apparent Arabidopsis homologs (GID1A, AT3G05120; GID1b, AT3G63010; and GID1c, At5G27320) of the rice GA receptor GID1 was recently determined, and it was found that the three GID1 genes have redundant functions in mediating GA responses (Griffiths et al., 2006). We also analyzed GA responses in T-DNA insertion mutants for each of the three Arabidopsis GID1 genes (Figure 2A). For our analysis, we selected three mutant alleles with in-gene in-exon T-DNA insertions, namely, gid1a-1, gid1b-1, and gid1c-2. Our gene and allele nomenclature is identical to the one used in the previous publications of these genes (Griffiths et al., 2006; Nakajima et al., 2006), and with the exception of the gid1c-2

![Diagram](image_url)

**Figure 2.** Loss of Arabidopsis GID1 GA Receptor Function Results in GA Insensitivity. (A) Schematic representation of the genomic organization of the three Arabidopsis GID1 orthologs GID1A to GID1C. Exons are shown as black boxes and introns as lines. The positions of T-DNA insertions and the names of the gid1 mutant alleles are indicated by arrows. (B) Seed germination rate after 4 d as evaluated by root emergence of the gid1a-1, gid1b-1, and gid1c-2 alleles and their double and triple mutant combinations (n = 100). In the case of the gid1 triple mutant, germination rate was calculated based on the germination rate of a homozygous gid1a gid1b double mutant segregating for the gid1c mutation. (C) Hypocotyl elongation in response to GA measured from 5-d-old seedlings grown on GA3-containing media as indicated. The gid1 triple mutant seedlings used in this experiment were manually removed from the seed coat (n = 10). (D) Phenotype of 4-week-old gid1 mutants as indicated in the panel. The average and SD of the height of 4-week-old gid1 mutants is indicated below the genotypes (n ≥ 8).
allele used in our study, identical mutant alleles were used for physiological analysis and genetic experiments. We found that while single mutants of each of the three GID1 genes do not have obvious defects in GA-controlled growth responses, such as germination, GA-induced hypocotyl elongation, elongation growth, or flowering time, gid1 double and triple mutants are partially (double mutants) or fully (triple mutants) impaired in these responses (Figures 2B to 2D). Therefore, our gid1 triple mutants display a complete suppression of GA responses and are phenotypically indistinguishable from severe GA biosynthesis mutants, such as ga1-3, in that they fail to germinate and, after manual removal of the seed coat, develop into dark-green severely dwarfed plants with a maximum rosette diameter of 1 cm (Figures 2B to 2D). In contrast with ga1-3, which is the most severe GA biosynthesis mutant described in the literature (Koornneef and van der Veen, 1980), and in contrast with the gid1 triple mutant described in a recent publication (Griffiths et al., 2006), our gid1 triple mutants never flower even in long-day conditions (8 h dark/16 h light), continuous light conditions, or when treated with GA3 (see Supplemental Figure 4 online). This difference in phenotype severity may be attributable to the fact that we used the allele gid1c-2 to generate the gid1a-1 gid1b-2 gid1c-2 triple mutant, whereas the above-mentioned report (Griffiths et al., 2006) made use of the allele gid1c-1. The T-DNA insertion of the gid1c-2 mutation (GABI_639F05) resides in the second exon of the GID1c gene, and this mutation may affect gene function more severely than the T-DNA insertion in gid1c-1 (SALK_023529), which is located in the GID1c intron (Figure 2A). Taken together, based on our genetic analyses and the biochemical analyses conducted by others (Griffiths et al., 2006; Nakajima et al., 2006), we conclude that the three Arabidopsis GID1 proteins have redundant functions as GA receptors and that gid1 triple mutants are insensitive to GA.

**gid1 Mutants Are GA Insensitive with Respect to GA-Promoted DELLA Protein Degradation and GA-Controlled Transcriptional Responses**

To examine whether the GA insensitivity observed in the gid1 triple mutant correlates with DELLA repressor stabilization, we tested RGA and GAI abundance in the gid1 mutants (Figure 3A). Interestingly, neither the gid1 single mutants nor the gid1 double mutants showed the increase in DELLA protein levels expected for mutants impaired in GA perception. Although minor differences in RGA and GAI abundance may be observed in the comparison of individual gid1 single and double mutants in the experiment shown in Figure 3A, these differences were generally not reproducible and cannot be considered significant. However, we observed a significant and reproducible accumulation of RGA but not of GAI in the gid1 triple mutant (Figure 3A). Furthermore, following GA treatments that cause complete degradation of these proteins in the wild type, RGA and GAI were found to be fully stabilized in the gid1 triple mutant (Figure 3B). We thus conclude that the gid1 triple mutants are GA insensitive with respect to DELLA protein degradation and that GAI and RGA accumulate to different levels in the gid1 receptor mutants.

Since we could show that gid1 triple mutants are GA insensitive at the physiological and biochemical level, we next examined whether their GA insensitivity also extends to GA-regulated transcription. To this end, we dissected ga1 and gid1 triple mutant seedlings from seeds, allowed the dissected seedlings to grow for 1 week on growth media, and subjected the phenotypically identical mutant seedlings for 1 h to 100 μM GA3 or a mock control treatment (three biological replicate samples for each mutant and each treatment). With the exception of the GA1 and GID1 genes mutated in the respective loss-of-function mutants, gene expression analyses with the Arabidopsis ATH1 gene chip identified only a single gene that is differentially expressed between mock-treated ga1 and mock-treated gid1a-c (data not shown). Thus, ga1 and gid1 mutants are not only identical at the phenotypic level but also at the level of gene expression. In turn, the analysis of the GA-treated ga1 mutant resulted in the identification of 120 GA-repressed and 28 GA-induced genes (Figure 3C; see Supplemental Table 1 online). In line with previous gene expression studies, this analysis identified GAI as being induced in response to GA and GID1A, GID1B, SLY1, and several genes encoding proteins required for GA biosynthesis as being repressed in response to GA (see Supplemental Table 1 online) (Cao et al., 2006; Griffiths et al., 2006). Importantly, the expression of the 148 genes regulated by GA in ga1 was not affected by the GA treatment in the gid1 triple mutants (Figure 3C; see Supplemental Table 1 online). Furthermore, the direct comparison of the expression profiles of mock-treated and GA-treated gid1 triple mutants did not lead to the identification of any GA-regulated genes in the gid1 triple mutant. Thus, our findings suggest that all GA-regulated transcriptional responses are mediated by the GID1 GA receptors in Arabidopsis, at least at the seedling stage.

**GID1 Receptors and the DELLA Repressors Interact at the Biochemical and Genetic Levels**

Next, we generated a transgenic line expressing a fusion protein of GID1A and green fluorescent protein (GFP) under control of the 3SS promoter of Cauliflower mosaic virus (3SS:GID1:GFP). Overexpression of GID1:GFP in the wild-type background resulted in a slight reduction of flowering time and correlated with a slight reduction in RGA protein levels when compared with wild-type plants (Figures 4A and 4C). Furthermore, the 3SS:Gid1:GFP transgene was able to complement the germination, elongation, and flowering time defects of the gid1 triple mutant, indicating that the fusion protein retained functionality (Figure 4B). As described for the rice GID1 protein, the GFP-tagged Arabidopsis GID1A protein localizes to the nucleus, the presumed site of action of the DELLA repressors, and to the cytoplasm. Furthermore, the localization of the GID1A receptor was not altered by GA treatments or by treatments of the GA biosynthesis inhibitor PAC (Figure 4D; data not shown) (Silverstone et al., 2001; Fleck and Harberd, 2002; Ueguchi-Tanaka et al., 2005).

To gain evidence for the in vivo interaction between GID1A and the DELLA repressors, we immunoprecipitated GID1:GFP with an anti-GFP affinity matrix. Using protein extracts of shy1-10 mutant seedlings, which accumulate the DELLA repressors, we were able to communoprecipitate RGA in a GA-dependent manner (Figure 3D). Our attempts to communoprecipitate GID1:GFP and GAI in a similar experiment were not successful, most likely due to the comparatively lower affinity of the anti-GAI antibody.
Nevertheless, this experiment indicates that GID1A can interact with DELLA proteins in vivo.

In rice, the introduction of a SLENDER RICE loss-of-function mutant completely suppresses the gid1 mutant phenotype. In a similar experiment, we introduced GAI and RGA loss-of-function alleles (gai-t6 and rga-24) into the Arabidopsis gid1 triple mutant background. In the resulting quintuple mutant, we observed a dramatic suppression of almost all aspects of the gid1 mutant phenotype, including the defects in germination, elongation growth, and flowering time (Figure 3E). Quadruple mutants lacking only GAI
or RGA suppressed the gid1 triple mutant phenotype to a comparatively minor extent, but the suppression by rga-24 was significantly stronger than that by gai-t6 (Figure 3E). Since the gai-t6 and rga-24 mutations are in a different Arabidopsis ecotype (Landsberg erecta) than the gid1 mutations (Columbia), we were concerned with the effect of the genetic background on the mutants’ phenotypes. Since the complexity of the genetic experiment and the severity of the mutant phenotypes prevented us from performing repeated backcrosses, we restricted our analysis to quadruple and quintuple mutants that were genotyped as being homozygous wild type for the Columbia allele of the ERECTA (ER) gene. ER is mutated in Landsberg erecta, and this mutation is at least in part responsible for the differences in elongation growth between the Columbia and Landsberg erecta ecotypes (Torii et al., 1996). A minimum of four plants was identified for each quadruple and quintuple mutant in the Columbia ER background, and all plants belonging to the same group were phenotypically indistinguishable. Taken together, our genetic and biochemical data strongly support the notion that GID1 proteins interact with DELLA proteins in a GA-dependent manner in vivo and that the DELLA repressors GAI and RGA are the major growth repressors in the absence of GA signaling in an Arabidopsis GA receptor mutant background.

The GAI DELLA Domain Mediates GID1A GA Receptor Interactions

Since mutations of the GAI DELLA domain and mutations of the GID1 GA receptors confer GA-insensitive growth (Figures 1 and 2), and since DELLA proteins and GID1 proteins interact in vivo (Figure 3), we reasoned that the GAI DELLA domain itself may mediate the interactions with the GID1A GA receptor. Following up on experiments made by others, we used the yeast two-hybrid system to demonstrate that GAI can interact with GID1A in a GA-dependent manner in yeast (Nakajima et al., 2006). We then tested the gai mutant variants in this interaction assay and found that all gai mutant variants with a partial or full deletion of the DELLA domain fail to interact with GID1A (Figure 5). This finding is in agreement with recent reports that the DELLA domain of RGA is required for GID1A interactions (Griffiths et al., 2006). Consistent with our observation that plants expressing GAI:Sn1D are partially impaired in GA responses, we detected an ~10-fold reduction of the interaction between the Sn1D mutant variant and GID1A over a range of GA concentrations (Figure 5C; data not shown). In a complementary experiment, we observed that the GAI DELLA domain alone (amino acids 1 to 73) is sufficient to mediate GA-dependent interactions with GID1A and that the presence of the adjacent VHYNP domain (amino acids 1 to 115) does not contribute to the strength of this interaction. Thus, our results support the notion that the DELLA domain serves as a receiver domain for the GA receptor following GA binding and that the loss of this interaction is the molecular cause for the GA insensitivity observed in the previously reported dwarfing alleles of DELLA repressor genes from a range of species.

DISCUSSION

We were interested in determining the molecular function of the DELLA domain of the DELLA repressor proteins. To this end, we introduced previously identified DELLA domain mutations from Arabidopsis, maize, wheat, and barley into Arabidopsis GAI (Figure 1A). Genetic evidence suggested that these mutations are responsible for GA-insensitive growth in the respective alleles (Peng et al., 1999b; Chandler et al., 2002). Transgenic Arabidopsis plants expressing GAI variants with a deletion of or in the DELLA domain were phenotypically indistinguishable GA-insensitive dwarf plants that express stabilized gai proteins (Figure 1; see Supplemental Figure 1B online). The single amino acid exchange mutation designed based on the barley Sn1D mutant allele showed an intermediate phenotype with respect to plant growth and mutant gai protein stabilization, and these data are entirely consistent with a previous study on the SLN1D protein from barley (Gubler et al., 2002). In turn, our interaction studies with the
Arabidopsis GID1A GA receptor and the GAI DELLA repressor
mutant proteins indicate that the loss (or reduction in the case of
Sin1D) of GA responsiveness can be explained by the loss (or
reduction) of GA-dependent GID1A binding (Figure 5). Since we
also found that the DELLA domain alone is sufficient to mediate
GA-dependent GAI interactions in the yeast two-hybrid system,
we propose that the DELLA domain functions as the receiver
domain for the activated GID1A receptor. Thus, loss of GA re-
ceptor interaction may be molecular cause for GA-insensitive
growth of the respective dwarfing alleles from wheat, maize, and
barley. At the same time, we of course realize that the ultimate
proof for this hypothesis needs to be furnished in the respective
crop species, as already done in the case of barley Sin1D (Gubler
et al., 2002). In the case of the wheat Rht1 alleles, another but
related mechanistic explanation to the one mentioned above
may be valid. Based on the nature of the Rht1 mutations, it may
be possible that these alleles express an N-terminally truncated
GAI ortholog, as used in our study, as well as a peptide con-
taining only the protein’s N terminus, including the DELLA
domain (Peng et al., 1999b). It cannot be excluded that the
expression of this DELLA domain-only peptide titrates out GID1
interaction partners and thereby causes indirectly the stabiliza-
tion of functional full-length DELLA repressors.

We characterized mutants lacking the three genes encoding
GID1 GA receptor function in Arabidopsis. To a large extent, our
phenotypic analysis is consistent with a report on the function of
these genes that was published recently by others (Griffiths et al.,
2006). In addition to the phenotypic analysis provided in this
other report, we could show that gid1 double mutants are im-
paired to the same extent in germination, while the gid1 triple
mutants are unable to germinate (Figure 2B). By contrast, we found
that GA-induced hypocotyl elongation and plant height of adult
plants are significantly more affected in gid1a gid1c mutants than
in double mutant combinations containing gid1b (Figures 2C and
2D). Analyses of the gid1 double mutants at the adult stage
further indicate that gid1a mutations make a stronger contribution
to the adult phenotype than the two other genes. These ob-
servations are also consistent with the observations published
recently (Griffiths et al., 2006). By contrast, however, we noted
that our gid1 triple mutant never flowered and therefore has a more severe phenotype than the previously described gid1 triple
mutant. We attribute this difference to the different gid1c alleles
that were used in the two studies. We made use of the gid1c-2
allele, which may be stronger than the gid1c-1 allele used in the
other study. gid1c-2 has an insertion in the large exon of the
GID1c gene, while the insertion in gid1c-1 is in the gene’s intron
and could potentially be removed by splicing (Figure 2A).

Our analyses also extend the understanding of the interaction
between the GID1 proteins and the DELLA repressors (Figure 3).
While we show that GAI and RGA are stabilized in the gid1 triple
mutant and that both proteins act as repressors in the gid1 tri-
ple mutant background (Figures 3B and 3E), we also show that
GA-regulated transcription is fully impaired in the gid1 triple
mutants (Figure 3C). In this experiment, we had envisioned three
different scenarios: First, GA-regulated transcription may be fully
repressed in the gid1 triple mutant, indicating that all transcrip-
tional GA responses are mediated by the three GID1 GA recep-
tors. Second, GA-regulated transcription may be globally reduced

Figure 5. The DELLA Domain Is Required and Sufficient for the Interaction with the GID1A Receptor.

(A) Schematic representation of the constructs used for the yeast two-
hybrid interaction study. The positions of the DELLA (D) and the VHYNP
(V) domains are indicated.

(B) Immunoblots with anti-GAI (AD constructs; anti-GAI antibodies) and anti-
GAL4 (DBD constructs; anti-GAL4 antibodies) demonstrating the expression of the respective fusion proteins
in yeast. The asterisk indicates a cross-reacting band. The expression levels of the AD:D and AD:D − V constructs in yeast could not be examined because
the anti-GAI peptide antibody does not recognize the GAI N terminus.

(C) Result of the yeast two-hybrid interaction study demonstrating that
the DELLA domain is required and sufficient for the GA-dependent
GID1A interactions. The first and third columns show yeast transform-
ants (growth control); the second and fourth columns show the result of a
qualitative LacZ filter lift assay conducted with yeast grown for 2 d in the
absence (left) and presence (right) of 100 μM GA3 (Duchefa). Indicated
on the right are the fold inductions of LacZ enzyme activity of DBD:GID1
AD-construct combinations grown on GA3 over untreated samples as
determined using the Galacton-Star luminescent reagent. In agreement with
the qualitative assays, the DBD AD-construct combinations (negative controls) did not result in differential LacZ activity in the absence and
presence of GA (data not shown).
but not fully repressed, a finding indicative of the existence of other proteins with a general role in GA perception and signal transduction. (An alternative explanation for this scenario could have been that the gid1 triple mutants are not null alleles.) Third, GA-regulated gene expression of a subset of GA-regulated genes can still occur, a finding indicative for the existence of other receptor proteins that control a subset of GA responses. Our gene expression analysis strongly supports the first scenario, suggesting that all transcriptional GA responses, at least at the growth stage examined, are mediated by the GID1 proteins.

While our study shows that the GAI DELLA domain alone (amino acids 1 to 73) is sufficient to mediate interactions with the GID1A receptor, the recently published study on the interaction between GID1 proteins and RGA suggests that the RGA DELLA domain (RGA amino acids 1 to 70) alone is not sufficient to mediate this interaction (Griffiths et al., 2006). In addition to the fact that these two studies use different vector systems, we would like to note that our GAI DELLA domain fragment (GAI amino acids 1 to 73) corresponds based on sequence homologies to the first 89 amino acids of RGA, due to the extended N terminus of RGA. Thus, our fragment covers a considerably longer part of the GAI N terminus downstream of what we define as the DELLA domain than the RGA DELLA domain fragment used in the other study (Figure 1A; see Supplemental Figure 1A online). The longer GAI DELLA domain fragment used in our study may provide additional flexibility to the DELLA domain and thereby permit interactions with the GA receptor. It may also be that the functional DELLA domain is longer than what is generally defined as the DELLA domain (Figure 1A; see Supplemental Figure 1A online) since, in the absence of structural protein data, it is difficult to delimit the boundaries of the DELLA domain solely based on the degree of sequence conservation.

In our experiments, we also detected an effect of GAI stabilization on the abundance of RGA. All lines expressing stabilized GAI protein had strongly reduced RGA levels. GA biosynthesis is under negative feedback control of GA, and this feedback control mechanism is known to be impaired in GA signaling mutants that fail to degrade GAI or RGA, such as gai-1 (Peng and Harberd, 1997). We therefore reasoned that an increase in GA levels promotes the accelerated degradation of RGA in plants expressing stabilized gai variants. We could substantiate this hypothesis by our finding that RGA levels increase in plants expressing stabilized gai when GA biosynthesis is inhibited with the GA biosynthesis inhibitor PAC. Thus, although we did not examine other levels of control, such as gene transcription, the reduction of RGA levels may at least in part be explained by increases in GA levels after GAI stabilization. Similar observations were made in previous studies where, for example, GAI protein levels were found to increase in the absence of RGA in ga1-3 rga-24 and sly-1/10 rga-24 loss-of-function mutants compared with the ga1-3 or sly-1/10 single mutants (Dill et al., 2004). It thus appears that the abundance of DELLA repressors is under global control and that a mechanism is in place that controls DELLA protein levels. While one element of this control mechanism is certainly SLY1-mediated GA-dependent DELLA protein turnover, the precise mode of regulation remains to be established. A differential accumulation of the DELLA repressors is also apparent in several of our mutant analyses. For example, RGA and GAI accumulate and are stabilized in the sly1-10 mutant, which lacks the F-box protein that promotes RGA and GAI degradation. In turn, only RGA but not GAI accumulates in the gid1 triple mutant, but neither protein responds to GA in the GA receptor mutant background (Figures 3A and 3B). This difference may be explained by a differential GA-independent turnover of GAI by SLY1. This hypothesis may be supported by the observation that SLY1 interacts significantly better with GAI than with RGA in the yeast two-hybrid system, notably in the absence of GA (Dill et al., 2004). Such a hypothetical GA-independent turnover may explain the differential accumulation of the two repressors in the gid1 triple mutant. Although we have not been able to resolve such a GAI turnover in our experiments, we cannot exclude its existence and consider it a suitable mechanism to explain the differences in basal DELLA protein levels.

Independent of the molecular mechanism that underlies the differential accumulation of GAI and RGA in specific mutants, it also needs to be said that there is a striking discrepancy in the severity of the phenotypes of the (weak) sly1-10 and the (strong) gid1 triple mutant, especially in relation to the (strong and weak) accumulation of the DELLA repressors (e.g., Figure 3A). Thus, there is no good correlation between DELLA protein accumulation, the ability to degrade the repressors, and the severity of a phenotype. It is therefore very likely that, besides GA-dependent protein degradation, alternative molecular mechanisms, such as posttranslational modifications, regulate DELLA repressor activity. Several reports point to a role of phosphorylation in controlling DELLA protein function, but the regulatory role of this modification with respect to DELLA repressor activity is unclear (Sasaki et al., 2003; Gomi et al., 2004; Itoh et al., 2005). Glycosylation by the glycosyl transferase SPINDLY is a second modification that may be implicated in DELLA repressor activity, or more specifically in repressor activation (Silverstone et al., 2007). The identification of the proteins that exert regulatory functions on the DELLA repressors and the identification of the site, nature, and function of DELLA protein modifications will further increase our understanding of the GA signaling pathway in the future.

METHODS

Plant Material

All gid1 alleles are from Arabidopsis thaliana (ecotype Columbia) and were obtained from the Nottingham Arabidopsis Stock Centre and the GABI-KAT facility (Max-Planck Institute for Plant Breeding Research) (Alonso et al., 2003; Rosso et al., 2003). Homozygous mutants were identified by PCR-based genotyping: gid1a-1 was genotyped using 5′-attB1-CGGATCTCCGACTGCCGAGCGTGAGAATTAC-3′ and 5′-attB2-CTGCAGTTAACTCCGCGTTTACAAACGCCG-3′ and 5′-CTTATTTCAGTAAGA-9 to test for the wild-type gene and 5′-CGGCCATTGGTGGTGGTAACGAAGTCAACC-3′ and 5′-CTAAAGGAGTAAAGGAGCACA-GAACCTTGAC-3′ to test for the insertion. gid1b-1 was genotyped using 5′-ATGGC-TGGTGTAACGAAGTCAACC-3′ and 5′-CTAAGGAGTAAAGGAGCACA-GAACCTTGAC-3′ to test for the wild-type gene and 5′-CTAAAGGAGTAAAGGAGCACA-GAACCTTGAC-3′ to test for the insertion. gid1c-2 was genotyped using 5′-ACCAGCTGATGCGACCTCACCAGGTG-3′ and 5′-GGCATCTGCAGTTACAATGAGGATTCCATGAGG-3′ to test for the wild-type gene and 5′-CACACAGTGTTCCCTCTCAATACA-3′ together with 5′-CCCATTTGGACGTTGAAATGGTACACGAC-3′ to test for the insertion. rga-24 was genotyped...
using 5'-GGTATTGGCAGTGTTG-3' and 5'-TCGGTTAGATTTGATTTAC-3' to amplify the wild-type gene fragment and 5'-CATGACCCATTAGCTACTAGTAGC-3' and 5'-TCCTGTTAGTTAGTCTACT-3' to amplify the mutant allele. gai-6 was genotyped using 5'-CTAGATCCACATTGGAA-3' and 5'-AGGCTCAACATGCAAAC-3' as an XhoI-BamHI fragment into pG400179. Subsequently, the 2-kb GAI downstream sequence was amplified using 5'-GGATCCATCGCCAATTTTGTTAAGAC-3' and 5'-GATGCCGGCAGTCCAC-3' to amplify the gai-6 insertion allele. The alleles gid1a-1, gid1d-1, and gid1c-2 were used to generate mutant combinations. The genotypes of important mutant combinations, such as the gid1a gid1b gid1c rga-24 gai-6 mutants, were confirmed three times using independent genomic DNA preparations.

T-DNA Constructs for Plant Transformation

To generate GAI-GAI, GAI-gai, etc., the open reading frames of GAI and gai mutant variants were obtained by overlap extension PCR and introduced into the vector pGREEN0179 containing 2-kb GAI upstream and downstream sequences (Helliens et al., 2000). All constructs were designed and cloned in an identical manner. In detail, the 2-kb GAI promoter fragment was amplified by PCR from ecotype Columbia genomic DNA using 5'-CTCTGATATTACTTCTTTAGAAAAATATGTTTGG-3' and 5'-GAATCTAGATGGTGGCTCAATGAATTGATC-3', respectively, and the insertion allele. The alleles were amplified by PCR from ecotype Columbia genomic DNA using 5'-GGATTTTCGACAT-3' and 5'-GGTGATTTTCACGGTGGTTG-3'. Deletions were then generated and used to replace part of the sequence: For GAI:Rht1, a PCR product was obtained with 5'-GAATTCTAGATGGTGGCTCAATGAATTGATC-3' and 5'-AGGCTCAACATGCAAAC-3' to amplify the gai-6 insertion allele. The alleles gid1a-1, gid1d-1, and gid1c-2 were used to generate mutant combinations. The genotypes of important mutant combinations, such as the gid1a gid1b gid1c rga-24 gai-6 mutants, were confirmed three times using independent genomic DNA preparations.

Microarray Analysis

For gene expression profiling, nongerminating ga1 and gid1 triple mutant seedlings were dissected and grown on solid growth medium for 1 week in continuous light. For each mutant, three replicate samples (biological replicates) were subjected to a mock spray treatment with water (uninduced control), and three replicate samples were subjected to a spray treatment with 100 μM GA3 (Duchefa). After 1 h, the plant material was frozen in liquid nitrogen, and RNA was extracted from each replicate using the RNeasy kit (Qiagen). Complementary RNA was prepared from 1 μg of total RNA from each sample as described using the MessageAmp II Biotin-Enhanced Signal Round aRNA amplification kit (Ambion). In brief, double-stranded artificial DNA was synthesized and biotin-labeled target artificial RNA was prepared by artificial DNA in vitro transcription in the presence of biotinylated UTP and CTP. After purification, artificial RNA was fragmented and used to hybridize the Arabidopsis ATH1 GeneChip array (Affymetrix). Hybridization, washing, staining, scanning, and data collection were performed for each replicate sample independently in an Affymetrix GeneChip Fluidics Station 450 and GeneArray Scanner. The microarray computational analysis was performed on CEL data files and analyzed using the robust multivariate average GC method (gcRMA) of the GeneSpring software (version 7.2; Agilent Technologies). Means of the three replicate values were analyzed for each data set. Data sets with expression levels below 50 were excluded from comparative analysis (noise level of expression cutoff). Genes were considered as induced or repressed if their mean expression level deviated >1.75 in a comparison of two samples. Statistical significance of gene expression was tested using a one-way analysis of variance test combined with a Benjamini and Hochberg false discovery rate multiple correction algorithm (GeneSpring version 7.2) with an adjusted P value <0.05 set as cutoff. The gene tree was generated with the 148 genes identified as being GA regulated using the Pearson correlation and average linkage algorithms of the GeneSpring software. The microarray data were submitted to the Gene Expression Omnibus and are available under the accession numbers GSM177119 to GSM177121 (ga1 mutant experiment), GSM177122 to GSM177124 (ga1 mutant + GA3), GSM177125 to GSM177127 ( gid1a-c mutant experiment), and GSM177128 to GSM177130 ( gid1a-c mutant experiment + GA3).

ImmunobLOTS and Immunoprecipitation

Specific anti-GAI and anti-RGA peptide antibodies were raised in rabbits and affinity-purified against the specific peptides GGDYTTTNKRLKC (amino acids 127 to 139 of GA1) and KRDHHQFGQRLSNHG (amino acids 2 to 16 of RGA; Eurogentec). Both peptide sequences are specific for the respective DELLA proteins. Immunoblots were performed using 45 μg of total plant protein extract prepared in extraction buffer (50 mM Tris-HCl, 150 mM NaCl, 0.5% Triton X-100, 10 μM MG132, 0.1 μM PMSF, and Sigma-Aldrich protease inhibitor cocktail, pH 7.5). For immunoprecipitation of GID1-GFP, 400 μg of plant extract from the wild type or a 35S: GID1-GFP-expressing line was preincubated with 20 μL of anti-GFP agarose (Vector Laboratories) in a binding buffer (50 mM Tris-HCl, 150 mM NaCl, 10 μM MG132, 0.1 mM PMSF, and Sigma-Aldrich protease inhibitor cocktail, pH 7.5). Following three washes in wash buffer (50 mM Tris-HCl and 150 NaCl), GID1:GFP fusion protein bound to the matrix was mixed with 200 μg of protein extract from 7-d-old stf1-10 mutant seedlings in the presence and absence of 100 μM GA3 and incubated for 3 h in binding buffer. Following three washes in wash buffer, the resin was resuspended in loading buffer, and equal volumes were loaded for immunoblot analysis. GID1:GFP was detected with an anti-GFP antibody (Invitrogen) or an anti-GID1 peptide antibody raised in rabbits and affinity-purified against the GID1A-specific peptide FGGNTERSEKSLDG (Eurogentec).

Microscopy

The intracellular distribution of GID1-GFP was examined in roots of 35S: GID1-GFP plants using a Leica TCS SP2 confocal microscope. Propidium iodide was used to outline the cell boundaries.

Yeast Two-Hybrid System

Yeast two-hybrid constructs were obtained by insertion of GAI and its mutant variants into the vector pGAD424 (+2) as BamHI-PstI fragments.
excised from the pGREEN0179-based GAI constructs described above to generate AD:GAI, AD:gai, etc. (Roder et al., 1996). Constructs expressing only the DELLA domain (AD:D) and the DELLA and VHYNP domains (AD:D-V) were constructed by insertion of a PCR fragment obtained with the primers 5′-GGATCCAGAGAGATCATCATCATC-3′ and 5′-CTCGAGTCAGCTTCAAGGCCGTAATTGAGA-3′ to generate AD: D or 5′-GGATCCAGAGAGATCATCATCATC-3′ and 5′-CTCGAGTCAAGC GAACTGTGAGGATCAGCTGTC-3′ to generate AD:D-V. The GID1A open reading frame was obtained by RT-PCR from Arabidopsis ecotype Columbia mRNA and inserted in frame into the yeast two-hybrid vector pGB9T. Yeast two-hybrid interaction studies were performed in the yeast strains Y187 and Y190 using standard protocols (Schwechheimer and Deng, 2002). In brief, DBD and DBD:GID1A constructs were transformed into Y190 and mated with Y187 strains harboring AD, AD:GAI, AD:gai, etc. DBD and AD plasmids were selected on dropout medium lacking Leu and Trp. LacZ filter lift assays were performed with yeast clones grown on dropout medium lacking Leu and Trp in the absence and presence of 100 μM GA3. Quantitative LacZ assays were conducted in a Mithras LB940 luminometer (Berthold) using protein extracts prepared from clones grown in liquid dropout medium lacking Leu and Trp in the absence and presence of 100 μM GA3 and the Galact-Star reagent (Tropix) as a luminescent substrate. Average relative light units/μg protein were determined from four replicates, and fold induction was calculated.

Accession Numbers
Arabidopsis Genome Initiative locus identifiers for the genes mentioned in this article are as follows: ER (AT2G26330), GID1A (AT3G05120), GID1b (AT3G63010), GID1c (AT5G27320), GAI (AT1G14920), RGA (AT2G01570), and SLY1 (AT4G24210).

Supplemental Data
The following materials are available in the online version of this article.

Supplemental Figure 1. Alignment of Arabidopsis GAI with Maize, Wheat, and barley Orthologs, and Transgenic GAI:gai Plants Mimic the gai-1 Gain-of-Function Mutant Phenotype.

Supplemental Figure 2. Anti-GAI and Anti-RGA Antibodies Specifically Recognize the Respective DELLA Protein.

Supplemental Figure 3. Mutants Expressing Stabilized GAI Variants Are GA Insensitive.

Supplemental Figure 4. Growth of ga1 GA Biosynthesis Mutants but Not That of gid1 Triple Mutants Can Be Normalized by GA Treatment.

Supplemental Table 1. Genes That Are Repressed or Induced by 100 μM GA3 Treatment (1 h) in a ga1 Allele (SALK_109115, Columbia Ecotype) and Their Expression in the gid1a-c Triple Mutant.

ACKNOWLEDGMENTS
We thank Gerd Jürgens and Erika Isono (Tübingen University) for critical comments on the manuscript. We also thank Nicholas Harberd (John Innes Centre, Norwich, UK) and Camille Steber (Washington State University, Pullman, WA) for sharing mutant seeds. This work is supported by grants from the Deutsche Forschungsgemeinschaft (SChw751/4 and Sch751/5) and the Sonderforschungsbereich SFB446 to C.S. and by a fellowship from the Landesgraduiertenföderung Baden-Württemberg to E.M.N.D.

Received March 2, 2007; revised March 2, 2007; accepted March 22, 2007; published April 6, 2007.

REFERENCES
Itoh, H., Ueguchi-Tanaka, M., Sato, Y., Ashikari, M., and Matsuoka, M.
(2002). The gibberellin signaling pathway is regulated by the appearance and disappearance of SLENDER RICE1 in nuclei. Plant Cell 14: 57–70.


The DELLA Domain of GA INSENSITIVE Mediates the Interaction with the GA INSENSITIVE DWARFIA Gibberellin Receptor of *Arabidopsis*

Björn C. Willige, Soumya Ghosh, Carola Nill, Melina Zourelidou, Esther M.N. Dohmann, Andreas Maier and Claus Schwechheimer

*Plant Cell*; originally published online April 6, 2007;
DOI 10.1105/tpc.107.051441

This information is current as of January 2, 2021

| Supplemental Data | /content/suppl/2007/03/23/tpc.107.051441.DC1.html |
| eTOCs | Sign up for eTOCs at: http://www.plantcell.org/cgi/alerts/ctmain |
| CiteTrack Alerts | Sign up for CiteTrack Alerts at: http://www.plantcell.org/cgi/alerts/ctmain |
| Subscription Information | Subscription Information for *The Plant Cell* and *Plant Physiology* is available at: http://www.aspb.org/publications/subscriptions.cfm |