The roles of the GA receptors GID1a, GID1b, and GID1c in sly1-independent GA signaling

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Gibberellin (GA) hormone signaling occurs through proteolytic and non-proteolytic mechanisms. GA binding to the GA receptor GID1 (GA-INSSENSITIVE DWARF1) enables GID1 to bind negative regulators of GA responses called DELLA proteins. In proteolytic GA signaling, the SLEEPY1 (SLY1) F-box protein targets DELLA proteins in the GID1-GA-DELLA complex for destruction through the ubiquitin-proteasome pathway. Non-proteolytic GA signaling in sly1 mutants where GA cannot target DELLA proteins for destruction, requires GA and GID1 gene function. Based on comparison of gid1 multiple mutants to sly1 gid1 mutants, GID1a is the primary GA receptor stimulating stem elongation in proteolytic and non-proteolytic signaling, and stimulating fertility in proteolytic GA signaling. GID1b plays the primary role in fertility, and a secondary role in elongation during non-proteolytic GA signaling. The stronger role of GID1b in non-proteolytic GA signaling may result from the fact that GID1b has higher affinity for DELLA protein than GID1a and GID1c.

Keywords: gibberellin signaling, GA, GA receptor, GID1, F-box, SLY1

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Submitted: 01/01/2014
Revised: 01/28/2014
Accepted: 01/28/2014
Published Online: 02/12/2014

Citation: Hauvermale AL, Ariizumi T, Steber CM. The roles of the GA receptors GID1a, GID1b, and GID1c in sly1-independent GA signaling. Plant Signaling & Behavior 2014; 9:e28030; PMID: 24521922; http://dx.doi.org/10.4161/psb.28030

Addendum to: Ariizumi T, Hauvermale AL, Nelson SK, Hanada A, Yamaguchi S, Steber CM. Lifting DELLLArepression of Arabidopsis seed germination by non-proteolytic gibberellin signaling. Plant Physiol 2013; 162:2125–39; PMID:23818171; http://dx.doi.org/10.1104/pp.113.219451

The plant hormone GA stimulates seed germination, stem elongation via cell division and elongation, and the transition to flowering and fertility. GA stimulates these processes by lifting repression by the DELLA domain transcriptional regulators, through both proteolytic and non-proteolytic mechanisms.1-3 Proteolytic DELLA destruction requires GA hormone biosynthesis, the 3 Arabidopsis GA receptors, and the SLY1 (SLEEPY1) gene (reviewed by Hauvermale et al.).4 GA stimulates the interaction of the GID1 receptors with DELLA proteins. GID1-GA-DELLA complex formation stimulates DELLA protein-protein interaction with SLY1, the F-box subunit of an SCF E3 ubiquitin ligase that polyubiquitinates DELLA proteins, thereby targeting them for destruction via the 26S proteasome. The sly1 loss-of-function mutants result in increased seed dormancy, dwarfism, and infertility associated with high level DELLA accumulation due to lack of DELLA proteolysis.5 If GA signaling resulted solely from DELLA destruction, then we would expect the severity of GA-insensitive phenotypes to correlate with the level of DELLA protein accumulation. Paradoxically, sly1 mutants accumulate more DELLA protein than the GA biosynthesis mutant ga1-3, and the GA receptor gid1a gid1b gid1c triple knockout lines, but the sly1 phenotypes are not as strong as those of ga1-3 and gid1a gid1b gid1c lines. The sly1 phenotypes can
be partly rescued by overexpression of each of the 3 GID1a, GID1b, and GID1c GA receptor genes on the CaMV 35S promoter, suggesting that the GIDI1 genes can trigger GA signaling without DELLA protein destruction.1,2 Non-proteolytic GA signaling requires both GA and GIDI1 since both the ga1-3 biosynthesis mutant and gid1 mutations exacerbate the syl1-2 phenotypes.

By comparing the previously published effects of gid1a-1, gid1b-1, and gid1c-2 mutations in the wild type Columbia (Col-0) to their effects in the syl1-2 mutant in the Col-0 background, this paper examines the relative roles of the 3 Arabidopsis GA receptor genes in proteolytic and non-proteolytic GA signaling, respectively (Table 1).1,6,7 The role of each GIDI1 gene in proteolytic GA signaling can be considered by reviewing the effects of gid1a, gid1b, and gid1c single, double, and triple mutants in previous studies.6-8 Because DELLA proteins are not destroyed in response to GA in syl1 mutants, the syl1-2 gid1 double and triple mutants generated by Arizumi et al.1 demonstrate the relative importance of each GIDI1 gene in GA responses in the absence of DELLA destruction—or non-proteolytic GA signaling.

GIDI1a and GIDI1b have important roles in seed germination. In the wild-type Col-0 background, the gid1a-1 gid1b-1 gid1c-2 triple mutant was unable to germinate unless the seed coat was cut.2 The gid1 single and double mutants in Col-0 were all able to germinate, suggesting that each GIDI1 gene can function in seed germination. However, a gid1b allele in the Nossen ecotype resulted in a decreased response to GA stimulation of seed germination, suggesting that GIDI1b is important for proteolytic GA signaling during seed germination.8 Neither the Nossen gid1a nor Col-0 gid1c alleles were found to alter GA response during seed germination. The syl1-2 mutant is highly dormant, but acquires the ability to germinate with long (1–2 y) dry after-ripening.9 The syl1-2 gid1a-1 double mutant completely failed to after-ripen in 20 mo, and the syl1-2 gid1c-2 double mutant showed reduced germination compared with syl1-2. The syl1-2 gid1b-1 double mutant seeds failed to germinate with 20 mo of after-ripening, but could not be well characterized due to limited sample size resulting from infertility. Thus, GIDI1a appears to be important for non-proteolytic GA signaling during syl1 seed germination. Published data suggest that GIDI1b may be important for both proteolytic and non-proteolytic GA signaling during seed germination. But it is difficult to draw a firm conclusion of its relative importance in proteolytic and non-proteolytic GA signaling due to ecotype differences.

Phenotypic comparison of gid1 loss-of-function mutants in the syl1-2 and Col-0 wild-type backgrounds provide important insights into the functional roles of GIDI1a, GIDI1b, and GIDI1c in stem elongation and fertility (Table 1). In Col-0, the gid1a-1 gid1c-2 and gid1a-1 gid1c-1 double mutants are shorter than the gid1a-1 gid1b-1 line.6,7 In contrast, gid1a-1 gid1b-1 mutations cause a stronger decrease in syl1-2 plant height than either gid1a-1 gid1c-1 or gid1b-1 gid1c-2. Thus, GIDI1a and GIDI1c play a stronger role in proteolytic GA signaling, whereas GIDI1a and GIDI1b together play a stronger role in non-proteolytic GA signaling during stem elongation. The gid1a-1 allele caused the strongest decrease in fertility in wild-type Col-0, whereas the gid1b-1 mutation resulted in a far stronger decrease in syl1-2 fertility than gid1a or gid1c. Thus, for fertility GIDI1a is more important during proteolytic, and GIDI1b during non-proteolytic GA signaling.

The functionality of the 3 GIDI1 genes can also be explored by examining how well HA:GIDI1 fusion constructs rescue syl1 phenotypes when overexpressed on the 35S promoter.1,2 While loss of GIDI1a function had the strongest effects on plant height and seed germination, HA:GIDI1b fusion protein overexpression (HA:GIDI1b-OE) was far more effective than HA:GIDI1a-OE and HA:GIDI1c-OE in rescuing the dwarfism and seed dormancy phenotypes of syl1-2 mutants. While loss of GIDI1b function caused the strongest decrease in syl1-2 fertility, HA:GIDI1c overexpression was most effective in rescuing the syl1-2 infertility phenotype. These results further support the idea that GIDI1b plays an important role in non-proteolytic GA signaling during germination and stem elongation. They also suggest that GIDI1c can be very effective in non-proteolytic regulation of DELLA proteins during syl1 flowering. HA:GIDI1b-OE also resulted in a greater increase in wild-type stem elongation in the Landsberg erecta (Ler) ecotype, suggesting that GIDI1b can influence both proteolytic and non-proteolytic GA signaling when overexpressed.2 This data demonstrates that the relative roles of the 3 GIDI1 genes based on loss- and gain-of-function phenotypes can be quite different from each other, suggesting that the transcriptional regulation of GIDI1a, GIDI1b, and GIDI1c likely contributes to determining the functional roles of GIDI1 genes.

The fact that HA:GIDI1b-OE had the strongest effect on plant height and seed germination may be explained by the fact that of the 3 GIDI1 proteins, GIDI1b has the strongest affinity for GA and DELLA protein.6,10,11 This was previously demonstrated using yeast 2-hybrid assays, GST pulldown assays, and in vitro binding assays with purified proteins. GIDI1b also shows some protein-protein interaction.
with DELLA in the absence of GA. Figure 1 illustrates this point using our HA:GID1-OE constructs. More DELLA RGA protein co-immunoprecipitated with HA:GID1b than with HA:GID1a and HA:GID1c in the syl1 mutant background. Although endogenous GA is present in syl1 protein extracts, the addition of more GA increased the interaction of HA:GID1a and HA:GID1c with DELLA RGA. Given that HA:GID1b has higher affinity for DELLA, it is interesting that HA:GID1c-OE resulted in better rescue of syl1-2 fertility than did HA:GID1b-OE. This together with the fact that the syl1-2 gid1b-1 double mutant was the least fertile syl1 gid1 double mutant, suggests that GID1b protein levels must be tightly regulated during Arabidopsis flowering as having too much or too little GID1b function reduces fertility.

While GID1 proteins are negative regulators of DELLA protein levels during proteolytic GA signaling, GID1 genes function as positive regulators of DELLA mRNA and protein accumulation in syl1 mutants. The gid1 mutations result in REDUCED DELLA protein and mRNA accumulation in syl1-2, suggesting that GIDI genes may normally function in positive feedback regulation of DELLA transcription. There are 5 DELLA genes in Arabidopsis. DELLA RGA (REPRESSOR OF GA1-3) regulates plant height but also participates in seed germination and fertility, whereas DELLA RGL2 (RGA-LIKE2) regulates seed germination, participates in regulating fertility, but does not regulate stem elongation. The accumulation of DELLA RGA and RGL2 proteins were examined in syl1-2 gid1 multiple mutants to examine which GIDI gene regulated each DELLA protein (Table 2). The gid1c-2 mutation resulted in the strongest decrease in DELLA RGA accumulation in syl1 flower buds. This is consistent with the observation that HA:GID1c overexpression best rescued syl1-2 fertility (Table 1). Loss of gid1a function resulted in the strongest decrease in DELLA RGA accumulation in 4 wk-old syl1-2 plants. This is paradoxical given that syl1-2 gid1a double mutants are considerably shorter than the syl1-2 mutant that has MORE DELLA RGA repressor of plant stem elongation. Thus, in the syl1 background it is clearly not the case that more DELLA repressor correlates with shorter plants. This emphasizes the need to better understand the mechanisms underlying non-proteolytic GA signaling.

Based on comparisons of syl1 gid1 multiple mutant phenotypes to gid1 multiple mutant phenotypes, different GIDI genes predominate in non-proteolytic vs. proteolytic GA signaling (Fig. 2). In both cases, the GID1a gene is the main GA receptor stimulating stem elongation. However, GID1c is more important in proteolytic while GID1b is more important in non-proteolytic GA signaling during stem elongation. GID1a is the primary GA receptor stimulating fertility during proteolytic GA signaling, whereas GID1b is the primary GA receptor required during non-proteolytic GA signaling. These data suggest that GID1b protein, with its higher affinity for DELLA protein, may become more important once it is no longer possible.

Table 1. DELLA Protein accumulation was measured in tissues harvested from the syl1-2 gid1 loss-of-function mutants in the Col-0 background. a = gid1a-1, b = gid1b-1, or c = gid1c-2 loss of function alleles result in REDUCED DELLA protein accumulation in the syl1-2 mutant. n.e. = Not Expressed in vegetative tissue.

| DELLA Protein | Vegetative | Flower Buds | Reference |
|---------------|------------|-------------|-----------|
| RGA           | a > b > c  | c > a > b   | 1         |
| RGL2          | a > c > b  |             | 1         |

Table 2. The effects of the gid1 loss-of-function on DELLA protein accumulation in syl1-2

| DELLA Protein | Vegetative | Flower Buds | Reference |
|---------------|------------|-------------|-----------|
| GID1a         | a > b > c  | c > a > b   | 1         |
| GID1b         | a > c > b  |             | 1         |

Figure 1. HA:GID1b co-immunoprecipitates (co-iP) more DELLA RGA than HA:GID1a and HA:GID1c. Co-iP of DELLA RGA with HA:GID1 was performed as in Ariizumi et al. Total protein extracted from 12 d-old syl1-10 35S:HA:GID1-OE seedlings was incubated with HA agarose in the presence of 0 µM, 1 µM, or 100 µM GA (0.1% ethanol). Protein blot analysis was performed using anti-DELLA (1:10,000), anti-HA (1:5000), Immuno Consultants Laboratory) and anti-CULLIN1 (1:10,000). 40 µg of total protein was loaded on an SDS-PAGE gel (input). A ponceau loading control, and short (1 min) and long (10 min) exposures of the RGA blot are shown. The CUL1 blot is a negative control demonstrating the specificity of the HA:GID1 interaction with RGA.

Figure 2. A diagram illustrating the relative roles of the GIDI genes during (A) proteolytic, and (B) non-proteolytic GA signaling, based on gid1 loss-of-function phenotypes in the wild-type Col-0 and syl1-2 mutant backgrounds, respectively. GIDI gene function is ranked according to the severity of the gid1 loss-of-function phenotypes with heavy black arrows indicating a primary role, heavy blue arrows a secondary role, and thin blue arrows a tertiary role in each GA response.
to downregulate DELLa proteins via the ubiquitin-proteasome pathway.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We would like to thank Dr T Sun for providing the RGA antibody, Dr X Deng for providing the CUL111 antibody, and Dr C Schwechheimer for providing the gid1 mutant alleles in the Col-0 background. This work was supported by the National Science Foundation (award no. 0850981 to C.M.S).

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