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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-INSENSITIVE 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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This paper reviews evidence that the 3 *Arabidopsis thaliana* gibberellin (GA) hormone receptors, *GID1a*, *GID1b*, and *GID1c* (*GIBBERELLIN-INSENSITIVE DWARF1*), have partially specialized functions both in proteolytic and in the non-proteolytic GA signaling that occurs in the *sly1-2* (*sleepy1-2*) F-box mutants (based on Ariizumi et al., and others).[1] 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

Table 1. The effects of *gid1* loss-of-function and *GID1-OE* on GA regulated responses.

	[4]Relative Phenotypic Effect on			
[1]Genotype	Germination	Elongation	Fertility	Reference
[2]wild-type sly1-2	N.A. a > (b) > c	a > c > b a > > b > c	a > b > > c b > > a > c	6, 7 1, 2
[3]wild-type	N.A.	B-OE > C-OE > A-OE	N.S.	2
sly1-2	B-OE > A-OE > C-OE	B-OE > A-OE > C-OE	C-OE > A-OE > B-OE	1

[1]Genetic background in which the effects of *GID1* loss-of-function or overexpression (OE) were examined. [2]Relative phenotypic effects of each *gid1* loss-of-function allele on germination, stem elongation, and fertility in wild-type and *sly1-2* in the Col-0 ecotype, where a = *gid1a-1*, b = *gid1b-1*, and c = *gid1c-2*. [3]Relative phenotypic effects of each *HA:GID1* overexpression allele in wild-type and *sly1-2* in the L*er* ecotype, where A-OE = *HA:GID1a -OE*, B-OE = *HA:GID1b-OE*, and C-OE = *HA:GID1c-OE;* [4]NA = no information available, NS = no statistically significant effect observed.

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 *GID1* genes can trigger GA signaling without DELLA protein destruction.[1,2] Non-proteolytic GA signaling requires both GA and *GID1* since both the *ga1-3* biosynthesis mutant and *gid1* mutations exacerbate the *sly1-2* 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 *sly1-2* 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 *GID1* 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 *sly1* mutants, the *sly1-2 gid1* double and triple mutants generated by Ariizumi et al.[1] demonstrate the relative importance of each *GID1* gene in GA responses in the absence of DELLA destruction—or non-proteolytic GA signaling.

GID1a and *GID1b* 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.[7] The *gid1* single and double mutants in Col-0 were all able to germinate, suggesting that each *GID1* 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 *GID1b* 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 *sly1-2* mutant is highly dormant, but acquires the ability to germinate with long (1–2 y) dry after-ripening.[9] The *sly1-2 gid1a-1* double mutant completely failed to after-ripen in 20 mo, and the *sly1-2 gid1c-2* double mutant showed reduced germination compared with *sly1-2*. The *sly1-2 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, *GID1a* appears to be important for non-proteolytic GA signaling during *sly1*seed germination. Published data suggest that *GID1b* may be important for both proteolytic and non-protetolytic 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 *sly1-2* and Col-0 wild-type backgrounds provide important insights into the functional roles of *GID1a*, *GID1b*, and *GID1c* 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 *sly1-2* plant height than either *gid1a-1 gid1c-1* or *gid1b-1 gid1c-2*.[1] Thus, *GID1a* and *GID1c* play a stronger role in proteolytic GA signaling, whereas *GID1a* and *GID1b* 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 *sly1-2* fertility than *gid1a* or *gid1c*. Thus, for fertility *GID1a* is more important during proteolytic, and *GID1b* during non-proteolytic GA signaling.

The functionality of the 3 *GID1* genes can also be explored by examining how well *HA:GID1* fusion constructs rescue *sly1* phenotypes when overexpressed on the 35S promoter.[1,2] While loss of *GID1a* function had the strongest effects on plant height and seed germination, *HA:GID1b* fusion protein overexpression (*HA:GID1b-OE*) was far more effective than *HA:GID1a-OE* and *HA:GID1c-OE* in rescuing the dwarfism and seed dormancy phenotypes of *sly1-2* mutants. While loss of *GID1b* function caused the strongest decrease in *sly1-2* fertility, *HA:GID1c* overexpression was most effective in rescuing the *sly1-2* infertility phenotype. These results further support the idea that *GID1b* plays an important role in non-proteolytic GA signaling during germination and stem elongation. They also suggest that *GID1c* can be very effective in non-proteolytic regulation of DELLA proteins during *sly1* flowering. *HA:GID1b-OE* also resulted a greater increase in wild-type stem elongation in the Landsberg *erecta* (L*er*) ecotype, suggesting that *GID1b* can influence both proteolytic and non-proteolytic GA signaling when overexpressed.[2] This data demonstrates that the relative roles of the 3 *GID1* genes based on loss- and gain-of-function phenotypes can be quite different from each other, suggesting that the transcriptional regulation of *GID1a*, *GID1b*, and *GID1c* likely contributes to determining the functional roles of *GID1* genes.

The fact that *HA:GID1b-OE* had the strongest effect on plant height and seed germination may be explained by the fact that of the 3 GID1 proteins, GID1b 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. GID1b 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-immunoprecipiated with HA:GID1b than with HA:GID1a and HA:GID1c in the *sly1* mutant background. Although endogenous GA is present in *sly1* 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 *sly1-2* fertility than did *HA:GID1b-OE*. This together with the fact that the *sly1-2 gid1b-1* double mutant was the least fertile *sly1 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 *sly1* mutants.[1] The *gid1* mutations result in REDUCED DELLA protein and mRNA accumulation in *sly1-2*, suggesting that *GID1* 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 *sly1-2 gid1* multiple mutants to examine which *GID1* gene regulated each DELLA protein (**Table 2**).[1] The *gid1c-2* mutation resulted in the strongest decrease in DELLA RGA accumulation in *sly1* flower buds. This is consistent with the observation that *HA:GID1c* overexpression best rescued *sly1-2* fertility (**Table 1**). Loss of *gid1a* function resulted in the strongest decrease in DELLA RGA accumulation in 4 wk-old *sly1-2* plants. This is paradoxical given that *sly1-2 gid1a* double mutants are considerably shorter than the *sly1-2* mutant that has MORE DELLA RGA repressor of plant stem elongation. Thus, in the *sly1* 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 *sly1 gid1* multiple mutant phenotypes to *gid1* multiple mutant phenotypes, different *GID1* 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

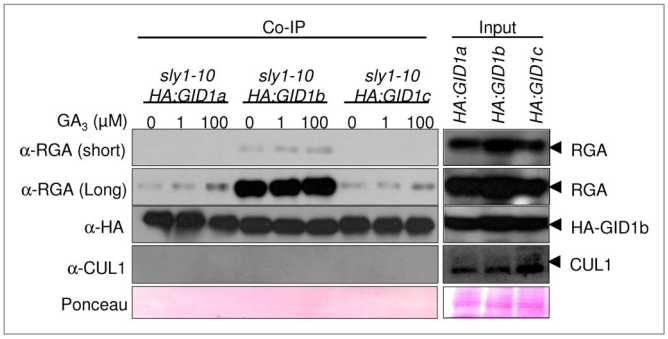

Figure 1. HA:GID1b co-immunoprecipitates (co-IP) more DELLA RGA than HA:GID1a and HA-GID1c. Co-IP of DELLA RGA with HA:GID1 was performed as in Ariizumi et al.[1] Total protein extracted from 12 d-old *sly1–10* 35S:*HA:GID1-OE* seedlings was incubated with HA agarose in the presence of 0 μM, 1 μM, or 100 μM GA_3 (0.1% ethanol). Protein blot analysis was performed using anti-RGA (1:10,000), anti-HA (1:5000, Immuno Consultants Laboratory) and anti-CULLIN1 (1:10,000).[12,13] 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.

Table 2. The effects of the *gid1* loss-of-function on DELLA protein accumulation in *sly1-2*

	[1]Effect on DELLA Protein Levels in		
DELLA Protein	**[2]Vegetative**	**Flower Buds**	**Reference**
RGA	[3]a > > b = c	c > > a > b	1
RGL2	4N.E.	a > > c > b	1

[1]DELLA protein accumulation was measured in tissues harvested from the *sly1-2 gid1* loss-of-function mutants in the Col-0 background. [2]Vegetative refers to 4 wk-old rosettes and stems. [3]The degree to which a = *gid1a-1*, b = *gid1b-1*, or c = *gid1c-2* loss of function alleles result in REDUCED DELLA protein accumulation in the *sly1-2* mutant. [4]N.E. = Not Expressed in vegetative tissue.

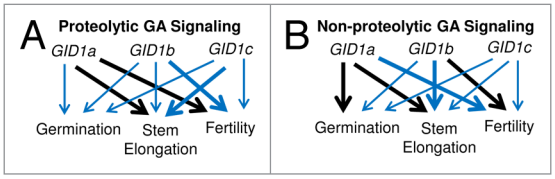

Figure 2. A diagram illustrating the relative roles of the *GID1* genes during (**A**) proteolytic, and (**B**) non-proteolytic GA signaling, based on *gid1* loss-of-function phenotypes in the wild-type Col-0 and *sly1–2* mutant backgrounds, respectively. *GID1* gene function is ranked according to the 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.

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