Foolish Seedlings and DELLA Regulators: The Functions of Rice SLR1 and Arabidopsis RGL1 in GA Signal Transduction

As every student of plant biology learns, gibberellin (GA) was first isolated in Japan in the 1930s from a fungus that causes a serious disease in rice, called “foolish seedling disease” (bakanae-byo) by Japanese farmers. Afflicted plants were characterized by excessive growth of seedlings and a decline in seed production. Researchers soon discovered that application of GA to certain dwarf varieties of numerous plant species could cause the plants to grow to normal height and sometimes even taller. The reader is referred to Taiz and Zeiger (1991) for a particularly dramatic illustration of the effect of GA on stem elongation.

In addition to its role in controlling cell and stem elongation, GA action is essential in various other stages of plant development, including seed germination and flower development. GA responses are regulated through control of the level of bioactive GA via the regulation of GA biosynthesis and catabolism (deactivation) and through transduction and perception of the active GA signal to various locations throughout the plant. Cell elongation is known to be affected by other hormones and signaling molecules, including cytokinin, ethylene, brassinosteroid, Ca\(^{2+}\), and sugars, but most of these effects ultimately may result from how these molecules impinge upon the biosynthesis, signal transduction, and/or perception of GA.

**GA MUTANTS**

GA signal transduction is an active area of research, and a number of putative transcription factors have been identified as regulators of GA signal transduction through mutational analyses. Mutants include the dominant or semidominant GA-insensitive dwarves, such as Arabidopsis GA insensitive (gai; Koornneef et al., 1985) and short internodes (shi; Fridborg et al., 1999), wheat Reduced height (Rht; Gale and Marshall, 1973), maize D8 and D9 (Harberd and Freeling, 1989), and the recessive GA constitutive response slender mutants, which include Arabidopsis spindly (spy; Jacobson and Olszewski, 1993), rice slr1 (Ikeda et al., 2001), pea sln (Reid et al., 1992) and lacry (Potts et al., 1985), and tomato procerosa (Jones, 1987). SHI, identified using a transposon tagging strategy in Arabidopsis, encodes a zinc finger transcription factor (Fridborg et al., 1999). Pea SLN encodes a GA 2-oxidase responsible for the deactivation of active GA (Martin et al., 1999), and similar GA 2-oxidases have been cloned from runner bean and Arabidopsis (Thomas et al., 1999). SPY encodes a protein with similarity to O-linked GlcNAc transferases (Jacobsen et al., 1996), a class of enzyme known to regulate protein activity via glycosylation.

**DELLA PROTEINS AND GA SIGNAL TRANSDUCTION**

Arabidopsis has five genes in the DELLA subfamily: GAI, RGA, RGL1, RGL2, and RGL3. GAI and RGA encode negative regulators of GA responses that appear to have partially redundant or overlapping functions (Dill and Sun, 2001). The gain-of-function gai mutant has a GA-insensitive dwarf phenotype. Peng et al. (1997) showed that GAI is a repressor of GA responses and that GA could release this repression, suggesting that GA modulates plant growth through derepression. The loss-of-function rga mutant was identified by its ability to repress the dwarf phenotype of the GA biosynthesis mutant ga1-3, which has extremely low levels of active GA (Silverstone et al., 1998). RGL1, RGL2, and RGL3 (for RGA-LIKE) encode candidate GA response proteins on the basis of their similarity to RGA (Sánchez-Fernández et al., 1998; Dill and Sun, 2001). In contrast to Arabidopsis, the rice genome appears to have just one DELLA gene (Ogawa et al., 2000; Ikeda et al., 2001).

The acidic N-terminal DELLA domain (named for the consensus amino acid sequence it encodes) of the proteins in this subfamily is of considerable interest because it is present in all plant GRAS genes that are known to be involved in GA signaling and appears to play a critical role in the GA response. For example, the semidominant gai-1 allele encodes a 17-carboxyl acid deletion that removes the DELLA domain (Peng et al., 1997), and a dominant GA-insensitive slr1 mutant was created in rice by introducing a deletion of the DELLA domain analogous to that of gai-1 in SLR1, the single DELLA gene in rice (Ikeda et al., 2001).
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In this issue of *The Plant Cell*, Itoh et al. (pages 57–70) and Wen and Chang (pages 87–100) describe features of the functions of rice SLR1 and Arabidopsis RGL1, respectively, in GA signal transduction. Itoh et al. investigated the subcellular localization of the SLR1 protein fused to green fluorescent protein (SLR1-GFP) and performed domain analysis of SLR1 by overexpressing various truncated SLR1 constructs in transgenic plants. Wen and Chang (2002) characterized the phenotypes of *rgl1* gain-of-function and loss-of-function mutants, analyzed expression patterns of *RGL1* and the four other Arabidopsis DELLA subfamily members in different plant tissues, and examined the subcellular localization of a GFP-RGL1 fusion protein.

FUNCTIONAL DOMAINS OF SLR1

Because rice has only one DELLA protein, it provides a good model system for the functional analysis of SLR1. Null alleles of *RGA* or *GAI* in Arabidopsis produce a normal or weak GA phenotype, presumably because of a degree of overlap among the functions of these genes in this species. In contrast, the *slr1* loss-of-function mutant shows a slender constitutive GA response phenotype (Figure 1).

Itoh and colleagues show that SLR1-GFP is localized in the nucleus and functions as a repressor of the GA signaling pathway, because its overproduction in the transgenic seedlings caused a dwarf phenotype. Second, they show that the SLR1 protein contains at least four different functional domains: a GA signal perception domain (which includes the DELLA motif), a repression domain that causes suppression of downstream GA responses, a dimer formation domain, and a regulatory domain.

The N-terminal DELLA domain and a nearby TVHYNP region are conserved in the DELLA subfamily of GA response proteins but are not found in other GRAS proteins. Itoh et al. found that transgenic plants overproducing SLR1 that lacked the DELLA domain (ΔDELLA), the TVHYNP domain (ΔTVHYNP), or a nonconserved spacer region between these domains (Δspace) exhibited a severe GA-insensitive dwarf phenotype (Figure 1). These truncated proteins retained their negative regulatory function but lacked the ability to respond to GA, strongly suggesting that these regions are critical for the perception and transmission of the GA signal. Using fusions with GFP, they showed that the wild-type SLR1 protein was localized to the nucleus but disappeared from the nucleus after treatment with exogenous GA. However, fusion proteins of GFP with ΔDELLA, ΔTVHYNP, or Δspace were retained in the nucleus after GA treatment.

Itoh and colleagues also tested the effect of the overproduction of three other truncated SLR1 proteins: ΔpolyS/T/V, lacking an 11-amino acid region rich in serine/threonine and valine; ΔLZ, lacking a leucine heptad repeat region; and ΔC-ter, lacking the C-terminal portion of the VHIID domain. The C-terminal half of GAI and RGA has been shown to be important for the suppressor function of these proteins (Peng et al., 1997; Silverstone et al., 1998). This was found to be the case as well for SLR1, because the ΔC-ter overproducers exhibited a slender phenotype like...
that of the slr1 loss-of-function mutant. Plants overproducing the ΔLZ protein did not show a detectable phenotype. However, the ΔLZ protein was unable to form a heterodimer with the intact SLR1 protein in a yeast two-hybrid assay, whereas the intact SLR1 protein readily formed a homodimer. Together, these results suggest that SLR1 functions as a dimer in vivo; proteins with mutations in the DELLA region or the C terminus produce a dominant negative dwarf or slender phenotype, respectively, because they retain the ability to form homodimers (and presumably heterodimers with endogenous SLR1). The ΔLZ protein, although overexpressed, produces no phenotype, presumably because it cannot form a functional dimer with itself or with endogenous SLR1.

Overproducers of the ΔpolyS/T/V-SLR1 protein showed a severe dwarf phenotype, which in this case was restored to wild type by the application of exogenous GA, suggesting that this region does not function in GA signal transduction but plays a role in regulating the GA response. Interestingly, the ΔpolyS/T/V-GFP fusion protein disappeared from the nucleus after GA treatment, as did the intact SLR1-GFP, whereas all other truncated SLR1-GFP fusion proteins lost GA responsiveness and were retained within the nucleus. These results suggest that derepression of the GA response in rice is coupled tightly to the movement of SLR1 out of the nucleus, and a similar phenomenon has been observed for the RGA protein in Arabidopsis (Dill et al., 2001; Silverstone et al., 2001).

The SLR1 domain analysis is consistent with the phenotypes of different gai and rga alleles in Arabidopsis, suggesting that it is a general feature of DELLA proteins. The semidominant GA-insensitive dwarf gai-1 mutant expresses a GAI protein that has lost the DELLA domain, and because of the loss of GA perception, it retains a negative regulatory function as a repressor of transcription, becoming a constitutive repressor of GA response genes. Loss-of-function or null alleles of RGA or GAI do not exhibit a pronounced phenotype in Arabidopsis, presumably because of the overlapping functions of the other DELLA proteins. A double mutant containing null alleles of both gai and rga, rga-24/gai-t6, exhibited a wild-type or GA-overdose phenotype, indicating that these two genes together regulate many aspects of the GA response in vegetative tissues in Arabidopsis (Dill and Sun, 2001). However, seed germination and flower development defects were not restored in the double mutant, suggesting that RGA and GAI may have minimal or reduced roles in these aspects of the GA response.

FUNCTIONAL ANALYSIS OF RGL1

Wen and Chang show that gain-of-function and loss-of-function phenotypes for rgl1 mutants are similar to those of gai and rga, with some important distinctions, suggesting that the different DELLA proteins in Arabidopsis have overlapping but distinct functions. Examination of rgl1 mutant phenotypes suggested that unlike gai and rga mutants, it has a role in floral and seed development. Wen and Chang created a GA-insensitive dwarf by overexpressing a mutated version of RGL1 (RLG1Δ17) lacking the 17–amino acid DELLA domain, as predicted by analogy to the gai-1 mutant allele, offering further confirmation of the general role of the DELLA domain in GA signal transduction. However, the rgl1Δ17 mutant displayed a floral phenotype that was similar to that of the GA biosynthesis mutant gai-3 but unlike that of other GA-insensitive mutants, such as gai-1. rgl1Δ17 flowers were severely underdeveloped (Figure 2) and were male sterile.

Wen and Chang also isolated progeny from an apparently cosuppressed rgl1Δ17 line that reverted to a wild-type phenotype. This line, designated rgl1Δ17-R, retained the transgene and kanamycin resistance but showed very low levels of both the rgl1Δ17 and the endogenous RGL1 transcript, suggesting that it could be considered a loss-of-function or pseudonull mutant. Like the null gai and rga mutants, rgl1Δ17-R lacked an obvious phenotype under normal growth conditions. However, under GA deficiency (like that induced by treatment with the GA biosynthesis inhibitor paclobutrazol [PAC]), rgl1Δ17-R exhibited GA-independent phenotypes that are distinct from the wild-type phenotype, including resistance to the PAC-induced effects of inhibition of seed germination and stem elongation, delayed flowering, and defective flower development. Importantly, some of these

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**Figure 2.** Gain-of-Function GA-Insensitive Arabidopsis rgl1 Mutant.

This transgenic plant overexpresses a mutated rgl1 gene, rgl1Δ17, which encodes a protein that lacks the 17–amino acid DELLA domain. Such plants exhibit a severe dwarf phenotype with small, dark green rosette leaves, poorly developed trichomes, and male-sterile flowers that produced stunted pistils protruding from underdeveloped sepals, petals, and stamens. Bar = 1 mm. (Figure courtesy of Caren Chang.)
effects also differed from those of the gai and rga mutants. For example, seed germination in rgl1-17-R was found to be highly resistant to PAC treatment, whereas seed from wild-type plants and from the gai-t6 null mutant was highly sensitive to PAC, indicating that RGL1 may have a unique function in seed germination.

Analysis of transcript levels of the five DELLA genes in various tissues suggested that RGL1 might play a role in floral development. RNA gel blot analysis revealed that the RGL1 transcript was most abundant in floral tissues and was weak or undetectable in rosette leaves, siliques, mature stems, and roots. RGL3 showed a pattern of expression similar to that of RGL1, whereas RGA and GAI transcripts were detected at varying levels in all tissues examined and the RGL2 transcript was undetected in any of the tissues.

Finally, Wen and Chang examined the subcellular localization of the GFP-RGL1 fusion protein. Like the SLR1 protein in rice (Itoh et al., 2002) and RGA in Arabidopsis (Silverstone et al., 2001), GFP-RGL1 was localized in the nucleus. Interestingly, in contrast to SLR1 and RGA, GFP-RGL1 was not observed to disappear from the nucleus after treatment with GA. This observation should be followed up with more detailed studies, because it suggests the possibility of a different mechanism for the regulation of RGL1 function in response to GA.

The work of Itoh et al. and Wen and Chang fills important gaps in our knowledge of GA signal transduction, but there remains much to learn about the functions of the DELLA proteins in GA responsiveness. How is GA perceived: does it bind directly at or near the DELLA domain, or are other proteins involved? How is the signal transduced from the DELLA domain to the repressor domain? What controls the movement of DELLA proteins in and out of the nucleus, or, in the case of RGL1, what regulates its function in the absence of movement in and out of the nucleus? The answers await further research on the functions of these important proteins.

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REFERENCES


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