Gibberellins (GAs) are diterpenoid plant hormones that promote a number of plant growth responses, including seed germination, stem elongation, leaf expansion, and flowering. GAs act by inducing the degradation of DELLA domain proteins that function as repressors of GA-dependent processes. Research over the past few years has uncovered the principal steps associated with GA perception and signal transduction in rice and Arabidopsis.

Two years ago, Ueguchi-Tanaka et al. (2005) identified a GA receptor in rice, GIBBERELLIN INSENSITIVE DWARF1 (GID1), which encodes a soluble protein with similarity to hormone-sensitive lipases. Nakajima et al. (2006) cloned three homologous genes in Arabidopsis, called GID1a, GID1b, and GID1c, and confirmed that all three genes encode proteins that bind GA in vitro and rescue the GA-insensitive dwarf phenotype when expressed (individually) in the rice gid1 mutant.

Recent work by Griffiths et al. (2006), Willige et al. (2007), and Iuchi et al. (2007) has shown that the gid1a gid1b gid1c triple mutant exhibits a severe GA-insensitive dwarf phenotype, demonstrating that these three proteins have overlapping functions as the major GA receptors in Arabidopsis. These studies and others have also shown that the GID1 receptors likely participate in a GA-dependent interaction with the DELLA protein SLR1 in rice and the five DELLA proteins RGA, GAI, RGL1, RGL2, and RGL3 in Arabidopsis. This interaction leads to degradation of the DELLA proteins via the proteasome pathway, thus relieving DELLA-mediated repression of GA-dependent growth processes (Figure 1). The F-box proteins GID2 in rice (Sasaki et al., 2003) and SLY1 in Arabidopsis (McGinnis et al., 2003) are components of SCF E3 ubiquitin ligases that are predicted to target the DELLA proteins for proteasome-mediated degradation in a GA-dependent fashion.

Previous work demonstrating a direct interaction between GID1-GA and SLR1 was based on yeast two-hybrid (Y2H) experiments. In this issue of The Plant Cell, Ueguchi-Tanaka et al. (pages 2140–2155) show that rice GID1 interacts directly with SLR1 in a GA-dependent manner in vivo. The authors also tested the affinity of different GAs for interaction with GID1 using the Y2H assay. GAs form a large family of compounds (136 GAs have been identified from plants, fungi, and bacteria), most of which do not show activity as plant growth regulators. GA4 and GA1 are considered the most physiologically active GAs in shoots and in germinating seeds (reviewed in Pimenta Lange and Lange, 2006). GA4 was found to have the highest affinity for GID1, in accordance with its level of activity in planta. In addition, the authors performed domain analyses of SLR1 and extensive site-directed Ala substitutions of GID1, allowing them to identify important regions of both proteins necessary for their interaction and to propose a molecular model for interaction between GA, GID1, and SLR1.

**GA, GID1, AND SLR1 INTERACT IN VIVO**

The authors sought to confirm the GA-dependent GID1–SLR1 interaction in vivo using two types of experiments. First, they used rice callus lines that overproduce green fluorescent protein (GFP)-tagged GID1. Two such transgenic lines were treated with or without GA4, and crude protein fractions were tested for coimmunoprecipitation of SLR1 with GFP-GID1. In these experiments, the SLR1 protein coimmunoprecipitated with GFP-GID1 in extracts of GA4-treated but not untreated callus. SLR1 occurs in both phosphorylated and nonphosphorylated forms, and both forms were found to interact with GA-GID1. In an earlier report, Sasaki et al. (2003) suggested that degradation of SLR1 via SCFGID2 was initiated by GA-dependent phosphorylation. However, Itoh et al. (2005) reported that both phosphorylated and nonphosphorylated SLR1 interact with GID1, which was confirmed by Ueguchi-Tanaka et al. in this issue. Itoh et al. (2005) concluded that phosphorylation of SLR1 is independent of its degradation and is dispensable for the interaction of SLR1 with GID2. The function of SLR1 phosphorylation has yet to be determined.

In a second set of experiments, the authors used bimolecular fluorescence complementation to confirm the GA-dependent interaction between GID1 and SLR1 in vivo.
IN THIS ISSUE

This method made use of constructs encoding GID1 linked to the N-terminal region of enhanced yellow fluorescent protein (N-EYFP) and SLR1 linked to the C-terminal region (C-EYFP). Agrobacterium cell suspensions carrying both constructs were infiltrated into Nicotiana benthamiana leaf epidermal cells. The YFP signal, which would occur only as a result of an interaction between N-EYFP-GID1 and C-EYFP-SLR1, was detected only in infiltrated leaves treated with GA4. In subsequent Y2H experiments, domain analysis of SLR1 showed that the DELLA and TVHYNP domains of SLR1 are required for the GID1–SLR1 interaction. Accordingly, no interaction was observed in bimolecular fluorescence complementation experiments when SLR1 constructs were used that carried deletions of the DELLA or TVHYNP regions.

GA BINDING PREFERENCE AGREES WITH GA BIOACTIVITY

Next, the authors conducted experiments to compare the in vitro preference of different GAs for GID1 with the physiological effectiveness of the GAs in planta. They used the Y2H assay to assess the selectivity of GID1 for different GAs and a leaf sheath elongation assay in seedlings of a GA-deficient rice mutant to test the bioactivity of the different GAs. In general, there was good correlation between selectivity for GID1 binding and bioactivity in the leaf sheath elongation assay. For example, GA4 and H2-GA4 showed high activity in both assays, whereas GA3 and GA4-Me showed the lowest activities in both assays. The main discrepancies were that GA3 was the most effective GA for leaf sheath elongation but showed intermediate activity for GID1 binding in the Y2H system, and GA4 showed higher activity in the Y2H assay than in the leaf sheath elongation experiment.

The authors considered that the greater response of leaf sheath elongation with GA3 compared with GA4 may be due to the stability of GA3 in planta, whereas GA4 is more rapidly inactivated in planta by GA-inactivating enzymes. This idea was tested by monitoring the GA dose response for SLR1 degradation in callus tissue, where GA-inactivating enzymes were found not to be expressed. This experiment confirmed that GA3 is the most bioactive GA among GA1, GA3, and GA4 in rice cells in the absence of GA-inactivating enzymes.

The authors speculated that GA3 showed higher activity than GA4 in the leaf sheath elongation assay because it is not inactivated by inactivating enzymes, whereas GA4 is rapidly inactivated (Sponsel and Hedden, 2004).

NEW MUTANT gid1 ALLELES

Ueguchi-Tanaka et al. (2005) previously reported four gid1 alleles, gid1-1 to -4, all of which showed similarly severe dwarf phenotypes. In this issue, the authors isolated another four mutant alleles, gid1-5 to -8 (Figure 2). Alleles showing severe phenotypes, gid1-1 to -6, contain deletions or replacements of amino acids that are conserved between rice and Arabidopsis GID1 proteins and are located in the central part of GID1. The mutated proteins produced by these mutant GID1 genes in Escherichia coli showed no GA binding activity in vitro. The two weak alleles, gid1-7 and -8, have one amino acid deletion near the C-terminal end and a single amino acid change near the N-terminus, respectively. Both of these mutant proteins showed GA binding activity, albeit with reduced levels in comparison to that of the intact GID1 protein. Interestingly, the mutated GID1-7 and GID1-8 proteins showed significantly higher activities than wild-type GID1 in the interaction with SLR1. Therefore, the weak phenotypes of these alleles could not be explained solely by weak GA binding activity or weak SLR1 interacting activity. It is hypothesized that the interaction of mutant GID1-7 and GID1-8 with SLR1 somehow interferes with the SLR1–GID2 interaction and subsequent degradation of SLR1. These new mutant alleles may provide good material for further investigating the SLR1–GID2 interaction.

SLR1 AND GID1 DOMAIN ANALYSIS

Finally, the authors conducted domain analysis of SLR1 and Ala-scanning mutagenesis of GID1 to identify regions of both proteins essential for the GA–GID1–SLR1 interaction. SLR1 domain analysis confirmed that the DELLA and TVHYNP domains of SLR1 are required for interaction with GID1. Ala-scanning mutagenesis of GID1 revealed that the amino acid residues important for SLR1 interaction completely overlapped the residues required for GA binding and were scattered throughout the
GID1 molecule. These residues were mapped onto the GID1 structure predicted by analogy with a crystal structure from a bacterial hormone-sensitive lipase family member, and many of these essential residues were found to be located at regions corresponding to the substrate binding pocket and lid. These observations, together with the results of experiments showing that SLR1 stabilizes the GA–GID1 interaction, suggest that the interaction of SLR1 with GA–GID1 closes the lid, effectively locking GA inside the substrate pocket.

The results of these experiments are in accordance with previous conclusions about GA perception and signaling but are significant in several respects. First, they provide evidence that the GA–GID1–SLR1 interaction observed in Y2H assays occurs in a similar fashion in planta. Second, they confirm that the conserved DELLA and TVHYNP motifs in the N-terminal region of SLR1 are required for this interaction, identify regions of GID1 that are required for binding to GA and to SLR1, and show that SLR1 stabilizes GA binding to GID1, allowing the authors to postulate a molecular model for the interaction. Although the model ultimately will need to be confirmed and refined by x-ray crystallography and/or other experiments, it provides a detailed picture of the essential interactions that take place in GA perception and signaling that may be used to guide further investigations.

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REFERENCES


