Rice GIBBERELLIN INSENSITIVE DWARF1 Is a Gibberellin Receptor That Illuminates and Raises Questions about GA Signaling

Recently, gibberellins (GAs) joined the list of plant hormones with a known receptor protein with the report by Ueguchi-Tanaka et al. (2005) that the GIBBERELLIN INSENSITIVE DWARF1 (GID1) protein of rice has the expected properties of the long-sought GA receptor. This exciting discovery not only provides a major new piece to the puzzle of the GA signal transduction pathway but also raises new questions and calls into question some long-held assumptions concerning the topology of the pathway.

GAs are tetracyclic diterpenoids that act at all stages in the plant life cycle by promoting germination, hypocotyl elongation, phase transitions, root, leaf, stem, and fruit growth, greening of leaves, flowering, and flower and seed development. In addition, they have been implicated in meristem function and recently have been shown to inhibit cytokinin action (Greenboim-Wainberg et al., 2005). GA action, biosynthesis, and signaling have been the subject of several recent reviews (Sponsel, 1995; Olszewski et al., 2002; Sakamoto et al., 2004; Sun and Gubler, 2004; Swain and Singh, 2005). In this essay, we summarize the steps leading to the discovery that GID1 functions as a GA receptor and discuss remaining questions concerning GA signal transduction.

GID1

In marked contrast with other loss-of-function GA response mutants, the gid1 mutant of rice appears to be completely unresponsive to GA. One of the best-characterized GA responses is the induction of α-amylase in the aleurone layer of cereal seeds (discussed below). This induction was not detectable in gid1-1 aleurone layers even when treated with 100 times more GA than is required for maximal induction in wild-type layers. Similarly, the second leaf sheath of gid1-1 does not elongate in response to treatment with large amounts of GA. Another feature of nonresponsive mutants is that they overaccumulate bioactive GA because GA signaling inhibits biosynthesis and promotes catabolism of these GAs. GA1, a bioactive GA of rice, accumulates in gid1-1 mutants up to 100-fold over the concentration in wild-type plants. The GID1 gene was cloned by chromosome walking and encodes a protein with similarity to hormone-sensitive lipases (HSLs). A GID1-green fluorescent protein (GFP) fusion expressed under the control of an actin promoter was primarily nuclear localized but was also present in the cytosol.

In animals, HSL hydrolyzes triacylglycerol, and, as the name implies, the activity of this enzyme is modified through hormone-regulated phosphorylation (Yeaman, 2004), but GID1 and the GID1-like proteins of other plants lack this regulatory domain. GID1 and the GID1-like proteins also lack a conserved His that is believed to be essential for enzyme activity, and GID1 does not have detectable lipase activity.

Ueguchi-Tanaka et al. (2005) found that GID1 binds 16,17-dihydro-GA₃ in a saturable manner. The affinity of different GAs for GID1 was estimated using a competition assay with 16,17-dihydro-GA₃. In this assay, the GAs that are bioactive in rice had at least 10-fold higher affinity for GID1 than the inactive GAs. While there was not a perfect correlation between the bioactivity of the GAs and their affinity for GID1, the estimated affinities were consistent with GID1 being a functional receptor, especially after taking into account that GAs are differentially catabolized by plants. Interestingly, an Asp and a Ser that are part of the HSL catalytic site are present in GID1 and GID1-like proteins, raising the possibility that they participate in GA binding. Based on the mechanism of HSL catalysis, the Ser could potentially form a covalent adduct with GA, but this does not seem to be the case because the binding is reversible.

gid1-1, -2, and -3 plants have no or greatly reduced responses to GAs. The gid1-1, -2, and -3 proteins, which have either a single amino acid change or a small deletion, did not bind GA, suggesting that GA binding is required for a GA response. Consistent with this hypothesis, overexpression of GID1 in rice increases the sensitivity of the second leaf sheath to GA.

One of the more informative discoveries is that in a yeast two-hybrid assay, GID1 interacts with the rice DELLA protein SLENDER RICE1 (SLR1) in a GA-dependent manner. This discovery provides additional evidence that GID1 is a GA receptor and suggests where GID1 functions in the signaling pathway. The GID1–SLR1 interaction was shown to be dependent on the presence of GA₃, a bioactive GA, but the activity of other GAs was not reported. A test of the hypothesis that the GID1–SLR1 interaction is central to GA signaling will be to determine if, as has already been shown for GA binding to GID1, the relative effectiveness of different GAs in promoting this interaction is correlated with the intrinsic bioactivity of GAs. In addition, it will be important to determine if GID1 and SLR1 interact in rice and if this interaction is GA dependent.

Arabidopsis has three potential GID1 orthologs. Like GID1, all three proteins lack the essential His of the catalytic triad,
DELLA PROTEINS

SLR1 is a member of a family of proteins called DELLA proteins that negatively regulate plant responses to GA. SLR1, which is also known as Os GAI, negatively regulates most if not all GA responses of rice and is a putative transcription factor (Ogawa et al., 2000; Ikeda et al., 2001; Itoh et al., 2002). In rice, barley, and Arabidopsis, GA signaling causes the rapid destruction of DELLA proteins by the proteasome pathway (Figure 1). Destruction of a protein by the proteasome is initiated when the protein interacts specifically with an F-box protein component of the SCF ubiquitin ligase complex. The protein is then ubiquitinated and degraded by the proteasome pathway. GID2 is the rice (Sasaki et al., 2003) and SLY and SNE are the Arabidopsis (McGinnis et al., 2003; Dill et al., 2004; Fu et al., 2004; Strader et al., 2004) F-box proteins involved in this process.

DO PLANTS HAVE ADDITIONAL GA RECEPTORS?

The hypothesis that GID1 is a GA receptor calls into question long-held views about the GA signaling pathway. While GID1 is primarily nuclear localized, there is significant experimental support for a membrane-localized GA receptor, and early action of heterotrimeric G proteins, calcium, and protein phosphorylation acting upstream of DELLA proteins (Figure 1).

Two types of experiments support the hypothesis that binding of GA to a plasma membrane–localized receptor is required for GA signaling. A classic system used to study GA signaling is the induction of α-amylase gene transcription in the aleurone layer of cereal seeds (Lovegrove and Hooley, 2000). During germination, embryos use GA to control the availability of nutrients. GA from the embryo induces the synthesis and release of hydrolytic enzymes from the aleurone layer. These enzymes act on storage macromolecules producing nutrients for the embryo. GA covalently bound to sepharose beads induces the expression of α-amylase genes in oat aleurone protoplasts but not in cells with an intact wall (Hooley et al., 1991). These results, along with additional control experiments, suggest that the response is not due to the release of GA from the sepharose. Since the size of the sepharose beads precludes entry into cells, GA must be perceived at the plasma membrane. An independent test of this hypothesis involved comparisons of the ability of extracellular and microinjected GA to induce GA responses (Gilroy and Jones, 1994). In these experiments, GA injected into barley aleurone cells did not elicit GA responses, including induction of α-amylase expression, while extracellular GA did. Since microinjected cells responded to external GA, trauma caused by injection is unlikely to compromise the ability of these cells to respond to microinjected GA.

In animals, transmembrane spanning proteins can act as hormone receptors, often directly transducing the signal through

How GA triggers the interaction of DELLA proteins with the F-box protein is an open question. Initially it was proposed that phosphorylation of DELLA proteins in a GA-dependent manner caused them to interact with the F-box proteins (Fu et al., 2002; Sasaki et al., 2003; Gomi et al., 2004), but recent results suggest that this may not be the case (Hussain et al., 2005; Itoh et al., 2005a). The discovery that in yeast cells GID1 interacts with SLR1 in a GA-dependent manner raises the possibility that the interaction with GID1 rather than phosphorylation causes SLR1 to interact with the F-box protein (Figure 1). Consistent with this hypothesis, slr1 is epistatic to gid1, and SLR1 protein accumulates in gid1 plants.

There is an interesting parallel between the auxin and GA receptors in that both appear to play a direct role in the destruction of signaling pathway proteins by promoting interaction with the SCF complex. However, because TIR1, the auxin receptor, is an F-box protein (Dharmasiri et al., 2005; Kepinski and Leyser, 2005; Woodward and Bartel, 2005), the receptors act by different mechanisms.

Figure 1. A Model of Rice GA Signaling from the Receptor to SLR1.

A previously proposed GA signaling pathway involved GA binding to an extracellular receptor with signal transduction possibly via a heterotrimeric G protein and/or Ca²⁺ (dashed arrows) triggering interaction of active SLR1 with the SCFGID2 complex and subsequent destruction by the proteasome. Posttranslational modification (M) of SLR1 by phosphorylation or addition of O-GlcNAc activates the repressive activity. The model in which binding of GA to GID1 triggers association of active SLR1 with the SCFGID2 complex (solid arrows) leading to destruction of SLR1 is also shown. The possibility that the extracellular receptor acts through GID1 is indicated by the dotted arrow from this receptor to GID1. Note that GID1 could bind GA in either the cytoplasm or nucleus.

suggesting that they will not have lipase activity. Screens for GA response mutants have not identified these proteins, suggesting that, if they are the Arabidopsis GA receptors, they have significant functional overlap.
CURRENT PERSPECTIVE ESSAY

heterotrimeric G proteins. Mutations and chemicals affecting the activity of plant heterotrimeric G proteins reduce but do not eliminate GA responses, suggesting a role for these proteins in GA signaling (Ueguchi-Tanaka et al., 2000; Ullah et al., 2002). Since inhibiting G protein signaling does not completely block GA signaling, G proteins must either function in one branch of redundant GA pathways or in another signaling pathway that regulates the GA pathway. In either case, G protein signaling is likely to act upstream of or at the DELLA protein(s) because slr1 is epistatic to a mutation affecting a rice G protein α-subunit (Ueguchi-Tanaka et al., 2000).

One of the fastest known GA responses is an increase in the concentration of cytosolic calcium, which is detectable 2 to 5 min following treatment of wheat aleurone cells (Bush, 1996). It is possible that DELLA proteins do not mediate this increase in cytosolic calcium because the fastest documented GA-induced decrease in a DELLA protein occurs 5 to 10 min after GA treatment (Gubler et al., 2002). If DELLA proteins do not mediate the increase in cytosolic calcium, GID1 must interact with other GA signaling proteins, perhaps an ion channel, or, alternatively, another GA receptor must be responsible for this effect. Therefore, it will be important to simultaneously examine the kinetics of calcium fluxes and DELLA destabilization and to determine if GID1 interacts with other proteins.

While the GA insensitivity of gid1 mutants suggests that GID1 is the only GA receptor, the results discussed above are difficult to reconcile with this model. If GID1 is the only receptor, aleurone cells should respond to microinjected GA. One possibility is that GID1 only signals when it binds GA at the external face of the plasma membrane. Since some of the GID1-GFP fusion protein is located in the cytosol, it is possible that GID1 is associated with the plasma membrane, but the lack of an identifiable membrane-spanning domain leaves open how GID1 can perceive extracellular GA. Moreover, for it to be the receptor that transduces the signal from GA conjugated to sepharose, it would need to bind the GA and undergo a stable change in conformation or be posttranslationally modified in such a way that it could then carry the signal from the plasma membrane to the nucleus as an unliganded receptor. Since these scenarios seem unlikely, it seems plausible to reject a single receptor model in favor of there being two independent receptors. However, since gid1 plants are insensitive to GA and microinjected GA is inactive, a simple model with a soluble GID1 receptor and a second plasma membrane–localized receptor is not supported. There are however several possible solutions to this conundrum. One solution is to discount the evidence for a membrane-bound receptor or to discount that GID1 is the receptor, but there are no obvious reasons for doing either. A second solution is to propose that GID1 plays multiple roles in GA signaling, with GID1 being the receptor of intracellular GA in some cells and a downstream component in other cells. Under this model, aleurone cells, which do not synthesize GA, perceive extracellular GA using a membrane-bound receptor that is not GID1, with GID1 instead acting as a downstream pathway component. To explain the inactivity of microinjected GA, it is also necessary to hypothesize that binding of GA to GID1 does not induce α-amylase expression. In cells that synthesize and respond to GA, GID1 could be the only functional receptor. A third possibility is that, since the studies discussed above employed different species, GA signaling may vary between species. However, the available evidence suggests that GA signaling is similar in different plants. Therefore, it is important to reexamine the site(s) of GA perception, the localization of GID1, and the possibility that it functions without binding GA.

FINDING NEW GA PATHWAY COMPONENTS

Based on what is known about GID1, efforts to identify new GA signaling pathway components should focus on proteins that interact with GID1 or SLR1 and the targets of SLR1. Interestingly, DELLA protein stability is affected by auxin and ethylene. The addition of ethylene (Achard et al., 2003) and disruption of auxin transport (Fu and Harberd, 2003) both delay the GA-induced degradation of DELLA proteins, suggesting that DELLA proteins moderate information from several hormone pathways. Recently, a number of experiments have suggested that the regulation of DELLA protein involves more than just destabilization by GA (Dill et al., 2001; King et al., 2001; Itoh et al., 2002, 2005b). Several lines of evidence suggest that the intrinsic activity of the DELLA proteins is regulated posttranslationally (Figure 1; Silverstone et al., 1998; Dill et al., 2001; Itoh et al., 2002). It has been noted that, whereas F-box mutants accumulate DELLA proteins to high levels, the reduction in GA response is not proportional to the amount of protein, suggesting that some of the protein is inactive (McGinnis et al., 2003; Ueguchi-Tanaka et al., 2005).

For example, gid1 plants accumulate less SLR1 than gid2 plants but have more severe GA defects (Ueguchi-Tanaka et al., 2005). Deletion of the poly-Ser/Thr/Val-rich domain of SLR1 makes it a stronger repressor of GA responses without affecting the destruction of SLR1 by GA (Itoh et al., 2002). The nature of the regulatory posttranslational modification(s) is unknown, but two candidates are phosphorylation and O-linked GlcNac (O-GlcNac). DELLA proteins are phosphoproteins (Fu et al., 2002; Sasaki et al., 2003; Gomi et al., 2004). Initially it was suggested that phosphorylation triggered the degradation of DELLA proteins, but recent inhibitor and site-directed mutant studies suggest that phosphorylation has no effect or stabilizes the proteins (Hussain et al., 2005; Itoh et al., 2005a). Perhaps phosphorylation also regulates protein activity. Mutations affecting SPINDLY suppress the repression of GA responses by DELLA proteins, suggesting that O-GlcNac modification activates DELLA proteins (Wilson and Somerville, 1995; Peng et al., 1997; Swain et al., 2001).

DELLA proteins are believed to regulate transcription, but the only evidence for this comes from transgenic spinach, where a DELLA GAL4 DNA binding domain fusion protein induced transcription of a reporter gene with a GAL4 binding site (Ogawa et al., 2000). Wild-type DELLA proteins have not been shown to
interact with chromatin. Therefore, it is important to learn if DELLA proteins interact with chromatin at specific genes and if this interaction affects the expression of these genes. In addition to providing insight into DELLA protein functions, these experiments will identify downstream GA signaling components. DELLA proteins regulating the transcription of a number of genes, each with a relatively small effect on GA responses, could explain why genetic screens have identified relatively few GA signaling genes. This hypothesis suggests that expression profiling of DELLA knockout and dominant mutants or chromatin immunoprecipitation experiments will be a productive avenue for finding additional components of the GA pathway.

Since GID1 interacts with SLR1 in a GA-dependent manner and SLR1 protein accumulates in a gid1 mutant, GID1 is likely to play a central role in controlling the stability and perhaps activity of SLR1. Therefore, a major focus of GA signaling research should be to understand the interaction between these proteins and the role of GA binding in this process.

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