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Gibberellin Signal Transduction in Rice

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Abstract

The rice *SLR1* gene is an ortholog of *Arabidopsis GA1* and *RGA*, which function as negative regulators of gibberellin (GA) signal transduction. Genetic, physiological and biochemical analyses have revealed that the SLR1 protein, which is localized in the nucleus, is degraded by an upstream GA signal to induce the downstream GA actions. In other words, SLR1 suppresses GA activity and the GA-dependent degradation of the SLR1 protein releases SLR1-suppressed GA action. We identified the link between SLR1 degradation and GA signaling when we were analyzing the rice *GA-insensitive dwarf* (GID2) mutant. The *GID2* gene encodes a putative F-box protein that is a component of an SCF complex associated with protein degradation via the 26S proteasome. Biochemical analyses of SLR1 and GID2 suggest that the proteasome-dependent degradation of SLR1 is initiated by phosphorylatian of SLR1 and mediated by an SCF complex involving GID2. In this review, we propose a model for GA signal transduction in rice.

Key words: Gibberellin; Signal transduction; Rice; Dwarf; Slender

Introduction

Gibberellins (GAs) are a large family of tetracyclic diterpenoid plant growth regulators that are associated with a number of plant growth and development processes such as seed germination, stem elongation, flowering, fruit development and regulation of gene expression in the cereal aleurone layer (Reid 1993; Hooley 1994; Davies 1995; Ross and others 1997). GA was originally identified in the process of studying a rice elongation disease called ''Bakanae-disease''. In 1898, Hori first reported that abnormal rice elongation is induced by infection of a fungus belonging to the genus *Fusarium* (Hori 1898). Following this, Sawada (1912) proposed that elongation in rice seedlings infected with bakanae fungus, *Gibberella fujikroi*, might be due to the stimulus of fungal hyphae. Kurosawa (1926) demonstrated that the abnormal stem elongation was due to a compound produced by the fungus. Yabuta and Sumiki (1938) succeeded in crystallizing the compound and named it gibberellin. To date, through the great effort of many researchers involved in the study of GA compounds, 126 GAs have been found in higher plants, fungi and bacteria (Hedden and Phillips 2000). A few of these have an important role in plant growth development.

In plants, many GA-related mutants have been isolated from various plant species (Hooley 1994;

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Ross and others 1997; Olszewski and others 2002) and can be roughly classified into 2 categories: GA-sensitive and GA-insensitive. These GA-related mutants show dwarf or elongation phenotypes, and are crucial for elucidating the regulatory mechanisms governing the GA biosynthetic and signal transduction pathways in plants. GA-sensitive mutants respond to exogenous GA because they cannot produce bioactive GA, or they produce it in insufficient quantities because of deficiencies in the genes encoding GA catalytic enzymes. Many genes encoding GA-catalytic enzymes have been identified using GA-deficient mutants, enabling researchers to build an almost complete picture of the GA biosynthetic process (Hedden and Phillips 2000; Olszewski and others 2002).

In contrast to GA-sensitive mutants, GA-insensitive mutants do not respond to exogenous GA, suggesting that the mutant genes may be involved in GA signal transduction. The mechanism by which GA triggers signal transduction is still poorly understood. The isolation and characterization of genes defining the dwarf mutation in GA-insensitive plants provide ideal tools to clarify not only the molecular mechanisms of plant growth and development, but also the signal transduction pathway of GAs. Recently, similar kinds of mutants associated with GA signal transduction have been screened and the corresponding genes have been isolated; including *gai* (Peng and others 1997) and *rga* (Silverstone and others 1998) in *Arabidopsis*, *D8* in maize, *Rht* in wheat (Peng and others 1999), and *slr1* in rice (Ikeda and others 2001; Itoh and others 2002). These are orthologous genes that work as negative regulators of GA signal transduction. In addition, we have isolated and characterized three novel genes, *D1*, *GID1* and *GID2*, using rice dwarf mutants associated with GA-signal transduction. Here, we describe the characterization of these genes and their function, and propose a putative GA signal transduction model in rice.

Rice Mutants are Useful for Analysis of GA Signal Transduction

Rice (*Oryza sativa* L.) is one of the most important staple food crop. It has been estimated to be as the main source of nutrition for 50% of the world population (White 1994). Rice is particularly important for people living in the monsoon areas of Asia where it has a long history of cultivation. For many years, rice has been the subject of numerous breeding studies aimed at developing higher yield or better taste. Over the past 100 years, rice breeders and geneticists have accumulated a large number of rice mutants and have developed a rice classical genetic map using phenotypic markers of these mutants (Nagao and Takahashi 1963; Iwata and others 1975; Kinoshita 1995). Rice has also become a useful plant for studying plant biology, as a model of monocotyledons (monocots), because of its genome synteny and compactness. Rice shows apparent genome synteny with many other important crops such as wheat, barley and maize (Gale and Devos 1998).

Rice has a small genome (430 Mb) relative to other cereal crops (about 1/10 of maize, or about 1/40 of wheat) and its genome size is about three times larger than that of *Arabidopsis*, a model plant of dicotyledons (dicots). The compact nature of the rice genome and genome synteny provide a distinct advantage in gene isolation and genomic sequencing as opposed to other cereal crops, and the results of rice genomics can be directly applied to cereal breeding due to their syntenies. For these reasons, rice has been selected as a model plant for cereal crops. Last decade, technological innovations in science enabled dramatic advancements in the field of plant genomics (genome science). Rice is the first food crop being completely sequenced and some rice genome projects have been launched which provide very useful information for plant biologists and breeders (Goff and others 2002; Yu and others 2002; Sasaki T and others 2002; Feng and others 2002).

Because dwarf characteristics are favored in plant breeding, many rice dwarf mutants have been identified and some have been used in the analysis of GA. For example, the GA-deficient mutants *sd1* and *d18*, which have mutations in the *GA20 oxidase* and *GA3 oxidase* genes, respectively, were used to elucidate the rice GA biosynthetic pathway (Ashikari and others 2002; Sasaki A and others 2002; Spielmeyer and others 2002; Itoh and others 2001). GA-deficient mutants have contributed not only to our understanding of GA analysis but also of plant breeding. The *sd1* mutant (mutation in *GA20 oxidase* gene) enabled a dramatic increase in rice yield and made a significant contribution to global food security in the 1960s. This remarkable achievement was referred to as the ''green revolution'' (Khush 1999; Ashikari and others 2002; Sasaki A and others 2002; Spielmeyer and others 2002). We have screened many rice GA-sensitive and insensitive dwarf mutants and have used them to analyze the GA biosynthetic and signal transduction pathways.

In *Arabidopsis*, GA signal genes are often redundant, making it difficult to isolate knockout

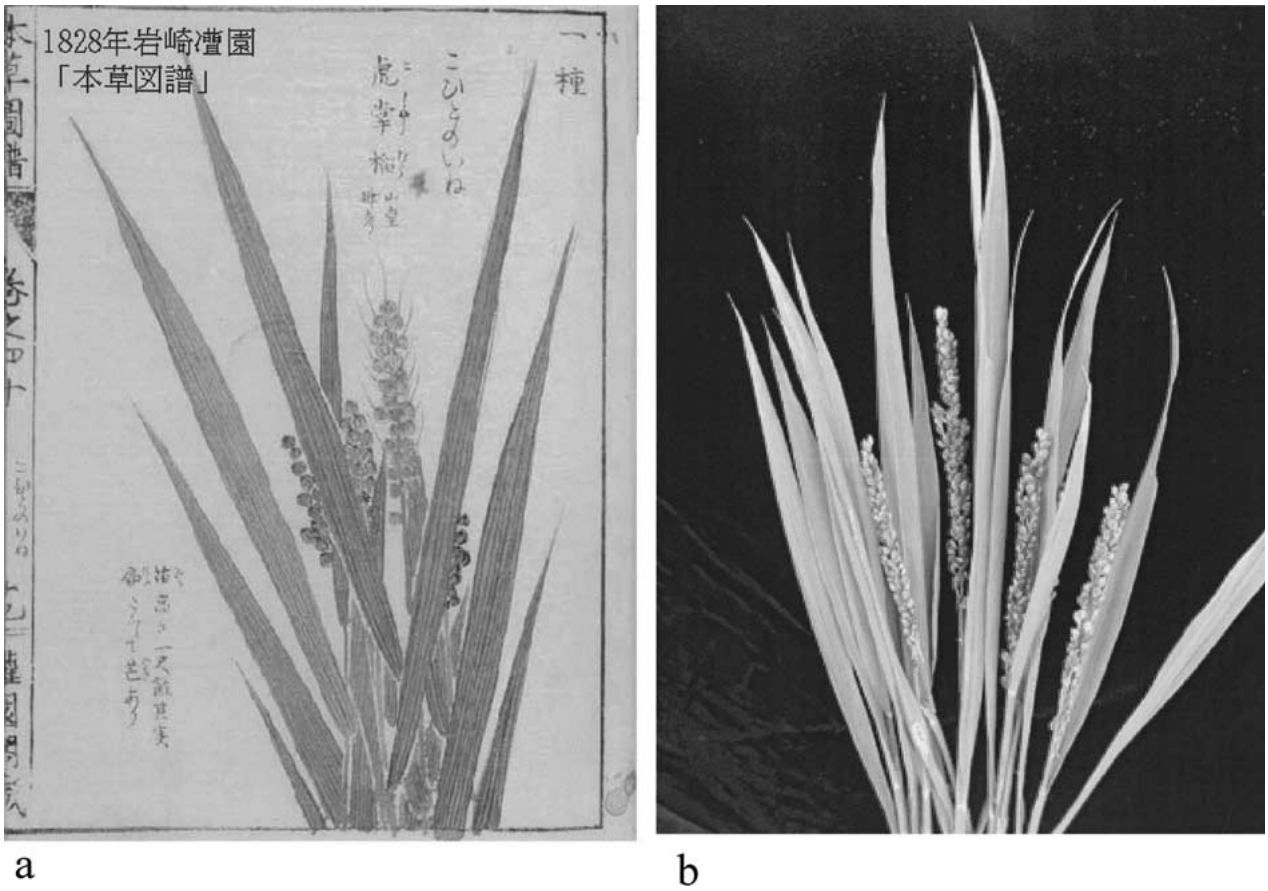

Figure 1. Gross morphology of *Daikoku Dwarf1* (*d1*). **a**. Illustrated rice *d1* mutant in Honzouzufu. **b**. Pictorial rice *d1* mutant.

mutants, for example, four *GAI* homologous genes, *RGA*/*RGL1*/*RGL2*/*RGL3*, are present (Dill and Sun 2001; Hussian and Peng in this issue). In contrast, rice has only one *GAI* ortholog gene, *SLR1*. In another example, the rice genome has one *gid1* (*gibberellin insensitive dwarf 1*) gene (see below), whereas the *Arabidopsis* genome has at least three *gid1* homologous genes (Matsuoka unpublished data). The non-redundancy of GA-related genes in rice provides an advantage for the study of GA signaling. In particular, the rice genome infrastructure enhances cloning and characterization of GA-insensitive dwarf genes for the study of the GA signal transduction pathway.

Rice GA Signal Transduction Mutants

Daikoku Dwarf (d1)

The rice dwarf mutant, Daikoku (*d1*), is one of the oldest rice mutants and is recorded in the "Honzouzufu", which is the first illustrated book of Japanese flora published in 1828 (Figure 1a). In 1925, Akemine (1925) first described the genetic characterization of *d1* by inheritance and crossing tests.

The *d1* mutant not only shows dwarfism, but also broad and dark green leaves, compact panicles, and short and round grains (Figures 1b, 2). All of these characteristics are induced in a pleiotropic manner by a recessive allele *d1*. *d1* also lacks the ability to induce α-amylase in cereal aleurone (Mitsunaga and others 1994; Ueguchi and others 2000), and consequently is classified as a GA-insensitive mutant since α-amylase induction in aleurone is one of the most typical GA-dependent events (Nolan and Ho 1988). The *D1* gene has been cloned by positional cloning and found to encode the α subunit of a heterotrimeric G-protein (Ashikari and others 1999; Fujisawa and others 1999). It is well recognized that G-proteins play an important role in signal transduction in animals and microbes (Neves and others 2002), and therefore the Gα protein may have a similar role in rice.

We have examined the GA-dependent biological actions in *d1* to investigate the role of the Gα protein in GA signaling (Ueguchi and others 2000). Exo-

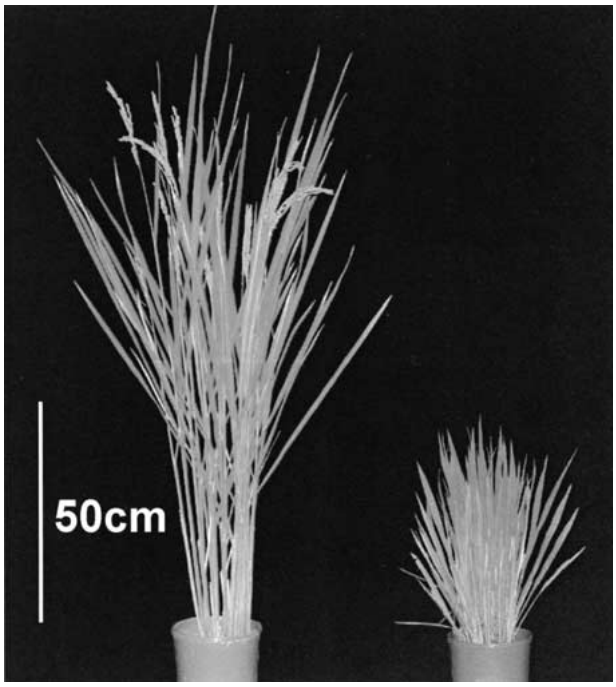

Figure 2. Comparison of plant morphology between wild and *d1*. **(Left)**. Wild plant. **(Right)**. *d1* mutant.

genous treatment with GA_3 does not induce expression of the α-amylase gene (*Ramy1A*), *GAMYB* (*OsGAMYB*), or the *Ca-ATP* gene in the *d1* aleurone layer, whereas the same treatment induces a high level of expression of these genes in wild-type plants. However, α-amylase induction occurs even in *d1* in the presence of high GA concentrations. The responsiveness of internode elongation to GA is much lower in *d1* than in wild-type plants. Furthermore, the expression of *OsGA20ox*, which encodes GA20 oxidase, is up-regulated and GA levels are elevated in the stunted internodes of *d1*.

These findings demonstrate that *D1* is involved in at least part of the GA signaling pathway, namely, the induction of α-amylase in the aleurone layer and internode elongation. In addition, analysis of a double mutant between *d1* and *slr1* (see below) has revealed that *SLR1* is epistatic to *D1*, supporting the notion that the Gα protein is involved in GA signaling. Gα is known to be associated with various kinds of signaling (Neves and others 2002; Jones 2002). Recently, it has been reported that rice Gα (*D1*) is also involved in disease resistance (Suharsono and others 2002).

It is also suggested that Gα in rice associates with other signals (see the section by Iwasaki and others in this issue). Taken together, these results suggest that the rice Gα protein is involved not only in the GA signal transduction but also in other signaling pathways. It is possible that Gα may function as an enhancer of various kinds of signaling pathways.

Slender Rice 1 (SLR1) Mutant

The *slr1* mutants show a slender phenotype with an elongated stem and leaf and reduced root number and length, which is similar to that of rice plants treated with GA_3 (Figure 3, left) (Ikeda and others 2001; Itoh and others 2002). The *slr1* mutant was first identified on the basis of its abnormal elongation phenotype at the seedling stage, which is similar to the appearance of wild-type rice plants infected by "Bakanae-disease". In fact, it is difficult to distinguish between *slr1* and "Bakanae-disease" plants. The *slr1* phenotype appears to be the result of saturation with GAs, however, the levels of endogenous GAs (GA_{19}, GA_{20} and GA_1) in *slr1* are actually lower than in the wild-type. Also, GA-inducible α-amylase (*Ramy1A*) is produced in the aleurone cells in the absence of GA application. However, the GA-saturation phenotype of *slr1* is not affected by treatment with uniconazole, a GA biosynthesis inhibitor (Ikeda and others 2001). These results indicate that *slr1* is a constitutive GA response mutant and that the SLR1 protein may be associated with GA signal transduction as a negative regulator (Ikeda and others 2001; Itoh and others 2002).

The *SLr1* gene has been isolated by linkage analyses between a rice gene homologous to *Arabidopsis GAI* and the slender phenotype. Some *slr1* alleles contain a nucleotide substitution or deletion that disrupts the open reading frame, therefore these are considered to be loss-of-function alleles. Actually, the introduction of the wild-type *SLR1* gene complements the slender mutation (Ikeda and others 2001). On the basis of these findings, the *SLR1* gene is regarded to be homologous *to Arabidopsis GAI*, which encodes a putative repressor protein for the GA signaling pathway.

The SLR1 protein shares high amino acid identity with *Arabidopsis* GAI (47.2%), RGA (41.2%), wheat RHT-D1a (77.2%) and maize d8 (80.3%). The *SLR1* gene is located on the long arm of rice chromosome 3, a region which shows the genome synteny with the wheat *Rht* locus of chromosome 4 and maize *D8* locus of chromosome 1, confirming that these genes of grass species are orthologous (Peng and others 1999; Ikeda and others 2001).

The deduced SLR1 protein has 625 amino acid residues and contains the DELLA, TVHYNP domain (called regions I and II in GAI) in the N-terminal region which is conserved among *Arabidopsis* GAI and RGA, wheat RHT and maize d8 (Peng and

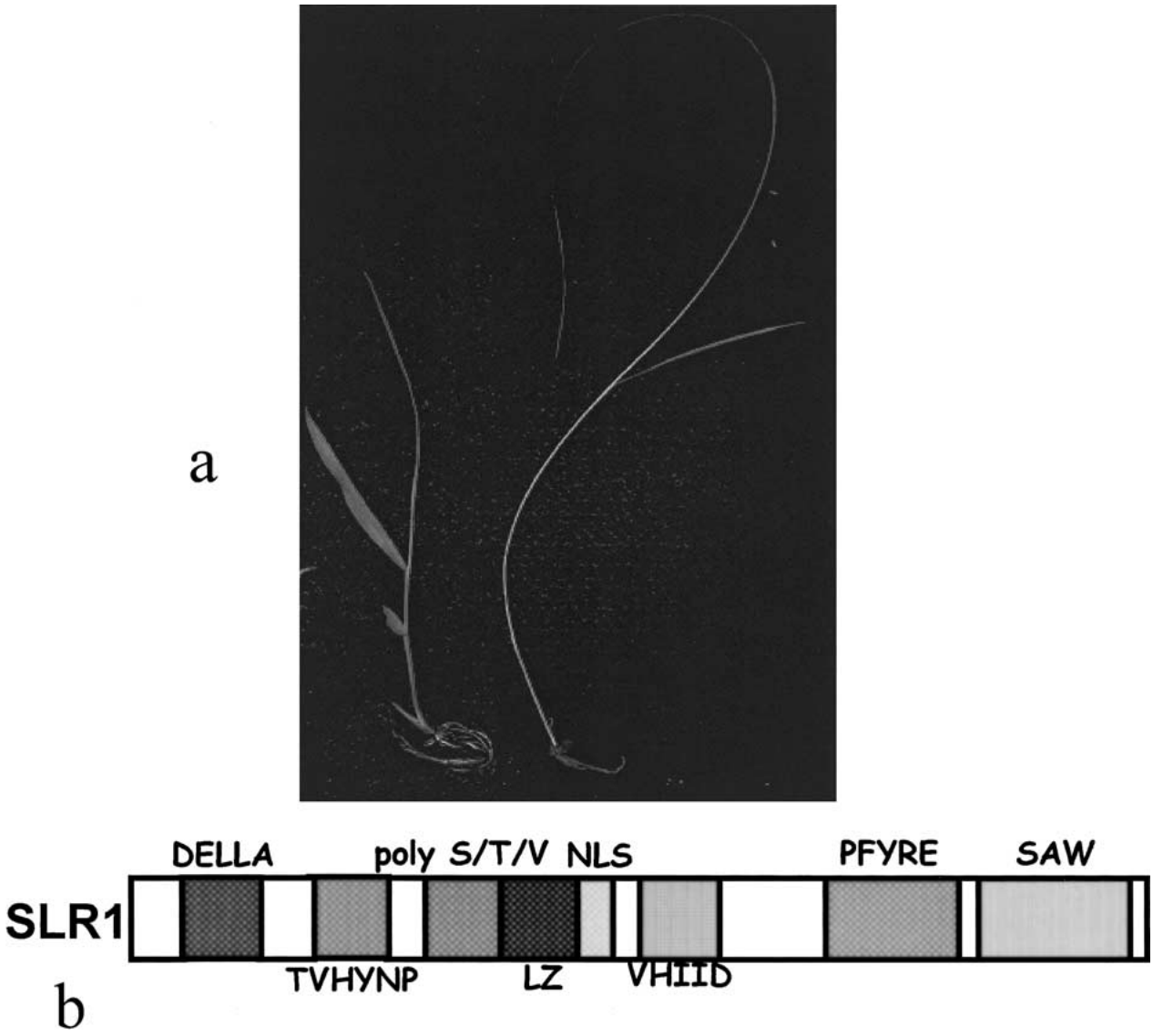

Figure 3. Gross morphology of *slender1* (*slr1*) and domain structure of SLR1. **a**. Left: wild-type, right: *slr1*, **b**. Domain structure of SLR1.

others 1999). SLR1 also contains other consensus domains at the C-terminal region, such as a leucine heptad repeat, NLS, VHIID, PFYRF and SAW, which belong to the GRAS family (Pysh and others 1999) (Figure 3b). Because proteins in the GRAS family, including *Arabidopsis* SCR (Laurenzio and others 1996), are considered to function as transcriptional factors, SLR1 may have a similar role. Biochemical analysis of SLR1, namely, nuclear localization and transcriptional activity, support this idea (Itoh and others 2002; Ogawa and others 2000).

To investigate the function of SLR1 in plants, we have generated transgenic rice plants that constitutively produce the SLR1-GFP protein under the control of the rice *Actin1* promoter. These transgenic plants show the dwarf phenotype, supporting the idea that SLR1 functions as a negative regulator of GA signaling (Itoh and others 2002). The GFP signal is localized in the nucleus but disappears following treatment with GA_3; this effect is accompanied by leaf and stem elongation. The disappearance of SLR1 in response to GA_3 treatment has been confirmed by immunoblot analysis using an anti-SLR1 antibody (Itoh and others 2002). Based on these results, we have proposed a model for SLR1 function whereby, in the absence of a GA signal, the SLR1 protein localized in the nucleus suppresses GA activity as a transcriptional regulator, but SLR1 rapidly degrades in response to a GA signal, thereby releasing the suppression of GA action (Itoh and

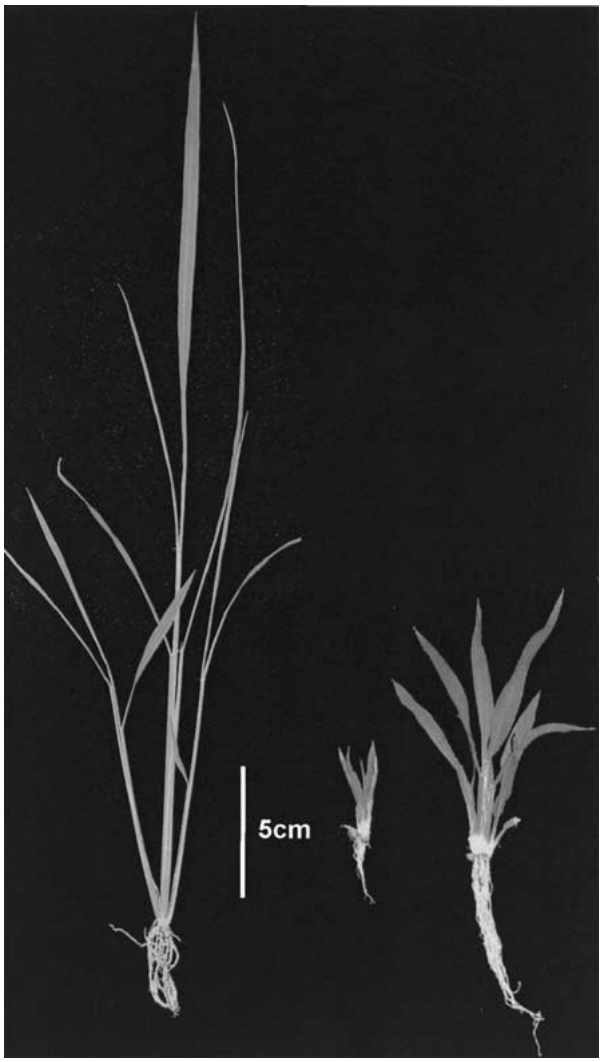

Figure 4. Gross morphology of *gid1* and *gid2*. Left: wild-type, center: *gid1*, *right*: *gid2*.

others 2002). Similar findings have also been reported for SLR1 homologous proteins: the *Arabidopsis* RGA protein and barley SLN protein are localized in the nuclei (Dill and Sun 2001; Silverstone and others 2001; Gubler and others 2002) and RGA and SLN disappear following the application of GA_3 (Dill and Sun 2001; Silverstone and others 2001; Fu and others 2002; Gubler and others 2002). This suggests that the suppressive action of SLR1, SLN1, and RGA in rice, barley, and *Arabidopsis*, respectively, is similar in the regulation of GA signaling.

Unlike SLR1, RGA and SLN1 proteins, the GAI and RGL1 (RGA-like1) proteins in *Arabidopsis* are not degraded by the GA treatment (Fleck and Harberd 2002; Wen and Chang 2002). There are two classes of the SLR1 orthologous proteins in *Arabidopsis*, one of which (RGA) disappears from the nucleus in response to GA treatment, the others (GAI and RGL1) do not (Fleck and Harberd 2002).

Dominant alleles in the *Arabidopsis gai*, wheat *Rht-B1*/*Rht-D1*, and maize *D8* loci confer GA-insensitive mutants with the dwarf phenotype (Koornneef and others 1985; Peng and others 1993; Peng and others 1997; Harberd and Freeling 1989; Winkler and Freeling 1994). Molecular cloning of *Arabidopsis GAI* has demonstrated that the in-frame deletion of its N-terminal domain, DELLA (region I), induces the *gai* mutant (Peng and others 1997). Similarly, wheat *Rht-B1*/*Rht-D1* and maize *D8* have mutations in their N-terminal domains, DELLA (region I) and TVHYNP (region II), as in *GAI* (Peng and others 1999). Transgenic plants that overproduce a SLR1 protein truncated in the DELLA domain have a dominant dwarf phenotype similar to *Arabidopsis gai* (Ikeda and others 2001; Itoh and others 2002). Interestingly, all of these mutants and transgenic plants that overproduce the truncated form of SLR1 show GA-insensitive characteristics. These results suggest that the N-terminal region involving the DELLA and TVHYNP domains may function as a receptor for upstream GA signals.

To examine the function of the conserved domain of SLR1, we have performed a domain analysis of SLR1 using transgenic plants that overproduce various truncated SLR1 proteins. Transformants overproducing ΔDELLA or ΔTVHYNP show a severe dwarf phenotype and lack GA responsiveness. Correspondingly, the ΔDELLA and ΔTVHYNP proteins do not degrade following GA treatment. These results strongly suggest that these N-terminal domains are involved in the perception of GA signals. In contrast to the N-terminal proteins, the C-terminal region containing the VHIID, PFYRE, and SAW domains is involved in the suppressive function of SLR1. This is supported by the finding that the null alleles of *slr1* often contain nucleotide substitutions or deletions in the C-terminal region. Domain analysis has also revealed that there are an additional two functional domains in SLR1, that is, a dimer formation domain and a regulatory domain. As its name suggests, the dimer domain is important for formation of a dimer of SLR1, and proteins lacking this domain (ΔLZ) do not retain their repressive function. Conversely, if a truncated SLR1 protein containing the dimer domain, but not the suppressive domain (ΔC-ter protein), is overproduced in the wild-type, the transformants show the slender phenotype, demonstrating the dominant negative function of the truncated SLR1 containing the dimer domain. The regulatory domain, which is rich in serine/threonine residues, may be involved in the regulation of

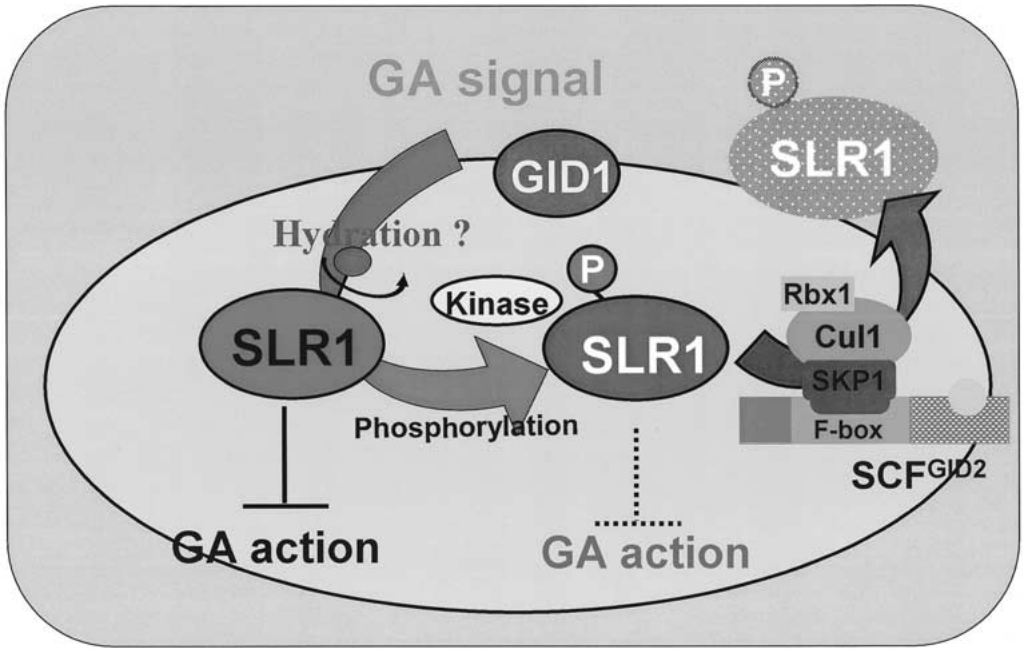

Figure 5. Putative model for the GA signal transduction pathway in rice.

SLR1 repression activity (Itoh and others 2002). In fact, it has been proposed that the activity or stability of SLR1 is regulated by *O*-GluNAcylation or phosphorylation via the action of the SPINDLY protein (Thornton and others 1999) or kinase, with the serine/threonine residues as the target site.

Dill and others (2001) have also performed a domain analysis of RGA in *Arabidopsis* using transgenic plants overproducing truncated RGA proteins. Transgenic plants with Δ*DELLA* show the GA-insensitive severe dwarf phenotype and the protein is resistant to degradation following GA treatment. This also demonstrates that the DELLA motif is essential for GA-induced RGA degradation.

Why do the loss-of-function alleles of RGA or GAI show an almost normal phenotype, even though rice *slr1* and barley *sln1* show the GA-constitutive response phenotype? For example, *gai-t6*, the loss-of-function allele of gai has wild-type features but has slightly increased resistance to paclobutrazol (PAC), an inhibitor of GA biosynthesis. This has been explained by a functional redundancy of GAI, RGA and other orthologous proteins. Indeed, *RGA* has a highly similar structure to that of *GAI*, and also works as a negative regulator of GA signaling (Silverstone and others 1998). Consequently, the loss-of-function of RGA does not result in a typical constitutive GA response phenotype but rather a partial suppression of the dwarf phenotype conferred by the GA-deficient mutation, *gal-3* (Silverstone and others 1997; Silverstone and others 1998). Double mutants *gai*/*gai*, *rga*/*rga* do not show the slender phenotype, but are slightly taller than wild-type plants. This is probably due to the presence of redundant genes, *RGLs* (*RGL1*, *RGL2* and *RGL3*) (Gill and Sun 2001). However, it has recently been reported that *RGL1* and *RGL2* play a larger role in seed germination than does *GAI* or *RGA* which are mainly associated with stem elongation (Wen and Chang 2002; Lee and others 2002). In contrast, the barley *sln1* mutant has the slender phenotype (Foster 1977) and induces α-amylase expression without GA treatment, as is the case in rice (Chandler 1988; Lanahan and Ho 1988; Croker and others 1990). The rice and barley genomes have only one gene encoding an orthologous protein to GAI/RGA (Chandler and others 2002). Such non-redundancy of GA-related genes in rice should provide an advantage for studying the GA signal transduction pathway.

Isolation Of New Genes Associated with GA Signaling

We have screened numerous rice GA-insensitive dwarf mutants from MNU, *r*-ray and T-DNA mutagenized M2 lines in order to clarify the mechanisms underlying the GA signal transduction pathway. From these, more than 50 mutants showing severe dwarfism, dark green leaves and sterility, which are similar to the phenotypes of the severe alleles of rice GA-deficient mutants, have been selected. We have

tested the GA sensitivity of these dwarf mutants using the three criteria of GA responsiveness, that is, elongation of the second leaf sheath, α-amylase induction in aleurone cells, and expression of *GA20 oxidase*. We have identified two *g*ibberellin-*i*nsensitive *d*warf (*gid*) mutants that show no second sheath elongation or α-amylase induction in response to GA treatment. High-level expression of the *GA20ox*, which is negatively regulated by active GA in a feedback manner (Xu and others 1999), also occurs in the mutants with or without GA treatment. We have characterized the *gid1* and *gid2* mutants to gain a better understanding of GA signaling.

Gid1 mutant. The *gid1* mutant was first isolated on the basis of its very severe dwarfism (Figure 4). Recently, we have identified 6 *gid1* alleles. In *gid1-1* to *gid1-6*, elongation of the second leaf sheath or α-amylase induction in aleurone cells is not observed, even at high GA concentrations. Moreover, *GA20ox* is highly expressed in *gid1* and consequently the level of GA_1 is 100 times higher than in wild-type plants. These findings demonstrate that *GID1* encodes a positive regulator of GA signal transduction. To elucidate the molecular function of *GID1*, we have cloned *GID1* by positional cloning and determined that *GID1* encodes a protein similar to members of the hydrolase proteins (unpublished results). We have also identified a double mutant *gid1*/*gid1*, *slr1*/*slr1*, indicating that *slr1* is epistatic to the *gid1*. Interestingly, a protein-protein interaction between the GID1 and SLR1 has been observed in yeast cells, indicating that GID1 may modify the stability or suppressive action of the SLR1 protein. We are now further investigating the molecular function of GID1 in GA signaling. The *Arabidopsis* genome carries at least three *GID1* homologous genes, suggesting that they may function in a redundant manner (unpublished results). Actually, our preliminary results indicate that the knockout allele of one of these homologous genes does not show an abnormal phenotype.

Gid2 mutant. The *gid2* mutant lines show a severe dwarf phenotype with wide leaf blades and dark green leaves (Figure 4), which are features of GA-related mutants such as *d1* and *d18* (Ashikari and others 1999; Itoh and others 2001). *gid2* does not show any GA-responsiveness when measured against the three criteria outlined above, that is, second leaf sheath elongation, α-amylase induction in aleurone, and feed-back expression of *GA20 oxidase*. Moreover, even though the *gid2* mutants have severe dwarfism, they accumulate more than 150 times the level of bioactive GA_1 than that in wild-type plants. Given the GA-insensitivity of the *gid2* mutant, we expect that the *GID2* gene encodes a positive regulator of GA signaling.

To clarify the molecular function of *GID2*, the gene has been isolated by positional cloning. Genetic analysis enabled us to narrow-down the *gid2* mutation to a 13kb region on rice chromosome 2. A comparison of the nucleotide sequence of this region between *gid2* and the wild-type revealed that all three *gid2* alleles have nucleotide substitutions or deletions in one putative gene that introduces novel stop codons, suggesting that these are null alleles. Introduction of a wild DNA fragment spanning the entire region of the candidate gene into the *gid2* mutant rescues the *gid2* phenotype to normal. The *GID2* gene encodes a 636bp open reading frame, capable of producing a polypeptide of 212 amino acid residues. The deduced amino acid sequence of *GID2* contains an F-box domain, which is a conserved motif of F-box proteins that form a component of an E3 ubiquitin-ligase complex. The F-box sequence in GID2 is well conserved in other F-box proteins from *Arabidopsis*, yeast, mold, and humans. Many F-box proteins contain a protein-protein interaction domain, such as leucine-rich repeat (LMR) or WD-40 repeat sequences outside the F-box (Dashaies 1999; Yang and others 1999; Li and Jonston 1997; Skowyra and others 1997; Winston and others 1999). However, we have not found any conserved motifs outside the F-box in the GID2 structure, but the structure of GID2 is similar to that of *Arabidopsis* SLY1 protein which is considered to be a positive regulator of GA signaling in *Arabidopsis* (Personal communication with C. Steber). It is very likely that the rice GID2 and *Arabidopsis* SLY1 are orthologous proteins.

As described above, the SLR1 protein functions as a repressor of GA signaling in rice and its degradation is essential for the downstream action of GA. Because the *GID2* gene encodes a F-box protein, which is a component of a SCF complex (E3 ubiquitin-ligase complex), we thought that the SLR1 protein might be targeted for degradation by the SCF complex in a GA-dependent manner. Immunoblot analysis with an anti-SLR1 antibody has revealed that the SLR1 protein accumulates at a high level in the *gid2* mutant, whereas it is only present at low levels in the wild-type. The immunoreactive SLR1 protein in the wild-type is degraded following GA_3 treatment, but this does not occur in the *gid2* mutant. These findings indicate that the GA-dependent degradation of SLR1 is defective in *gid2* and therefore SCF^{GID2} may directly target the SLR1 protein for degradation through ubiquitination.

Interestingly, there are two immunoreactive bands with different mobilities on SDS-PAGE in the *gid2* mutant whereas only one band is detected in the wild-type (A. Sasaki and others unpublished results). In *gid2*, the band with higher mobility (Form I) has the same mobility as the protein synthesized in *E. coli*, indicating that this band corresponds to the nascent protein of SLR1. We suspect that the band with lower mobility (Form II) may be an intermediate in the SLR1 degradation process (A. Sasaki and others unpublished results). Actually, the band with higher mobility is not detected under natural SCF^{GID2} functional conditions. The appearance of a band with higher mobility has also been noted in the barley *sln1d* mutant (Gubler and others 2002), and therefore may be a common part of the degradation process of the SLR1/RGA/SLN1 proteins.

Treatment of a crude extract of *gid2* with calf intestine alkaline phosphatase (CIP) prior to immunobloting leads to the disappearance of Form II SLR1. This suggests that Form II is a phosphorylated form of the SLR1 protein. Phosphorylation of SLR1 has also been examined by *in vivo* labeling with radioactive phosphate, $^{32}PO_4^-$ (A. Sasaki and others unpublished results). When the wild-type plants were incubated in the presence of$^{32}PO_4^-$, we detected one faint radioactive SLR1 band, which disappeared following GA_3 treatment. In contrast, one strong radioactive band was observed when the *gid2* plants were treated with $^{32}PO_4^-$, and its intensity was increased by GA_3 treatment. These results suggest that GA increases the phosphorylated form of SLR1 and leads to its degradation by interacting with the SCF^{GID2} complex. In *gid2*, the phosphorylation of SLR1 also occurs following GA_3 treatment but the degradation does not occur due to the loss-of-function of the GID2 protein, and consequently the SLR1 protein accumulates (A. Sasaki and others unpublished results). This model is consistent with previous findings in yeast, mammals and plant, that is, phosphorylation of a target protein triggers the degradation process (Deshaies 1999; Pozo and others 2002). A recent publication describes the inhibition of barley SLN1 protein degradation by a proteasome inhibitor (Fu and others 2002). This supports the notion that the SLR1 protein is degraded through the proteasome.

Conclusions and Prospects

Based on the results described in this review, we conclude that SLR1 functions as a molecular switch in GA signaling in rice plants. Actually, whether GA activity occurs or not is readily determined by the absence or presence, respectively, of the functional SLR1 protein in the nucleus.

GID2 encodes an F-box protein that may be a component of an SCF ubiquitin-ligase complex. The fact that *GID2* encodes an F-box protein and SLR1 is highly accumulated in the *gid2* mutant led us to speculate that GA-dependent degradation of SLR1 is mediated by the SCF^{GID2} complex. This is supported by the finding that a phosphorylated form of the SLR1 protein also accumulates in *gid2*. There are previous reports that phosphorylation of target proteins triggers SCF-mediated degradation. Our results also indicate that GA-dependent phosphorylation of SLR1 triggers the ubiquitin-mediated degradation (Figure 5) in a manner similar to the SCF-mediated pathway in plant, yeast and animals. On the other hand, the mechanism by which SLR1 perceives the GA signal is still unknown. It is possible that the other GA-insensitive dwarf gene, *GID1*, modifies the molecular structure of the SLR1 protein.

Unlike other plant hormones, the GA receptor has not yet been identified. Identification of new mutants associated with GA signaling will be important for elucidating the mechanism of the GA signal transduction pathway, including identification of the GA receptor. As in the case of *SLR1* and *GID1*, there is a tendency for the rice genome to have a single gene associated with GA signaling. This non-redundant relationship of GA signal-related genes in rice plants should facilitate the study of the GA signal transduction pathway.

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