OsRAR1 and OsSGT1 Physically Interact and Function in Rice Basal Disease Resistance

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The RAR1 and SGT1 proteins function synergistically or antagonistically in plant innate immune responses. Here, we show that the rice orthologs OsRAR1 and OsSGT1 physically interact in vivo and in yeast. They displayed conserved roles in *Arabidopsis* disease resistance through ectopic expression in the *Arabidopsis rar1* and *sgt1* mutants. Overexpression of *OsRar1* and *OsSGT1* in rice significantly increased basal resistance to a virulent bacterial blight *Xanthomonas oryzae* pv. *oryzae* PXO99 but not to another virulent strain DY89031, suggesting race-specific-like basal resistance conferred by *OsRar1* and *OsSGT1*. *OsRar1*-OE and *OsSGT1*-OE plants also enhanced resistance to all four virulent blast fungal *Magnaporthe oryzae* races. Overexpression of the *OsSGT1*-green fluorescent protein (GFP) fusion most likely caused a dominant negative phenotype which led to race-specific-like basal resistance. Transgenic plants overexpressing *OsSGT1*-GFP show enhanced resistance to DY89031 but decreased resistance to PXO99, implying that *OsSGT1* might be the target of a component required for DY89031 virulence or *OsSGT1*-GFP might stabilize weak resistance proteins against DY89031. Consistent with the hypothesis of the dominant negative regulation, we observed the reduced sensitivity to auxin of *OsSGT1*-GFP plants compared with the wild-type ones, and the curling-root phenotype in *OsSGT1*-OE plants. These results collectively suggest that *OsRar1* and *OsSGT1* might be differentially required for rice basal disease resistance. Our current study also provides new insight into the roles of *OsSGT1* in basal disease resistance.

Additional keywords: development, R-mediated resistance.

During their long-time co-evolution with microbes, plants have developed a sophisticated innate immune system that responds to infection through recognition of both general pathogen- and microbe-associated molecule patterns (PAMPs and MAMPs) by pattern recognition receptors (PRRs), and specific pathogen-encoded molecules (effectors) by disease resistance (R) proteins, resulting in defense responses (Abramovitch et al. 2006; Ausubel 2005; Chisholm et al. 2006; Dangl 2007).

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Although it does not lead to strong disease resistance compared with R-mediated specific resistance, basal defense provides the first line of defense against pathogen attack. One such PRR is the well-characterized flagellin receptor kinase FLS2 that recognizes the highly conserved flg22 peptide, leading to immediate defense responses (Asai et al. 2002; Gomez-Gomez et al. 2001; Zipfel et al. 2004). Moreover, the recognition of flg22 induces FLS2 internalization associated with defense responses (Robatzek et al. 2006). In addition, some other important components involved in basal defense have been identified, of which EDS1 and its interacting protein PAD4 are required by both R resistance and basal defense (Falk et al. 1999; Feys et al. 2001; Jirage et al. 1999; Parker et al. 1996). Recently, a key regulator of R resistance, RIN4, also has been shown to modulate flg22 signaling (Kim et al. 2005). Collectively, both basal and specific immune signaling pathways appear to be continuum-linked.

As a key component involved in diverse R resistance, RAR1 is a novel eukaryotic zinc-binding protein. It is required for race-specific resistance to powdery mildew in barley (Halterman et al. 2001; Halterman and Wise 2004; Lahaye et al. 1998; Shen et al. 2003; Shirasu et al. 1999). Genetic screening also discovered that RAR1 is a key component for diverse R-mediated resistance in *Arabidopsis* (Holt et al. 2005; Muskett et al. 2002; Tornero et al. 2002). The *Rar1* gene also plays an important role in plant basal defense: mutations in *Rar1* enhance susceptibility to virulent pathogens in *Arabidopsis* and barley (Holt et al. 2005; Jarosch et al. 2005). Furthermore, AtRAR1 is a target of the *Pseudomonas syringae* effector AvrB that suppresses PAMP-triggered immunity (Shang et al. 2006). RAR1 contains two conserved CHORD domains required for physical interaction with the partner protein SGT1, which also plays an essential role in *Mla12*-mediated specific powdery mildew resistance in barley (Azevedo et al. 2002). Similarly, SGT1 protein is required by multiple R-mediated resistance and nonhost resistance in many species, including *Arabidopsis*, tobacco, wheat, pepper, and tomato (Austin et al. 2002; Leister et al. 2005; Liu et al. 2002; Peart et al. 2002; Schornack et al. 2004; Scofield et al. 2005; Tor et al. 2002; Zhang et al. 2004). It has been demonstrated that RAR1 and SGT1 directly interact with each other in planta, and the interaction of RAR1 and SGT1 is suggested to be essential for their functions in plant defense (Austin et al. 2002; Liu et al. 2002; Tor et al. 2002). RAR1 and SGT1 function synergistically in most R resistance. However, the *Arabidopsis* AtRAR1 and AtSGT1b play antagonistic roles in accumulation of some R proteins and in basal defense (Holt et al. 2005). A recent report further suggested that the antagonis-

tic roles of AtRAR1 and AtSGT1b might result from the presence of two copies of SGT1, AtSGT1a and AtSGT1b, in *Arabidopsis*. AtSGT1a and AtSGT1b accumulate at different steady-state levels and diverse R proteins require different threshold of SGT1 to trigger effective resistance (Azevedo et al. 2006). Therefore, arguments arise on the precise mechanisms of RAR1 and SGT1 roles in plant disease resistance.

Silencing of the *Rar1* homolog *chp* in *Caenorhabditis elegans* by RNA interference (RNAi) resulted in semisterility and embryo lethality, indicating a role of RAR1 in development (Shirasu et al. 1999). In tobacco, both NbRAR1 and NbSGT1 are associated with the COP9 signalosome and the components of the Skp1/Cullin/F-box protein (SCF)-type E3 ubiquitin ligase complex, which is involved in protein degradation mediated by the COP9 signalosome (Liu et al. 2006). Furthermore, AtSGT1 is required for SCF^{TIR1}-mediated degradation of Aux/indole-3-acetic acid (IAA) proteins and is involved in the auxin-related processes, including auxin inhibition of root growth, lateral root development, hypocotyl elongation at high temperature, and apical dominance (Gray et al. 2003). Therefore, RAR1 and SGT1 link disease resistance to developmental processes. However, questions still remain as to the regulation of RAR1 and SGT1 in the cross-talk of plant defense and development.

Rice, as a monocot plant with known genome sequence, has been a model for studying the molecular mechanism of defense responses in cereal crops. The rice genome contains single-copy *Rar1* and one *SGT1* homologous genes, *OsRar1* and

OsSGT1. Whether the two genes are involved in disease resistance remains unknown. Here, we report that the rice orthologs OsRAR1 and OsSGT1 physically interact with each other and function in basal disease resistance to rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* and fungal blast caused by *Magnaporthe oryzae* in a race-specific-like basal resistance manner. We also present evidence that OsSGT1 functions in the cross-talk between defense and development, and that expression of the OsSGT1-green fluorescent protein (GFP) fusion might dominate or negatively regulate defense and root growth.

RESULTS

OsRAR1 and OsSGT1 interact with each other in vivo and in yeast.

It was reported that the barely and *Arabidopsis* RAR1 and SGT1 proteins interact with each other via their CHORDII and CS domains (Azevedo et al. 2002). We first determined subcellular localization of the OsRAR1 and OsSGT1 proteins. Both DsRED-OsRAR1 and OsSGT1-GFP fusion proteins localize ubiquitously in the cytoplasm and nucleus (Fig. 1A and B). Furthermore, co-expression of OsSGT1-GFP and DsRED-OsRAR1 showed that they were co-localized (Fig. 1C), suggesting that they might physically interact with each other in vivo. We further performed a bimolecular fluorescence complementation (BiFC) assay in which the yellow fluorescent protein (YFP)

Fig. 1. Subcellular localization and physical interaction of OsRAR1 and OsSGT1 in onion epidermal cells. **A**, Cytoplasm and nuclear localization of the OsSGT1-green fluorescent protein (GFP) fusion protein. **B**, Cytoplasm and nuclear localization of the DsRED-OsRAR1 fusion protein. **C**, Co-localization of DsRED-OsRAR1 and OsSGT1-GFP in a single cell. Green and red fluorescence were detected in the same cell, together with bright field image of cells (A–C). Merged image of green and red fluorescence generated yellow fluorescence image (left). **D**, Schematic illustration of the constructs for analysis of bimolecular fluorescence complementation (BiFC). The N and C termini of YFP were fused with OsSGT1 (YN-OsSGT1) and OsRAR1 (YC-OsRAR1), respectively. **E**, Fluorescence was restored when YN-OsSGT1 and YC-OsRAR1 were co-expressed. Bright field image of cells was shown. **F**, Co-localization of OsSGT1-GFP and DsRED-OsRAR1 in the transgenic rice root. OsSGT1-GFP rice was crossed with DsRED-OsRAR1 plant to obtain hybrid (F1) plants. Green (left) and red (middle) fluorescence were detected in the same cells. Merged image of green and red fluorescence generated yellow fluorescence image (right). The lengths of scale bars are given in 100 μ m in each panel.

was split into two polypeptides (Fig. 1D), with the N terminus fused with OsSGT1 (YN-OsSGT1) and the C terminus with OsRAR1 (YC-OsRAR1). Co-expression of YN-OsSGT1 and YC-OsRAR1 reconstituted YFP fluorescence (Fig. 1E), whereas co-expression of YN-OsSGT1 and YC or YC-OsRAR1 and YN

did not produce any fluorescence (data not shown). Collectively, we demonstrated that OsRAR1 and OsSGT1 physically interact with each other in vivo. We further examined the interacting domains of OsSGT1 and OsRAR1 using the yeast two-hybrid system. The result showed that OsRAR1 and OsSGT1 interact

Fig. 2. Determination of interaction domains of OsRAR1 and OsSGT1 in yeast two-hybrid experiments. The full-length and partial coding sequences of *OsSGT1* and *OsRAR1* were inserted into the vectors pGAD-T7 (activator domain fusion, AD), pGBK-T7 (binding domain fusion, BD), or both, as indicated. These constructs were introduced into yeast AH109 cells with empty vectors as controls, and interactions were detected in high stringent screen medium with X- α -gal according to the protocol for the GAL4 system.

Fig. 3. Conserved function of OsRAR1 and OsSGT1 in *Arabidopsis*. **A**, Levels of OsRAR1 and OsSGT1 were detected by Western blot in the transformed *Arabidopsis rar1* and *sgt1* mutants. Note that OsRAR1 accumulated to only a trace amount. **B**, Resistance gene-mediated resistance to DC3000 (*AvrRpt2*) was restored in the *rar1* mutant expressing the *AtRAR1-OsRAR1* chimera. **C**, Basal resistance to DC3000 was restored in the *rar1* mutant expressing the *AtRAR1-OsRAR1* chimera. **D**, Significantly ($P < 0.05$) enhanced basal resistance to DC3000 in the *sgt1b* mutant overexpressing *OsSGT1*. Each two representative lines of *OsRAR1* and *OsSGT1* transformants were shown. All experiments were repeated at least once with similar results.

with each other via the CHORDII domain of OsRAR1 and the CS domain of OsSGT1 (Fig. 2), consistent with the previous observation (Azevedo et al. 2002).

***OsRar1* and *OsSGT1* exhibit conserved functions in *Arabidopsis* disease resistance.**

Mutations in *AtRar1* lead to impairment of multiple R-mediated resistance and basal defense (Holt et al. 2005; Muskett et al. 2002; Tornero et al. 2002). High identity in sequences predicts that *OsRar1* and *AtRar1* could be functionally conserved. To test this hypothesis, we expressed *OsRar1* driven by the *AtRar1* promoter in the *Atrar1-11* mutant, as indicated by protein levels (Fig. 3A). The introduction of *OsRar1* restored the RPS2-mediated resistance of *Atrar1* to avirulent *P. syringae* pv. *tomato* DC3000 (*AvrRpt2*) to the same level of the wild-type *Ler* (Fig. 3B). In addition, *OsRar1* rescued basal disease resistance of *Atrar1* to virulent *P. syringae* pv. *tomato* DC3000 (Fig. 3C), indicating that *OsRar1* functions in basal defense as well.

Single mutation of neither *AtSGT1a* nor *AtSGT1b* affects the basal defense of *Arabidopsis*. We constitutively expressed *OsSGT1* (*OsSGT1*-OE) in the *Atsgt1b-1* mutant (Fig. 3A). Inoculation with *P. syringae* pv. *tomato* DC3000 showed that the *OsSGT1*-OE lines statistically significantly decreased the growth of bacteria compared with the wild-type and *Atsgt1b* mutant (Fig. 3D). We also observed that the *OsSGT1*-OE lines restored the RPP5-mediated resistance to *Peronospora parasitica* race Noco2 (Table 1). Therefore, *OsSGT1*, like *OsRar1*, is also the rice ortholog and functions in both R and basal disease resistance in transgenic *Arabidopsis*. These results demonstrate that the rice ortholog *OsRar1* and *OsSGT1* have conserved functions in *Arabidopsis* defense.

***OsRar1* and *OsSGT1* function in rice basal disease resistance to bacterial blight.**

To determine roles of *OsRar1* and *OsSGT1* in rice disease resistance, we generated transgenic rice constitutively expressing

Fig. 4. Enhanced basal disease resistance to bacterial blight in *OsRar1*-OE and *OsSGT1*-OE plants. **A**, Northern blot detection of the *OsRar1* and *OsSGT1* transcripts in transgenic plants. **B**, Levels of the OsRAR1 and OsSGT1 proteins detected by Western blot in each two representative transgenic lines, with Rubisco staining as a control. Note that both proteins were hardly detectable in the wild-type TP309. Disease resistance to virulent PXO99 was significantly enhanced ($P < 0.05$) in **C**, *OsRar1*-OE and **D**, *OsSGT1*-OE plants compared with the wild-type and vector control. Basal disease resistance in **E**, *OsRar1*-OE and **F**, *OsSGT1*-OE plants to virulent *Xanthomonas oryzae* pv. *oryzae* DY89031. No reliable difference was observed in disease resistance in these plants compared with the wild-type and vector control.

OsRar1 and *OsSGT1* (*OsRar1*-OE and *OsSGT1*-OE). RNA and Western blotting revealed that both *OsRar1* and *OsSGT1* were overexpressed in the homozygous progeny plants at the transcript and protein levels (Fig. 4A and B). We inoculated transgenic plants with virulent *X. oryzae* pv. *oryzae* PXO99 and DY89031, in comparison with the wild-type TP309, vector control, and the transgenic TP309 line 106 (Xa21-106) harboring the resistance gene *Xa21* to PXO99 but not DY89031 (Song et al. 1995; Yuan et al. 2007). All *OsRar1*-OE lines exhibited a statistically significant ($P < 0.05$) alleviation of disease symptom to PXO99 in comparison with the controls (Fig. 4C). Enhanced resistance to PXO99 was also observed for the *OsSGT1* overexpressing lines (Fig. 4D). Intriguingly, none of the *OsRar1*-OE and *OsSGT1*-OE lines showed significant change in resistance to another virulent *X. oryzae* pv. *oryzae* strain, DY89031, with repeated experiments of two generations (Fig. 4E and F). These results suggest that *OsRar1* and *OsSGT1* function in rice basal defense to bacterial blight in a race-specific-like manner (discussed below). Together with the data from transgenic *Arabi-*

dopsis (Fig. 3D), we propose that SGT1 is also a positive regulator of plant basal defense.

OsRar1 and *OsSGT1* function in rice basal disease resistance to blast fungus.

We further tested basal resistance of *OsRar1*-OE and *OsSGT1*-OE plants to blast fungus (*M. oryzae*). Unlike to *X. oryzae* pv. *oryzae*, both types of transgenic plants exhibited enhanced resistance to all the four tested virulent fungal races compared with the wild-type and vector transgenic controls (Fig. 5A and B), suggesting different requirements for *OsRar1* and *OsSGT1* in basal resistance to *X. oryzae* pv. *oryzae* and blast. Because there is no available *Osrar1* and *Ossgt1* knockout mutants, we also extensively conducted RNA interference with both double-stranded RNA technology that has been successfully used to knockdown the *OsNPR1* gene in our previous study (Yuan et al. 2007) and antisense expression, but we did not manage to generate *OsRar1* and *OsSGT1* knockdown or knockout plants (Supplemental Figure 1). It is possible that the regions of *OsRAR1* and *OsSGT1* chosen do not work well with the interference approach. It remains unknown what phenotypes, if any, are associated with knockout mutants of *OsRar1* and *OsSGT1*.

OsSGT1-GFP fusion probably acts as a dominant negative form and confers a race-specific-like basal resistance.

We generated rice plants (*OsSGT1*-GFP) constitutively expressing the *OsSGT1*-GFP fusion, in which both the *OsSGT1*-GFP fusion protein and the endogenous *OsSGT1* protein were

Table 1. *OsSGT1* restores *RPP5*-mediated resistance to *Peronospora parasitica* Noco2 in the *Atsgt1b* mutant

Line	Diseased plants/total plants
<i>Ler</i>	0/24
<i>Atsgt1b-1</i>	24/24
<i>Atsgt1b/OsSGT1-OE 2</i>	0/13
<i>Atsgt1b/OsSGT1-OE 50</i>	0/24
<i>Atsgt1b/OsSGT1-OE 106</i>	0/21
<i>Atsgt1b/OsSGT1-OE 170</i>	0/24

Fig. 5. Enhanced basal disease resistance to *Magnaporthe oryzae* in *OsRar1*-OE and *OsSGT1*-OE plants. Leaves were spot inoculated with spore suspensions of four blast races (CH131, OS9649, CH155, and SY22) and disease symptoms were recorded at 5 days postinoculation. Each two representative lines of **A**, *OsRar1*-OE and **B**, *OsSGT1*-OE plants were shown, together with the controls. Note that lesion development was inhibited in the transgenic plants compared with the wild-type and vector controls in independent inoculation experiments.

produced (Fig. 6A, upper panel). OsSGT1-GFP accumulation was further confirmed by Western blotting with a commercial anti-GFP antibody (Fig. 6A, lower panel). The OsSGT1-GFP fusion also ubiquitously localized in the cytoplasm and nucleus of transgenic rice root cells (Fig. 1F). We observed that the basal disease resistance to PXO99 was significantly compromised in OsSGT1-GFP plants (Fig. 6B). Surprisingly, resistance to DY89031 was greatly increased in all lines to a level similar to *Xa21*-triggered resistance to PXO99 with repeated experiments of two generations (Fig. 6C and D). Because TP309 does not contain known *R* genes to either PXO99 or DY89031, we propose that OsSGT1-GFP most likely acts as a dominant negative form that inhibits the endogenous OsSGT1-mediated resistance to PXO99 but positively regulates resistance to DY89031. We also generated transgenic rice lines (DsRED-OsRar1) constitutively expressing the *DsRED-OsRar1* fusion that express both the DsRED-OsRAR1 and endogenous OsRAR1 proteins (Fig. 7A). The DsRED-OsRAR1 fusion protein also was co-localized with OsSGT1-GFP fusion in the root cells of the transgenic hybrid plant (Fig. 1F), suggesting that the fusions do not change their physical interaction in the rice cell as in the onion cell. However, no changes were observed in resistance to both *X. oryzae* pv. *oryzae* strains in DsRED-OsRar1 plants (Fig. 7B and C). Whether the DsRED-OsRAR1 fusion has lost its function remains to be investigated.

OsSGT1-OE plants did not display obvious morphological changes. Interestingly, their roots exhibited a dramatic curling pattern under culture conditions (Supplemental Figure 2), similar to the *Arabidopsis Atmlo* mutants (Sandra et al. 2007). We further measured root responses to auxins and found that OsSGT1-GFP plant roots were less sensitive to 2,4-dichlorophenoxyacetic acid (2,4-D) in comparison with wild-type roots, and no obvious difference was observed in responses to indole 3-acetic acid (IAA) between OsSGT1-GFP and wild-type plants. In light of SGT1 functions in the auxin-mediated development (Gray et al. 2003; Liu et al. 2002), these results indicate that OsSGT1 also is involved in the auxin-mediated pathways and that the SGT1-GFP fusion protein also might cause a dominant negative regulation on development mediated by the uncharacterized auxin pathway in rice.

DISCUSSION

Our study has demonstrated the conserved functions of OsRAR1 and OsSGT1 in rice immunity. The functions of RAR1 and SGT1 in defense responses likely are fulfilled through their physical interaction in a complex (Azevedo et al. 2002; Dodds and Schwechheimer 2002; Muskett and Parker 2003). We performed detailed experiments to visualize the molecular interaction between OsRAR1 and OsSGT1 (Fig. 1). We also showed that this interaction is via the CHORD II and CS do-

Fig. 6. Race-specific-like basal disease resistance in OsSGT1-green fluorescent protein (GFP) plants. **A**, Both the endogenous OsSGT1 protein and the OsSGT1-GFP fusion were expressed in two representative OsSGT1-GFP lines. **B**, Significant decreased ($P < 0.05$) resistance to virulent *Xanthomonas oryzae* pv. *oryzae* PXO99 in the OsSGT1-GFP lines compared with TP309, with Xa21-106 as the resistant control. **C**, High resistance to virulent *X. oryzae* pv. *oryzae* DY89031 in the OsSGT1-GFP lines, in comparison with TP309, Xa21-106, and OsSGT1-OE plants. **D**, Lesion lengths of OsSGT1-GFP, TP309, and Xa21-106 inoculated with *X. oryzae* pv. *oryzae* DY89031.

mains of OsRAR1 and OsSGT1 (Fig. 2). Therefore, our study supports that the physical interaction is a common feature of RAR1 and SGT1 in diverse plant species.

RAR1 is required for basal defense in *Arabidopsis* and barley, probably through its contribution to R protein accumulation (Holt et al. 2005; Jarosch et al. 2005). We showed that overexpression of *OsRar1* and *OsSGT1* restricted growth of one of the two virulent bacterial strains and all fungal races tested (Figs. 4 and 5). The difference in basal resistance of *OsRar1*-OE and *OsSGT1*-OE plants to *X. oryzae* pv. *oryzae* and *M. oryzae* probably resulted from distinct pathways by which rice responds to *X. oryzae* pv. *oryzae* and *M. oryzae*, as suggested by our microarray assay (Li et al. 2006). Furthermore, we found that constitutively expressing *OsSGT1* in an *Arabidopsis sgt1b* mutant also increased resistance to its virulent pathogen (Fig. 3D), indicating that *SGT1*, like *Rar1*, also plays a positive role in plant basal defenses in addition to its involvement in R and nonhost disease resistance (Peart et al. 2002).

It is surprising that *OsSGT1*-OE and *OsRar1*-OE plants exhibited race-specific-like basal resistance to PXO99 and DY89031, suggesting that the two strains acquire divergent virulent components. Furthermore, when overexpressed as a GFP fusion, *OsSGT1* appeared to cause a dominant negative phenotype that impaired basal disease resistance to PXO99 but induced a race-specific-like resistance to DY89031 (Fig. 6).

Fig. 7. Basal disease resistance in DsRED-OsRar1 plants. **A**, Both endogenous OsRAR1 and DsRED-OsRAR1 fusion were expressed in two representative DsRED-OsRar1 lines. Asterisks indicate nonspecific proteins detected by the OsRAR1 antibodies. **B**, Disease resistance to PXO99 in DsRED-OsRar1, TP309, and Xa21-106 plants. **C**, Disease resistance to DY89031 in DsRED-OsRar1, TP309, and vector control plants. No reliable difference was observed in disease resistance in these plants compared with the wild-type and vector control.

Similar dominant negative effects of GFP fusion proteins were observed in the GFP-phragmoplastin fusion protein on cell plate formation of tobacco BY-2 cells (Gu and Verma 1997) and the ASK1-GFP on *Arabidopsis* growth and fertility (Wang and Yang 2006).

These observations have not been reported in plants. The complicated phenotype may indicate different requirements for the SGT1 protein or its complex in basal defense to diverse bacterial strains, because SGT1 is required differently for diverse *R* genes and nonhost resistance (Austin et al. 2002; Leister et al. 2005; Schornack et al. 2004; Scofield et al. 2005; Tor et al. 2002). Most likely, the *OsSGT1*-GFP fusion titrates out and the wild-type *OsSGT1* (as well as *OsRAR1*) stabilizes weak *R* proteins that recognize uncharacterized effectors or pathogen determinants of PXO99. In this scenario, weak *R* proteins recognizing effectors of DY89031 may be different from those of PXO99 and the *OsSGT1*-GFP fusion might stabilize weak *R* proteins against DY89031. Alternatively, *OsSGT1* may be the target of a component required for DY89031 virulence, and the *OsSGT1*-GFP fusion could have titrated out the component, similar to AtRAR1 as the target of the *P. syringae* AvrB (Shang et al. 2006). Consistent with our hypothesis, DY89031 contains mutations in the genes required for AvrXa21 activity to XA21 (Goes da Silva et al. 2004), which is proposed to be a secreted peptide acting as a quorum-sensing molecule with features of PAMP and Avr elicitors (Burdman et al. 2004; Lee et al. 2006). Further evidence that PAMPs derived from different bacterial strains induce strain-specific defense responses comes from the study that flagellin of *P. avenae* functions as a specific elicitor in rice (Che et al. 2000). A recent report also showed that sequence variation of flagellin among *X. campestris* pv. *campestris* strains affects FLS2-dependent pathogen recognition by *Arabidopsis* (Sun et al. 2006).

Our current study indicates that, although *OsRAR1* and *OsSGT1* physically interact with each other in planta and both function in basal disease resistance, mechanisms by which *OsRAR1* and *OsSGT1* regulate basal defense might be different, because the DsRED-OsRAR1 fusion protein appeared to not alter resistance to *X. oryzae* pv. *oryzae* (Fig. 7). Consistent with this idea, RAR1 and SGT1 in other plant species also function differently or even antagonistically in some *R*-gene-mediated resistance (Holt et al. 2005; Leister et al. 2005). It will be interesting to further investigate the mechanism of fine-tuning the RAR1–SGT1 interaction and additional components in the RAR1–SGT1 complex as previously proposed (Dodds and Schwechheimer 2002; Muskett and Parker 2003). *OsSGT1* loss-of-function data is not available currently; therefore, the dominant negative-like phenotype of *OsSGT1*-GFP needs to be further characterized genetically.

The *OsSGT1*-GFP fusion function is supported by the developmental phenotype. Because SGT1 has been shown to act cooperatively with the SCF^{TIR1}-mediated auxin pathway and the COP9 signalosome (Gray et al. 2003; Liu et al. 2006), our data point strongly to the possibility that *OsSGT1* and *OsSGT1*-GFP differently regulate the rice COP9 signalosome and SCF^{TIR1} complex that interactively play key roles in plant development through ubiquitin-proteasome-mediated protein degradation (Wei and Deng 2003). Due to lacking functional information of the rice COP9 and SCF^{TIR1} complexes (Yamamoto et al. 2003), we currently do not know how the *OsSGT1*-GFP-mediated dominant negative regulation occurs. Our preliminary data showed that *OsSGT1*-GFP roots were less sensitive to 2,4-D but not to IAA in comparison with wild-type roots. It is possible that the *OsSGT1*-GFP fusion might affect a particular auxin transport system differentially required for 2,4-D and IAA responses, as previously reported (Zhuang et al. 2006). Interestingly, the

curling root phenotype also is associated with the *Atmlo* mutants (Sandra et al. 2007). Overexpression of *OsSGT1* leads to this phenotype in rice, probably through regulating the interaction between SGT1 and RAR1, because the latter has been shown to be associated with the MLO function and rice contains a functional MLO ortholog (Elliott et al. 2002). The detailed proteomic and genetic analysis, and the identification of the SGT1-interacting components that is required for DY89031 virulence and probably for COP9/SCF^{TIR1}-mediated development as well, would provide a clue for the *OsSGT1*-mediated phenotypes.

MATERIALS AND METHODS

Gene cloning and plasmid construction.

Using publicized genome sequences of rice, we isolated the 1.2-kb full-length *OsSGT1* cDNA (GenBank accession number AF192467) from a rice cDNA library, which was inserted into *KpnI* and *BamHI* sites of the binary vector 35S-C1301 (provided by P. Ronald) to form the *OsSGT1* overexpression construct *OsSGT1-OE*. The full-length cDNA of *OsRar1* (GenBank accession number C28356) was obtained from the National Institute of Agrobiological Sciences (Tsukuba, Japan) and was inserted into *ApaI* and *SacI* sites of 35S-C1301 to form the overexpression construct *OsRar1-OE*. For complementation of the *Arabidopsis rar1* mutant, the *OsRar1* cDNA was placed under the promoter of *AtRar1* to generate the *AtRar1-OsRar1* chimera that was inserted into pCambia1301 for *Arabidopsis* transformation. The construct *OsSGT1-OE* also was transformed into the *Atsgt1b* mutant. For RNAi, a 600-bp *OsRar1* or *OsSGT1* cDNA fragment was reversely inserted into 35S-C1301 to generate antisense expression plasmid, or was inserted into the double-stranded RNA interference vector 1300S to form RNAi plasmid (Yuan et al. 2007).

Rice and *Arabidopsis* transformation.

Rice and *Arabidopsis* transformation was performed with the *Agrobacterium*-mediated method. The rice cv. Taipei 309 (TP309) was used as recipient to produce more than 20 independent transgenic lines for each construct. All transformants were confirmed by genomic DNA polymerase chain reaction and β -glucuronidase staining, and the homozygous progenies (T2 to T4) were used for all assays. The *Arabidopsis* mutants *rar1-11* and *sgt1b-1* (Austin et al. 2002; Muskett et al. 2002) were transformed with *AtRar1-OsRar1* and *OsSGT1-OE* for complementation experiments. Transformants were screened with hygromycin and homozygous progeny lines were used for pathogen inoculation.

Pathogen inoculation.

Seven-week-old plants of independent transgenic lines and controls were inoculated with *X. oryzae* pv. *oryzae* races P6 (strain PXO99) and K1 (strain DY89031) using the leaf clip method (Song et al. 1995). After 2 weeks, lesion lengths were recorded. For blast inoculation, four virulent races (OS9649, Sy22, CH155, and CH131) and a spot-inoculation method were applied (Xiong and Yang 2003). Leaves were pierced with a needle, and a droplet of spore suspension (10 μ l containing approximately 50 spores) was inoculated on the punctured sites. Inoculated leaves were kept in a growth chamber with light intensity of 240 μ m m⁻² s⁻¹ at 26°C, and disease symptoms were recorded at 5 and 7 days postinoculation. *P. syringae* inoculation on *Arabidopsis* was performed with the syringe injection method. The bacterial pathogen growth curve was measured with independent assays. *Arabidopsis* inoculation with *Peronospora parasitica* race Noco2 was performed as previously described (Bowling et al. 1997).

Subcellular localization and BiFC analysis.

The full-length coding regions of *OsSGT1* and *OsRar1* were in-frame fused with *mGFP* (GenBank accession number U87973) and *DsRED* (GenBank accession number AF168419) in 35S-C1301 with appropriate sites (Supplemental Table 1). The *OsSGT1*-GFP and *DsRED*-*OsRar1* fusions were expressed transiently in onion epidermal cells by particle bombardment. Transgenic rice also was generated with the fusion constructs. For analysis of BiFC of *OsSGT1* and *OsRar1* interaction, the fragments corresponding to the N (YN1-155) and C (YC156-239) termini of YFP (GenBank accession number AF292557) were fused in-frame with *OsSGT1* and *OsRar1*, respectively, to generate BiFC expression constructs YN-*OsSGT1* and YC-*OsRar1* in 35S-C1301 with appropriated sites, which were co-introduced into onion epidermal cells by particle bombardment. Fluorescent images were observed with a confocal laser microscopy (Zeiss LSM510).

Yeast two-hybrid assay.

The full-length and partial coding regions of *OsSGT1* and *OsRar1* were recombined in frame into pGAD-T7 and pGBK-T7 (Clontech, Palo Alto, CA, U.S.A.), respectively, to construct yeast two-hybrid pairs with appropriate restriction enzyme sites. Yeast transformation and protein interaction were performed according to the manufacturer's protocol.

RNA preparation and Northern blotting.

Total RNA was prepared from leaf tissues using TRIzol reagent according to the manufacturer's instructions (Gibco BRL, Gaithersburg, MD, U.S.A.). RNA samples (20 μ g) were separated on a 1% formaldehyde-agarose gel, and then blotted onto Hybond-N membranes (Amersham, Tokyo). The 1.1-kb *OsRar* and a 1.2-kb *OsSGT1* fragments were labeled with [α -³²P] dCTP using a random primer labeling kit (Takara) for hybridization and autoradiography.

Protein analysis.

The full-length coding regions of *OsRar1* and *OsSGT1* were ligated into pET-32a to produce the fusion proteins in the *Escherichia coli* BL21(DE3). Antibodies (antiserum) against the *OsRar1* and *OsSGT1* fusion proteins were raised with standard procedure. Western blotting was performed using the SuperSignal West chemiluminescence kit according to the manufacturer's protocol (Pierce, Rockford, IL, U.S.A.).

Auxin responses.

For analysis of auxin responses, 2,4-D and IAA of different concentrations were added to 1/2 Murashige Skoog medium. Sterilized rice seed were germinated on the media at 60 μ m m⁻² s⁻¹ light intensity, 26°C, and day and night length of 14 and 10 h, respectively. Root length was measured at day 7 after germination.

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LITERATURE CITED

Abramovitch, R. B., Anderson, J. C., and Martin, G. B. 2006. Bacterial elicitation and evasion of plant innate immunity. *Nat. Rev. Mol. Cell. Biol.* 7:601-611.

- Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W. L., Gomez-Gomez, L., Boller, T., Ausubel, F. M., and Sheen, J. 2002. MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* 415:977-983.
- Austin, M. J., Muskett, P., Kahn, K., Feys, B. J., Jones, J. D., and Parker, J. E. 2002. Regulatory role of SGT1 in early R gene-mediated plant defenses. *Science* 295:2077-2080.
- Ausubel, F. M. 2005. Are innate immune signaling pathways in plants and animals conserved? *Nat. Immunol.* 6:973-979.
- Azevedo, C., Sadanum, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K., and Schulze-Lefert, P. 2002. The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. *Science* 295:2073-2076.
- Azevedo, C., Betsuyaku, S., Peart, J., Takahashi, A., Noel, L., Sadanum, A., Casais, C., Parker, J., and Shirasu, K. 2006. Role of SGT1 in resistance protein accumulation in plant immunity. *EMBO (Eur. Mol. Biol. Organ.) J.* 25:2007-2016.
- Bowling, S. A., Clarke, J. D., Liu, Y., Klessig, D. F., and Dong, X. 1997. The cpr5 mutant of *Arabidopsis* expresses both NPR1-dependent and NPR1-independent resistance. *Plant Cell* 9:1573-1584.
- Burdman, S., Shen, Y., Lee, S. W., Xue, Q., and Ronald, P. 2004. RaxH/RaxR: a two-component regulatory system in *Xanthomonas oryzae* pv. *oryzae* required for AvrXa21 activity. *Mol. Plant-Microbe Interact.* 17:602-612.
- Che, F. S., Nakajima, Y., Tanaka, N., Iwano, M., Yoshida, T., Takayama, S., Kadota, I., and Isogai, A. 2000. Flagellin from an incompatible strain of *Pseudomonas avenae* induces a resistance response in cultured rice cells. *J. Biol. Chem.* 275:32347-32356.
- Chisholm, S. T., Coaker, G., Day, B., and Staskawicz, B. J. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* 124:803-814.
- Dangl, J. L. 2007. Nibbling at the plant cell nucleus. *Science* 5815:1088-1089.
- Dodds, P. N., and Schwachheimer, C. 2002. A breakdown in defense signaling. *Plant Cell (Suppl.)* 14:S5-8.
- Elliott, C., Zhou, F., Spielmeier, W., Panstruga, R., and Schulze-Lefert, P. 2002. Functional conservation of wheat and rice Mlo orthologs in defense modulation to the powdery mildew fungus. *Mol. Plant-Microbe Interact.* 15:1069-1077.
- Falk, A., Feys, B. J., Frost, L. N., Jones, J. D., Daniels, M. J., and Parker, J. E. 1999. EDS1, an essential component of R gene-mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. *Proc. Natl. Acad. Sci. U.S.A.* 96:3292-3297.
- Feys, B. J., Moisan, L. J., Newman, M. A., and Parker, J. E. 2001. Direct interaction between the Arabidopsis disease resistance signaling proteins, EDS1 and PAD4. *EMBO (Eur. Mol. Biol. Organ.) J.* 20:5400-5411.
- Goes da Silva, F., Shen, Y., Dardick, C., Burdman, S., Yadav, R., Sharma, P., and Ronald, P. 2004. Bacterial genes involved in type I secretion and sulfation are required to elicit the rice *Xa21*-mediated innate immune response. *Mol. Plant-Microbe Interact.* 17:593-601.
- Gomez-Gomez, L., Bauer, Z., and Boller, T. 2001. Both the extracellular leucine-rich repeat domain and the kinase activity of FLS2 are required for flagellin binding and signaling in *Arabidopsis*. *Plant Cell* 13:1155-1163.
- Gray, W. M., Muskett, P. R., Chuang, H. W., and Parker, J. E. 2003. *Arabidopsis* SGT1b is required for SCF(TIR1)-mediated auxin response. *Plant Cell* 15:1310-1319.
- Gu, X., and Verma, D. P. 1997. Dynamics of phragmoplastin in living cells during cell plate formation and uncoupling of cell elongation from the plane of cell division. *Plant Cell* 9:157-169.
- Halterman, D., Zhou, F., Wei, F., Wise, R. P., and Schulze-Lefert, P. 2001. The MLA6 coiled-coil, NBS-LRR protein confers AvrMla6-dependent resistance specificity to *Blumeria graminis* f. sp. *hordei* in barley and wheat. *Plant J.* 25:335-348.
- Halterman, D. A., and Wise, R. P. 2004. A single-amino acid substitution in the sixth leucine-rich repeat of barley MLA6 and MLA13 alleviates dependence on RAR1 for disease resistance signaling. *Plant J.* 38:215-226.
- Holt, B. F., Belkadir, Y., and Dangl, J. L. 2005. Antagonistic control of disease resistance protein stability in the plant immune system. *Science* 309:929-932.
- Jarosch, B., Collins, N. C., Zellerhoff, N., and Schaffrath, U. 2005. RAR1, ROR1, and the actin cytoskeleton contribute to basal resistance to *Magnaporthe grisea* in barley. *Mol. Plant-Microbe Interact.* 18:397-404.
- Jirage, D., Tootle, T. L., Reuber, T. L., Frost, L. N., Feys, B. J., Parker, J. E., Ausubel, F. M., and Glazebrook, J. 1999. *Arabidopsis thaliana* PAD4 encodes a lipase-like gene that is important for salicylic acid signaling. *Proc. Natl. Acad. Sci. U.S.A.* 96:13583-13588.
- Kim, M. G., da Cunha, L., McFall, A. J., Belkadir, Y., DebRoy, S., Dangl, J. L., and Mackey, D. 2005. Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in *Arabidopsis*. *Cell* 121:749-759.
- Lahaye, T., Shirasu, K., and Schulze-Lefert, P. 1998. Chromosome landing at the barley *Rar1* locus. *Mol. Gen. Genet.* 260:92-101.
- Lee, S. W., Han, S. W., Bartley, L. E., and Ronald, P. C. 2006. Unique characteristics of *Xanthomonas oryzae* pv. *oryzae* AvrXa21 and implications for plant innate immunity. *Proc. Natl. Acad. Sci. U.S.A.* 103:18395-18400.
- Leister, R. T., Dahlbeck, D., Day, B., Li, Y., Chesnokova, O., and Staskawicz, B. J. 2005. Molecular genetic evidence for the role of SGT1 in the intramolecular complementation of Bs2 protein activity in *Nicotiana benthamiana*. *Plant Cell* 17:1268-1278.
- Li, Q., Chen, F., Sun, L., Zhang, Z., Yang, Y., and He, Z. 2006. Expression profiling of rice genes in early defense responses to blast and bacterial blight pathogens using cDNA microarray. *Physiol. Mol. Plant Pathol.* 68:51-60.
- Liu, Y., Schiff, M., Serino, G., Deng, X. W., and Dinesh-Kumar, S. P. 2002. Role of SCF ubiquitin-ligase and the COP9 signalosome in the N gene-mediated resistance response to *Tobacco mosaic virus*. *Plant Cell* 14:1483-1496.
- Muskett, P., and Parker, J. 2003. Role of SGT1 in the regulation of plant R gene signalling. *Microbes Infect.* 5:969-976.
- Muskett, P. R., Kahn, K., Austin, M. J., Moisan, L. J., Sadanum, A., Shirasu, K., Jones, J. D., and Parker, J. E. 2002. *Arabidopsis* RAR1 exerts rate-limiting control of R gene-mediated defenses against multiple pathogens. *Plant Cell* 14:979-992.
- Parker, J. E., Holub, E. B., Frost, L. N., Falk, A., Gunn, N. D., and Daniels, M. J. 1996. Characterization of eds1, a mutation in *Arabidopsis* suppressing resistance to *Peronospora parasitica* specified by several different RPP genes. *Plant Cell* 8:2033-2046.
- Peart, J. R., Lu, R., Sadanandom, A., Malcuit, I., Moffett, P., Brice, D. C., Schausser, L., Jaggard, D. A., Xiao, S., Coleman, M. J., Dow, M., Jones, J. D., Shirasu, K., and Baulcombe, D. C. 2002. Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. *Proc. Natl. Acad. Sci. U.S.A.* 99:10865-10869.
- Robatzek, S., Chinchilla, D., and Boller, T. 2006. Ligand-induced endocytosis of the pattern recognition receptor FLS2 in *Arabidopsis*. *Genes Dev.* 20:537-542.
- Sandra, N., Jones, A. M., Chen, Z., and Panstruga, R. 2007. In: 18th Int. Conf. *Arabidopsis* Res. Beijing.
- Schornack, S., Ballvora, A., Gurlbeck, D., Peart, J., Baulcombe, D., Ganai, M., Baker, B., Bonas, U., and Lahaye, T. 2004. The tomato resistance protein Bs4 is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of AvrBs4 and overexpressed AvrBs3. *Plant J.* 37:46-60.
- Scotfield, S. R., Huang, L., Brt, A. S., and Gill, B. S. 2005. Development of a virus-induced gene-silencing system for hexaploid wheat and its use in functional analysis of the *Lr21*-mediated leaf rust resistance pathway. *Plant Physiol.* 138:2165-2173.
- Shang, Y., Li, X., Cui, H., He, P., Thilmony, R., Chintamanani, S., Zwiesler-Vollick, J., Gopalan, S., Tang, X., and Zhou, J. 2006. RAR1, a central player in plant immunity, is targeted by *Pseudomonas syringae* effector AvrB. *Proc. Natl. Acad. Sci. U.S.A.* 103:19200-19205.
- Shen, Q. H., Zhou, F., Bieri, S., Haizel, T., Shirasu, K., and Schulze-Lefert, P. 2003. Recognition specificity and RAR1/SGT1 dependence in barley Mla disease resistance genes to the powdery mildew fungus. *Plant Cell* 15:732-744.
- Shirasu, K., Lahaye, T., Tan, M. W., Zhou, F., Azevedo, C., and Schulze-Lefert, P. 1999. A novel class of eukaryotic zinc-binding proteins is required for disease resistance signaling in barley and development in *C. elegans*. *Cell* 99:355-366.
- Song, W. Y., Wang, G. L., Chen, L. L., Kim, H. S., Pi, L. Y., Holsten, T., Gardner, J., Wang, B., Zhai, W. X., Zhu, L. H., Fauquet, C., and Ronald, P. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science* 270:1804-1806.
- Sun, W., Dunning, F. M., Pfund, C., Weingarten, R., and Bent, A. F. 2006. Within-species flagellin polymorphism in *Xanthomonas campestris* pv. *campestris* and its impact on elicitation of *Arabidopsis* FLAGELLIN SENSING2-dependent defenses. *Plant Cell* 18:764-779.
- Tor, M., Gordon, P., Cuzick, A., Eulgem, T., Sinapidou, E., Mert-Turk, F., Can, C., Dangl, J. L., and Holub, E. B. 2002. *Arabidopsis* SGT1b is required for defense signaling conferred by several downy mildew resistance genes. *Plant Cell* 14:993-1003.
- Tornero, P., Merritt, P., Sadanum, A., Shirasu, K., Innes, R. W., and Dangl, J. L. 2002. RAR1 and NDR1 contribute quantitatively to disease resistance in *Arabidopsis*, and their relative contributions are dependent on the R gene assayed. *Plant Cell* 14:1005-1015.
- Wang, Y., and Yang, M. 2006. The ARABIDOPSIS SKP1-LIKE1 (ASK1)

- protein acts predominately from leptotene to pachytene and represses homologous recombination in male meiosis. *Planta* 223:613-617.
- Wei, N., and Deng, X. W. 2003. The COP9 signalosome. *Annu. Rev. Cell Dev. Biol.* 19:261-286.
- Xiong, L., and Yang, Y. 2003. Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *Plant Cell* 15:745-759.
- Yamamoto, T., Kimura, S., Mori, Y., Uchiyama, Y., Ishibashi, T., Hashimoto, J., and Sakaguchi, K. 2003. Interaction between proliferating cell nuclear antigen and JUN-activation-domain-binding protein 1 in the meristem of rice, *Oryza sativa* L. *Planta* 217:175-183.
- Yuan, Y., Zhong, S., Li, Q., Zhu, Z., Lou, Y., Wang, L., Wang, J., Li, Q., Wang, M., Yang, D., and He, Z. 2007. Functional analysis of rice *NPRI*-like genes reveals that *OsNPRI/NH1* is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. *Plant Biotechnol. J.* 5:313-324.
- Zhang, Y., Dorey, S., Swiderski, M., and Jones, J. D. 2004. Expression of RPS4 in tobacco induces an AvrRps4-independent HR that requires EDS1, SGT1 and HSP90. *Plant J.* 40:213-224.
- Zhuang, X., Jiang, J., Li, J., Ma, Q., Xu, Y., Xue, Y., Xu, Z., and Chong, K. 2006. Over-expression of OsAGAP, an ARF-GAP, interferes with auxin influx, vesicle trafficking and root development. *Plant J.* 48: 581-591.
- Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E., Jones, J.D.G., Felix, G., and Boller, T. 2004. Bacterial disease resistance in *Arabidopsis* through flagellin perception. *Nature* 428:764-767.