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RACK1 Functions in Rice Innate Immunity by Interacting with the Rac1 Immune Complex W OA

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A small GTPase, Rac1, plays a key role in rice (*Oryza sativa*) innate immunity as part of a complex of regulatory proteins. Here, we used affinity column chromatography to identify rice RACK1 (for Receptor for Activated C-Kinase 1) as an interactor with Rac1. RACK1 functions in various mammalian signaling pathways and is involved in hormone signaling and development in plants. Rice contains two *RACK1* genes, *RACK1A* and *RACK1B*, and the RACK1A protein interacts with the GTP form of Rac1. Rac1 positively regulates *RACK1A* at both the transcriptional and posttranscriptional levels. *RACK1A* transcription was also induced by a fungal elicitor and by abscisic acid, jasmonate, and auxin. Analysis of transgenic rice plants and cell cultures indicates that RACK1A plays a role in the production of reactive oxygen species (ROS) and in resistance against rice blast infection. Overexpression of RACK1A enhances ROS production in rice seedlings. RACK1A was shown to interact with the N terminus of NADPH oxidase, RAR1, and SGT1, key regulators of plant disease resistance. These results suggest that RACK1A functions in rice innate immunity by interacting with multiple proteins in the Rac1 immune complex.

INTRODUCTION

Plants have a number of defense mechanisms to protect them from infection by nematodes, fungi, bacteria, and viruses (Chisholm et al., 2006; Jones and Dangl, 2006). Two systems of plant disease resistance have been extensively studied: pathogen-associated molecular pattern (PAMP)–triggered innate immunity (PTI) and effector-triggered immunity. PTI is induced by recognition of PAMPs, or elicitors, produced by pathogens. The classes of PAMP molecules that stimulate plant defense responses are similar to those that stimulate nonspecific immunity in mammals and include lipopolysaccharides, chitin, and transglutaminase (Zipfel and Felix, 2005; Chisholm et al., 2006). Receptors for plant PAMPs have recently been identified (Zipfel, 2008).

Effector-triggered immunity is specifically induced by the interaction of disease resistance (R) proteins and cognate effectors produced by pathogens. This type of resistance is characterized by hypersensitive cell death in infected plants (Chisholm et al., 2006; Jones and Dangl, 2006). Recognition of specific effectors by nucleotide binding Leu-rich repeat (NB-LRR)–type R proteins, the major class of R proteins, can be either direct or indirect. In an indirect recognition, plant proteins other than R protein are used as sensors for the entry of protein effectors into plant cells (Chisholm et al., 2006). In *Arabidopsis thaliana–Pseudomonas syringae* interactions, resistance signaling is initiated by changes that occur in host protein RIN4 that are caused by interaction with effectors (Mackey et al., 2002, 2003; Axtell and Staskawicz, 2003). The changes in RIN4 are recognized by R proteins, leading to activation of resistance signaling. Direct interactions between effectors and NB-LRR proteins have been observed between the rice (*Oryza sativa*) NB-LRR protein Pi-ta and its corresponding effector (Jia et al., 2000) and between flax R protein and effectors from flax rust fungus (Dodds et al., 2006).

Neither the mechanisms that allow direct or indirect recognition of effectors nor the mechanisms by which NB-LRR proteins are activated by effectors are known. It is also not known how the immune response is initiated by activated R proteins. R proteins are imported into the nucleus where they are apparently active, suggesting that R proteins may function at multiple sites in the cytoplasm and the nucleus (Burch-Smith et al., 2007; Shen et al., 2007; Wirthmueller et al., 2007).

Rac/Rop small GTPases constitute the sole group of Rho family small GTPases in plants and have diverse functions in many important cellular activities, such as polar growth, cell differentiation, and stress responses (Yang and Fu, 2007). The roles of the Rac/Rop small GTPases in plant innate immunity have been studied in rice, barley (*Hordeum vulgare*), and other

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species (Gu et al., 2004; Assmann, 2005; Berken, 2006; Nibau et al., 2006). Rice and *Arabidopsis* contain seven and 11 Rac genes, respectively (Yang, 2002; Miki et al., 2005), and Os Rac1 is involved in basal and R protein–mediated resistance to rice blast fungus (*Magnaporthe grisea*) and bacterial blight (Ono et al., 2001; Suharsono et al., 2002). Os Rac1 is involved in the immune response induced by *N*-acetylchitooligosaccharide and sphingolipids, which act as PAMPs (Ono et al., 2001; Suharsono et al., 2002).

In rice, Rac1 regulates cell death, reactive oxygen species (ROS) production, activation of pathogenesis-related gene expression, and phytoalexin production (Kawasaki et al., 1999). It also regulates lignin production by interacting with and activating cinnamoyl CoA reductase (Kawasaki et al., 2006). Furthermore, PAMP-induced activation of rice MAPK6 requires Rac1, and MAPK6 coimmunoprecipitates with Rac1 protein in rice cell extracts (Lieberherr et al., 2005). A proteomic analysis of proteins induced by the constitutively active (CA) form of Rac1 in cultured rice cells indicates that the majority of proteins induced by sphingolipid elicitors (SEs) are also induced by CA-Rac1 (Fujiwara et al., 2006). Rac1 forms a complex with RAR1, HSP90, and HSP70, which are conserved components of plant innate immunity as part of its pathogen defense response (Thao et al., 2007).

The dominant-negative (DN) form of Rac1, the form that suppresses its endogenous activity, inhibits *N* gene–mediated resistance to tobacco mosaic virus infection in tobacco (*Nicotiana tabacum*; Moeder et al., 2005). Similarly, Rac/Rop GTPase is involved in ROS production in *Arabidopsis* (Park et al., 2000). Barley RacB is required for susceptibility to powdery mildew fungus (Schultheiss et al., 2002, 2003). Thus, Rac/Rop GTPases play important roles in innate immunity by regulating a number of downstream components of the immune response. However, how Rac/Rop GTPase acts in the early stages of plant innate immunity signaling is not well understood.

To further understand the role of Rac/Rop GTPase and the molecular components of rice innate immunity, we applied a proteomic approach to identify Rac1-interacting proteins in rice. Among the selected Rac1-interacting proteins, we selected RACK1, which has been extensively studied and has been shown to be involved in many cellular activities in animals. RACK1 interacts with many signaling proteins in animals (Schechtman and Mochly-Rosen, 2001; Yaka et al., 2002; Patterson et al., 2004; Lopez-Bergami et al., 2005) and based on its structure (McCahill et al., 2002) is considered to be a scaffolding protein in a number of signaling pathways. In addition to its signaling roles, it is associated with 80S ribosomes and is involved in translational regulation in yeasts and animals (Link et al., 1999; Ceci et al., 2003; Sengupta et al., 2004). Recently it was also shown that RACK1 is associated with cytosolic ribosomes in *Arabidopsis* (Chang et al., 2005; Giavalisco et al., 2005). Thus, it is evident that RACK1 plays multiple roles in the cellular activities of eukaryotes. We have previously shown that rice RACK1 plays a key role in the production of ROS and disease resistance and binds RAR1 and SGT1, two important regulators of plant innate immunity (Shirasu and Schulze-Lefert, 2003). Here, we show that RACK1 is positively regulated by Rac1 at both the transcriptional and posttranscriptional levels, that it interacts with other proteins in the Rac1 immune complex, and that it is involved in resistance to rice blast infection.

RESULTS

Isolation of RACK1 by Rac1 Affinity Column Chromatography

To understand the molecular mechanisms of disease resistance induced by Rac GTPase, an affinity method successfully used for the isolation of Rho GTPase-interacting proteins in mammals (Amano et al., 1996) was adapted to isolate Rac1-interacting proteins using GST-Rac1 affinity column chromatography. A simplified methodology for Rac1 affinity column chromatography is shown in Figure 1A. Affinity columns were loaded with GST, GDPβS-GST-Rac1, or GTPγS-GST-Rac1 (GTPγS is a nonhydrolyzable GTP analog), and protein extracts obtained from rice cell cultures treated with a sphingolipid elicitor were passed through the columns. To obtain proteins with various functions, both GTP and GDP forms of Rac1 were used. Bound proteins were eluted with 500 mM NaCl (Figure 1B). A total of 21 proteins were identified by mass spectrometry (Table 1; see Supplemental Tables 1 to 3 online), of which seven may play a role in rice innate immunity (Table 1). Five were identified as NB-LRR type proteins, which are the largest class of plant R proteins (Dangl and Jones, 2001). In addition, proteins homologous to RACK1 and stress-inducible protein 1 (STI1), which are involved in cellular signaling in animals and other organisms were found. STI1 (also known as Hop in mammalian cells) is a cochaperone for the HSP90 and HSP70 chaperones (Pratt and Toft, 2003) and has three tetratricopeptide repeat domains (Smith, 2004). None of these identified proteins were previously shown to interact with Rac/Rop GTPase. We chose RACK1 for further study since its role in plant innate immunity has not been studied in any system.

Rac1 Interacts with WD40 Repeats 1 and 2 of RACK1A

RACK1 was originally isolated as a receptor for activated C-kinase 1, and it has homology with the G protein β-subunit (Ron et al., 1994). RACK1 contains seven WD40 repeats and serves as an adaptor protein by binding with protein kinases, phosphatases, transcription factors, and membrane receptors in various mammalian signaling pathways (Schechtman and Mochly-Rosen, 2001; Yaka et al., 2002; Patterson et al., 2004; Lopez-Bergami et al., 2005). In rice, a RACK1 homolog was isolated as a homolog of Gβ and designated RWD (Iwasaki et al., 1995), but its function has not been studied. Rice has two copies, and *Arabidopsis* has three copies of the RACK1 homologs (Chen et al., 2006). To follow the nomenclature used in *Arabidopsis* (Chen et al., 2006), we have used *RACK1A* and *RACK1B* for the two rice *RACK1* genes in this article. RACK1A and RACK1B share 82% amino acid identity (Figure 2A). Rac1 affinity chromatography identified RACK1A but not RACK1B (Figure 2A).

Since rice RACK1A was isolated from the affinity columns bound with both GDPβS-GST-Rac1 and GTPγS-Rac1, we examined interactions of RACK1A and RACK1B with Rac1 by yeast two-hybrid assays (Figure 2B). The CA form of Rac1 could bind

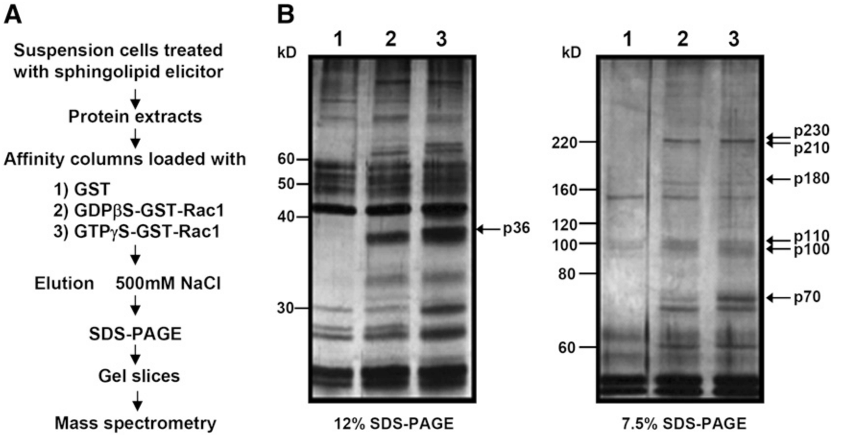

Figure 1. Rac1 Affinity Column Chromatography and Identification of Rac1-Interacting Proteins.

(A) Simplified Rac1 affinity column chromatography methodology.

(B) Separation of proteins eluted from affinity columns by SDS-PAGE. Proteins were eluted from affinity columns bound with GST (lane 1), GDPβS-GST-Rac1 (lane 2), and GTPγS-GST-Rac1 (lane 3). Eluted protein sizes determined by mass spectrometry are indicated at the right side of the gel (see Table 1).

both RACK1A and RACK1B, but RACK1A interacted with CA-Rac1 with higher affinity (Figure 2B), consistent with the finding that only RACK1A was isolated using affinity chromatography (see boxed peptides in Figure 2A). The DN form of Rac1 did not interact with RACK1A or RACK1B, indicating that only RACK1A is an effector of Rac1 and thus may be involved in Rac GTPase signaling in rice. Analysis using various fragments of RACK1A showed that WD40 repeats 1 and 2 interacted with Rac1 (Figure 2C). RACK1A also binds CA-Rac1 with higher affinity than RACK1B, as measured using WD40 repeats 1 and 2 (Figure 2D). The other six *Rac* genes present in the rice genome (Miki et al., 2005) were also tested for interactions with RACK1A using the split ubiquitin yeast two-hybrid system (Kim et al., 2002) that detects interaction at the periphery of the plasma membrane. In addition to Rac1, CA forms of Rac3 and Rac6 clearly interacted with RACK1A, and Rac5 and Rac7 weakly interacted with RACK1A (Figure 2E). Rac4 did not interact with RACK1A.

Intracellular Localization of RACK1A

To test whether Rac1 associates with RACK1A in rice cell extracts, we performed coimmunoprecipitation experiments using extracts from transgenic rice cell cultures expressing myc-CA-Rac1, myc-DN-Rac1, or myc-C212S-Rac1 (a construct in which the C-terminal Cys was exchanged with Ser to block the plasma membrane localization of myc-CA-Rac1), all under the control of the constitutive maize (*Zea mays*) *Ubiquitin* promoter (Lieberherr et al., 2005). The RACK1 antibody used in this study preferentially recognized RACK1A (see Supplemental Figure 1 online). RACK1A coimmunoprecipitated with myc-CA-Rac1 but not with DN or CS mutant Rac1 protein, indicating that RACK1A associates with the GTP form of Rac1 in rice cell extracts (Figure 3A), consistent with the yeast two-hybrid assay results. Endogenous Rac1 could not be immunoprecipitated with Rac1-specific antibody (Lieberherr et al., 2005) (data not shown).

Table 1. Proteins Identified by GST-Rac1 Affinity Column Chromatography

MW	Homologous Protein	GTP/GDP[a]	Accession Number	Mascot Score	No. of Peptides[b]
p230	NB-LRR protein	GTP	gi 16905161	121	2
p210	NB-LRR protein	GTP	gi 115443833	103	3
P180	NB-LRR protein	GTP	gi 15422169	87	2
p180	NB-LRR protein	GTP	gi 55770687	125	3
p110	NB-LRR protein	GTP	gi 14589374	65	2
p70	Stress-induced protein 1 (STI1/Hop) like protein (Os STI1a, Os STI1b)	GTP	gi 115447567	79	2
p36	WD repeat–containing receptor of activated C kinase 1 (RACK1) homolog (RACK1A)	GTP	gi 1346109	162	3

[a] GTP and GDP indicate affinity columns bound to GTP-Rac1 and GDP-Rac1, respectively, identified in the corresponding protein.

[b] Numbers of matching peptides identified in the amino acid sequence of assigned protein.

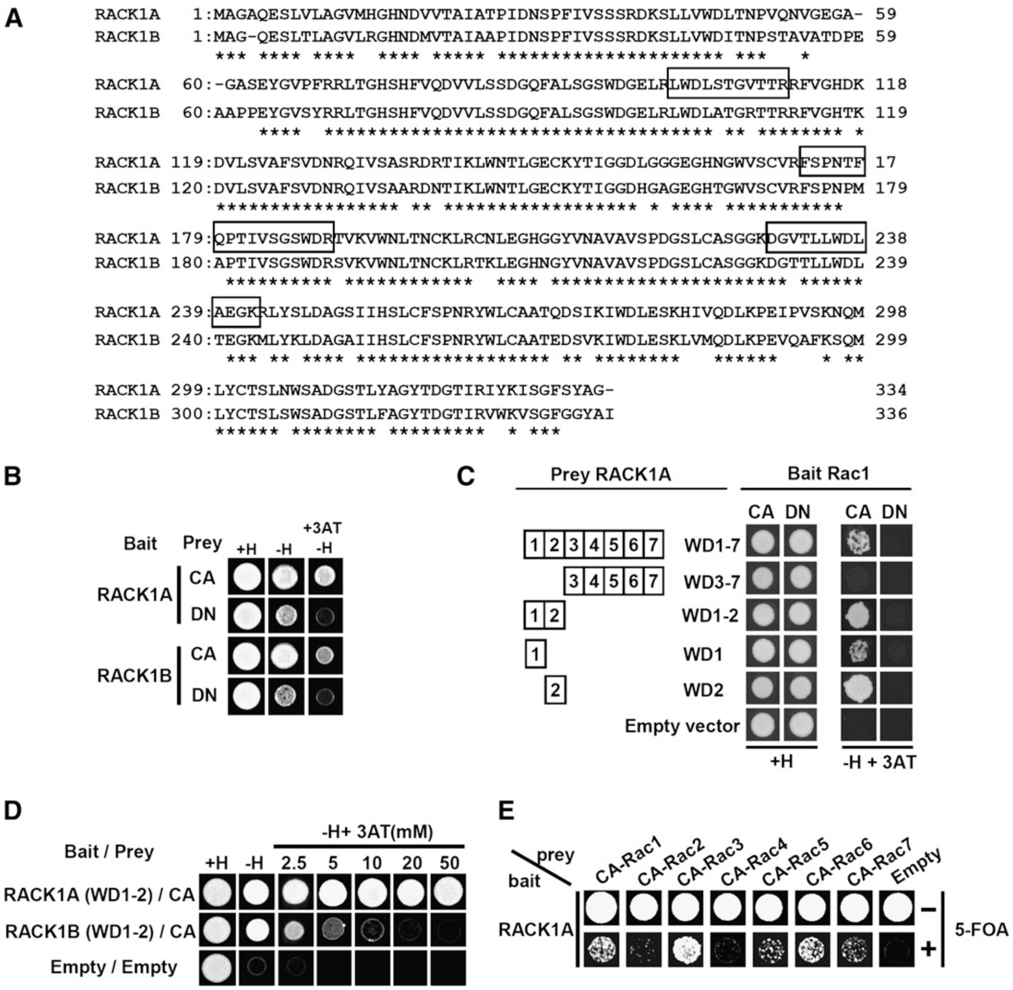

Figure 2. Interaction of RACK1 and Rac1 in Yeast Two-Hybrid Assays.

(A) Alignment of the amino acid sequences of rice RACK1A and RACK1B. The peptide fragments identified by mass spectrometry are boxed. Asterisks represent amino acids conserved in RACK1A and RACK1B.

(B) Interaction of RACK1A and RACK1B with CA- and DN-Rac1 in yeast two-hybrid assays using a LexA-VP16 system. This two-hybrid system detects the protein–protein interaction in nuclei. Since the C-terminal Cys of Rac1, which is required for plasma membrane localization, was considered to inhibit transfer of Rac1 into nuclei, the Cys residue was exchanged with Ser. Growth on selective plates without His (−H) or without His plus 3-aminotriazole (3AT) indicates a positive interaction.

(C) Determination of specific WD40 repeats that interact with Rac1 using yeast two-hybrid assays.

(D) Difference of binding activities of RACK1A and RACK1B to Rac1.

(E) Interaction of RACK1A and the CA form of Rac family proteins in split-ubiquitin two-hybrid assays. Growth on selective plates with 5-fluroorotic acid (5-FOA) indicates a positive interaction.

To determine the intracellular localization of RACK1A in rice cells, we isolated proteins from the cytosolic and microsomal fractions of rice cell cultures expressing CA, DN, and the C212S mutant form of Rac1 and immunoblotted with anti-RACK1A antibody (Figure 3B). RACK1A protein was found in both cytosolic and microsomal fractions of the wild type and in the three mutant transgenic cell culture lines. RACK1A–green fluorescent protein (GFP) fluorescence was mainly detected in the cytoplasm (Figure 3C). RACK1-GFP localization in rice cells is similar to mammalian cells (Parent et al., 2008).

RACK1A Expression Is Induced by an Elicitor and Plant Hormones in Rice Cell Cultures

To study the function of RACK1A, its expression was studied in wild-type and transgenic rice cell cultures expressing CA-Rac1 (Figure 4). *RACK1A* was induced in CA-Rac1 cell cultures by an SE (Figure 4A) that has been previously shown to induce a variety of defense responses in rice (Koga et al., 1998; Suharsono et al., 2002). However, there was no induction of *RACK1A* expression by SE in wild-type cell cultures (Figure 4A). These results show that *RACK1A* expression is induced by an elicitor in CA-Rac1 cell cultures but not in wild-type cell cultures, suggesting that RACK1A expression may be positively regulated by the elicitor and active Rac1.

Since T-DNA insertion mutants of *RACK1A*, one of three *RACK1* homologs in *Arabidopsis*, were shown to have altered sensitivity to multiple hormones (Chen et al., 2006), we tested the effects of plant hormones on *RACK1A* expression in cell culture. Methyl jasmonate, indole-3-acetic acid (IAA), and abscisic acid (ABA) all induced *RACK1A* expression in wild-type cell cultures (Figures 4B to 4D), suggesting that *RACK1A* expression is not only induced by an elicitor but phytohormones in rice cell culture. The mechanisms of RACK1 induction appear to be different, since RACK1 induction by the three phytohormones did not

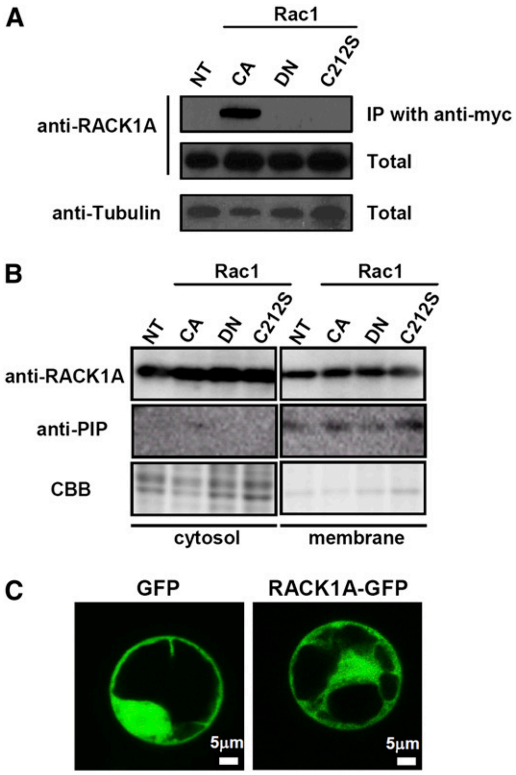

Figure 3. Intracellular Localization of RACK1A.

(A) RACK1A coimmunoprecipitates with Rac1 in a GTP-dependent manner. Total protein extracts from transgenic cell cultures expressing Rac1 mutant proteins (CA, DN, or C212S) were incubated with anti-myc antibody and protein A Sepharose beads. Precipitates were washed, collected by centrifugation, and separated by SDS-PAGE. Total extracted and immunoprecipitated samples from nontransformed (NT) cell cultures were used. Immunoblot analyses were performed with anti-RACK1A and antitubulin antibodies. RACK1A was detected in CA immune complexes but not in DN or C212S-Rac1.

(B) Intracellular localization of RACK1A. Soluble and microsomal membrane fractions were prepared from transgenic *Rac1* cell cultures and subjected to immunoblot analysis. PIP is a marker for the plasma membrane. The bottom panel shows protein staining by CBB (Coomassie blue).

(C) Localization of RACK1A-GFP in rice protoplasts. Bars = 5 μm.

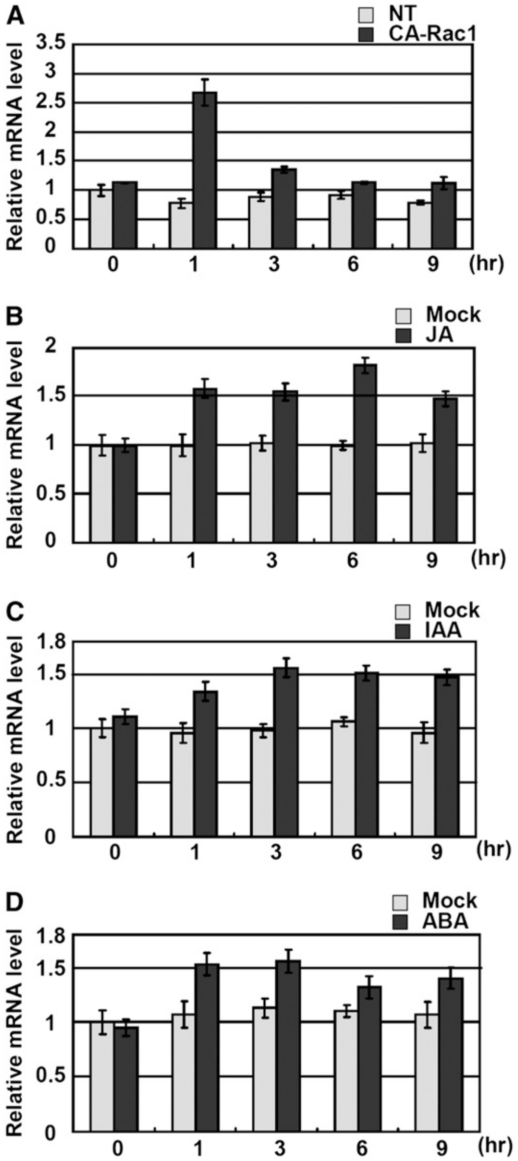

Figure 4. *RACK1A* mRNA Expression Is Induced by SE and Plant Hormones.

(A) Transcript levels for RACK1A were measured by real time RT-PCR in nontransgenic rice cell cultures (NT) and transgenic cell cultures expressing CA-Rac1 after treatment with SE.

(B) to **(D)** Transcript levels for RACK1A were measured by real time RT-PCR in nontransgenic rice cell cultures with plant hormones jasmonic acid **(B)**, IAA **(C)**, and ABA **(D)**. Real time RT-PCR data were normalized to *Ubiquitin* (*Ubq*) transcript and shown as relative to that of RACK1A transcript in nontransgenic rice cell cultures without any treatments. Bars represent the means ± SD calculated using three biological replicates, where each biological replicate consists of two technical replicates.

require the presence of active Rac1, whereas the elicitor response did.

RACK1A Regulates ROS Production and Defense Gene Expression in Rice Cell Culture

To examine the possible functions of RACK1A in rice, *RACK1A*-RNA interference (RNAi) cell cultures were produced in which *RACK1A* was specifically suppressed by the target gene 3′ untranslated region (Figure 5A). Though *RACK1B* RNA was not suppressed, RACK1 protein levels were strongly reduced, suggesting that anti-RACK1A antibody did not appreciably detect RACK1B protein (Figure 5B; see Supplemental Figure 1 online). No plants could be generated from >50 independently transformed *RACK1A*-RNAi calli. Shoots were occasionally obtained from transformed calli, but they became necrotic and did not grow further, suggesting that *RACK1A* has critical functions for the regeneration of plants from rice callus. Therefore, analysis of RACK1A function was limited to *RACK1A*-RNAi cell cultures.

SE induction of H_2O_2 was strongly reduced in each of the three *RACK1A*-RNAi cell cultures examined (Figure 5C), indicating that RACK1A is a positive regulator of ROS production in rice. Expression of *PBZ1*, a rice pathogen defense gene, was reduced in three *RACK1A*-RNAi cell cultures tested (Figure 5D). These results indicate that RACK1A contributes to ROS production and defense gene expression in rice cell cultures.

RACK1A Plays a Role in Resistance to Rice Blast Infection

Transgenic rice plants overexpressing *RACK1A* (*RACK1A ox*; Figures 6A and 6B) had increased resistance to a compatible race of rice blast fungus compared with the wild type (Figures 6C and 6D). Since *RACK1A*-RNAi plants could not be regenerated, leaky-but-viable *RACK1A* mutants may be needed to test the role of RACK1A in incompatible interactions. Levels of *Rac1* and *PBZ1* expression were also much higher than in the wild type in the absence of pathogen infection (Figures 6E and 6F). These results suggest that *RACK1A* overexpression increases levels of *Rac1* mRNA, which in turn induce various defense responses, including increased *PBZ1* expression, leading to increased resistance to rice blast infection.

To test whether *RACK1A* overexpression influences ROS production in seedlings, T1 seedlings of *RACK1A ox* plants were stained with nitroblue tetrazolium (NBT), a pale yellow dye that becomes dark blue when reduced by free radicals and is used to detect superoxide production. There was more intense NBT staining in the scutellum and roots than in nontransformed controls (Figure 6G), consistent with the results obtained from *RACK1A*-RNAi cell cultures (Figure 5). It is thus likely that RACK1A plays a role in rice innate immunity, including as a regulator of ROS production in rice.

Rac1-RNAi Decreases Levels of RACK1A Protein and *PBZ1* Expression in Rice Cell Cultures

Our analysis of RACK1A indicates that it functions in ROS production and innate immunity in rice, and it is possible that RACK1A function in rice innate immunity is dependent on Rac1. These observations prompted an analysis of the influence of Rac1 on *RACK1A* transcription and translation using *Rac1*-RNAi cell cultures (Miki et al., 2005). As reported previously, *Rac1* transcription was decreased in the *Rac1*-RNAi cell cultures (Figure 7A), and, interestingly, *RACK1A* transcription was also significantly lower (Figure 7B). Furthermore, RACK1A protein levels were reduced to a barely detectable level, suggesting that Rac1 also regulates RACK1 posttranscriptionally. *PBZ1* expression was also reduced in Rac1-RNAi cell cultures (Figure 7D). Together, these results indicate that RACK1A expression is positively regulated by Rac1 at both the transcriptional and translational levels, which would make Rac1 a key regulator of its effector, RACK1A.

RACK1A Interacts with RAR1 and SGT1 and the N Terminus of Rboh

In *Arabidopsis* and other species, RAR1 and SGT1 play critical roles in R protein–mediated disease resistance (Shirasu and Schulze-Lefert, 2003). Recent studies indicate that they may function as molecular chaperones to stabilize NB-LRR type R proteins (Bieri et al., 2004; Holt et al., 2005; Leister et al., 2005; Azevedo et al., 2006), though their biochemical functions are not well understood. It was recently found that RAR1 and HSP90 complex with Rac1 and are involved in rice innate immunity (Thao et al., 2007), suggesting that RAR1, SGT1, and HSP90 could also form a complex with RACK1A. Therefore, we examined whether RACK1A directly interacts with RAR1, SGT1, and HSP90. Results of two-hybrid assays indicate that RACK1 interacts with RAR1 and SGT1 but not with HSP90 (Figure 8A). These results were confirmed by split ubiquitin two-hybrid assays in yeast (see Supplemental Figure 2 online). We further analyzed the interaction of RACK1A with RAR1 and SGT1 by dissecting RACK1A. We found that both RAR1 and SGT1 interacted with WD40 repeats 1 and 2 (Figure 8B). Rice RAR1 and SGT1 interact with each other (Wang et al., 2008), as has been shown in other plants (Azevedo et al., 2002; Liu et al., 2002).

To determine whether Rac1 could interact with SGT1 and RAR1 in rice cell extracts, we performed coimmunoprecipitation experiments using wild-type and *Rar1*-RNAi cell cultures (Thao et al., 2007). RACK1A coimmunoprecipitated with RAR1, but only a very faint band was detected in the control experiments using *Rar1*-RNAi cell cultures, confirming that RAR1 interacts with RACK1A (Figure 8C). Similar results were obtained for SGT1 (Figure 8D), though we were not able to use *SGT1*-RNAi cell cultures (Thao et al., 2007), presumably because rice has a single *SGT1* gene and *SGT1*-RNAi cell cultures were nonviable. These results showed that both SGT1 and RAR1 interact with RACK1A in rice cells.

Because we have recently shown that Rac1 directly interacts with the N terminus of the NADPH oxidase (Rboh) and stimulates ROS generation (Wong et al., 2007), we tested whether RACK1A interacts with the N terminus of rice RbohB in split ubiquitin two-hybrid assays. The results indicate that they interact with each other (Figure 8E). We tested whether interaction of RACK1A with RbohB is influenced by Rac1 and found that neither the CA nor the DN form of Rac1 affects interaction of RACK1A and RbohB (Figure 8E). Full-length RbohB did not interact with RACK1A,

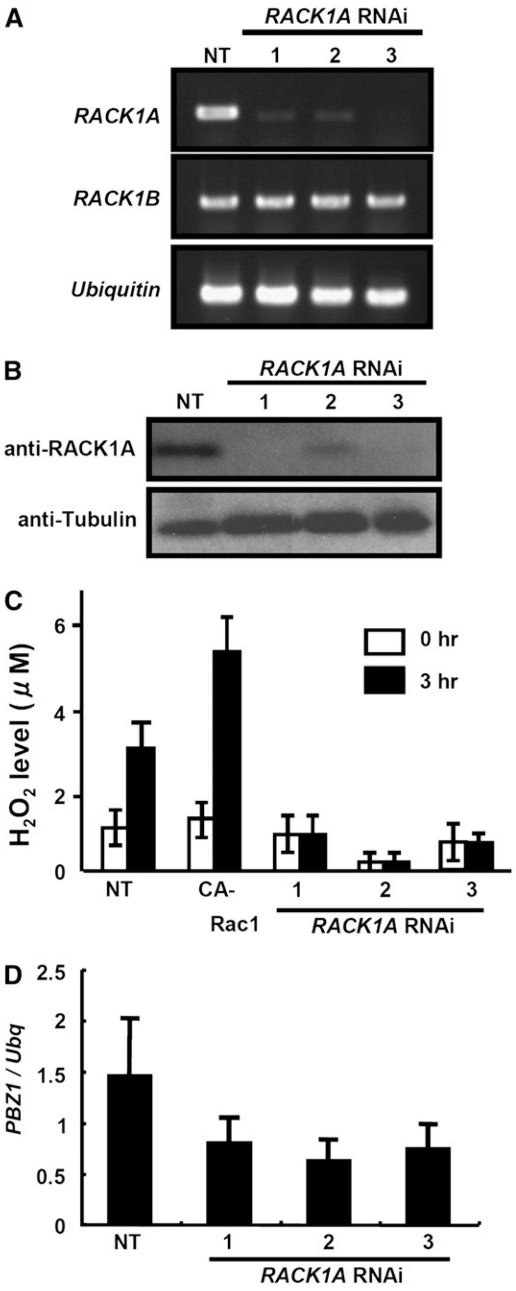

Figure 5. *RACK1A*-RNAi Rice Cell Cultures.

(A) Transcript levels for RACK1A and RACK1B were measured by RT-PCR in *RACK1A*-RNAi rice cell cultures and nontransgenic rice cell cultures (NT). Numbers 1 to 3 indicate independent RNAi transgenic lines. *Ubq* is used as an internal control.

(B) Protein levels of RACK1A in *RACK1A*-RNAi cell cultures. RACK1A protein levels were analyzed by immunoblotting with anti-RACK1A antibody, and an antitubulin antibody was used as a loading control.

(C) Reduction of H_2O_2 induction by SE in RACK1A-RNAi rice cell cultures. Numbers 1 to 3 indicate independent transgenic lines. Bars represent the means ± SD of four independent experiments.

(D) Reduction of *PBZ1* mRNA induction by SE in *RACK1A*-RNAi rice cell cultures. Numbers 1 to 3 indicate independent transgenic lines. Transcript levels were normalized to *Ubq* transcript. Bars represent the means ± SD calculated using three biological replicates, where each biological replicate consists of two technical replicates.

suggesting that the C terminus of RbohB has an inhibitory effect on the interaction (see Supplemental Figure 3 online). Thus, RACK1A functions in ROS production by directly interacting with the N terminus of Rboh, and this interaction is not dependent on Rac1.

DISCUSSION

Rac1 Affinity Column Chromatography

A method originally used for the isolation of interacting proteins of the small GTPase Rho in mammals (Amano et al., 1996) was modified to isolate Rac1-interacting proteins. A number of proteins that could have roles in innate immunity were identified in the screen, including NB-LRR proteins and Sti1 (Table 1). Affinity column chromatography may thus be a promising method for the isolation of protein interactors in plants, particularly for small GTPases. We have previously used a yeast two-hybrid screen with CA-Rac1 to isolate Rac1-interacting proteins and found that cinnamoyl-CoA reductase (CCR) acts as an effector (Kawasaki et al., 2006). However, no other important interactors were identified in the previous yeast two-hybrid screen (K. Hasegawa and K. Shimamoto, unpublished results). Therefore, with respect to the efficiency of the identification of Rac1 effectors, affinity column chromatography may be an alternative and superior screen for interactors, though a direct and robust comparison of the methods is difficult.

RACK1A Is a Key Effector of Rac GTPase

We show here that RACK1A specifically binds a GTP-bound form of Rac1. RACK1A contributes to ROS production, defense gene expression, and disease resistance; all of which are regulated by Rac1 (Kawasaki et al., 1999). Thus, RACK1A is apparently an important effector of Rac1 function in rice innate immunity. Though numerous proteins have been identified as interactors with RACK1 in mammalian cells (McCahill et al., 2002; Yaka et al., 2002; Patterson et al., 2004; Lopez-Bergami et al., 2005; Liu et al., 2007; Parent et al., 2008), Rho-type GTPase apparently is not an interactor. By contrast, we have shown that Rac1 is a Rho GTPase that does interact with RACK1 in rice. We have previously shown that CCR is an effector of Rac1 and that CCR has increased enzymatic activity when bound to Rac1, thus stimulating lignin production during pathogen infection (Kawasaki et al., 2006). Effectors of Rac/Rop GTPase have been isolated from *Arabidopsis*, and RICs (Rop-interactive CRIB motif–containing proteins), which contain CRIB (cdc42/Rac interactive binding) domains, are involved in the regulation of the cytoskeleton in pavement cells and pollen tubes (Fu et al., 2005; Gu et al., 2005). ICR1, another effector of Rac/Rop GTPase, was recently isolated (Lavy et al., 2007). ICR1 interacts with GTP-bound Rops, and it is required for the maintenance of cell polarity, root-stem

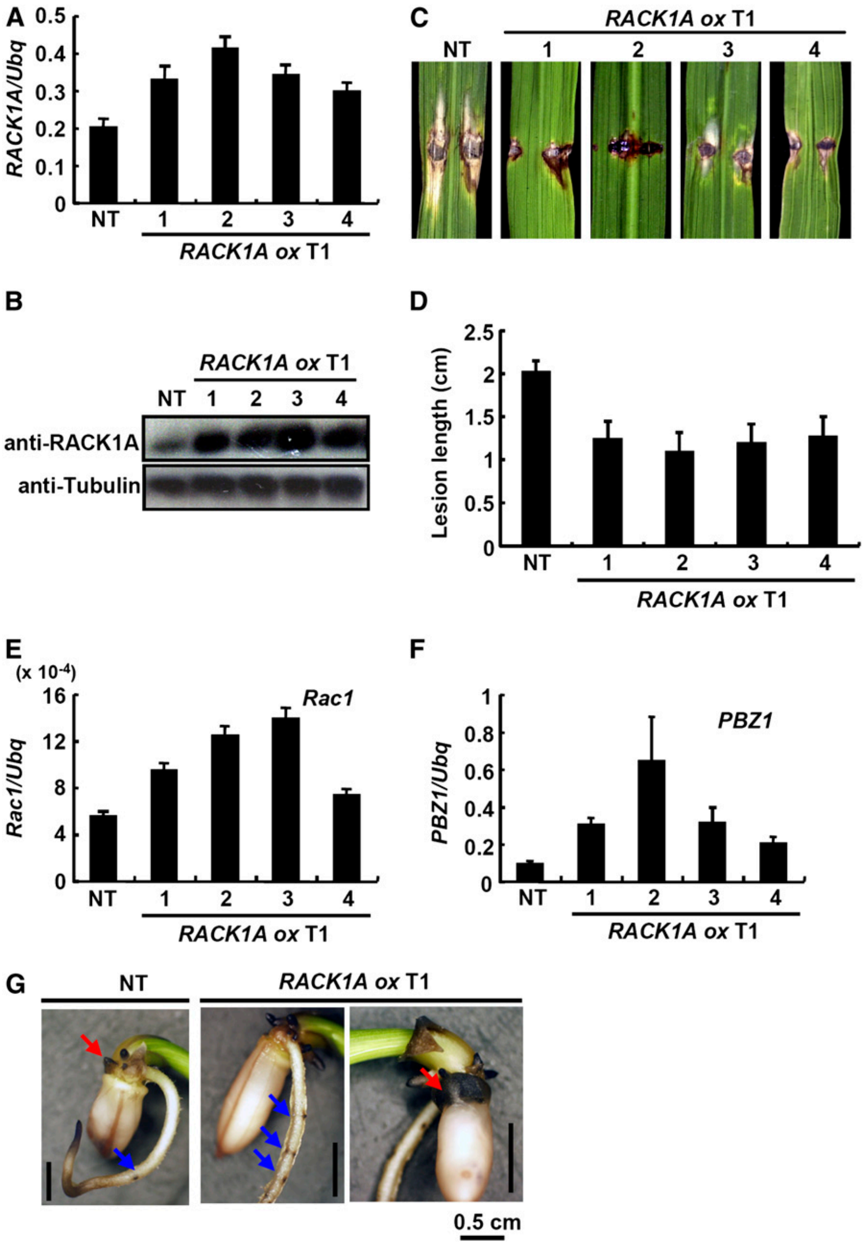

Figure 6. *RACK1A*-Overexpressing Rice Plants.

(A) Expression of *RACK1A* in T1 *RACK1A-ox* and in nontransgenic (NT) rice plants. *RACK1A* mRNA levels were measured by real time PCR. Numbers 1 to 4 indicate independent transgenic lines. Transcript levels were normalized to *Ubq* transcript. Bars represent the means ± SD calculated using three biological replicates, where each biological replicate consists of two technical replicates.

(B) Expression of RACK1A protein in T1 *RACK1A-ox* rice plants. Levels of RACK1A protein were measured by immunoblotting with anti-RACK1A antibody. Numbers 1 to 4 indicate independent transgenic lines.

(C) Increased resistance of T1 *RACK1A-ox* plants to infection by a compatible race of rice blast fungus 2 weeks after infection. Numbers 1 to 4 indicate independent transgenic lines.

(D) Quantitative analysis of increased resistance of T1 *RACK1A-ox* plants to infection by a compatible race of rice blast fungus. Numbers 1 to 4 indicate the independent transgenic lines shown in **(C)**. Bars represent the means ± SD calculated using >15 independent inoculated regions for each line.

(E) Expression of *Rac1* mRNA in T1 *RACK1A-ox* plants. Levels of *Rac1* mRNA were measured by real-time RT-PCR. Numbers 1 to 4 indicate independent transgenic lines. Transcript levels were normalized to *Ubq* transcript. Bars represent the means ± SD calculated using three biological replicates, where each biological replicate consists of two technical replicates.

(F) Expression of *PBZ1* mRNA in T1 *RACK1A-ox* transgenic rice in the absence of elicitor. Levels of *PBZ1* mRNA were measured by real-time RT-PCR. Numbers 1 to 4 indicate independent transgenic lines. Transcript levels were normalized to *Ubq* transcript. Bars represent the means ± SD calculated using three biological replicates, where each biological replicate consists of two technical replicates.

(G) ROS production in T1 *RACK1A-ox* rice seedlings. T1 *RACK1A-ox* seeds were germinated for 5 d and stained with NBT for ROS production. Arrows indicate sites of increased ROS production. NT, nontransgenic control.

cell integrity, and vesicle trafficking. It can interact with multiple proteins, thus potentially functioning as a scaffold within protein complexes. Our study shows that RACK1A interacts with Rac1, RAR1, and SGT1; thus, it could act as a scaffolding protein in plants. These three proteins seem to interact with WD40 repeats

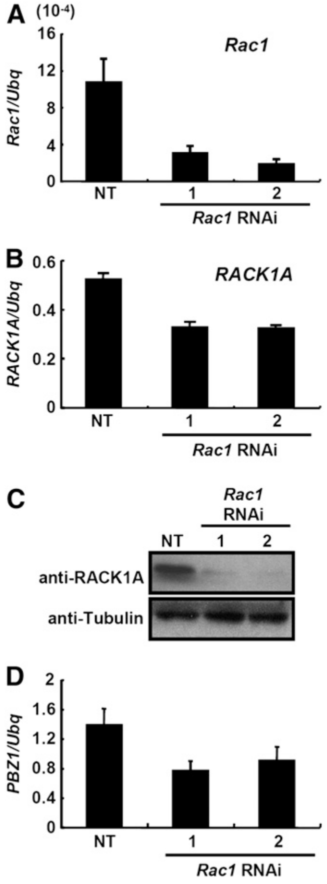

Figure 7. RACK1A Expression in *Rac1*-RNAi Cell Cultures.

(A) Transcript levels for Rac1 were measured by real time RT-PCR in *Rac1*-RNAi rice cell cultures and nontransgenic rice cell cultures (NT). Numbers 1 and 2 indicate independent RNAi transgenic lines. Transcript levels were normalized to *Ubq* transcript. Bars represent the means ± SD calculated using three biological replicates, where each biological replicate consists of two technical replicates.

(B) Transcript levels for *RACK1A* were measured by real time RT-PCR in *Rac1*-RNAi rice cell cultures. Transcript levels were normalized to *Ubq* transcript. Bars represent the means ± SD calculated using three biological replicates, where each biological replicate consists of two technical replicates.

(C) Levels of RACK1A protein were measured by immunoblotting with specific antibodies. Numbers 1 and 2 indicate independent transgenic lines. NT, nontransgenic rice cell cultures. 1-2 indicates independent RNAi transgenic lines.

(D) Expression of *PBZ1* in *Rac1*-RNAi rice cell cultures in the absence of elicitor treatment. Levels of *PBZ1* mRNA were measured by real-time RT-PCR. Numbers 1 and 2 indicate independent transgenic lines. Transcript levels were normalized to *Ubq* transcript. Bars represent the means ± SD calculated using three biological replicates, where each biological replicate consists of two technical replicates.

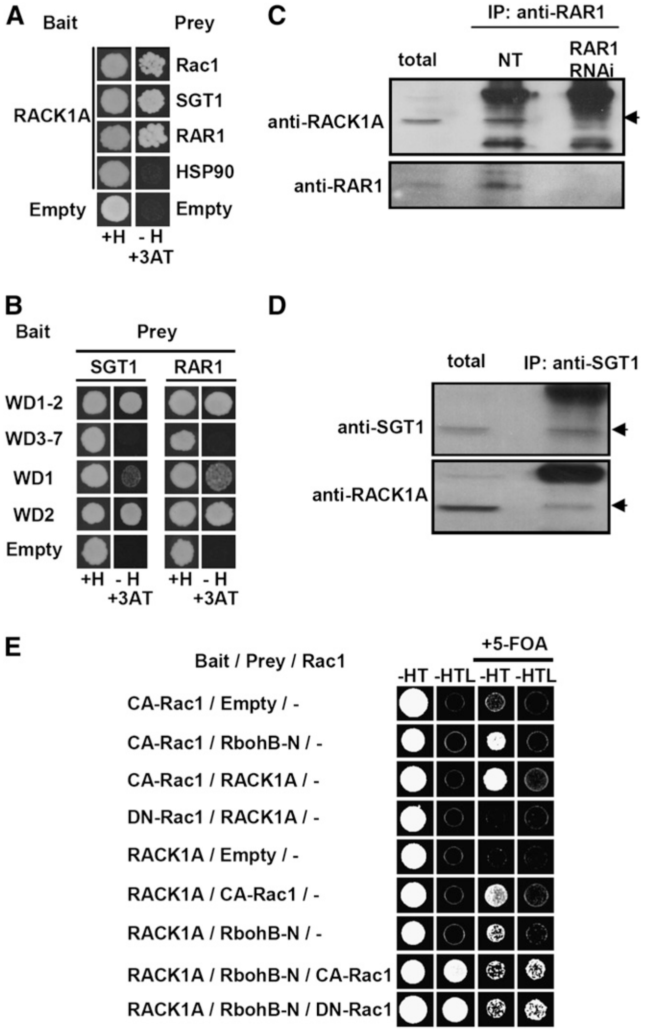

Figure 8. Interaction of RACK1A with RbohB, RAR1, and SGT1 in Yeast Two-Hybrid Assays.

(A) Interaction of RACK1A with Rac1, SGT1, and RAR1 but not with cytosolic HSP90 in yeast two-hybrid assays. SGT1 and RAR1 are both single-copy genes in rice.

(B) Determination of the WD40 repeats of RACK1A that are involved in interaction with SGT1 and RAR1.

(C) RACK1A coimmunoprecipitated with RAR1 in extracts from wild-type rice cell cultures. A very faint band was detected in *RAR1*-RNAi cell cultures.

(D) RACK1A coimmunoprecipitated with SGT1 in extracts from wild-type rice cell cultures. *SGT1*-RNAi cell culture was nonviable since *SGT1* is a single-copy gene in rice. Control *SGT1*-RNAi cultures were thus not available for the analysis.

(E) Split-ubiquitin two/three-hybrid assays of RACK1A, RbohB-N, and Rac1. RbohB-N contains the N-terminal region (amino acids 1 to 355) of RbohB, which interacts with Rac1 (Wong et al., 2007). The bait and prey vectors were selected on plates without His (−H) and Trp (−T), respectively. The pDEST32 vectors carrying Rac1 were selected by removal of Leu (−L) from the plates. Growth on selective plates with 5-fluroorotic acid (5-FOA) indicates a positive interaction.

1 and 2 of RACK1A. RACK1A that is already bound with the active form of Rac1 may thus not be able to interact with RAR1 or SGT1. However, since RACK1 forms a homodimer (see Supplemental Figure 4 online) and potentially interacts with two different proteins by its recognition of the same motif (Thornton et al., 2004; Liu et al., 2007), RACK1A could possibly interact with two or three interactors simultaneously. Furthermore, WD40 repeats 1 and 2 of RACK1A could interact with distinct proteins. Whether RACK1A indeed interacts with multiple proteins simultaneously remains to be studied.

Many mammalian RACK1-interacting proteins interact with RACK1 on repeats 4, 5, 6, and 7 (McCahill et al., 2002), but the three intracellular loops of the thromboxane A2 receptor (TPβ) interact with RACK1 at WD40 repeats 1 to 3, and the C terminus of TPβ interacts with WD40 repeats 3 and 7 (Parent et al., 2008). As more plant proteins that interact with RACK1 are found, specific WD40 repeats that preferentially interact with other proteins will be clarified.

RACK1A Function in Rice Innate Immunity

Based on the results obtained in our study, two functions of RACK1 in rice innate immunity can be envisaged (Figure 9). One is that it constitutes a component of the Rac1 complex consisting of Rac1, RAR1, SGT1, HSP90, and HSP70 and functions as a scaffolding protein for the immune complex. We have previously postulated that all of these proteins could form a protein complex (Thao et al., 2007). The abundance of each of the cochaperones (RAR1, HSP90, and HSP70) present in the immune complex may need to be finely regulated to ensure a rapid and stable response to pathogen attack. However, whether these components are always present in a complex or whether some components associate with the complex only transiently remain to be studied. Another hypothesis is that RACK1A constitutes a component of the NADPH oxidase complex together with Rac1 and regulates ROS production at an early stage of immune responses since RACK1A interacts with the N-terminal region of NADPH oxidase (Rboh) (Figure 8E). How these functions of RACK1A are regulated or how its interactions with other proteins are temporally and spatially regulated after pathogen infection remain to be studied in the future (Figure 9).

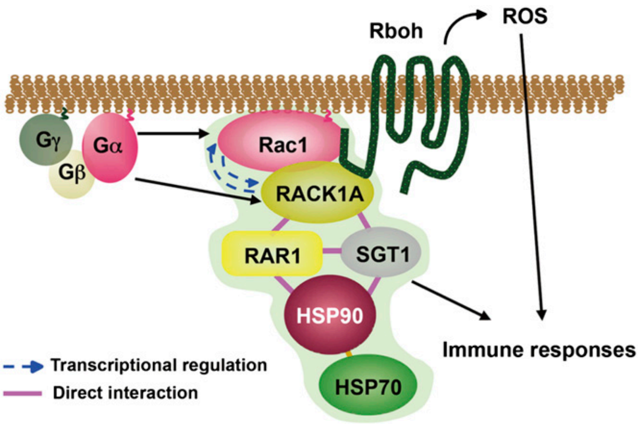

Figure 9. Model for the RACK1A-Containing Immune Complex in Rice Innate Immunity.

RACK1A preferentially binds the active form of Rac1 and interacts with the N terminus of Rboh (NADPH oxidase), RAR1, and SGT1. SGT1, HSP90, and HSP70 could form an immune complex at the plasma membrane (Thao et al., 2007). Rac1 transcriptionally regulates RACK1 and vice versa. RACK1A could contribute to ROS production by binding with the N terminus of RbohB protein (NADPH oxidase). RACK1A could also regulate the integrity of the Rac1 immune complex (indicated by light green) as a scaffolding protein. Heterotrimeric G proteins regulate expression of *Rac1* (Suharsono et al., 2002) and *RACK1* (Komatsu et al., 2005); thus, they influence rice innate immunity through their effects on these two molecules.

One recent development in the study of plant innate immunity is that the NB-LRR type R proteins are translocated to the nucleus and function there in immunity (Burch-Smith et al., 2007; Shen et al., 2007; Wirthmueller et al., 2007). Thus, it is possible that some components of the initial immune complex thought to be present at the periphery of the plasma membrane may move to the nucleus. In this regard RACK1 may be an interesting adaptor protein since it functions in various intracellular compartments in mammalian cells, including the plasma membrane, the nucleus, and the endoplasmic reticulum, and it is able to interact with cell surface receptors, protein kinases, transcription factors, and transcription-associated proteins (Schechtman and Mochly-Rosen, 2001; Yaka et al., 2002; Patterson et al., 2004; Lopez-Bergami et al., 2005; Liu et al., 2007; Parent et al., 2008). A possible function of RACK1 in plant immune responses may be that it facilitates translocation of components present in the immune complex, which is initially present at the periphery of the plasma membrane, to various subcellular compartments where it would stimulate or complete immune responses. Alternatively, as shown in the study of the effect of RACK1 on the TPβ receptor, it could function to transport membrane receptors from the endoplasmic reticulum to the cell surface (Parent et al., 2008). Studies on the function of RACK1A in various steps in innate immune responses will be the subjects of future studies.

Regulation of RACK1A Expression by Rac1

Our results indicate that Rac1 regulates *RACK1A* expression at the transcriptional as well as posttranscriptional levels (Figure 7B) and possibly vice versa (Figure 6E). We have previously found positive transcriptional regulation of *RAR1* and *SGT1* by Rac1 in rice (Thao et al., 2007). Rac1 exerts posttranscriptional regulation of *RAR1* as it does on RACK1A. These results suggest that Rac1 regulates the expression levels of RACK1A, RAR1, and SGT1 in the rice immune complex and that this could be a means to balance abundance of components in the immune complex during defense responses.

RACK1 in Phytohormone and Immune Signaling

RACK1 homologs have been isolated from several plant species (Ishida et al., 1993; Iwasaki et al., 1995; McKhann et al., 1997; Perennes et al., 1999). However, in all previous studies, *RACK1* expression patterns in different tissues and the effects of a variety of exogenous factors on expression have been the principle focus. More recently, T-DNA insertion mutants of *RACK1A*, one of three RACK1 homologs in *Arabidopsis*, were shown to exhibit altered sensitivity to multiple hormones (Chen et al., 2006). *Arabidopsis rack1a* mutants showed reduced sensitivity to GA during seed germination and hypersensitivity to ABA during seed germination and early seedling growth. Furthermore, *rack1a* mutants are hypersensitive to auxin during root formation. Our analysis showed that *RACK1A* expression is induced by methyl jasmonate, ABA, and IAA (Figure 4).

Arabidopsis G protein complex mutant proteins are hypersensitive to ABA responses (Pandey et al., 2006). A recent proteomic study of the rice *dwarf1* mutant lacking the Gα subunit of heterotrimeric G protein indicates that, in the embryo at least, RWD (RACK1A) protein levels are reduced compared with the wild type (Komatsu et al., 2005). Heterotrimeric G proteins regulate innate immunity through Rac1 (Suharsono et al., 2002) and hormone signaling (Fujisawa et al., 2001) in rice. Therefore, it is possible that RACK1A function in rice innate immunity may be partly mediated through heterotrimeric G proteins (Figure 9). A recent study on the role of yeast RACK1 in the regulation of heterotrimeric G proteins and Rho-type small GTPase may help to shed light on the signaling pathway involving these three groups of proteins (Zeller et al., 2007). The yeast RACK1 ortholog Asc1 was found to associate with one of the two Gα proteins in a GDP-dependent manner, indicating that it acts as a negative regulator of G protein signaling in response to glucose. Interestingly, yeast RACK1 has another role in glucose signaling. It inhibits the Cdc42-mediated MAPK pathway by suppressing Ste20 kinase, which is positively regulated by Cdc42 (Zeller et al., 2007). Therefore, in the G protein signaling of the glucose response in budding yeast, RACK1 suppresses Gα and Cdc42-regulated Ste20 kinase, two different proteins (Zeller et al., 2007). RACK1 may thus also be involved in both Rac/Rop-mediated innate immunity and hormone signaling.

METHODS

Affinity Chromatography and Mass Spectrometry

Rice (*Oryza sativa*) cells were homogenized in TEDM buffer (20 mM Tris/HCl, pH 7.5, 1 mM DTT, 5 mM EDTA, and 10 mM $MgCl_2$) containing 1 μg/mL leupeptin and 10% sucrose and filtered through four layers of gauze. The homogenate was centrifuged at 6000*g* for 30 min at 4°C to remove cellular debris, and the supernatant was clarified by centrifugation at 100,000*g* for 60 min at 4°C. The soluble proteins were used for affinity chromatography. The GST-fused rice Rac1 protein was purified as described previously (Kawasaki et al., 2006). Guanine nucleotide-bound forms of GST-Rac1 were made by incubating purified protein for 1 h at 30°C with GDPβS or GTPγS (Sigma-Aldrich) in a reaction buffer (20 mM Tris/HCl, pH 7.5, 1 mM DTT, 10 mM EDTA, and 5 mM $MgCl_2$). The GTPγS- or GDPβS-bound GST-Rac1 proteins were immobilized on glutathione Sepharose 4B beads (GE Healthcare). The soluble proteins were incubated with glutathione Sepharose 4B beads to remove the native rice GST proteins and then loaded onto a GST-Rac1 affinity column, washed with a TEDM buffer, and eluted three times with an elution buffer (20 mM Tris/HCl, pH 7.5, 10 mM EDTA, 1 mM DTT, 5 mM $MgCl_2$, and 500 mM NaCl).

Eluted proteins were separated by 7.5 and 12% SDS-PAGE and stained with silver nitrate. The silver staining was performed without glutaraldehyde fixation as described by Shevchenko et al., (1996). Protein bands that interacted specifically with GTP- or GDP-bound Rac1 were excised from the gels, washed twice with HPLC-grade water containing 30% acetonitrile (Wako), washed with 100% acetonitrile, and dried in a vacuum concentrator. The dried gel pieces were treated with 2 μL of a 0.5 μg/μL trypsin (Promega) in 50 mM ammonium bicarbonate (Shevchenko, 2001) and incubated at 37°C for 16 h. The digested peptides in the gel pieces were extracted twice with 20 μL of 5% formic acid in 50% acetonitrile, combined, and dried in a Speedvac evaporator. Trypsin-digested peptides were separated by HPLC using a PEPMAPC18 column (5 μm, 75-μm internal diameter, 15 cm; Dionex) in the CapLC system (Waters). Buffers were 0.1% HCOOH in water (A) and 0.1% HCOOH in acetonitrile (B). A linear gradient from 5 to 45% B for 25 min was applied, and peptides eluted from the column were introduced directly into a Q-TOF Ultima mass spectrometer (Waters) with a flow rate of 100 nL/min. Ionization was performed with a potential of 2200 V applied to a PicoTip nanospray source (New Objective). For the survey scan, mass spectrometry (MS) spectra were acquired for mass-to-charge ratios of 400 to 1500, and MS/MS spectra were acquired for the two most intense ions from the precursor ion scan. For collision-induced dissociation, the collision energy was set automatically according to the mass and charge state of the precursor peptides. MS/MS spectra were uploaded to the MASCOT server (Matrix Science, version 2.0) and compared against a protein database (NCBI_20030405) from the National Center for Biotechnology Information (NCBI). The NCBI's taxonomy used was *O. sativa*. To remove weak matching peptides, the threshold was set at 0.05 in the ion score cut off during the MASCOT search. The following parameters were used for database searches: peptide tolerance at ±0.4 D and MS/MS tolerance at ±0.2 D; peptide charge of 2+ or 3+; trypsin as enzyme allowing up to one missed cleavage; carbamidomethylation on Cys residues as a fixed modification and oxidation on Met as a variable modification.

Yeast Two-Hybrid Assay Using a LexA-VP16 System

Bait vectors carrying CA and DN mutants of rice *Rac1* have been described previously (Kawasaki et al., 2006). This two-hybrid system detects the protein–protein interaction in nuclei. Since the C-terminal Cys of Rac1, which is required for plasma membrane localization, was considered to inhibit transfer of Rac1 into nuclei, the Cys residue was exchanged with Ser. The bait and prey vectors containing different fragments of *RACK1A* were made using pBYM116 and pVP16, respectively (Kawasaki et al., 2006). Prey vectors containing rice SGT1, rice RAR1, and barley (*Hordeum vulgare*) HSP90 (Takahashi et al., 2003) were cloned into pVP16. Combinations of bait and prey vectors were introduced into cells of *Saccharomyces cerevisiae* L40. The interaction was analyzed based on the requirement of His for growth as described previously (Kawasaki et al., 2006).

Split-Ubiquitin Two/Three-Hybrid Assay

CA mutants of six members (Rac2 to Rac7) of the rice Rac family were produced by base substitution so that their amino acids corresponded to the CA mutant of Rac1. The resultant mutants were designated as Rac2-G16V, Rac3-G17V, Rac4-G17V, Rac5-G15V, Rac6-G15V, and Rac7-G15V. The coding regions of rice *RACK1A*, *RACK1B*, *RAR1*, *SGT1*, CA-*Rac1-7*, and DN-*Rac1* were cloned into the pMet-GWY-Cub-RUra3A bait vector or pCup-NuI-GWY-Cyc1 prey vector through the gateway system (Kim et al., 2002). Split-ubiquitin two-hybrid system assays were performed according to the published protocol (Kim et al., 2002). A split-ubiquitin three-hybrid system was modified from the split-ubiquitin two-hybrid system by introducing a third pDEST32 (Invitrogen) construct carrying *Rac1* into yeast host strain DJ53. Yeast cells expressing two (bait and prey) or three (bait, prey, and pDEST32) constructs were grown to 5 × 10^6 cells/μL and spotted in serial dilutions on selective agarose plates with or without 5-fluoroorotic acid. At least three independent colonies were repeated for each two-hybrid pair.

Plasmid Constructs and Rice Transformation

To produce transgenic plants that overexpress *RACK1A*, the protein coding region was amplified by PCR using the primer set (forward, 5′-CACCATGGCCGGCGCGCAGGAGTCT-3′; reverse, 5′-CTAGCCGGCGTAGCTGAAACC-3′). The amplified fragment was cloned into the pENTR/D-TOPO cloning vector (Invitrogen) and transferred into the p2K1 vector by an LR clonase reaction. Expression of *RACK1A* is under the control of the maize (*Zea mays*) *Ubp1* promoter (Miki and Shimamoto, 2004). For the RNAi construct of *RACK1A*, a 328-bp *RACK1A* cDNA region covering the C-terminal region (73-bp upstream of the stop codon) and a 255-bp fragment of the 3′ untranslated region was amplified using the primer set (forward, 5′-CACCCTCTATGCTGGTTACAC-3′; reverse, 5′-AAAAAAGAGAGAAGCACCATG-3′) and cloned in inverse orientation into the pANDA vector (Miki and Shimamoto, 2004).

For the RNAi construct of *RACK1A*, a 451-bp cDNA fragment covering the C-terminal region (214-bp upstream of the stop codon) and a 234-bp fragment of the 3′ untranslated region were amplified using specific primers (forward, 5′-TCTGAATGAGCCTAGGGTTTG-3′; and reverse 5′-GACCGAAGTCTCCACACACA-3′). The amplified fragment was cloned into pENTR/D-TOPO (Invitrogen) and subcloned into pANDA in the inverse orientation by one LR reaction. *Agrobacterium tumefaciens*–mediated transformation of rice calli was performed as described previously (Hiei et al., 1994). The rice suspension cell cultures expressing RAR1 RNAi was described previously (Thao et al., 2007).

Elicitor Treatment, Real-Time RT-PCR, and Quantification of ROS

Cultured rice cells expressing *CA-Rac1*, *RACK1A*, and *RACK1A* RNAi were collected after treatment with 5 μg/mL of an SE prepared from *Magnaporthe grisea* (Koga et al., 1998; Umemura et al., 2002). Total RNA was extracted using the RNAeasy plant mini kit (Qiagen) and treated with DNase I (Invitrogen). cDNA was synthesized from total RNA using Superscript II reverse trascriptase (Invitrogen) and used for quantitative analysis of gene expression performed with SYBR Green PCR master mix (Applied Biosystems) with the gene-specific primers listed in Supplemental Table 2 online. Data were collected using the ABI PRISM 7000 sequence detection system according to the instruction manual. Quantification of SE-induced hydrogen peroxide was performed as described previously (Wong et al., 2004). Superoxides generated during seedling stages were detected by staining with 0.1% (w/v) nitroblue tetrazolium.

Pathogen Inoculation

Rice blast fungus (*M. grisea*) strain 2403-1 (race 007) is virulent and TH67-22 (race 031) is avirulent on cv Kinmaze. The growth conditions of blast fungus and punch infection of leaf blades were performed as described previously (Takahashi et al., 1999; Ono et al., 2001). Photographs of disease lesions were taken 2 weeks after inoculation.

Antibody, Immunoblot Analysis, and Coimmunoprecipitation Assays

To produce His-tagged RACK1A protein, *RACK1A* cDNA was cloned into pET15b (Novagen). The recombinant protein was produced in *Escherichia coli* and purified with a HiTrap chelating HP column according to the manufacturer's instructions (GE Healthcare) to be used as an antigen to raise antibodies in rabbits. To purify the antibody, GST-fused RACK1A protein was prepared by cloning *RACK1A* cDNA into pGEX-4T-1 (GE Healthcare). A 10-mL aliquot of antiserum was incubated at 25°C for >4 h with a membrane containing bound recombinant GST- RACK1A protein. The membrane was then washed five times with Tris-buffered saline buffer (137 mM NaCl, 2.68 mM KCl, and 25 mM Tris, pH 7.4). RACK1A-specific antibody was eluted with 2 mL of 0.1 M glycine/0.15 M HCl, pH 2.5∼3.0, and neutralized with 450 μL of 0.5 M HEPES/NaOH, pH 8.5.

Coimmunoprecipitation and immunoblotting experiments were performed using transgenic rice cells expressing myc-CA-Rac1, myc-DN-Rac1, myc-C212S-Rac1, or RACK1A, and transgenic rice cells suppressing expression of *Rac1* or *RACK1A* by RNAi. Rice cells were homogenized as above. Total proteins were prepared by removing insoluble debris by centrifugation at 2000*g* for 10 min at 4°C. Soluble and microsomal membrane proteins were separated by centrifugation at 100,000*g* for 1 h. The proteins were separated by SDS-PAGE and electrotransferred onto an Immobilon-P membrane (Millipore) for immunoblot detection. The membrane was blocked for 1 h in PBS (137 mM NaCl, 8.1 mM Na_2HPO_4, 2.68 mM KCl, and 1.47 mM KH_2PO_4) containing 5% skim milk, washed three times with PBS, and incubated for 1 h with purified anti-RACK1A, anti-α-tubulin, anti-RAR1 (Thao et al., 2007), or anti-SGT1 antibodies (Azevedo et al., 2002). After washing with PBS containing 0.1% Tween, the membranes were incubated for 1 h with anti-rabbit IgG conjugated to horseradish peroxidase (GE Healthcare). Detection was performed using ECL protein gel blot detection reagents (GE Healthcare). Coimmunoprecipitation assays with anti-myc, anti-SGT1, or anti-RAR1 antibodies were performed as described previously (Lieberherr et al., 2005).

Transient Expression in Rice Protoplasts

The *GFP* sequence derived from sGFP-S65T (Chiu et al., 1996) was fused to the C terminus of RACK1A. Expression of the *GFP* control and *RACK1A-GFP* was driven by the maize ubiquitin promoter. Protoplast isolation and electroporation were performed as described previously (Wong et al., 2004). After a 24-h incubation at 30°C, the protoplasts were examined under a confocal microscope (LSM510; Carl Zeiss).

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under the following accession numbers: Os Rac1 (AB029508), Os Rac2 (AB029509), Os Rac3 (AB029510), Os Rac4 (AK061102), Os Rac5 (AK067504), Os Rac6 (AK100842), Os Rac7 (AK058414), Os RACK1A (D38231), Os RACK1B (AK121567), Os SGT1 (AAF18438), Os RAR1 (AK111881), PBZ1 (D38170), and barley HSP90 (AY325266).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. The anti-RACK1A Antibody Showed Much Lower Affinity for the RACK1B Protein.

Supplemental Figure 2. RAR1 and SGT1 Interact with RACK1A in a Split-Ubiquitin Two-Hybrid Assay.

Supplemental Figure 3. Interaction between RACK1A and RbohB in a Split-Ubiquitin Two-Hybrid Assay.

Supplemental Figure 4. Dimerization of RACK1A and RACK1B.

Supplemental Table 1. List of Proteins Identified by GST-Rac1 Affinity Column Chromatography.

Supplemental Table 2. MS Data of Identified Proteins.

Supplemental Table 3. List of Other Candidate Proteins.

Supplemental Table 4. Primers Used in This Study.

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REFERENCES

Amano, M., Mukai, H., Ono, Y., Chihara, K., Matsui, T., Hamajima, Y., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996). Identification of a putative target for Rho as the serine-threonine kinase protein kinase N. Science **271:** 648–650.

Assmann, S.M. (2005). G protein regulation of disease resistance during infection of rice with rice blast fungus. Sci. STKE **2005:** cm13.

Axtell, M.J., and Staskawicz, B.J. (2003). Initiation of RPS2-specified disease resistance in Arabidopsis is coupled to the AvrRpt2-directed elimination of RIN4. Cell **112:** 369–377.

Azevedo, C., Betsuyaku, S., Peart, J., Takahashi, A., Noel, L., Sadanandom, A., Casais, C., Parker, J., and Shirasu, K. (2006). Role of SGT1 in resistance protein accumulation in plant immunity. EMBO J. **25:** 2007–2016.

Azevedo, C., Sadanandom, 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.

Berken, A. (2006). ROPs in the spotlight of plant signal transduction. Cell. Mol. Life Sci. **63:** 2446–2459.

Bieri, S., Mauch, S., Shen, Q.H., Peart, J., Devoto, A., Casais, C., Ceron, F., Schulze, S., Steinbiss, H.H., Shirasu, K., and Schulze-Lefert, P. (2004). RAR1 positively controls steady state levels of barley MLA resistance proteins and enables sufficient MLA6 accumulation for effective resistance. Plant Cell **16:** 3480–3495.

Burch-Smith, T.M., Schiff, M., Caplan, J.L., Tsao, J., Czymmek, K., and Dinesh-Kumar, S.P. (2007). A novel role for the TIR domain in association with pathogen-derived elicitors. PLoS Biol. **5:** e68.

Ceci, M., Gaviraghi, C., Gorrini, C., Sala, L.A., Offenhauser, N., Marchisio, P.C., and Biffo, S. (2003). Release of eIF6 (p27BBP) from the 60S subunit allows 80S ribosome assembly. Nature **426:** 579–584.

Chang, I.F., Szick-Miranda, K., Pan, S., and Bailey-Serres, J. (2005). Proteomic characterization of evolutionarily conserved and variable proteins of Arabidopsis cytosolic ribosomes. Plant Physiol. **137:** 848–862.

Chen, J.G., Ullah, H., Temple, B., Liang, J., Guo, J., Alonso, J.M., Ecker, J.R., and Jones, A.M. (2006). RACK1 mediates multiple hormone responsiveness and developmental processes in Arabidopsis. J. Exp. Bot. **57:** 2697–2708.

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.

Chiu, W., Niwa, Y., Zeng, W., Hirano, T., Kobayashi, H., and Sheen, J. (1996). Engineered GFP as a vital reporter in plants. Curr. Biol. **6:** 325–330.

Dangl, J.L., and Jones, J.D. (2001). Plant pathogens and integrated defence responses to infection. Nature **411:** 826–833.

Dodds, P.N., Lawrence, G.J., Catanzariti, A.M., Teh, T., Wang, C.I., Ayliffe, M.A., Kobe, B., and Ellis, J.G. (2006). Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. Proc. Natl. Acad. Sci. USA **103:** 8888–8893.

Fu, Y., Gu, Y., Zheng, Z., Wasteneys, G., and Yang, Z. (2005). Arabidopsis interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis. Cell **120:** 687–700.

Fujisawa, Y., Kato, H., and Iwasaki, Y. (2001). Structure and function of heterotrimeric G proteins in plants. Plant Cell Physiol. **42:** 789–794.

Fujiwara, M., Umemura, K., Kawasaki, T., and Shimamoto, K. (2006). Proteomics of Rac GTPase signaling reveals its predominant role in elicitor-induced defense response of cultured rice cells. Plant Physiol. **140:** 734–745.

Giavalisco, P., Wilson, D., Kreitler, T., Lehrach, H., Klose, J., Gobom, J., and Fucini, P. (2005). High heterogeneity within the ribosomal proteins of the *Arabidopsis thaliana* 80S ribosome. Plant Mol. Biol. **57:** 577–591.

Gu, Y., Fu, Y., Dowd, P., Li, S., Vernoud, V., Gilroy, S., and Yang, Z. (2005). A Rho family GTPase controls actin dynamics and tip growth via two counteracting downstream pathways in pollen tubes. J. Cell Biol. **169:** 127–138.

Gu, Y., Wang, Z., and Yang, Z. (2004). ROP/RAC GTPase: An old new master regulator for plant signaling. Curr. Opin. Plant Biol. **7:** 527–536.

Hiei, Y., Ohta, S., Komari, T., and Kumashiro, T. (1994). Efficient transformation of rice (*Oryza sativa* L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. Plant J. **6:** 271–282.

Holt III, B.F., Belkhadir, Y., and Dangl, J.L. (2005). Antagonistic control of disease resistance protein stability in the plant immune system. Science **309:** 929–932.

Ishida, S., Takahashi, Y., and Nagata, T. (1993). Isolation of cDNA of an auxin-regulated gene encoding a G protein beta subunit-like protein from tobacco BY-2 cells. Proc. Natl. Acad. Sci. USA **90:** 11152–11156.

Iwasaki, Y., Komano, M., Ishikawa, A., Sasaki, T., and Asahi, T. (1995). Molecular cloning and characterization of cDNA for a rice protein that contains seven repetitive segments of the Trp-Asp forty-amino-acid repeat (WD-40 repeat). Plant Cell Physiol. **36:** 505–510.

Jia, Y., McAdams, S.A., Bryan, G.T., Hershey, H.P., and Valent, B. (2000). Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. EMBO J. **19:** 4004–4014.

Jones, J.D., and Dangl, J.L. (2006). The plant immune system. Nature **444:** 323–329.

Kawasaki, T., Henmi, K., Ono, E., Hatakeyama, S., Iwano, M., Satoh, H., and Shimamoto, K. (1999). The small GTP-binding protein Rac is a regulator of cell death in plants. Proc. Natl. Acad. Sci. USA **96:** 10922–10926.

Kawasaki, T., Koita, H., Nakatsubo, T., Hasegawa, K., Wakabayashi, K., Takahashi, H., Umemura, K., Umezawa, T., and Shimamoto, K. (2006). Cinnamoyl-CoA reductase, a key enzyme in lignin biosynthesis, is an effector of small GTPase Rac in defense signaling in rice. Proc. Natl. Acad. Sci. USA **103:** 230–235.

Kim, M.C., Panstruga, R., Elliott, C., Muller, J., Devoto, A., Yoon, H. W., Park, H.C., Cho, M.J., and Schulze-Lefert, P. (2002). Calmodulin interacts with MLO protein to regulate defence against mildew in barley. Nature **416:** 447–451.

Koga, J., Yamauchi, T., Shimura, M., Ogawa, N., Oshima, K.,

Umemura, K., Kikuchi, M., and Ogasawara, N. (1998). Cerebrosides A and C, sphingolipid elicitors of hypersensitive cell death and phytoalexin accumulation in rice plants. J. Biol. Chem. **273:** 31985–31991.

Komatsu, S., Abbasi, F., Kobori, E., Fujisawa, Y., Kato, H., and Iwasaki, Y. (2005). Proteomic analysis of rice embryo: An approach for investigating Galpha protein-regulated proteins. Proteomics **5:** 3932–3941.

Lavy, M., Bloch, D., Hazak, O., Gutman, I., Poraty, L., Sorek, N., Sternberg, H., and Yalovsky, S. (2007). A Novel ROP/RAC effector links cell polarity, root-meristem maintenance, and vesicle trafficking. Curr. Biol. **17:** 947–952.

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.

Lieberherr, D., Thao, N.P., Nakashima, A., Umemura, K., Kawasaki, T., and Shimamoto, K. (2005). A sphingolipid elicitor-inducible mitogen-activated protein kinase is regulated by the small GTPase OsRac1 and heterotrimeric G-protein in rice. Plant Physiol. **138:** 1644–1652.

Link, A.J., Eng, J., Schieltz, D.M., Carmack, E., Mize, G.J., Morris, D. R., Garvik, B.M., and Yates III, J.R. (1999). Direct analysis of protein complexes using mass spectrometry. Nat. Biotechnol. **17:** 676–682.

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.

Liu, Y.V., Baek, J.H., Zhang, H., Diez, R., Cole, R.N., and Semenza, G.L. (2007). RACK1 competes with HSP90 for binding to HIF-1alpha and is required for O(2)-independent and HSP90 inhibitor-induced degradation of HIF-1alpha. Mol. Cell **25:** 207–217.

Lopez-Bergami, P., Habelhah, H., Bhoumik, A., Zhang, W., Wang, L. H., and Ronai, Z. (2005). RACK1 mediates activation of JNK by protein kinase C. Mol. Cell **19:** 309–320.

Mackey, D., Belkhadir, Y., Alonso, J.M., Ecker, J.R., and Dangl, J. L. (2003). Arabidopsis RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. Cell **112:** 379–389.

Mackey, D., Holt, B.F., Wiig, A., and Dangl, J.L. (2002). RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell **108:** 743–754.

McCahill, A., Warwicker, J., Bolger, G.B., Houslay, M.D., and Yarwood, S.J. (2002). The RACK1 scaffold protein: A dynamic cog in cell response mechanisms. Mol. Pharmacol. **62:** 1261–1273.

McKhann, H.I., Frugier, F., Petrovics, G., de la Pena, T.C., Jurkevitch, E., Brown, S., Kondorosi, E., Kondorosi, A., and Crespi, M. (1997). Cloning of a WD-repeat-containing gene from alfalfa (*Medicago sativa*): A role in hormone-mediated cell division? Plant Mol. Biol. **34:** 771–780.

Miki, D., Itoh, R., and Shimamoto, K. (2005). RNA silencing of single and multiple members in a gene family of rice. Plant Physiol. **138:** 1903–1913.

Miki, D., and Shimamoto, K. (2004). Simple RNAi vectors for stable and transient suppression of gene function in rice. Plant Cell Physiol. **45:** 490–495.

Moeder, W., Yoshioka, K., and Klessig, D.F. (2005). Involvement of the small GTPase Rac in the defense responses of tobacco to pathogens. Mol. Plant Microbe Interact. **18:** 116–124.

Nibau, C., Wu, H.M., and Cheung, A.Y. (2006). RAC/ROP GTPases: 'Hubs' for signal integration and diversification in plants. Trends Plant Sci. **11:** 309–315.

Ono, E., Wong, H.L., Kawasaki, T., Hasegawa, M., Kodama, O., and Shimamoto, K. (2001). Essential role of the small GTPase Rac in disease resistance of rice. Proc. Natl. Acad. Sci. USA **98:** 759–764.

Pandey, S., Chen, J.G., Jones, A.M., and Assmann, S.M. (2006). G-protein complex mutants are hypersensitive to abscisic acid regulation of germination and postgermination development. Plant Physiol. **141:** 243–256.

Parent, A., Laroche, G., Hamelin, E., and Parent, J.L. (2008). RACK1 regulates the cell surface expression of the G protein-coupled receptor for thromboxane A(2). Traffic **9:** 394–407.

Park, J., Choi, H.J., Lee, S., Lee, T., Yang, Z., and Lee, Y. (2000). Rac-related GTP-binding protein in elicitor-induced reactive oxygen generation by suspension-cultured soybean cells. Plant Physiol. **124:** 725–732.

Patterson, R.L., van Rossum, D.B., Barrow, R.K., and Snyder, S.H. (2004). RACK1 binds to inositol 1,4,5-trisphosphate receptors and mediates Ca^{2+} release. Proc. Natl. Acad. Sci. USA **101:** 2328–2332.

Perennes, C., Glab, N., Guglieni, B., Doutriaux, M.P., Phan, T.H., Planchais, S., and Bergounioux, C. (1999). Is arcA3 a possible mediator in the signal transduction pathway during agonist cell cycle arrest by salicylic acid and UV irradiation? J. Cell Sci. **112:** 1181–1190.

Pratt, W.B., and Toft, D.O. (2003). Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp. Biol. Med. **228:** 111–133.

Ron, D., Chen, C.H., Caldwell, J., Jamieson, L., Orr, E., and Mochly-Rosen, D. (1994). Cloning of an intracellular receptor for protein kinase C: A homolog of the beta subunit of G proteins. Proc. Natl. Acad. Sci. USA **91:** 839–843.

Schechtman, D., and Mochly-Rosen, D. (2001). Adaptor proteins in protein kinase C-mediated signal transduction. Oncogene **20:** 6339–6347.

Schultheiss, H., Dechert, C., Kogel, K.H., and Huckelhoven, R. (2002). A small GTP-binding host protein is required for entry of powdery mildew fungus into epidermal cells of barley. Plant Physiol. **128:** 1447–1454.

Schultheiss, H., Dechert, C., Kogel, K.H., and Huckelhoven, R. (2003). Functional analysis of barley RAC/ROP G-protein family members in susceptibility to the powdery mildew fungus. Plant J. **36:** 589–601.

Sengupta, J., Nilsson, J., Gursky, R., Spahn, C.M., Nissen, P., and Frank, J. (2004). Identification of the versatile scaffold protein RACK1 on the eukaryotic ribosome by cryo-EM. Nat. Struct. Mol. Biol. **11:** 957–962.

Shen, Q.H., Saijo, Y., Mauch, S., Biskup, C., Bieri, S., Keller, B., Seki, H., Ulker, B., Somssich, I.E., and Schulze-Lefert, P. (2007). Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. Science **315:** 1098–1103.

Shevchenko, A. (2001). Evaluation of the efficiency of in-gel digestion of proteins by peptide isotopic labeling and MALDI mass spectrometry. Anal. Biochem. **296:** 279–283.

Shevchenko, A., Wilm, M., Vorm, O., and Mann, M. (1996). Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal. Chem. **68:** 850–858.

Shirasu, K., and Schulze-Lefert, P. (2003). Complex formation, promiscuity and multi-functionality: Protein interactions in disease-resistance pathways. Trends Plant Sci. **8:** 252–258.

Smith, D.F. (2004). Tetratricopeptide repeat cochaperones in steroid receptor complexes. Cell Stress Chaperones **9:** 109–121.

Suharsono, U., Fujisawa, Y., Kawasaki, T., Iwasaki, Y., Satoh, H., and Shimamoto, K. (2002). The heterotrimeric G protein alpha subunit acts upstream of the small GTPase Rac in disease resistance of rice. Proc. Natl. Acad. Sci. USA **99:** 13307–13312.

Takahashi, A., Casais, C., Ichimura, K., and Shirasu, K. (2003). HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in Arabidopsis. Proc. Natl. Acad. Sci. USA **100:** 11777–11782.

Takahashi, A., Kawasaki, T., Henmi, K., Shi, I.K., Kodama, O., Satoh, H., and Shimamoto, K. (1999). Lesion mimic mutants of rice with alterations in early signaling events of defense. Plant J. **17:** 535–545.

Thao, N.P., Chen, L., Nakashima, A., Hara, S., Umemura, K., Takahashi, A., Shirasu, K., Kawasaki, T., and Shimamoto, K. (2007). RAR1 and HSP90 form a complex with Rac/Rop GTPase and function in innate-immune responses in rice. Plant Cell **19:** 4035–4045.

Thornton, C., Tang, K.C., Phamluong, K., Luong, K., Vagts, A., Nikanjam, D., Yaka, R., and Ron, D. (2004). Spatial and temporal regulation of RACK1 function and N-methyl-D-aspartate receptor activity through WD40 motif-mediated dimerization. J. Biol. Chem. **279:** 31357–31364.

Umemura, K., Ogawa, N., Koga, J., Iwata, M., and Usami, H. (2002). Elicitor activity of cerebroside, a sphingolipid elicitor, in cell suspension cultures of rice. Plant Cell Physiol. **43:** 778–784.

Wirthmueller, L., Zhang, Y., Jones, J.D., and Parker, J.E. (2007). Nuclear accumulation of the Arabidopsis immune receptor RPS4 is necessary for triggering EDS1-dependent defense. Curr. Biol. **17:** 2023–2029.

Wang, Y., Gao, M., Li, Q., Wang, L., Wang, J., Jeon, J.S., Qu, N., Zhang, Y., and He, Z. (2008). OsRAR1 and OsSGT1 physically interact and function in rice basal disease resistance. Mol. Plant Microbe Interact. **21:** 294–303.

Wong, H.L., Pinontoan, R., Hayashi, K., Tabata, R., Yaeno, T., Hasegawa, K., Kojima, C., Yoshioka, H., Iba, K., Kawasaki, T., and Shimamoto, K. (2007). Regulation of rice NADPH oxidase by binding of Rac GTPase to its N-terminal extension. Plant Cell **19:** 4022–4034.

Wong, H.L., Sakamoto, T., Kawasaki, T., Umemura, K., and Shimamoto, K. (2004). Down-regulation of metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. Plant Physiol. **135:** 1447–1456.

Yaka, R., Thornton, C., Vagts, A.J., Phamluong, K., Bonci, A., and Ron, D. (2002). NMDA receptor function is regulated by the inhibitory scaffolding protein, RACK1. Proc. Natl. Acad. Sci. USA **99:** 5710–5715.

Yang, Z. (2002). Small GTPases: Versatile signaling switches in plants. Plant Cell **14:** S375–S388.

Yang, Z., and Fu, Y. (2007). ROP/RAC GTPase signaling. Curr. Opin. Plant Biol. **10:** 490–494.

Zeller, C.E., Parnell, S.C., and Dohlman, H.G. (2007). The RACK1 ortholog Asc1 functions as a G protein beta subunit coupled to glucose responsiveness in yeast. J. Biol. Chem. **282:** 25168–25176.

Zipfel, C. (2008). Pattern-recognition receptors in plant innate immunity. Curr. Opin. Immunol. **20:** 10–16.

Zipfel, C., and Felix, G. (2005). Plants and animals: A different taste for microbes? Curr. Opin. Plant Biol. **8:** 353–360.