Cell Host & Microbe
Article

The Hop/Sti1-Hsp90 Chaperone Complex Facilitates the Maturation and Transport of a PAMP Receptor in Rice Innate Immunity

Letian Chen,[1] Satoshi Hamada,[1] Masayuki Fujiwara,[1] Tingheng Zhu,[1,5] Nguyen Phuong Thao,[1,6] Hann Ling Wong,[1] Priti Krishna,[2] Takashi Ueda,[3] Hanae Kaku,[4] Naoto Shibuya,[4] Tsutomu Kawasaki,[1] and Ko Shimamoto[1,*]
[1]Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma 630-0192, Japan
[2]Department of Biology, University of Western Ontario, London, ON N6A 5B7, Canada
[3]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan
[4]Department of Life Sciences, Faculty of Agriculture, Meiji University, 1-1-1 Higashi-mita, Tama-ku, Kawasaki, Kanagawa 214-8571, Japan
[5]Present address: College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou, Zhejiang 310014, China
[6]Present address: International University, Ho Chi Minh City 700000, Vietnam
*Correspondence: simamoto@bs.naist.jp
DOI 10.1016/j.chom.2010.02.008

SUMMARY

Recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) represents a critical first step of innate defense in plants and animals. However, maturation and transport of PRRs are not well understood. We find that the rice chitin receptor OsCERK1 interacts with Hsp90 and its cochaperone Hop/Sti1 in the endoplasmic reticulum (ER). Hop/Sti1 and Hsp90 are required for efficient transport of OsCERK1 from the ER to the plasma membrane (PM) via a pathway dependent on Sar1, a small GTPase which regulates ER-to-Golgi trafficking. Further, Hop/Sti1 and Hsp90 are present at the PM in a complex (designated the "defensome") with OsRac1, a plant-specific Rho-type GTPase. Finally, Hop/Sti1 was required for chitin-triggered immunity and resistance to rice blast fungus. Our results suggest that the Hop/Sti1-Hsp90 chaperone complex plays an important and likely conserved role in the maturation and transport of PRRs and may function to link PRRs and Rac/Rop GTPases.

INTRODUCTION

Pattern recognition receptors (PRRs) are the first layer of defense against pathogen infection at the cell surface (Jones and Dangl, 2006). Pathogen-specific molecules recognized by PRRs are called pathogen-associated molecular patterns (PAMPs) (Chisholm et al., 2006; Zipfel, 2008). In plants, host perception of PAMPs activates rapid defense responses such as calcium influx, production of reactive oxygen species (ROS), induction of defense-related genes, and accumulation of antimicrobial compounds (Jones and Dangl, 2006). Most plant PAMP receptors characterized to date are receptor-like kinases (RLKs) or receptor-like proteins (RLPs). RLKs possess an extracellular domain, a transmembrane (TM) domain, and a kinase domain (KD), whereas RLPs lack the intracellular KD. Protein structural analyses indicate that RLKs perceive signals through their extracellular domain and transmit signals via their intracellular KD. *Arabidopsis* and rice encode more than 600 and 1100 RLK/Ps, respectively (Shiu et al., 2004), which are involved in numerous cellular signaling and developmental events.

RLKs function in plant-microbe interactions and defense responses. FLS2 and EFR, for example, are receptors for bacterial flagellin and elongation factor Tu (EF-Tu), respectively (Gomez-Gomez and Boller, 2000; Zipfel et al., 2006), while CEBiP and LysM-type CERK1 are receptors for fungal chitin (Kaku et al., 2006; Miya et al., 2007; Wan et al., 2008). Recently, BRI-associated kinase (BAK1) was found to be important for innate immunity as well as cell death (Chinchilla et al., 2007; He et al., 2007; Kemmerling et al., 2007), suggesting that different RLK/P-mediated signaling pathways share common components. RLK/Ps are located in the PM and are assumed to move to endosomes through endocytosis (Russinova et al., 2004; Geldner and Robatzek, 2008). However, the modes of maturation, trafficking, and PM localization of RLK/Ps are largely unknown.

Rac/Rop small GTPases are plant-specific Rho-type GTPases which act as molecular switches in many biological processes, including plant innate immunity, in rice and other species (Berken, 2006; Brembu et al., 2006; Nibau et al., 2006). OsRac1 plays a role in basal resistance and is involved in the immune response induced by two PAMPs derived from fungal pathogens, N-acetylchitooligosaccharide (chitin) and sphingolipids (Ono et al., 2001; Suharsono et al., 2002; Fujiwara et al., 2006). OsRac1 controls cell death, the activation of *PR* gene expression, and phytoalexin production (Kawasaki et al., 1999; Ono et al., 2001; Suharsono et al., 2002; Wong et al., 2004). It also regulates ROS production by interacting directly with the N-terminal region of NADPH oxidase (Wong et al., 2007), and lignin production by interacting with cinnamoyl CoA reductase (Kawasaki et al., 2006). Furthermore, PAMP-induced activation of rice MAPK6 requires OsRac1, which forms a complex with MAPK6 in rice cell extracts (Lieberherr et al., 2005). Dominant-negative (DN) OsRac1 was shown to diminish *N* gene-mediated resistance to TMV infection in tobacco (Moeder et al., 2005).

Similarly, Rac/Rop GTPases have been shown to be involved in ROS production in *Arabidopsis* (Park et al., 2004; Jones et al., 2007), and in immune responses in a variety of species (Schiene et al., 2000; Pathuri et al., 2008). Taken together, these previous studies indicate that Rac/Rop GTPases are involved in plant innate immunity by regulating a number of important downstream components of the immune response.

Cytoplasmic Hsp90 chaperone plays critical roles in plant innate immunity together with the cochaperone-like proteins RAR1 and SGT1 (Hubert et al., 2003, 2009; Lu et al., 2003; Takahashi et al., 2003; Liu et al., 2004; Shirasu, 2009). We recently demonstrated that RAR1, Hsp90, and Hsp70 are present in the OsRac1 complex, but none of them appear to interact directly with OsRac1 (Thao et al., 2007). The OsRac1-interacting scaffold protein RACK1A directly interacts with SGT1 and RAR1, but not with Hsp90 (Nakashima et al., 2008). In mammalian cells, Hsp90 and Hsp70 form a complex through the cochaperone Hop (Pratt and Toft, 2003). Plant-encoded Hop, which is also known as stress-induced protein 1 (Sti1), interacts with Hsp90 in vitro (Torres et al., 1995; Zhang et al., 2003), although its biological function has not yet been elucidated. We have identified Hop/Sti1 as an OsRac1interactor (Nakashima et al., 2008).

In this study, we demonstrate that Hop/Sti1 interacts with the rice chitin receptor OsCERK1. OsCERK1 also interacts with the cytoplasmic Hsp90. Moreover, Hop/Sti1a and Hsp90 are required for efficient transport of OsCERK1 from the ER to the PM via the Sar1-dependent trafficking system. We further provide evidence that Hop/Sti1a and Hsp90 are present in an OsRac1 immune complex, designated the defensome, at the PM.

RESULTS

The Hsp90 Cochaperone Hop/Sti1 Interacts with OsRac1 and Is Localized to the ER

Based on affinity chromatography and database analysis, we identified two highly similar Hop/Sti1s in rice (Nakashima et al., 2008), hereafter designated Hop/Sti1a and Hop/Sti1b, which share 80.4% amino acid identity (see Figures S1A and S1B available online). Three Hop/Sti1 homologs are found in *Arabidopsis* (Figure S1A). Sequence analysis suggested that the rice Hop/Sti1 proteins share a conserved structure with yeast and mammalian Hops (Figure S1B; Nicolet and Craig, 1989).

Since Hop/Sti1 was identified through affinity chromatography with OsRac1, we first used in vitro binding assays to confirm the interaction of Hop/Sti1 with OsRac1. Hop/Sti1 interacted with wild-type (WT), constitutively active (CA), and DN OsRac1 (Figure 1A). These results were further substantiated by coimmunoprecipetation assays with crude lysates of cultured cells (Nakashima et al., 2008) (Figure S1C). Moreover, the split-ubiquitin two-hybrid system (SUS) showed that Hop/Sti1a, one of the two rice homologs, interacted with CA-OsRac1, but not with DN-OsRac1 (Figure 1B). Although the reasons for the differences between the results of the two-hybrid and in vitro binding assays remain unclear, these results indicate that Hop/Sti1 interacts with OsRac1 both in vitro and in vivo.

We next analyzed the interactions of Hop/Sti1 with known defense-related (co-)chaperones in the yeast two-hybrid (Y2H) system. Hop/Sti1a did not interact with RAR1 or SGT1 (Figure 1C). A strong interaction between Hop/Sti1a and cytoplasmic Hsp90 was observed, while the interaction of Hop/Sti1b, the other rice homolog, with Hsp90 was weak (Figure 1C, Figures S1E and S1F). As previously reported for *Arabidopsis*, RAR1 interacted with SGT1, and both interacted with Hsp90 (Figure 1C). Hop/Sti1 did not interact with MAPK6, RbohB, or RACK1, components of the OsRac1 immune complex (Nakashima et al., 2008). OsRac1 did not directly interact with RAR1, SGT1, or Hsp90 (Figures S1E and S1G). Since Hop/Sti1a strongly interacted with OsRac1 and Hsp90 (Figures S1E and S1F), we chose Hop/Sti1a for further studies.

We examined the intracellular localization of Hop/Sti1 by expressing the YFP-tagged Hop/Sti1a protein in rice protoplasts. Hop/Sti1a-YFP colocalized with an ER marker, showing a typical ER pattern (Figure 1D). We performed aqueous two-phase partitioning experiments and found Hop/Sti1a in the endomembrane-rich and PM-rich fractions, in addition to the soluble fraction (Figure 1E). Hsp90 was present mostly in the soluble fraction, but small amounts were also detected in the endomembrane-rich and PM-rich fractions (Figure 1E). OsRac1 was present in the same membrane-enriched fractions as Hop/Sti1a, suggesting that these three proteins can interact in the membranes (Figure 1E).

Hop/Sti1a Is Involved in Chitin-Triggered Immune Responses and Rice Blast Resistance

To examine the function of Hop/Sti1 in rice innate immunity, we produced transgenic rice cell cultures and plants in which *Hop/Sti1* expression was silenced by RNAi, as well as cells and plants that overexpressed Hop/Sti1a (Figure S2A). In *Hop/Sti1a*-RNAi cell lines, *Hop/Sti1a* was strongly reduced at mRNA and protein levels (Figure 2A, Figures S2B and S5A). *Hop/Sti1a*-RNAi and WT cell cultures were treated with chitin, and chitin-induced defense-related gene expression was monitored by quantitative PCR (qPCR). Chitin-induced defense-related gene expression of *PAL1* and *PBZ1* was strongly reduced and was delayed for 1–2 hr in *Hop/Sti1a*-RNAi cells compared to the WT cells (Figure 2B), indicating that Hop/Sti1a plays a role in chitin-triggered immunity. Consistent with this phenotype, rice cultivar Kinmaze possesses the chitin receptor OsCERK1 (Figure S2E), a rice ortholog of the *Arabidopsis* chitin receptor AtCERK1 that is involved in chitin-triggered immune responses (T. Shimizu, H.K., and N.S., unpublished data). However, since cv. Kinmaze lacks the flg22 receptor OsFLS2, flg22-triggered immune responses are absent (Takai et al., 2008).

In *Hop/Sti1ab*-RNAi plants, levels of both *Hop/Sti1a* and *Hop/Sti1b* mRNAs were strongly reduced (Figure S2C). In contrast, *Hop/Sti1a* expression was significantly increased in *Hop/Sti1a*-OX plants (Figure S2D). In infection assays with a virulent strain of the rice blast fungus, *Hop/Sti1ab*-RNAi plants were more susceptible than WT plants to rice blast infection (Figure 2C), whereas *Hop/Sti1a*-OX plants were more resistant (Figure 2D). Taken together, these results demonstrate that Hop/Sti1a is involved in chitin-triggered immune responses and resistance to blast infection in rice.

Hop/Sti1a and Hsp90 Interact with the Rice Chitin Receptor OsCERK1 via Its Transmembrane Domain

Since the Hop/Sti1-Hsp90 (co-)chaperone complex is involved in PAMP-triggered immune responses in rice, we wondered

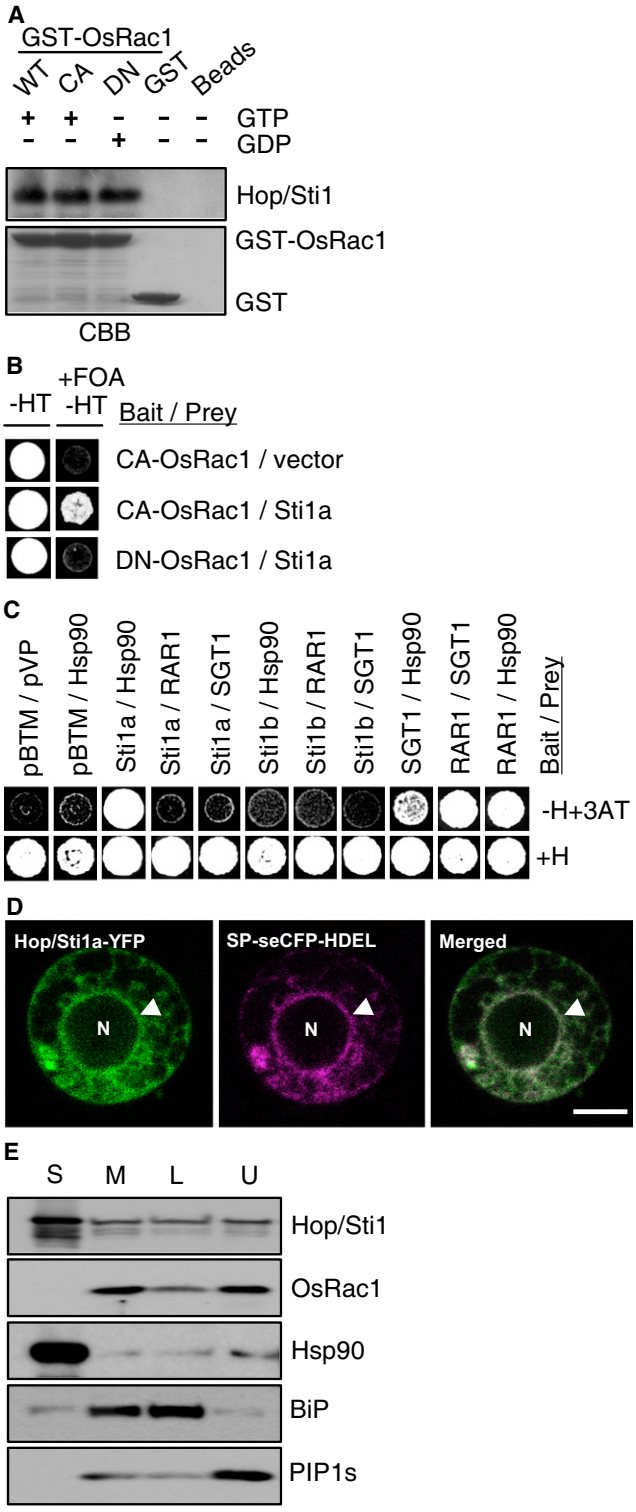

Figure 1. The Hsp90 Cochaperone Hop/Sti1 Interacts with OsRac1 and Is Localized to the ER

(A) Interaction of Hop/Sti1a and OsRac1 in in vitro binding assays. Upper image, purified GST-tagged OsRac1 and His-tagged Hop/Sti1a were subjected to pull-down assays with anti-GST beads and detected with anti-Hop/Sti1a antibody. Lower image, Coomassie Brilliant Blue (CBB)-stained gel after blotting.

(B) Two-hybrid assays of Hop/Sti1a and OsRac1 in SUS.

whether rice RLKs could also interact with the Hop/Sti1-Hsp90 complex. We chose the rice chitin receptor OsCERK1 to test this idea. Interestingly, Hop/Sti1 and Hsp90, but not CA-OsRac1, interacted with OsCERK1 (Figure 3A). Similarly, rice homologs of two other RLKs, OsFLS2 and OsBAK1, also interacted with Hop/Sti1a (Figure 3A). We further studied the interaction between Hop/Sti1a and OsCERK1 using truncated fragments of OsCERK1 and found that the TM domain of OsCERK1, but not the extracellular LysM domain (ED) or the KD, interacted with Hop/Sti1a (Figures 3B and 3C). Similarly, Hsp90 interacted with the TM domain of OsCERK1 (Figures 3B and 3C). A Hop/Sti1a deletion assay showed that either the TPR1 or the TPR2A domain of Hop/Sti1a alone was sufficient for its interaction with all three interacting proteins (Figure 3D). OsRac1 also interacted weakly with the DP1 domain of Hop/Sti1a. These results showed that Hop/Sti1a and Hsp90 interact with the TM region of OsCERK1, thus connecting an RLK with OsRac1 and its signaling partners in rice.

OsCERK1 Matures in the ER and Is Transported to the PM through a Vesicle Trafficking Pathway

It has been suggested that RLK receptors are PM proteins whose localization is regulated by endocytosis (Russinova et al., 2004; Geldner and Robatzek, 2008). However, the processes of maturation and transport of plant RLKs and PAMP receptors are largely unknown. The finding that Hop/Sti1a localizes to the ER and physically interacts with a PAMP receptor (Figures 1D, 1E, and 3) raises the question of how Hop/Sti1a, an ER protein, interacts with the PM-localized RLK. To address this question, we used GFP-fused intact OsCERK1 (OsCERK1-GFP) to investigate the subcellular localization of OsCERK1 in rice protoplasts.

OsCERK1-GFP was found predominantly in the PM. However, some signals were also detected in vesicle-like organelles and typical ER structures. To analyze GFP signals more precisely in transformed protoplasts, the cells were categorized into four types (P1–P4) according to the distribution of the OsCERK1-GFP signal in different organelles (Figure 4A), and the frequency of each type was determined (Figure 4B). Under normal conditions, P1, in which the GFP signal was observed only in the PM, contributed 4% of the total transformed cells; P2, in which signals were detected in the PM and in dot-like vesicles, comprised 50%; and P3, in which signals were detected in the PM, vesicles, and the ER network, accounted for 46%. No P4 cells, in which the signal was observed only in the ER, were found (Figure 4B, upper panel).

These results suggested that OsCERK1 may be transported from the ER to the PM through a vesicular trafficking pathway. Consistent with this, brefeldin A (BFA), a well-known inhibitor of

(C) A survey of Hop/Sti1-interacting proteins among known (co-)chaperones in the Y2H system.

(D) Subcellular localization of Hop/Sti1a protein. Hop/Sti1a-YFP was colocalized predominantly with the ER marker SP-seCFP-HDEL (middle). The arrowheads indicate the typical ER ring structure surrounding the perinuclear membrane. N, nucleus; scale bars, 5 μm.

(E) Hop/Sti1 protein distribution in two-phase partitioning. S, soluble fraction; M, microsomal fractions; U, upper phase of partitioning; L, lower phase of partitioning. Western blotting was performed with antibodies against the proteins indicated to the right of each panel. PIP1s, PM marker; BiP, ER marker.

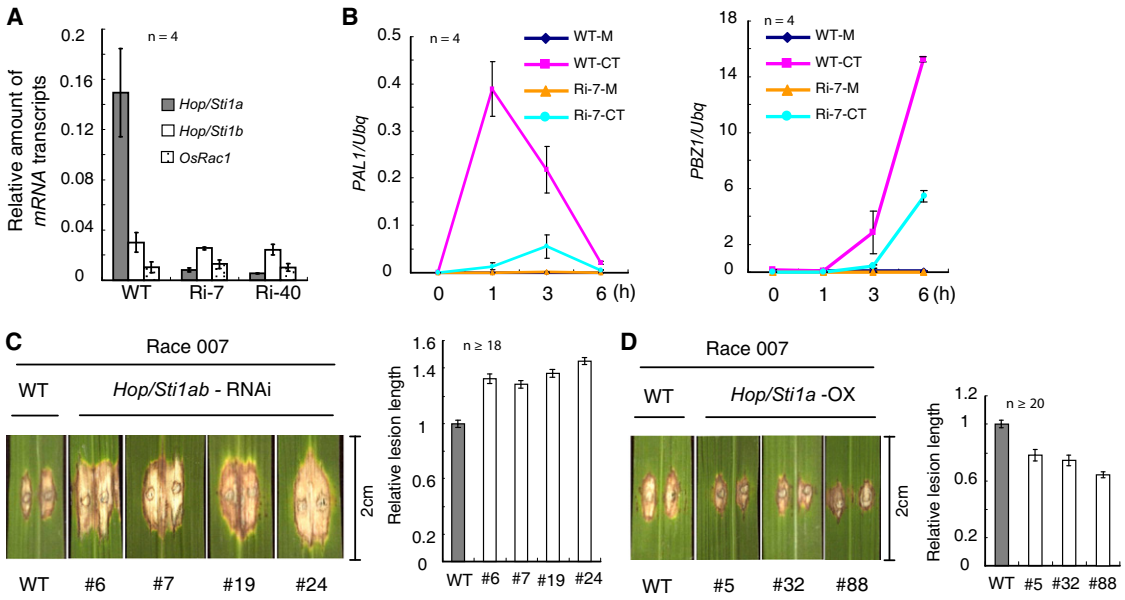

Figure 2. Hop/Sti1a Is Involved in the Chitin-Triggered Immune Response and Rice Blast Resistance

(A) Expression of *Hop/Sti1* and *OsRac1* in *Hop/Sti1a*-RNAi-cultured cells Ri-7 and Ri-40 was analyzed by qPCR and normalized with endogenous *Ubq*. Data shown are means ± SE; n = 4.

(B) Induction of defense-related genes, *PAL1* (left) and *PBZ1* (right), in *Hop/Sti1a*-RNAi (Ri-7) and WT cells, following chitin treatment (CT), was monitored at the indicated time points using qPCR. M, mock. Data are means ± SE; n = 4.

(C and D) Infection assays of *Hop/Sti1ab*-RNAi (C) and *Hop/Sti1ab*-OX (D) plants with the virulent rice blast fungus (race 007). Pictures (left) show typical phenotypes of WT and representative transgenic plants. Relative lesion lengths (right; WT = 1) are shown with means ± SE; n ≥ 18.

vesicle trafficking (Satiat-Jeunemaitre et al., 1996), dramatically changed the OsCERK1-GFP localization pattern. In 87% of the protoplasts examined, OsCERK1-GFP was confined to the ER (P4 type), while the remaining 13% of the protoplasts were type P3, with signals detected in the ER and the PM (Figure 4B, lower panel). No P1 or P2 cells were detected in the presence of BFA.

We observed dynamic changes in the subcellular localization of OsCERK1-GFP, which depended on the time after transformation and the presence of BFA. OsCERK1-GFP became visible at 3.5–4 hr after transformation. The GFP signal appeared mainly in the ER around the nucleus, while only a faint signal was detected in the PM at 4 hr after transformation (Figure 4C, upper left). The GFP signal in the PM became distinct by 7 hr after transformation (Figure 4C, upper right). These results showed that OsCERK1-GFP occurred first in the ER, where it was probably synthesized, before it reached the PM.

When BFA was added to protoplasts at 3 hr after transformation and the protoplasts were further incubated for 1 hr, the pattern was similar to the control (Figure 4C, lower left). However, after 4 hr of BFA treatment, the GFP fluorescence increased in the ER, whereas no such increase was detectable in the PM (Figure 4C, lower right). These results suggest that OsCERK1-GFP was synthesized normally in the presence of BFA but then accumulated in the ER due to the inhibition of vesicle trafficking by BFA.

We next examined whether the exit of OsCERK1 from the ER depends on COPII-mediated ER-to-Golgi traffic. AtSar1 is a small GTPase which regulates the ER-to-Golgi trafficking in *Arabidopsis* (Takeuchi et al., 2000). A constitutively active Sar1 (CA-AtSar1) mutant inhibits the transport of the Golgi membrane protein AtRer1B from the ER to the Golgi (Takeuchi et al., 2000). We cotransformed *OsCERK1-CFP*, *GFP-AtRer1B*, and either WT-*AtSar1* or CA-*AtSar1* into rice protoplasts and examined their fluorescence signals. OsCERK1-CFP predominantly localized to the PM, while AtRer1B-GFP was found in the Golgi-like organelles when WT-*AtSar1* was cotransformed (Figure 4D, upper panel). In contrast, when CA-*AtSar1* was cotransformed, the AtRer1B-GFP signal was restricted to the ER, as was the coexpressed OsCERK1-CFP signal (Figure 4D, lower panel). It was noted that when WT-*AtSar1* was cotransformed, GFP-AtRer1B signals overlapped punctate organelles, most probably the endosomes, en route to the PM (Figure 4D, upper panel). Together, these results indicate that OsCERK1 matures in the ER and is subsequently transported from the ER to the PM through a Sar1-dependent vesicle trafficking pathway.

Maturation and Transport of the OsCERK1 Receptor Are Regulated by the Hop/Sti1a-Hsp90 Chaperone Complex

To characterize proteins that may be involved in maturation and transport of OsCERK1, we studied protein interactions by bimolecular fluorescence complementation (BiFC) assays (Hu et al., 2002). When BiFC constructs were transformed into rice protoplasts, they sometimes generated background fluorescence, as has been reported previously (Walter et al., 2004). To quantify protein interactions in BiFC assays, we measured the frequency of reconstituted YFP-positive protoplasts in each combination of constructs and found that OsCERK1/Hsp90, Sti1a/Hsp90, OsCERK1/Sti1a, and CA-OsRac1/Sti1a all provided unambiguous evidence for protein interactions (Figure 5A; Figure 5B, lower right; and Figure 5E, lower right).

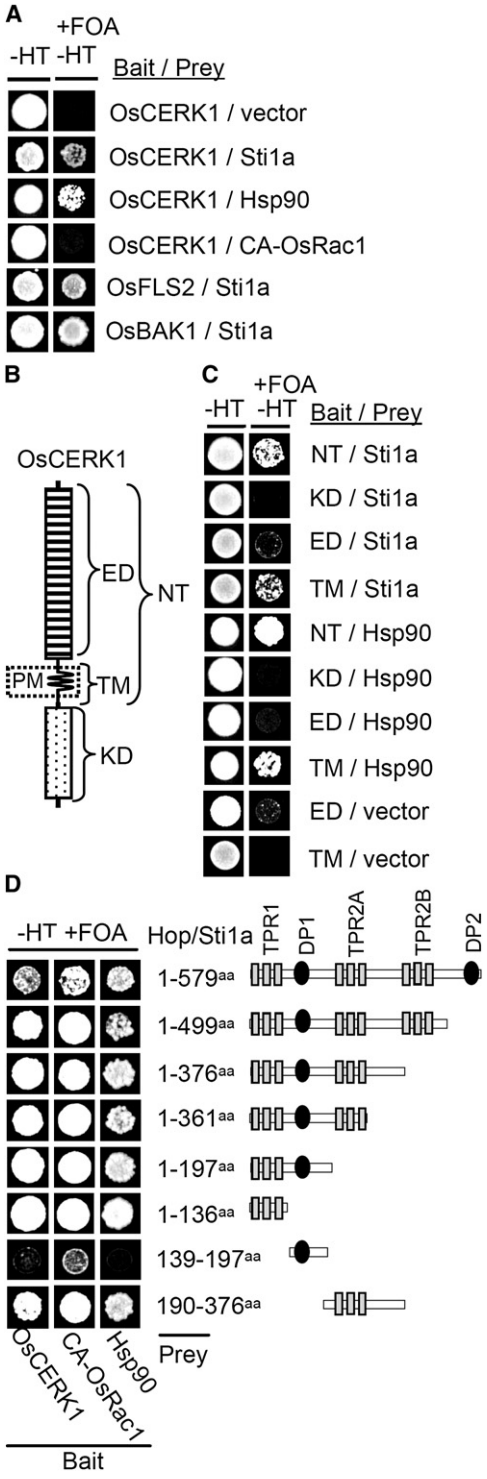

Figure 3. Hop/Sti1a Interacts with Rice Chitin Receptor via Its Transmembrane Domain

(A) Interactions of Hop/Sti1a, Hsp90, and CA-OsRac1 with the rice RLK PAMP receptors in SUS.

(B) Schematic diagram of OsCERK1 and its four deletion mutants used for SUS assays.

(C) Interactions of OsCERK1 deletion mutants with Hop/Sti1a and Hsp90 in SUS assays.

(D) Interactions of a series of Hop/Sti1a deletion mutants with OsCERK1, CA-OsRac1, and Hsp90 in SUS assays. A schematic diagram of the Hop/Sti1a deletion mutants is shown on the right. Numbers indicate the first and last amino acids of Hop/Sti1a (intact, 579 amino acids; top line) that are retained in the mutants.

Hop/Sti1a-OsCERK1 complexes were primarily detected in the PM, but also in the ER and dot-like organelles (Figure 5Ba), which resembled the pattern of OsCERK1-GFP localization (Figure 4B, upper panel). Addition of BFA to the BiFC assays prevented the transport of Hop/Sti1a-OsCERK1 complexes to the PM (Figure 5C). However, BFA had no effect on the localization of the nuclear protein GenL-GFP or CFP-GenL (Moritoh et al., 2005; Figures 5Ba and 5C). Thus, Hop/Sti1a appears to form a complex with OsCERK1 which is transported from the ER to the PM through punctate intermediate organelles. Furthermore, the NT fragment of OsCERK1, which contains both the ED and TM domains (Figure 3B), formed a complex with Hop/Sti1a in the ER and the PM (Figure 5Bb). In contrast, when the TM domain alone was used in BiFC assays with Hop/Sti1a, the Hop/Sti1a-OsCERK1-TM complex was mainly restricted to the ER, with no clear signal from the PM (Figure 5Bc). These results imply that the ED is mainly responsible for the proper ER exit of OsCERK1; consistent with this, deletion of the ED caused retention of the truncated OsCERK1 in the ER (Figure S3).

We next analyzed subcellular localization of Hsp90 and its interaction with OsCERK1 by BiFC assays. Hsp90 mainly localized to the cytoplasm, as expected from the results of the biochemical analysis (Figure 1E), but could also be detected in the ER (Figure 5D). However, the BiFC signal for the Hsp90-OsCERK1 complex was predominantly localized to the PM (Figure 5Ea). The interactions of the NT and TM fragments of OsCERK1 with Hsp90 were also examined. The intracellular localization of the BiFC signals (Figures 5Eb and 5Ec) resembled that obtained with Hop/Sti1a (Figures 5Bb and 5Bc), indicating that Hsp90 also interacts with the TM domain of OsCERK1. When CA-*AtSar1* was cotransformed in the BiFC assay for Hsp90 and OsCERK1, the fluorescence was mainly detected in the ER (Figure 5F), suggesting that the OsCERK1-Hsp90 complex is transported from the ER to the PM through the Sar1-dependent trafficking system. We also analyzed Hop/Sti1a-CA-OsRac1 interactions by BiFC and found low levels of fluorescence signals in the ER, besides the cytoplasm and the PM (Figure 5G). A similar subcellular localization pattern was observed for Hop/Sti1a-Hsp90 interactions (Figure 5H).

Since Hop/Sti1a and Hsp90 interacted with OsCERK1 in the ER and at the PM (Figure 5B, 5E), and since *Hop/Sti1a*-RNAi impaired chitin-triggered defense gene induction (Figure 2B), we investigated whether an impairment of Hop/Sti1a or Hsp90 function affected the transport of OsCERK1 from the ER to the PM by determining the percentage of OsCERK1-GFP-expressing protoplasts in which ER-localized GFP signals were detected. In *Hop/Sti1a*-RNAi protoplasts, the frequency of protoplasts with ER-localized OsCERK1-GFP signals was higher than that in WT protoplasts (Figure 5I), suggesting that Hop/Sti1a is required for the correct targeting of OsCERK1 to the PM. Next, we examined Hsp90 function in the transport of OsCERK1 using an Hsp90 inhibitor geldanamycin (GDA)

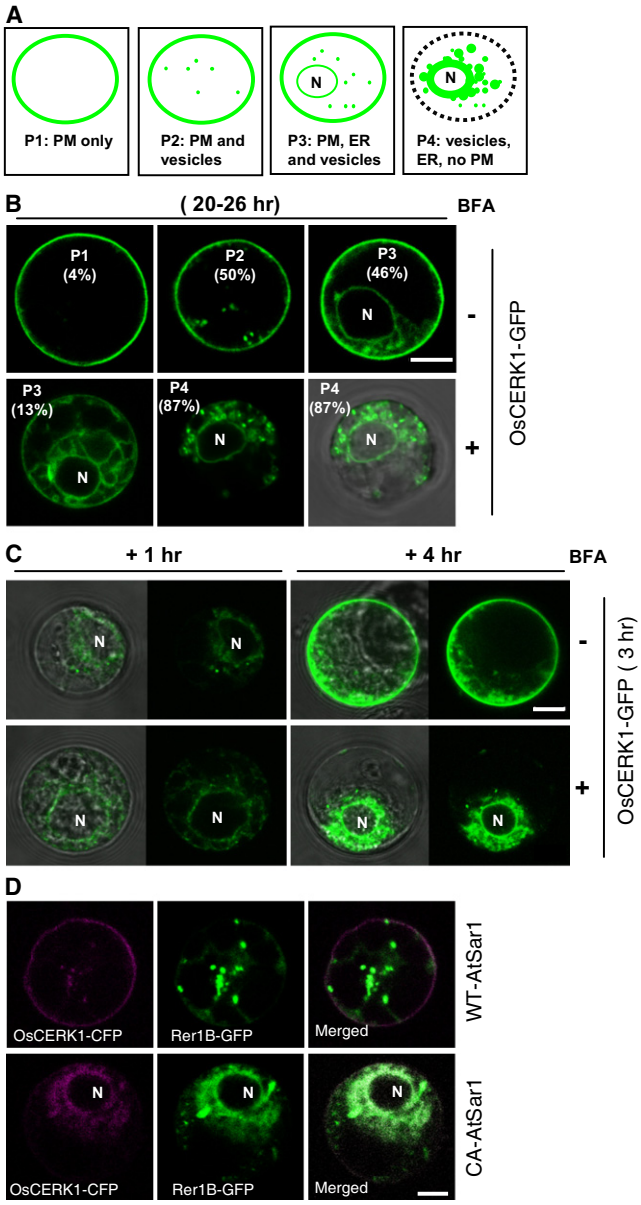

Figure 4. OsCERK1 Matures in the ER and Is Subsequently Transported to the PM by a Vesicle Trafficking Pathway

(A) Four types of OsCERK1-GFP localization. Green color indicates GFP signal. N, nucleus.

(B) Subcellular localization of OsCERK1-GFP in the absence (upper panel) or presence (lower panel) of BFA.

(C) OsCERK1-GFP localization pattern at the early stage of its biogenesis. Protoplasts transformed with OsCERK1-GFP were preincubated for 3 hr before BFA was added to the medium. Microscopic observation was performed at 1 and 4 hr after BFA treatment.

(D) OsCERK1-CFP localization pattern in the presence of WT- or CA-AtSar1. WT-AtSar1-Rer1B-GFP or CA-AtSar1-Rer1B-GFP was cotransformed with OsCERK1-CFP into protoplasts. N, nucleus; scale bars in (B)–(D), 5 μm.

(Richter and Buchner, 2001). Levels of ER signals were similar in GDA-treated WT and *Hop/Sti1a*-RNAi protoplasts (Figure 5I). However, when *Hop/Sti1a*-RNAi protoplasts were treated with GDA, no additive effect on the percentage of protoplasts showing ER signals was found (Figure 5I), suggesting that Hop/Sti1a and Hsp90 function in the same pathway to facilitate ER exit of OsCERK1. Together, these results indicate that both Hop/Sti1a and Hsp90 interact with OsCERK1 and regulate its transport from the ER to the PM.

OsCERK1, Hop/Sti1a, Hsp90, and OsRac1 May Form a Complex in the ER

Since our BiFC assays revealed that OsCERK1 can form a complex with Hop/Sti1a and Hsp90 in the ER as well as at the PM (Figures 5A, 5B, and 5E), we further analyzed the colocalization of these three proteins by a BiFC-based method (Figures 6A and 6C). First, a pair of OsCERK1 and Hop/Sti1a BiFC constructs was transformed into rice protoplasts together with CFP-WT-OsRac1, and the YFP and CFP signals arising respectively from the OsCERK1-Hop/Sti1 interaction and from OsRac1 were monitored. A BiFC-generated YFP signal was detected in the ER and at the PM, and colocalized with CFP, indicating that the OsCERK1-Hop/Sti1a complex and OsRac1 were present in the same subcellular compartments (Figure 6B). Similarly, OsCERK1-Hsp90 and Hop/Sti1a were present in the same regions (Figure 6D), which was consistent with the other interaction experiments. Taken together, these findings suggest that OsCERK1 interacts with Hop/Sti1a and Hsp90, and possibly with OsRac1 through Hop/Sti1a, in the ER, and that these interactions may be important for the efficient targeting of OsCERK1 to the PM where it functions as a PAMP receptor.

Hop/Sti1 Is Associated with the OsRac1 Immune Complex at the PM

Based on a number of protein-protein interaction and functional studies (Lieberherr et al., 2005; Thao et al., 2007; Wong et al., 2007; Nakashima et al., 2008) and the current study, we proposed that a protein complex at the PM, containing the PAMP receptor OsCERK1, OsRac1, Hop/Sti1a, Hsp90, Hsp70, RAR1, SGT1, RACK1A, RbohB, and MAPK6, plays a role in PAMP-triggered immunity in rice (Figure 7A). We name this complex present at the PM the "defensome."

To confirm that Hop/Sti1a is part of the defensome, we analyzed extracts of transgenic cultured cells expressing Myc-tagged CA-OsRac1 by size-exclusion chromatography. Hop/Sti1a, Hsp90, and Hsp70 were found in fractions containing proteins of 200–500 kDa, while CA-OsRac1 was detected in fractions of 250–350 kDa (Figure 7B). These results suggest that Hop/Sti1a, Hsp90, and Hsp70 form a complex of about 250–350 kDa with CA-OsRac1 at the PM and in the ER during PAMP-triggered immunity in rice. The low specificity of the OsCERK1 antibodies and the low level of OsCERK1 expression precluded its detection in the fractionation experiment shown in Figure 7B. Detailed biochemical and cell-biological studies of the defensome complex will be required to understand the molecular mechanisms of PAMP-triggered innate immunity in rice.

DISCUSSION

Importance of the Hop/Sti1-Hsp90 Chaperone Complex in PAMP-Triggered Immunity in Plants

In animals, Hop/Sti1 is best known as one of the cochaperones for the cytoplasmic Hsp90 chaperone, which participates in a

complex that regulates steroid hormone receptor biogenesis and maturation (Pratt and Toft, 2003). In yeast, Hop/Sti1 has been shown to stabilize the Hsp90 complex with a MAPKKK and to promote MAPK signaling (Lee et al., 2004). In plants, in vitro binding assays indicated that soybean Hop/Sti1 interacts with Hsp90 (Zhang et al., 2003).

Although the involvement of the cytoplasmic Hsp90 chaperone complex and other cochaperone-like proteins, RAR1 and SGT1, in plant innate immunity has been well established, the molecular mechanisms of their functions are not yet understood (Hubert et al., 2009; Shirasu, 2009). We have shown previously that GDA treatment suppresses PAMP-triggered immune responses in rice cells and disrupts OsRac1-Hsp90 complex formation (Thao et al., 2007). It is possible that the OsRac1-Hsp90 complex analyzed in our previous study is a component of a larger PM defensome complex, which contains RLK, Hop/Sti1, and the PM-anchored OsRac1. Since we showed that *Hop/Sti1a*-RNAi and GDA decreased the efficiency of the PM targeting of OsCERK1, and thereby impaired chitin-triggered defense gene expression (Figures 2B and 5I), it seems possible that the Hsp90 chaperone complex, including Hop/Sti1a, has a dual function in rice innate immunity: one function is related to efficient export from the ER and PM localization of PAMP receptors, and the other to signaling in the defensome at the PM.

Roles of the ER Maturation and Trafficking of PAMP Receptors in Plant Innate Immunity

Plant PAMP receptors possess an extracellular hydrophobic LRR or LysM region and putative N-glycosylation sites. Hydrophobic and glycosylated proteins often require chaperones for proper folding and assembly in the ER and are exported from the ER by the coatomer complex II (COPII) machinery (Gurkan et al., 2006). Our results indicate that maturation of the rice chitin receptor OsCERK1 occurs in the ER, and that the receptor is then transported to the PM through the ER and the Golgi (Figure 4).

Endosome localization of plant RLKs has been reported recently (Russinova et al., 2004; Geldner and Robatzek, 2008). Analysis of the OsCERK1-GFP localization patterns in a large protoplast population showed that although OsCERK1-GFP was mostly present in the PM, very few cells (4%) showed OsCERK1-GFP fluorescence exclusively in the PM. In the majority of protoplasts (96%), GFP fluorescence was also observed in punctate organelles or the ER (Figure 4B, upper panel). These results, together with the findings in the presence of BFA and CA-Sar1, strongly suggest that the ER is the site for OsCERK1 biogenesis and maturation (Figure 7C). Our results from Y2H and BiFC assays indicate that Hop/Sti1a also interacts with OsFLS2 and OsBAK1 (Figure 3A and Figure S4). OsFLS2 is a functional ortholog of AtFLS2, which perceives flg22 PAMP signals in some rice cultivars (Takai et al., 2008). Therefore, it is possible that Hop/Sti1a functions as a general facilitator of the ER exit and transport of PAMP receptors to the PM.

The trafficking system is known to be important for plant innate immunity (Collins et al., 2003; Wang et al., 2005; Nomura et al., 2006; Kwon et al., 2008; Speth et al., 2009), but how trafficking affects innate immunity is largely unknown. Recent studies have indicated that key components of the ER quality control (ERQC) system are involved in BRI1-mediated hormone signaling and innate immune responses in plants (Jin et al., 2007; Hong et al., 2008; Caplan et al., 2009; Li et al., 2009; Nekrasov et al., 2009; Saijo et al., 2009). Our results suggest that Hop/Sti1 and Hsp90 regulate OsCERK1 maturation by assembling a complex (or complexes) with OsRac1 in the ER and subsequently transporting OsCERK1 from the ER to the PM (Figure 7C). How the ERQC system cooperates with the Hsp90-Hop/Sti1 chaperone machinery to regulate the maturation, ER export, and trafficking of OsCERK1 will be an interesting topic for the future.

Conservation of the Hsp90 Chaperone System for Maturation and Trafficking of Cell-Surface Proteins in Animals and Plants

The role of the Hsp90 chaperone system in the maturation of nuclear steroid receptors is well established in animals (reviewed by Wegele et al., 2004), and more general functions of the system in the maturation, trafficking, and expression of animal cell surface proteins have recently begun to emerge. For example, the Hsp90 chaperone system helps to stabilize the chloride channel, cystic fibrosis TM conductance regulator (CFTR), and promotes its efficient transport to the cell surface (Wang et al., 2006). Downregulation of the Hsp90 cochaperone Aha1 rescues a transport-defective CFTR mutation, and modulation of ER exit by the Hop-containing Hsp90 chaperone complex may regulate the transport of CFTR to the cell surface (Wang et al., 2006). Maturation of the human cardiac potassium channel ether-a-gogo-related protein (hERG) in the ER is also facilitated by cytoplasmic Hsp90 and Hsp70, and GDA treatment suppresses hERG cell-surface targeting (Ficker et al., 2003). Furthermore, Hsp90 cochaperone FKBP38 is involved in the maturation and cell-surface targeting of hERG; Hop was also identified as an interactor of hERG in the study, but its role in hERG transport and function was not analyzed (Walker et al., 2007). All available studies highlight the importance of the cytoplasmic Hsp90 chaperone system in protein folding and maturation in the ER, and in the efficient cell-surface localization of physiologically important animal proteins.

We have shown here that Hop/Sti1 and Hsp90 are likewise involved in the maturation, trafficking, and PM targeting of the rice PAMP receptor OsCERK1 (Figures 5, 6, and 7C) and that they interact with the TM domain of OsCERK1. These results imply a function for the Hop/Sti1-Hsp90 chaperone complex in the ER exit of OsCERK1. Since the specific interacting regions of CFTR and hERG with Hsp90 and its cochaperones have not yet been identified, our results shed light on the questions of which regions of animal cell-surface proteins are likely to interact with the cytoplasmic Hsp90 chaperone complex, and of how these interactions contribute to protein maturation in the ER and cell-surface targeting. Furthermore, our observation that OsRac1 colocalizes with the OsCERK1 complex in the ER suggests that Rac or other small GTPases may likewise participate in the regulation and trafficking of CFTR, hERG, and other physiologically important mammalian cell-surface proteins. We also have demonstrated the involvement of Sar1 GTPase in the trafficking of OsCERK1 (Figures 4D and 7C); a similar observation regarding the trafficking of the hERG channel in mammalian cells was reported recently (Delisle et al., 2009), suggesting that trafficking systems for cell-surface proteins are highly conserved between animals and plants.

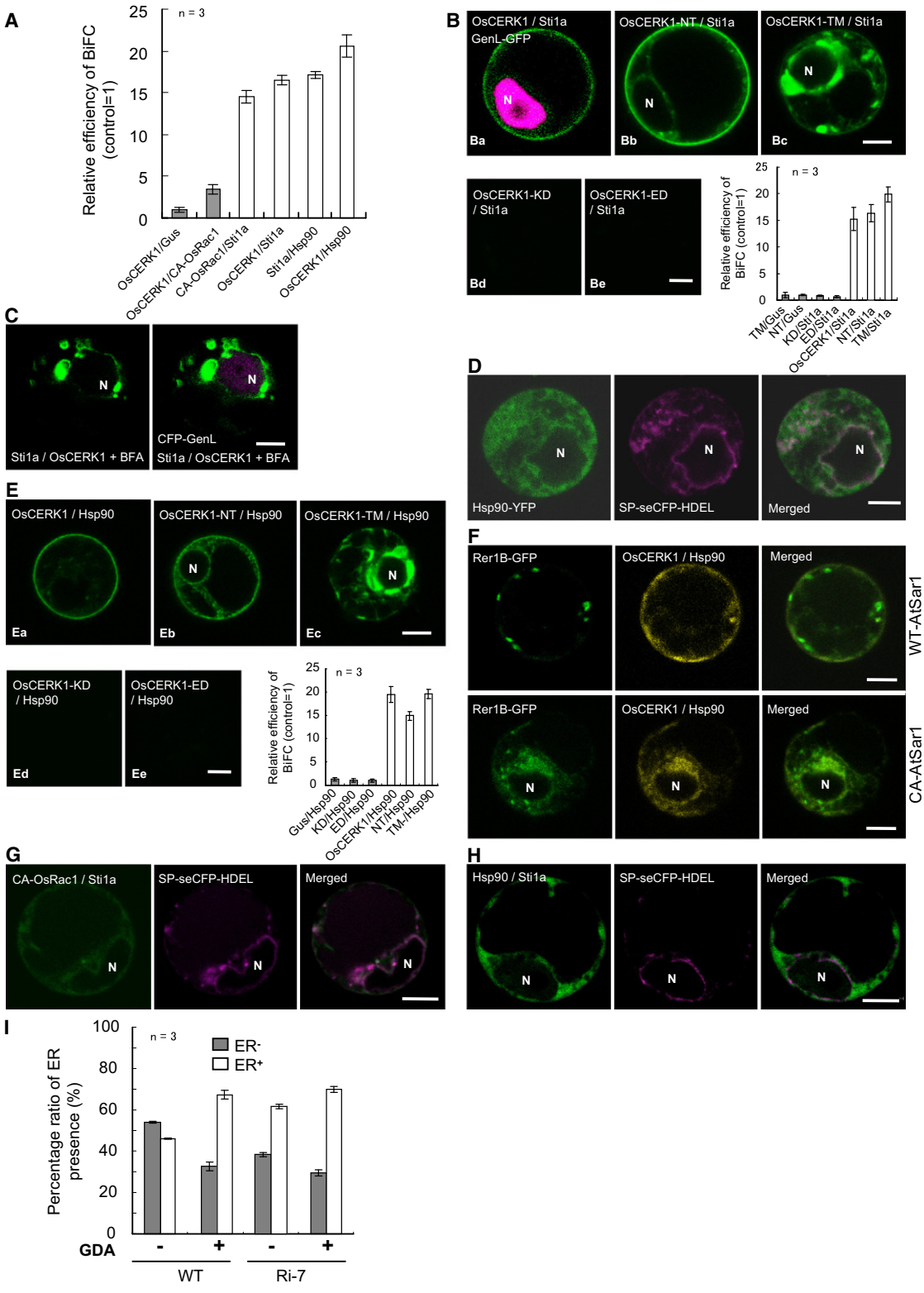

Figure 5. Maturation and Transport of the OsCERK1 Receptor Is Regulated by the Hop/Sti1a-Hsp90 Chaperone Complex

(A) Quantitative evaluation of the five BiFC pairs. Data presented are means ± SD. OsCERK1/Gus serves as a negative control.

(B) BiFC assays of Hop/Sti1a and OsCERK1. NT and MT deletion mutants of OsCERK1 are shown in Figure 3B. (Ba)–(Be) represent typical patterns of BiFC-reconstituted YFP fluorescence. GenL-GFP, nuclear marker. The graph (lower right) is a quantitative evaluation of each BiFC pair analyzed. TM/Gus and NT/Gus serve as negative controls. Bars in (B)–(H), 5 μm; N, nucleus.

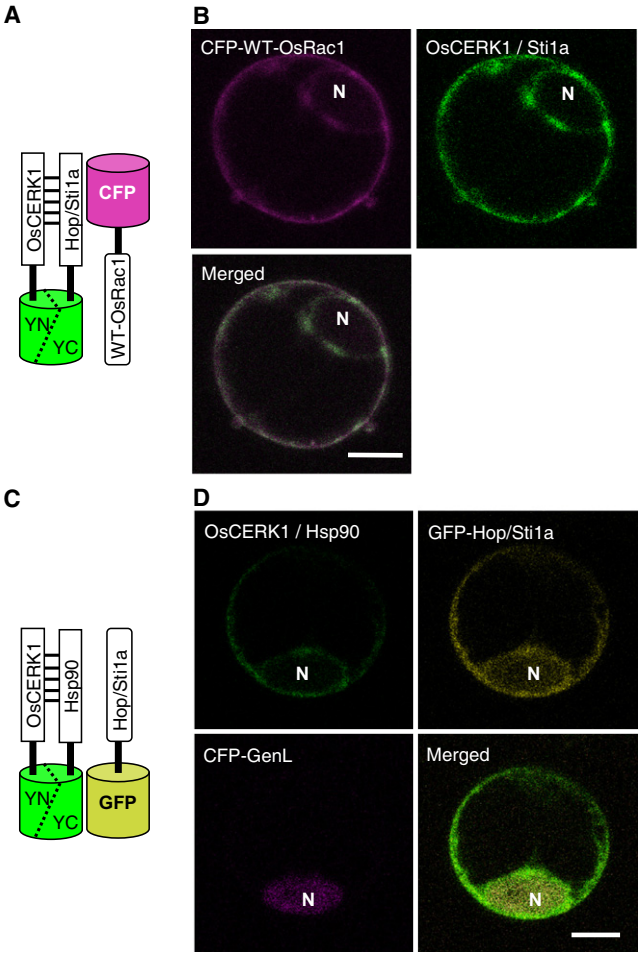

Figure 6. OsCERK1, Hop/Sti1a, Hsp90, and OsRac1 May Form a Complex in the ER

(A) OsCERK1-YN, Hop/Sti1a-YC, and CFP-WT-OsRac1 constructs used for BiFC-based colocalization assay.

(B) Colocalization of OsCERK1, Hop/Sti1a, and OsRac1 at the PM and the ER. Scale bars in (B) and (D), 5 μm; N, nucleus.

(C) OsCERK1-YN, Hsp90-YC, and Hop/Sti1a-GFP constructs used for BiFC-based colocalization assays.

(D) Colocalization of OsCERK1, Hsp90, and Hop/Sti1 at the PM and the ER. CFP-GenL, nuclear marker.

A Defensome Model for PAMP-Triggered Innate Immunity in Rice

Extending our previous studies on the interactions of a number of proteins during rice immune responses, we propose the existence of a protein complex, the defensome, which regulates rice innate immunity (Figures 7A and 7B). Two major components of the defensome are the PAMP receptor OsCERK1 and OsRac1, which is a molecular switch activating most of the downstream signaling components in PAMP-triggered immunity. These two key components are linked by Hop/Sti1. Thus, Hop/Sti1 has a critical role in connecting the RLK receptor with both the OsRac1 molecular switch and the Hsp90 chaperone complex, the latter including SGT1 and RAR1, which are required for innate immune responses. We propose that the components of the defensome have two functions: some, mainly the cochaperone proteins, contribute to receptor maturation/transport and the formation of the signaling complex, while others are recruited later to act as signaling components at the PM. It is also possible that OsCERK1 and OsRac1 interact independently with the Hop/Sti1-Hsp90 complex. *Hop/Sti1*-RNAi did not affect OsRac1 expression at the transcriptional level (Figure 2A and Figure S2B), but reduction of Hop/Sti1 protein might destabilize the defensome as exemplified by the decrease of OsRac1 (Figure S5B). This could be one factor which affected the defense response in the *Hop/Sti1*-RNAi line.

We have recently found that OsRac1 is activated within a few minutes of exposure to chitin, and also identified a guanine nucleotide exchange factor (GEF) that is specific for OsRac1 (A. Akamatsu, H.L.W., K. Nishide, K. Imai, and K.S., unpublished data). OsCERK1 may activate this GEF upon perception of chitin, thereby leading to OsRac1 activation at the PM; these events appear to occur in the defensome, whose components may thus undergo rapid modifications after pathogen infection. Future analysis of defensome dynamics is likely to illuminate the molecular mechanisms that underlie signaling in rice innate immunity.

EXPERIMENTAL PROCEDURES

BiFC Assays in Rice Protoplasts

Rice *Hop/Sti1a* and *Hop/Sti1b* cDNAs were provided by the Rice Genome Resource Center, Tsukuba, Japan. The BiFC system used for this study was described previously (Kakita et al., 2007). Gus, OsCERK1, OsCERK1-KD, OsCERK1-NT, OsCERK1-TM, OsCERK1-ED, OsRac1, and Hop/Sti1a were cloned into the pVN/gw vector for N-terminal fusion using LR reactions. Gus, Hsp90, Hop/Sti1a, and OsRac1 were cloned into the pVC/gw vector for C-terminal fusion in the same way. Protoplasts isolated from rice Oc suspension cell cultures were adjusted to 2.5 × 10^6 cells/ml. Plasmid DNAs (5 μg DNA of each construct) were mixed with 100 μl aliquots of suspended protoplasts in each transformation experiment. BFA (50 μg/ml) was added immediately to protoplasts. Confocal microscopy (LSM510-META, Carl Zeiss) was carried out after the protoplasts had been incubated for 24 hr at 30°C. For quantitative assays, cells with visible fluorescence were considered positive, and the number of positive cells was counted using a fluorescent microscope. The data were normalized with the mean of the negative control.

(C) Hop/Sti1a-OsCERK1 BiFC signals in the presence of BFA. CFP-GenL, nuclear marker.

(D) Subcellular localization of Hsp90-YFP in protoplasts. SP-seCFP-HDEL, ER marker.

(E) BiFC assays of Hsp90 and OsCERK1. NT and TM fragments of OsCERK1 are shown in Figure 3B. (Ea)–(Ee) represent typical patterns of BiFC-reconstituted YFP fluorescence. The graph (lower right) is a quantitative evaluation of each BiFC pair analyzed. Gus/Hsp90 serves as a negative control.

(F) Effect of CA-Sar1 on OsCERK1-Hsp90 complex localization in BiFC assays. WT-Sar1-Rer1B-GFP or CA-Sar1-Rer1B-GFP was cotransformed with OsCERK1-Hsp90 BiFC constructs into protoplasts.

(G) BiFC pattern of CA-Rac1 and Hop/Sti1a.

(H) BiFC pattern of Hsp90 and Hop/Sti1a.

(I) OsCERK1-GFP subcellular localization patterns in WT and *Hop/Sti1a*-RNAi line Ri-7 in the presence or absence of the Hsp90 inhibitor GDA. Gray and white bars indicate the percentages of cells without (–) and with (+) GFP signal in the ER, respectively. Data shown are means ± SD.

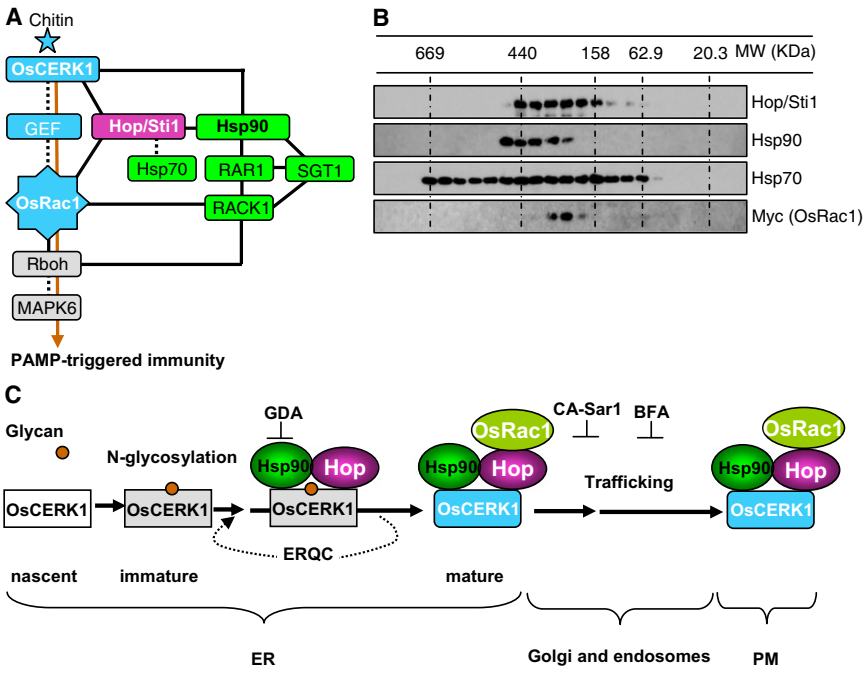

Figure 7. Protein Network in the OsRac1 Immune Complex and Model of Hop/Sti1-Hsp90 Chaperone Function in Maturation and Trafficking of OsCERK1

(A) Defensome protein network involved in PAMP-triggered innate immunity in rice. The defensome is present at the PM. A chitin signal (blue star) is received by OsCERK1 and transmitted to a putative GEF for OsRac1 activation. Activated OsRac1 induces activation of Rboh for ROS production, MAPK cascade triggering, and activation of other downstream components. Hsp90 and the cochaperones Hop/Sti1, RAR1, SGT1, and Hsp70 interact with OsRac1 and OsCERK1 through Hop/Sti1 and the scaffolding protein RACK1. Solid lines linking two proteins indicate established direct interactions, while dotted lines indicate possible interactions that remain to be demonstrated. The brown arrow indicates signal transduction.

(B) Gel filtration profiles of Hop/Sti1, Hsp90, Hsp70, and CA-OsRac1.

(C) A model for the maturation, trafficking, and PM localization of OsCERK1. Nascent OsCERK1 is glycosylated and folded in the ER. The Hop/Sti1-Hsp90 chaperone complex binds OsCERK1 in the ER and assists its ER exit. OsRac1 and other proteins may form a complex with mature OsCERK1 (blue), which then exits the ER and is transported to the PM via the Sar1-dependent vesicle trafficking system, while misfolded protein may be recycled by the ERQC system. At the PM, OsCERK1-Hop/Sti1-Hsp90 forms the defensome together with several (co-)chaperones and other signaling proteins for PAMP reception and signal transduction.

Chitin Treatment and Quantitative Real-Time PCR Analysis

Cultured rice cells were treated with 20 μg/ml chitin, hexa-N-acetylchitohexaose (Seikagaku, Japan), and harvested at the indicated times after chitin treatment. Total RNA was extracted using the RNeasy Plant Mini Kit (QIAGEN) and treated with DNase I (Invitrogen). cDNA was synthesized from 1 μg of total RNA using SuperScript II reverse transcriptase (Invitrogen). Quantitative real-time PCR was performed with samples of cDNA and standard plasmids using SYBR Green PCR master mix (Applied Biosystems) and the gene-specific primers listed in Table S1. Data were collected using the ABI PRISM 7000 sequence detection system according to the manufacturer's instructions.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures, one table, Supplemental Experimental Procedures, and Supplemental References and can be found with this article online at doi:10.1016/j.chom.2010.02.008.

ACKNOWLEDGMENTS

We thank Drs. Dave Piston for mCerulean, Seiji Takayama for the BiFC system, Yoichiro Fukao for the ER marker SP-seCFP-HDEL, and Ken Shirasu for the barley Hsp90 antibody. We are grateful to Ian Smith for critical reading of the manuscript. We also thank Masako Kanda, Yukiko Konomi, Junko Naritomi, and Yuko Tamaki for excellent technical assistance. This research was supported by Grants-in-Aid from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Rice Genome Project IP4001) and the Japan Society for the Promotion of Science (13G0023) to K.S.; and the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN) to T.K.

Received: September 24, 2009
Revised: January 14, 2010
Accepted: February 19, 2010
Published: March 17, 2010

REFERENCES

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

Brembu, T., Winge, P., Bones, A.M., and Yang, Z. (2006). A RHOse by any other name: a comparative analysis of animal and plant Rho GTPases. Cell Res. *16*, 435–445.

Caplan, J.L., Zhu, X., Mamillapalli, P., Marathe, R., Anandalakshmi, R., and Dinesh-Kumar, S.P. (2009). Induced ER chaperones regulate a receptor-like kinase to mediate antiviral innate immune response in plants. Cell Host Microbe *6*, 457–469.

Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J.D., Felix, G., and Boller, T. (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature *448*, 497–500.

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.

Collins, N.C., Thordal-Christensen, H., Lipka, V., Bau, S., Kombrink, E., Qiu, J.L., Huckelhoven, R., Stein, M., Freialdenhoven, A., Somerville, S.C., and Schulze-Lefert, P. (2003). SNARE-protein-mediated disease resistance at the plant cell wall. Nature *425*, 973–977.

Delisle, B.P., Underkofler, H.A., Moungey, B.M., Slind, J.K., Kilby, J.A., Best, J.M., Foell, J.D., Balijepalli, R.C., Kamp, T.J., and January, C.T. (2009). Small GTPase determinants for the Golgi processing and plasmalemmal expression of human ether-a-go-go related (hERG) K+ channels. J. Biol. Chem. *284*, 2844–2853.

Ficker, E., Dennis, A.T., Wang, L., and Brown, A.M. (2003). Role of the cytosolic chaperones Hsp70 and Hsp90 in maturation of the cardiac potassium channel HERG. Circ. Res. *92*, e87–e100.

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.

Geldner, N., and Robatzek, S. (2008). Plant receptors go endosomal: a moving view on signal transduction. Plant Physiol. *147*, 1565–1574.

Gomez-Gomez, L., and Boller, T. (2000). FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol. Cell *5*, 1003–1011.

Gurkan, C., Stagg, S.M., Lapointe, P., and Balch, W.E. (2006). The COPII cage: unifying principles of vesicle coat assembly. Nat. Rev. Mol. Cell Biol. *7*, 727–738.

He, K., Gou, X., Yuan, T., Lin, H., Asami, T., Yoshida, S., Russell, S.D., and Li, J. (2007). BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell-death pathways. Curr. Biol. *17*, 1109–1115.

Hong, Z., Jin, H., Tzfira, T., and Li, J. (2008). Multiple mechanism-mediated retention of a defective brassinosteroid receptor in the endoplasmic reticulum of Arabidopsis. Plant Cell *20*, 3418–3429.

Hu, C.D., Chinenov, Y., and Kerppola, T.K. (2002). Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. Mol. Cell *9*, 789–798.

Hubert, D.A., Tornero, P., Belkhadir, Y., Krishna, P., Takahashi, A., Shirasu, K., and Dangl, J.L. (2003). Cytosolic HSP90 associates with and modulates the Arabidopsis RPM1 disease resistance protein. EMBO J. *22*, 5679–5689.

Hubert, D.A., He, Y., McNulty, B.C., Tornero, P., and Dangl, J.L. (2009). Specific Arabidopsis HSP90.2 alleles recapitulate RAR1 cochaperone function in plant NB-LRR disease resistance protein regulation. Proc. Natl. Acad. Sci. USA *106*, 9556–9563.

Jin, H., Yan, Z., Nam, K.H., and Li, J. (2007). Allele-specific suppression of a defective brassinosteroid receptor reveals a physiological role of UGGT in ER quality control. Mol. Cell *26*, 821–830.

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

Jones, M.A., Raymond, M.J., Yang, Z., and Smirnoff, N. (2007). NADPH oxidase-dependent reactive oxygen species formation required for root hair growth depends on ROP GTPase. J. Exp. Bot. *58*, 1261–1270.

Kakita, M., Murase, K., Iwano, M., Matsumoto, T., Watanabe, M., Shiba, H., Isogai, A., and Takayama, S. (2007). Two distinct forms of M-locus protein kinase localize to the plasma membrane and interact directly with S-locus receptor kinase to transduce self-incompatibility signaling in Brassica rapa. Plant Cell *19*, 3961–3973.

Kaku, H., Nishizawa, Y., Ishii-Minami, N., Akimoto-Tomiyama, C., Dohmae, N., Takio, K., Minami, E., and Shibuya, N. (2006). Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proc. Natl. Acad. Sci. USA *103*, 11086–11091.

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.

Kemmerling, B., Schwedt, A., Rodriguez, P., Mazzotta, S., Frank, M., Qamar, S.A., Mengiste, T., Betsuyaku, S., Parker, J.E., Mussig, C., et al. (2007). The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. Curr. Biol. *17*, 1116–1122.

Kwon, C., Neu, C., Pajonk, S., Yun, H.S., Lipka, U., Humphry, M., Bau, S., Straus, M., Kwaaitaal, M., Rampelt, H., et al. (2008). Co-option of a default secretory pathway for plant immune responses. Nature *451*, 835–840.

Lee, P., Shabbir, A., Cardozo, C., and Caplan, A.J. (2004). Sti1 and Cdc37 can stabilize Hsp90 in chaperone complexes with a protein kinase. Mol. Biol. Cell *15*, 1785–1792.

Li, J., Zhao-Hui, C., Batoux, M., Nekrasov, V., Roux, M., Chinchilla, D., Zipfel, C., and Jones, J.D. (2009). Specific ER quality control components required for biogenesis of the plant innate immune receptor EFR. Proc. Natl. Acad. Sci. USA *106*, 15973–15978.

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.

Liu, Y., Burch-Smith, T., Schiff, M., Feng, S., and Dinesh-Kumar, S.P. (2004). Molecular chaperone Hsp90 associates with resistance protein N and its signaling proteins SGT1 and Rar1 to modulate an innate immune response in plants. J. Biol. Chem. *279*, 2101–2108.

Lu, R., Malcuit, I., Moffett, P., Ruiz, M.T., Peart, J., Wu, A.J., Rathjen, J.P., Bendahmane, A., Day, L., and Baulcombe, D.C. (2003). High throughput virus-induced gene silencing implicates heat shock protein 90 in plant disease resistance. EMBO J. *22*, 5690–5699.

Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H., and Shibuya, N. (2007). CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proc. Natl. Acad. Sci. USA *104*, 19613–19618.

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.

Moritoh, S., Miki, D., Akiyama, M., Kawahara, M., Izawa, T., Maki, H., and Shimamoto, K. (2005). RNAi-mediated silencing of OsGEN-L (OsGEN-like), a new member of the RAD2/XPG nuclease family, causes male sterility by defect of microspore development in rice. Plant Cell Physiol. *46*, 699–715.

Nakashima, A., Chen, L., Thao, N.P., Fujiwara, M., Wong, H.L., Kuwano, M., Umemura, K., Shirasu, K., Kawasaki, T., and Shimamoto, K. (2008). RACK1 functions in rice innate immunity by interacting with the Rac1 immune complex. Plant Cell *20*, 2265–2279.

Nekrasov, V., Li, J., Batoux, M., Roux, M., Chu, Z.H., Lacombe, S., Rougon, A., Bittel, P., Kiss-Papp, M., Chinchilla, D., et al. (2009). Control of the pattern-recognition receptor EFR by an ER protein complex in plant immunity. EMBO J. *28*, 3428–3438.

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.

Nicolet, C.M., and Craig, E.A. (1989). Isolation and characterization of STI1, a stress-inducible gene from Saccharomyces cerevisiae. Mol. Cell. Biol. *9*, 3638–3646.

Nomura, K., Debroy, S., Lee, Y.H., Pumplin, N., Jones, J., and He, S.Y. (2006). A bacterial virulence protein suppresses host innate immunity to cause plant disease. Science *313*, 220–223.

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.

Park, J., Gu, Y., Lee, Y., Yang, Z., and Lee, Y. (2004). Phosphatidic acid induces leaf cell death in Arabidopsis by activating the Rho-related small G protein GTPase-mediated pathway of reactive oxygen species generation. Plant Physiol. *134*, 129–136.

Pathuri, I.P., Zellerhoff, N., Schaffrath, U., Hensel, G., Kumlehn, J., Kogel, K.H., Eichmann, R., and Huckelhoven, R. (2008). Constitutively activated barley ROPs modulate epidermal cell size, defense reactions and interactions with fungal leaf pathogens. Plant Cell Rep. *27*, 1877–1887.

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. (Maywood) *228*, 111–133.

Richter, K., and Buchner, J. (2001). Hsp90: chaperoning signal transduction. J. Cell. Physiol. *188*, 281–290.

Russinova, E., Borst, J.W., Kwaaitaal, M., Cano-Delgado, A., Yin, Y., Chory, J., and de Vries, S.C. (2004). Heterodimerization and endocytosis of Arabidopsis brassinosteroid receptors BRI1 and AtSERK3 (BAK1). Plant Cell *16*, 3216–3229.

Saijo, Y., Tintor, N., Lu, X., Rauf, P., Pajerowska-Mukhtar, K., Haweker, H., Dong, X., Robatzek, S., and Schulze-Lefert, P. (2009). Receptor quality control in the endoplasmic reticulum for plant innate immunity. EMBO J. *28*, 3439–3449.

Satiat-Jeunemaitre, B., Cole, L., Bourett, T., Howard, R., and Hawes, C. (1996). Brefeldin A effects in plant and fungal cells: something new about vesicle trafficking? J. Microsc. *181*, 162–177.

Schiene, K., Puhler, A., and Niehaus, K. (2000). Transgenic tobacco plants that express an antisense construct derived from a Medicago sativa cDNA encoding a Rac-related small GTP-binding protein fail to develop necrotic lesions upon elicitor infiltration. Mol. Gen. Genet. *263*, 761–770.

Shirasu, K. (2009). The HSP90-SGT1 chaperone complex for NLR immune sensors. Annu. Rev. Plant Biol. *60*, 139–164.

Shiu, S.H., Karlowski, W.M., Pan, R., Tzeng, Y.H., Mayer, K.F., and Li, W.H. (2004). Comparative analysis of the receptor-like kinase family in Arabidopsis and rice. Plant Cell *16*, 1220–1234.

Speth, E.B., Imboden, L., Hauck, P., and He, S.Y. (2009). Subcellular localization and functional analysis of the Arabidopsis GTPase RabE. Plant Physiol. *149*, 1824–1837.

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.

Takai, R., Isogai, A., Takayama, S., and Che, F.S. (2008). Analysis of flagellin perception mediated by flg22 receptor OsFLS2 in rice. Mol. Plant Microbe Interact. *21*, 1635–1642.

Takeuchi, M., Ueda, T., Sato, K., Abe, H., Nagata, T., and Nakano, A. (2000). A dominant negative mutant of Sar1 GTPase inhibits protein transport from the endoplasmic reticulum to the Golgi apparatus in tobacco and Arabidopsis cultured cells. Plant J. *23*, 517–525.

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.

Torres, H.J., Chatellard, P., and Stutz, E. (1995). Isolation and characterization of gmsti, a stress-inducible gene from soybean (Glycine max) coding for a protein belonging to the TPR (tetratricopeptide repeats) family. Plant Mol. Biol. *27*, 1221–1226.

Walker, V.E., Atanasiu, R., Lam, H., and Shrier, A. (2007). Co-chaperone FKBP38 promotes HERG trafficking. J. Biol. Chem. *282*, 23509–23516.

Walter, M., Chaban, C., Schutze, K., Batistic, O., Weckermann, K., Nake, C., Blazevic, D., Grefen, C., Schumacher, K., Oecking, C., et al. (2004). Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. Plant J. *40*, 428–438.

Wan, J., Zhang, X.C., Neece, D., Ramonell, K.M., Clough, S., Kim, S.Y., Stacey, M.G., and Stacey, G. (2008). A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis. Plant Cell *20*, 471–481.

Wang, D., Weaver, N.D., Kesarwani, M., and Dong, X. (2005). Induction of protein secretory pathway is required for systemic acquired resistance. Science *308*, 1036–1040.

Wang, X., Venable, J., LaPointe, P., Hutt, D.M., Koulov, A.V., Coppinger, J., Gurkan, C., Kellner, W., Matteson, J., Plutner, H., et al. (2006). Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. Cell *127*, 803–815.

Wegele, H., Muller, L., and Buchner, J. (2004). Hsp70 and Hsp90—a relay team for protein folding. Rev. Physiol. Biochem. Pharmacol. *151*, 1–44.

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.

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.

Zhang, Z., Quick, M.K., Kanelakis, K.C., Gijzen, M., and Krishna, P. (2003). Characterization of a plant homolog of hop, a cochaperone of hsp90. Plant Physiol. *131*, 525–535.

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

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J.D., Boller, T., and Felix, G. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell *125*, 749–760.