Title

A rice kinase-protein interaction map.

Journal

Plant physiology, 149(3)

ISSN

0032-0889

Authors

Ding, Xiaodong
Richter, Todd
Chen, Mei
et al.

Publication Date

2009-03-24

Peer reviewed

A Rice Kinase-Protein Interaction Map[1][W][OA]

Xiaodong Ding, Todd Richter, Mei Chen, Hiroaki Fujii, Young Su Seo, Mingtang Xie, Xianwu Zheng, Siddhartha Kanrar, Rebecca A. Stevenson, Christopher Dardick, Ying Li, Hao Jiang, Yan Zhang[2], Fahong Yu, Laura E. Bartley, Mawsheng Chern, Rebecca Bart, Xiuhua Chen, Lihuang Zhu, William G. Farmerie, Michael Gribskov, Jian-Kang Zhu, Michael E. Fromm, Pamela C. Ronald, and Wen-Yuan Song*

Department of Plant Pathology (X.D., Y.Z., X.C., W.-Y.S.) and Interdisciplinary Center for Biotechnology Research (F.Y., W.G.F.), University of Florida, Gainesville, Florida 32611; Department of Plant Pathology, University of California, Davis, California 95616 (T.R., Y.S.S., L.E.B., M.C., R.B., P.C.R.); Plant Science Initiative, University of Nebraska, Lincoln, Nebraska 68588 (M.C., M.E.F.); Botany and Plant Sciences, University of California, Riverside, California 92521 (H.F., M.X., X.Z., S.K., R.A.S., J.-K.Z.); United States Department of Agriculture-Agricultural Research Service, Appalachian Fruit Research Station, Kearneysville, West Virginia 25430 (C.D.); Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907 (Y.L., H.J., M.G.); and Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China (L.Z.)

Plants uniquely contain large numbers of protein kinases, and for the vast majority of the 1,429 kinases predicted in the rice (*Oryza sativa*) genome, little is known of their functions. Genetic approaches often fail to produce observable phenotypes; thus, new strategies are needed to delineate kinase function. We previously developed a cost-effective high-throughput yeast two-hybrid system. Using this system, we have generated a protein interaction map of 116 representative rice kinases and 254 of their interacting proteins. Overall, the resulting interaction map supports a large number of known or predicted kinase-protein interactions from both plants and animals and reveals many new functional insights. Notably, we found a potential widespread role for E3 ubiquitin ligases in pathogen defense signaling mediated by receptor-like kinases, particularly by the kinases that may have evolved from recently expanded kinase subfamilies in rice. We anticipate that the data provided here will serve as a foundation for targeted functional studies in rice and other plants. The application of yeast two-hybrid and TAPtag analyses for large-scale plant protein interaction studies is also discussed.

Protein kinases play a key role in regulating nearly all aspects of cellular processes, including growth, differentiation, and defense. After sequencing the genomes of Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*), 1,027 and 1,429 protein kinases, respectively, have been identified, representing more than 3% of the annotated proteins encoded by each genome (http://plantsp.genomics.purdue.edu/; http://rkd.ucdavis.edu/). The number of protein kinases identified in rice alone is almost three times the number of those found in the human (*Homo sapiens*) genome (Manning et al., 2002) and is similar to the combined total of the 1,800 protein kinases found in human, mouse (*Mus musculus*), fruit fly (*Drosophila melanogaster*), nematode (*Caenorhabditis elegans*), and yeast (*Saccharomyces cerevisiae*) genomes (http://kinase.com/). Therefore, rice offers a rich source of material for studying kinase-regulated cell signaling as well as for the molecular mechanisms of kinome expansion (Shiu et al., 2004). Although plant kinomes are large, only a limited number of kinases have been functionally characterized. Examples include a number of receptor-like kinases (RLKs) and receptor-like cytoplasmic kinases (RLCKs), which control pathogen recognition and plant development (Morris and Walker, 2003; Morillo and Tax, 2006; Chinchilla et al., 2007a; Afzal et al., 2008). Other examples are several SNF1-related kinases (SnRKs) involved in the regulation of metabolism, development, and stress responses (Gong et al., 2004; Lu et al., 2007; Polge and Thomas, 2007) and a few mitogen-activated protein kinases (MAPKs) and their regulatory kinases (Jonak et al., 2002; Hamel et al., 2006; Colcombet and Hirt, 2008).

It has been well documented that many kinases function through their interactions with other proteins. For instance, the Arabidopsis receptor kinases BRI1 and BAK1 bind to each other, and both are involved in brassinosteroid signal transduction (Li

[1] This work was supported by the National Science Foundation (Plant Genome grant no. DBI–0217312).

[2] Present address: Plant Biotech Institute, NRC, 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada S7N 0W9.

* Corresponding author; e-mail wsong@ifas.ufl.edu.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Wen-Yuan Song (wsong@ifas.ufl.edu).

[W] The online version of this article contains Web-only data.

[OA] Open Access articles can be viewed online without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.108.128298

et al., 2002; Nam and Li, 2002). In addition to BRI1, BAK1 forms a complex with the receptor kinase FLS2 in response to the stimulation of bacterial flagellin (Chinchilla et al., 2007b; Heese et al., 2007). In the resistance to the bacterial pathogen *Xanthomonas oryzae* pv *oryzae*, XA21-mediated resistance is regulated by a number of the XA21-binding proteins (Wang et al., 2006; Park et al., 2008; Peng et al., 2008). During salt stress, the SnRK protein SOS2 interacts with SOS3, a novel EF-hand Ca^{2+} sensor with a sequence similar to the regulatory calcineurin B subunit from yeast (Halfter et al., 2000; Gong et al., 2004). In *Brassica*, the ARC1 protein interacts with the *S* receptor kinase to control self-incompatibility (Gu et al., 1998). Aside from these studies, the precise function and the protein binding partners for the vast majority of plant kinases are unknown.

A major focus of functional genomics is mapping cellular protein-protein interaction networks on a large scale. The information generated from these studies not only establishes links between well-characterized proteins but also reveals potential functions of uncharacterized proteins when their interacting partners have known functions: the ''guilt by association'' principle (Hazbun and Fields, 2001). To generate such databases of protein interactions, a number of high-throughput approaches have been developed (Phizicky et al., 2003; Zhu et al., 2003). Among them, yeast two-hybrid analysis is a robust method for detecting pair-wise protein-protein interactions in a cellular setting (Nodzon and Song, 2004; Parrish et al., 2006). It has been estimated that over half of the protein interactions reported in the literature were originally identified by yeast two-hybrid analyses (Xenarios et al., 2002). Notably, all of the protein-kinase interactions mentioned above can be detected by yeast two-hybrid analysis. The viability of this approach relies on low cost, simple manipulation, and sensitive detection. To date, yeast two-hybrid analysis has been used to systematically analyze protein-protein interactions in several model organisms, including yeast, bacteria, *Drosophila*, *C. elegans*, *Plasmodium falciparum*, and human (Uetz et al., 2000; Ito et al., 2001; Rain et al., 2001; Giot et al., 2003; Li et al., 2004; LaCount et al., 2005; Rual et al., 2005; Lim et al., 2006; Parrish et al., 2007; Sato et al., 2008; Shimoda et al., 2008). The resulting databases of protein-protein interactions continue to be a valuable resource for understanding signaling networks in various organisms.

Unlike gene expression studies, protein-protein interaction data sets are notoriously difficult to verify, yeast two-hybrid interaction being no exception. This has been attributed in part to the complexity and sometimes transient nature of protein-protein interactions themselves and not necessarily to inherent error in the methods used. Yeast two-hybrid screening is particularly notorious for producing high rates of false positives based on both the inability to reproduce identified interactions upon yeast retransformation and to confirm those using alternative methods such as coimmunoprecipitation. While the former can be controlled through additional screening steps, the latter poses a practical limitation that hinders the calculation of actual false-positive rates. Therefore, it is critical to eliminate as many false positives as possible by testing the reproducibility of all candidate interactions in retransformed yeast cells (Vidalain et al., 2004). However, many large-scale yeast two-hybrid analyses do not include these verification steps because the yeast plasmid isolation, propagation in *Escherichia coli*, and retransformation into yeast are costly and time-consuming when performed on a large scale. We previously developed a cost-effective yeast two-hybrid recovery/retransformation system using rolling-circle amplification (RCA; Ding et al., 2003, 2004, 2007). This system allows one to inexpensively and efficiently test reproducibility and specificity of the interactions on a large scale in yeast. We have conducted two-hybrid analyses with 204 representative rice kinases using this system. Among them, 22 kinases autoactivated the *His3* reporter in the absence of a prey plasmid and 66 kinases yielded no interactors. Here, we report a yeast two-hybrid-based protein interaction network for 116 rice kinases consisting of 378 kinase-protein interactions. These interactions support a number of hypotheses for the functions of these kinases and their interacting partners.

RESULTS AND DISCUSSION

Selection of Kinase Targets and Bait Design

The rice kinome is composed of kinases from six of the seven known protein kinase groups: AGC (PKA, PKG, and PKC kinases), CAMK (calcium/calmodulin-dependent protein kinase), CK1 (casein kinase 1), CMGC (containing the CDK, MAPK, GSK3, and CLK families), STE (homologs of yeast sterile 7, sterile 11, and sterile 20 kinases), TKL (tyrosine kinase-like, which includes MLKs [mixed lineage kinases], transforming growth factor-β receptor kinases, and Raf kinases), and other kinases (Manning et al., 2002; Shiu et al., 2004; Dardick and Ronald, 2006). Plants lack known members of the tyrosine kinase group. Approximately 75% (1,068) of rice kinases, within the TKL group, are members of the RLK/Pelle family, which is related to the *Drosophila* Pelle and the human interleukin-1 receptor-associated kinase (IRAK) families (Shiu et al., 2004). The rice RLK/Pelle family (also called the IRAK family) includes both RLKs and RLCKs, which have been further subdivided into nearly 70 subfamilies based on conserved domains and phylogenetic relationships (Shiu et al., 2004; Dardick and Ronald, 2006). A total of 204 representative kinases (15% of the kinome) were selected from all six kinase groups based, in part, on their proportion to the entire kinome and on their expression in leaves (Table I). Among these 204 kinases, 130 belong to the RLK/Pelle family (Table I).

These kinase-encoding open reading frames were amplified by reverse transcription-PCR and cloned

Table I. *Summary of the rice kinases chosen and the interactions identified by yeast two-hybrid screen*

Group	Family	No. of Kinases Studied	No. of Kinases Autoactivating His3	No. of Kinases Screened	No. of Kinases without Interactors	No. of Kinases with Interactors	No. of Interactions Identified
AGC	AGC_other	2	1	1	0	1	1
	PVPK	3	0	3	1	2	2
CAMK	CAMK_like	6	0	6	5	1	4
	KIN1/SNF1/Nim1	19	1	18	1	17	83
CK1	CK1	2	0	2	0	2	17
CMGC	CDK	4	1	3	0	3	5
	CLK	1	0	1	0	1	12
	GSK	6	0	6	0	6	26
	MAPK/ERK	10	2	8	3	5	25
	CMGC_other	3	1	2	1	1	1
Other	CK2	1	0	1	0	1	6
	NimA	1	0	1	0	1	2
	IRE	1	1	0	0	0	0
STE	Ste7	2	0	2	1	1	6
	Ste11	1	0	1	1	0	0
	Ste20	3	1	2	0	2	2
TKL	RLK/Pelle	130	11	119	52	67	170
	Raf	9	3	6	1	5	16
Total		204	22	182	66	116	378

into the Gateway entry vector pDONR207 as described previously (Rohila et al., 2006). For RLKs, only the intracellular kinase domains (including the juxtamembrane domain) were cloned. Through recombination, the cDNAs were individually fused in frame to the GAL4 DNA-binding domains in the bait vectors pXDGATcy86 and pXDGATU86 (see Ding et al., 2007, for vector details).

RCA-Based Yeast Two-Hybrid Screening

We conducted a large-scale yeast two-hybrid analysis using 204 representative rice kinases. Among the 204 rice kinases, 22 autoactivated the *His3* reporter in the absence of a prey plasmid (Table I; Supplemental Table S1). Half of the 22 kinases (11) are members of the RLK/Pelle family. These 22 bait kinases were removed from further yeast two-hybrid analysis, and the remaining 182 were subjected to screening rice cDNA libraries to determine their interacting proteins (see ''Materials and Methods''). To verify interactors identified from the initial library screening, we amplified the bait (on the pXDGATcy86 vector) and prey plasmids from the yeast cells capable of growing on selective medium using RCA. The amplified DNA was retransformed into fresh yeast cells containing either the empty pXDGATU86 vector or the same bait kinase carried by pXDGATU86. Both 2-amino-5-fluorobenzoic acid and the cycloheximide counterselections were used to eliminate the original pXDGATcy86-derived bait constructs (Ding et al., 2007). Only the prey that specifically bound to a bait kinase, not the empty GAL4 activation domain, was scored as a positive interactor. Using these criteria, almost two-thirds of the initial interactions were removed. Most of the discarded clones (approximately 80%) contain a rice cDNA that is not fused in frame to the activation domain of the GAL4 transcription factor in the prey vector. Therefore, they were unlikely to be genuine interactors. About 15% of these clones were putative DNA-binding proteins or transcription factors. The remaining 5% of the clones did not fall into either category. Among the 182 kinases used for library screenings, 116 yielded at least one interactor (Table I; Supplemental Table S2). The other 66 kinases that generated no interactors are listed in Supplemental Table S3.

A Rice Kinase-Protein Interaction Map

A total of 378 interactions composed of 254 distinct kinase interactors were identified (Supplemental Table S2). A kinase-protein interaction map was generated using the software Cytoscape 2.3.2 (http://www.cytoscape.org/; Supplemental Fig. S1A). The number of interactors for each bait kinase varies considerably, ranging from those having just a single interactor to those having up to 26 for a single bait kinase. For example, we identified 24 and 26 interactors for the SnRKs Os03g17980 and Os05g45420, respectively. Through the putative transcription factor OsEBP-98 (Os03g08460) and a putative DNA-binding protein (Os02g57200), these two SnRKs were linked to a network composed of 68 bait kinases and their 166 interacting proteins. The other 48 bait kinases and their 89 partners form discrete interaction networks. Many interactions in this map are supported by previous studies in rice and in other heterologous systems (see below for more discussion). Additional interactions include functionally uncharacterized kinases and/or unknown interacting proteins, which provides a resource to explore potentially novel associations in kinase-mediated signaling in the future. The sequences of the identified interacting partners also provide information on the

domains that are responsible for binding to the kinases (Supplemental Table S2). Among the 254 interactors, 15% are putative DNA binding/transcription factors, 11% are protein kinases, and 9% are various metabolic enzymes (Supplemental Fig. S1B), suggesting broad roles for rice kinases in the regulation of gene expression, kinase cascades, and metabolic pathways. Three percent of the identified interactors were categorized as E3 ubiquitin ligases (Supplemental Fig. S1B), some of which interacted with multiple members of the RLK/Pelle family (see below for more discussion).

In an attempt to assess the quality of our map, we examined six of the kinase-protein interactions in rice cells using a bimolecular fluorescence complementation (BiFC) system. This system is based on the formation of a fluorescent complex through the interaction of two proteins that are fused to nonfluorescent fragments of the yellow fluorescent protein (YFP; Hu and Kerppola, 2003). The BiFC system has been used to monitor protein-protein interactions in living plant cells in real time with high specificity and low background (Bracha-Drori et al., 2004; Bhat et al., 2006). Table II summarizes the BiFC results for the six kinase-interactor pairs that we selected for testing. Each protein was fused downstream (N-terminal fusions) to one of the two halves of YFP (YFPN-term and YFPC-term) and transiently expressed in protoplasts derived from etiolated rice seedlings (Bart et al., 2006). Complex formation between the two interacting proteins is indicated by reconstitution of YFP. As shown in Supplemental Figure S2, we detected interactions between four of the six pairs. Different pairs required different amounts of time to mature and give a clear YFP signal. For example, pair 1 showed a very strong signal in the cytoplasm and around the nucleus by 24 h after transformation, whereas pair 4 showed a weak interaction only at 24 h after transformation. For negative controls, we tested each fusion protein in the presence of the other half of YFP alone. That is, each YFPN-term interactor fusion construct was tested with YFPC-term (no fusion) and each YFPC-term interactor fusion construct was tested with YFPN-term (no fusion). Only one of the two negative controls is shown in Supplemental Figure S2, but for the pairs shown, no interactions between the unfused YFP segments were detected with either interactor. For pair 5 (Table II), we were unable to test the interaction, since the YFPC-term-bait fusion gives a YFP signal with the unfused YFPN-term construct, albeit a less intense one than when both interactors are present (data not shown). For pair 6 (Table II), we were unable to detect an interaction between this bait and prey with the N-terminal split-YFP fusions (data not shown). We tested both orientations without detecting an interaction (i.e. YFPN-term- and YFPC-term-bait with YFPC-term- and YFPN-term-prey). While these results do not necessarily provide an accurate estimate of the error in our interaction maps, they do provide evidence that a significant number of kinase-protein interactions identified by our yeast two-hybrid system are likely to also occur in rice cells.

RLK/Pelle Family Member-E3 Ubiquitin Ligase Interactions in Rice

The RLK/Pelle family represents the largest group of kinases in rice and Arabidopsis. The number of predicted rice RLK/Pelle family members, however, is nearly twice as many as that of Arabidopsis. The additional rice members are thought to result from recent lineage-specific expansions of resistance/defense-related genes (Shiu et al., 2004; Dardick and Ronald, 2006). Part of the support for this hypothesis comes from the fact that three cloned rice disease resistance genes, *Xa21*, *Xa3/Xa26*, and *Pi-d2*, encode RLKs (Song et al., 1995; Sun et al., 2004; Chen et al., 2006; Xiang et al., 2006). It has been recognized that these three resistance proteins fall into the non-RD class of kinases, which lack the conserved arginine in kinase subdomain VI. An association between the non-RD motif and a role in pathogen recognition has been demonstrated, although some RD RLKs are also involved in resistance/defense response (Scheer and Ryan, 2002; Godiard et al., 2003; Diener and Ausubel, 2005; Hu et al., 2005; Llorente et al., 2005; Dardick and Ronald, 2006). To study RLK/Pelle-mediated signaling in rice, we selected 130 kinases representing 47 RLK/Pelle subfamilies and conducted two-hybrid screenings with 119 kinases (the remaining 11 can autoactivate the reporter; Table I). A total of 170 interactions were detected (Table I).

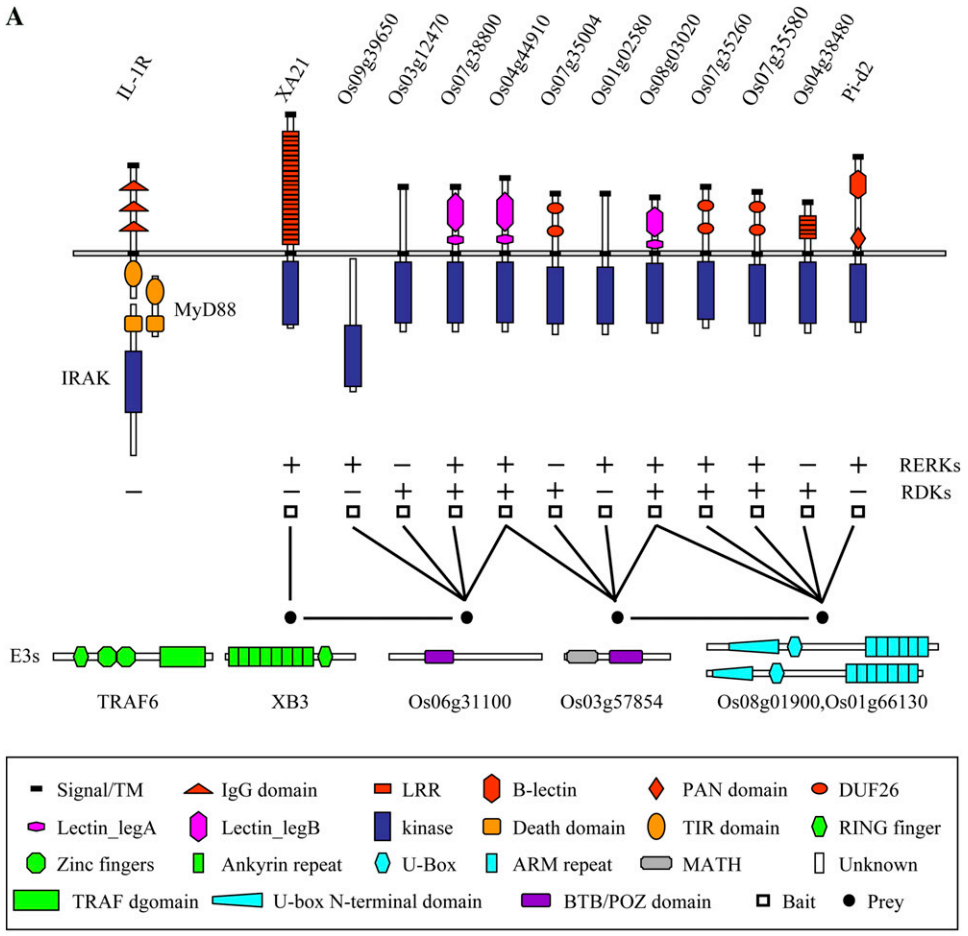
One pattern that emerged from the identified interactions is that four phylogenetically distant RLKs

Table II. *BiFC results for six kinase-interactor pairs identified by yeast two-hybrid screen*

*, Clone length in amino acids if the complete coding sequence was not tested. **, Negative control failed. Bait in p735 interacts with pSY736 to reconstitute YFP.

Pair No.	Bait ID (Function)	Prey ID (Function)	Split-YFP Results
1	Os01g32660 (OsMKK6)	Os10g38950 (OsMPK4)	Very strong
2	Os01g64970 (SAPK4)	Os01g64000 (OREB1)	Strong
3	Os05g32600 (R2)	Os06g22820 (CDK-activating kinase 1At-like)	Strong
4	Os05g45420 (SnRK1)	Os05g41220 (160–290*) (SNF1/AMPK/SnRK1 β-subunit)	Weak
5	Os03g02680 (Cdc2Os1)	Os03g05300 (CDK regulatory subunit)	**Bait alone forms YFP
6	Os01g62080 (LAMMER kinase)	Os08g41710 (61–160*) (FHA domain-containing protein)	No interaction detected

(Os04g38480, Os07g35580, Os07g35260, and Os08g03020) bind to two closely related U-box/ARM proteins (Os08g01900 and Os01g66130; Fig. 1A). These RLKs contain extracellular Leu-rich repeat (Os04g38480), Duf26 (Os07g35580 and Os07g35260), and lectin (Os08g03020) domains, respectively. Most of their intracellular kinase domains share less than 46% amino acid identity, with the exception of 66% and 69% identities between those of Os07g35580/Os07g35260 and Os04g44910/Os07g38800, respectively (Supplemental Table S4). The U-box/ARM proteins interacting with these RLKs belong to the Plant U-Box (PUB) family of putative E3 ubiquitin ligases (Mudgil et al., 2004). The overall identity between these two proteins is 50% (66% similarity), but their ARM repeats, a well-known protein-protein interaction domain (Andrade et al., 2001; Samuel et al., 2006), are much more conserved, with 74% identity (83% similarity). A truncated version of Os08g01900 containing mainly the ARM domain was recovered from the library screening using the kinase domain of Os08g03020 as bait (Supplemental Table S2). This is consistent with the previous observation that the ARM domain of the *Brassica* ARC1 protein is sufficient for binding to the *S* receptor kinase that controls self-incompatibility (Gu et al., 1998). Thus, the same kinase-binding specificity of the PUB-ARM proteins can be attributed to their conserved ARM domains. The PUB motif of these two proteins is also highly conserved (78% identity, 86% similarity). Such conservation does not seem to be necessary for supporting E3 activity only, because the PUB motif in many other functional E3 ubiquitin ligases appears to be divergent, with the exception of a few conserved Cys and His residues. It would be interesting to determine if the PUB motifs of



B

JM Domain

XA21	$_{677}$HKRTKKGA**P**SRTSMK**G**HPLVSYSQLVKATDG$_{707}$
Pi-d2	$_{460}$KRKRH**P**PPSQDDA**G**SSEDD**G**FLQTIS**G**APVRFTYRELQDATSN$_{502}$
Os04g38480(OsSERK1)	$_{267}$RRRK**P**EEHFFDV**P**AEEDPEVHLGQLKRFSLRELQVATDN$_{305}$
Os07g35580	$_{322}$RRRR**P**EKTPPPG**P**LRSASRSEDFESIESLFLDLSTLRIATDN$_{363}$
Os07g35260	$_{343}$KRKTERARK**P**SIADPTD**P**ADIESIDSLILSISTLRVATNN$_{382}$
Os08g03020	$_{311}$RRRQRKKMREEEEDDSEGD**P**IVEIEM**G**TGPRRF**P**YHILVNATKS$_{354}$

Figure 1. Interactions between RLK/Pelle kinases and putative E3 ubiquitin ligase complexes in rice. A, Connectivity of 12 rice RLK/Pelle kinases with their binding E3 ubiquitin ligases. Interacting proteins (black circles) are connected with solid lines to their bait kinases (white squares). Domains of the proteins are indicated. For comparison, part of the conserved interleukin-1-mediated pathway in animal innate immunity is adapted from Wu and Arron (2003). The interaction between XA21 and XB3 is from Wang et al. (2006). Recently expended rice kinases (RERKs) and RD kinases (RDKs) are indicated by plus signs, whereas non-RERKs and non-RDKs are labeled by minus signs. IL-1R, Interleukin-1 receptor. B, Juxtamembrane (JM) sequences of the six rice RLKs containing a putative proteolytic cleavage motif (P/GX$_{5-7}$P/G) in A. The motifs are underlined, and the conserved Pro (P) and Gly (G) residues are in boldface red. X denotes any amino acid. Numbers indicate amino acid positions in the full-length proteins.

Os08g01900 and Os01g66130 have a role in specific protein-protein interactions.

It has been reported that Arabidopsis PUB-ARM proteins interact with Arabidopsis and *Brassica* S-domain receptor kinases, and some of these interacting modules have been implicated in hormone response (Samuel et al., 2008). The function of the rice PUB-ARM proteins Os08g01900 and Os01g66130 is still unclear; however, they also interacted with the kinase domain of Pi-d2 (Fig. 1A). Pi-d2 confers resistance to rice blast disease caused by the fungal pathogen *Magnaporthe grisea* (Chen et al., 2006). Parallel to these interactions, we previously found that the rice ankyrin-RING protein XB3 interacts in yeast and in planta with XA21, which confers resistance to the bacterial pathogen *X. oryzae* pv *oryzae* (Wang et al., 2006). The ankyrin domain of XB3 alone is sufficient for binding to the kinase domain of XA21, whereas the RING finger motif in XB3 can mediate autoubiquitination (Wang et al., 2006). Therefore, we propose that the PUB-ARM proteins Os08g01900 and Os01g66130 may function equivalently to XB3 in the Pi-d2-mediated immune response. Interestingly, all six E3 binding RLKs (XA21, Pid2, Os04g38480, Os07g35580, Os07g35260, and Os08g03020) contain a putative proteolytic cleavage motif ($P/GX_{5-7}P/G$) in their intracellular juxtamembrane domain (Fig. 1B). This motif has been suggested as a cause of XA21 protein instability (Xu et al., 2006). Because instability appears to be a common feature of a number of plant disease resistance proteins (Mackey et al., 2002; Tornero et al., 2002; Hubert et al., 2003; Bieri et al., 2004; Holt et al., 2005; Xu et al., 2006; Wang et al., 2006), it is attractive to speculate that the RLKs Os04g38480, Os07g35580, Os08g03020, and Os07g35260 may be involved in rice immunity. Indeed, RLK Os04g38480 (OsSERK1, for rice somatic embryogenesis receptor-like kinase 1) is known to be associated with the processes of embryogenesis and defense response (Hu et al., 2005). The *OsSERK1* gene is significantly induced at the RNA level by rice blast fungus with higher levels during the incompatible interaction and by a number of defense signaling molecules, including salicylic acid, benzothiadiazole, jasmonic acid, and abscisic acid (ABA). Down-regulation of *OsSERK1* expression in rice calli reduces the shoot regeneration rate (Hu et al., 2005). By contrast, overexpression of *OsSERK1* enhances rice shoot regeneration and resistance against the blast fungus. These observations reinforce our hypothesis that the PUB-ARM proteins Os08g01900 and Os01g66130 may function as convergence points for various signals perceived and transduced by corresponding RLKs, including Pi-d2 and OsSERK1, in antifungal immunity.

In addition to the PUB-ARM proteins, our yeast two-hybrid screens identified two BTB/POZ (for bric-a-brac, tramtrack, and broad complex/pox virus and zinc finger) domain-containing proteins (Os06g31100 and Os03g57854) as common interactors of two sets of RLKs/RLCKs (Fig. 1A). The Os03g57854 protein possesses an additional protein-protein interaction domain, MATH (for meprin and TRAF homology). Emerging evidence suggests that BTB domain-containing proteins may generally be involved in E3 ubiquitin ligase complexes (van den Heuvel, 2004; Gingerich et al., 2007). For instance, in the CUL-3 ubiquitin ligase complex of *C. elegans*, the BTB protein MEL-26 functions as a substrate-specific adaptor that interacts with the substrate MEI-1 through its N-terminal MATH domain (Furukawa et al., 2003; Pintard et al., 2003; Xu et al., 2003). To test whether these two rice BTB domain-containing proteins also form protein complexes, we performed pair-wise yeast two-hybrid analyses and found that Os06g31100 interacted with the RING finger-containing protein XB3, whereas Os03g57854 bound to the PUB-ARM proteins Os08g01900 and Os01g66130 (Fig. 1A). These results suggest that Os06g31100 and Os03g57854 might be involved in E3 ubiquitin ligase complexes that function in rice RLK/Pelle-mediated immunity. Strikingly, the observed RLK-E3 ubiquitin ligase interactions in rice appear to be parallel to the proximal portion of the conserved innate immunity pathway in animals. Here, the RING finger-containing E3 ubiquitin ligase TRAF6 functions downstream of the receptor/kinase complex that consists of the interleukin-1 receptor, Myd88, and IRAK (Fig. 1A; Wu and Arron, 2003). Although the mechanism of how the E3s function in rice immunity remains to be determined, two possible models have been proposed previously (Wang et al., 2006).

Taken together, we have identified 11 E3-binding RLK/Pelles (excluding XA21), which represent more than 9% of the 119 rice RLK/Pelle family members that have been subjected to our yeast two-hybrid screening in this study. Notably, eight of these 11 (73%) belong to recently expanded rice kinase subfamilies (RERKs), as compared with the fact that less than 43% of the 119 RLK/Pelles are RERKs (Fig. 1A; Supplemental Table S2; see supplemental table SVI in Dardick and Ronald [2006] for RERKs). Given that the rice kinome contains a total of 1,069 RLK/Pelle family members, we propose that a large number of RLK/Pelles may interact with a limited number of E3 ubiquitin ligases, including XB3, Os06g31100, Os08g01900, Os01g66130, and Os03g57854. Most of these kinases may have evolved from recent lineage-specific expansions, and many of them may be related to rice immunity.

Interactions between Components in the Rice MAPK Cascade

The MAPK cascade is composed of three sequentially activated kinases: MAPKKK, MAPKK, and MAPK (Hamel et al., 2006). We found that the MAPKK OsMKK6 (Os01g32660) interacted with OsMPK4 (Os10g38950) and OsMPK6 (Os06g06090), which belong to groups A and B of the MAPK family, respectively (Fig. 2). The OsMKK6 and OsMPK4 interactions were further confirmed by reciprocal screening and by

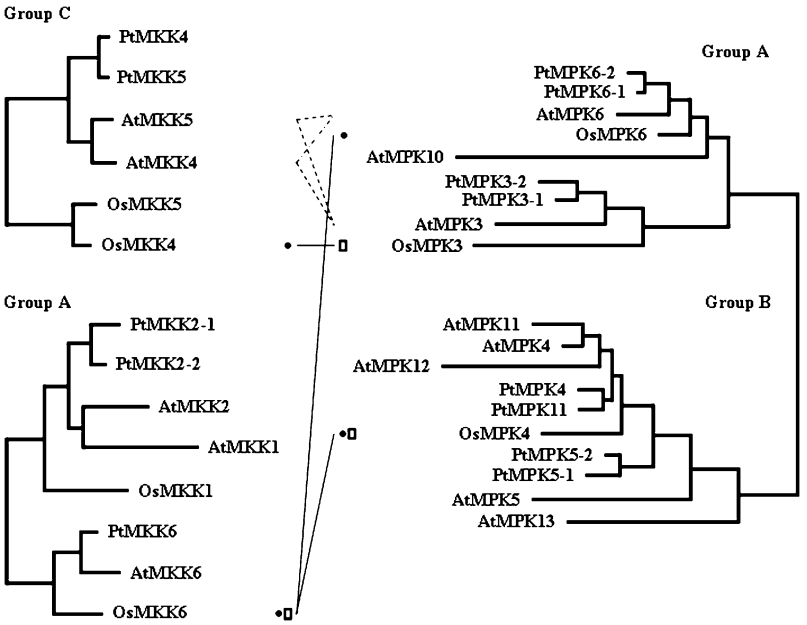


Figure 2. Interactions between MAPKKs and MAPKs. Interacting kinases (black circles) are connected with solid lines to their bait kinases (white squares). Dashed lines denote the connections of Arabidopsis MAPKKs and MAPKs described by Asai et al. (2002). Phylogenetic trees of rice, Arabidopsis, and poplar (*Populus trichocarpa*) MAPKKs (groups A and C) and MAPKs (groups A and B) are adapted from Hamel et al. (2006).

the BiFC system in vivo (Table II; Supplemental Fig. S2). Notably, the results from BiFC assays suggest that these interactions occur in the nucleus. OsMPK6 is orthologous to Arabidopsis AtMPK6, which has been linked to defense signal transduction pathways (Fig. 2; Asai et al., 2002; Menke et al., 2004). In cell culture suspensions, OsMPK6 was activated by the treatment of a sphingolipid elicitor isolated from the fungal pathogen *M. grisea* (Lieberherr et al., 2005). It is possible that OsMKK6 is an upstream kinase that activates OsMPK4 and OsMPK6 in the elicitor-induced defense response.

In a separate yeast two-hybrid screen, OsMKK4 (Os02g54600) was linked to OsMPK3 (Os03g17700), which has been implicated in stress tolerance and host resistance (Fig. 2; Xiong and Yang, 2003). Thus, OsMKK4 might function as an upstream regulator for OsMPK3. In support of this hypothesis, OsMKK4 is orthologous to the Arabidopsis AtMKK4, which has been identified as the upstream kinases of AtMPK3 and AtMPK6 in the flagellin-triggered pathway (Fig. 2; Asai et al., 2002). Given that OsMPK3 is orthologous to AtMPK3 (Fig. 2; Hamel et al., 2006), the proposed OsMKK4-OsMPK3 cascade in rice appears to be parallel to the AtMKK4-AtMPK3/AtMPK6 pathway that may function in Arabidopsis innate immunity (Asai et al., 2002).

A Cyclin-Dependent Kinase Cascade in Rice

Cyclin-dependent kinases (CDKs) are key molecules in mediating cell cycle progression (Potuschak and Doerner, 2001). CDK activity is regulated by multiple mechanisms, including phosphorylation by CDK-activating kinases (CAK), association with cyclin subunits, and degradation of CDK inhibitors that associate with CDKs. We found that rice R2 (Os05g32600), the first plant CAK isolated (Hata, 1991), interacted with the rice kinase Os06g22820, which is orthologous to the Arabidopsis kinase CAK1At (Fig. 3; Umeda et al., 1998). These interactions were confirmed in vivo (Table II; Supplemental Fig. S2). Both Os06g22820 and CAK1At contain a unique insertion sequence (amino acids 178–289 in Os06g22820), present in the T-loop region (Supplemental Fig. S3A; Umeda et al., 1998). It has been demonstrated that CAK1At can phosphorylate the T-loop of CAK2At and CAK4At in vitro and activates the CTD kinase activity of CAK4At in root protoplasts, indicating that CAK1At may be a CAK-activating kinase (Shimotohno et al., 2004). Therefore, the rice kinase Os06g22820 might function as an R2-activating kinase (Fig. 3).

R2 can specifically phosphorylate Thr-161 and Thr-160 in the T-loop of the rice CDK Cdc2Os1 (Os03g02680; Yamaguchi et al., 1998; Vandepoele et al., 2002). When Cdc2Os1 and a nearly identical kinase (Os03g01850; 98% identity, 98% similarity) were used as bait, a rice protein (Os03g05300) orthologous to the evolutionarily conserved CDK subunits (CKS) was identified to interact with these two kinases (Fig. 3; Supplemental Fig. S3B). We were unable to confirm the Cdc2Os1-Os03g05300 interactions with the BiFC system because Cdc2Os1 is capable of generating a YFP signal (Table II). However, previous studies in animals have demonstrated that Cks1 directs the ubiquitin-mediated degradation of the CDK2-associated inhibitor protein $p27^{Kip1}$ by the ubiquitin ligase complex SCF^{skp2} (Ganoth et al., 2001; Spruck et al., 2001). Given that two CDK inhibitors (Os02g52480 and Os10g33310) have been identified in the Cdc2Os1 complex using the TAPtag approach (Rohila et al., 2006), we propose that the CKS protein Os03g05300 might play a similar role in regulating Cdc2Os1 kinase activity through mediating the degradation of the Cdc2Os1-bound inhibitors

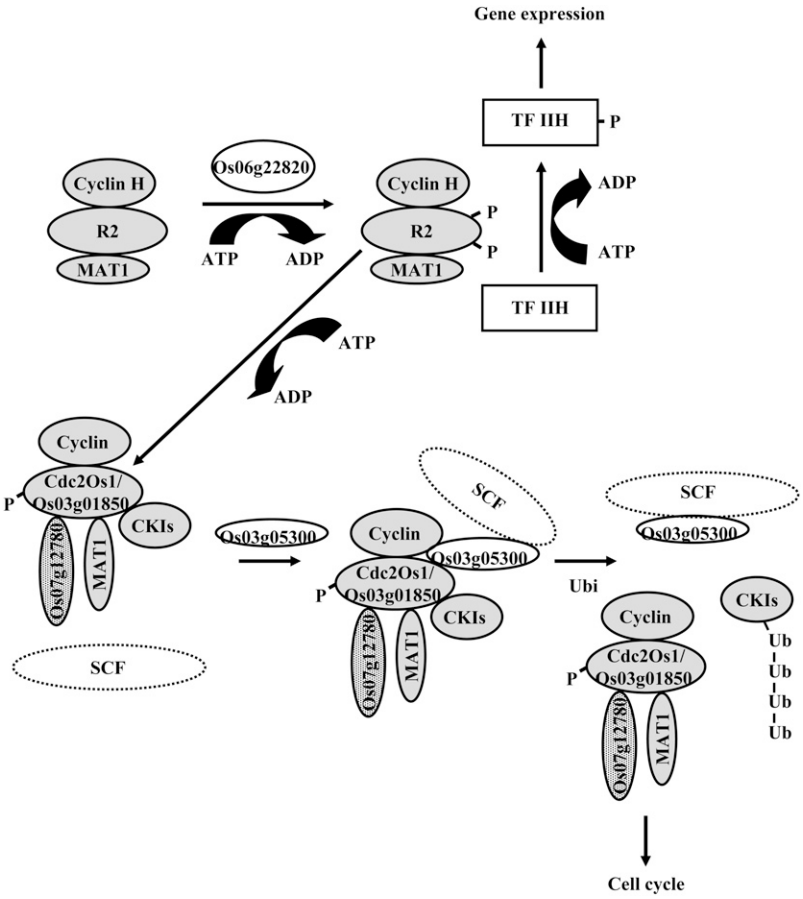


Figure 3. Model for CAK- and CDK-mediated signaling pathways in rice. The R2 and Cdc2Os1 kinase complexes (gray ovals) and phosphorylation of Cdc2Os1 and TFIIH by R2 are based on the studies by Rohila et al. (2006) and Yamaguchi et al. (1998), respectively. The protein Os07g12780 (dotted oval) was identified by both TAPtag and yeast two-hybrid analyses. A hypothetical SCF E3 ubiquitin ligase complex, presumably recruited by the conserved CKS protein Os03g05300 to the Cdc2Os1 kinase complex, is shown as a dashed oval. Protein phosphorylation (P) and ubiquitination (Ub) events are also indicated. Os06g22820 is a putative kinase orthologous to the Arabidopsis CAK1At (Umeda et al., 1998).

(Os02g52480 and Os10g33310; Fig. 3). Taken together, by combining the yeast two-hybrid and TAPtag data, a large portion of the putative components in the rice R2-mediated gene expression signaling have been identified (Fig. 3).

Proteins Associated with Rice SnRKs

The yeast SNF1 and its mammalian homolog AMP-activated protein kinase (AMPK) function as metabolic sensors to protect cells from environmental and nutritional stresses (Polge and Thomas, 2007). SNF1 and AMPK form stable heterotrimeric complexes consisting of a catalytic subunit (SNF1/AMPKα) and two regulatory subunits (SIP1/SIP2/GAL83/AMPKβ and SNF4/AMPKγ). Unlike yeast and mammals, plants carry a large family of SNF1-related kinases classified into three subgroups (SnRK1, SnRK2, and SnRK3; Hrabak et al., 2003). Eighteen rice SnRKs were used for library screening and yielded 82 interactions (Fig. 4).

As expected, two members of the SnRK1 family, Os03g17980 and Os05g45420, interacted with two proteins related to the SNF1/AMPK/SnRK1 β-subunits (Os05g41220 and Os09g20010), and the interactions between Os05g45420 and Os05g41220 were confirmed in vivo (Fig. 4; Table II; Supplemental Fig. S2). Os05g41220 is homologous to Arabidopsis AKINβ1 (At5g21170; 50% identity, 67% similarity), with the conserved KIS (for kinase interaction sequence) and ASC (for association with the SNF1 complex) domains, whereas Os09g20010 is similar to Arabidopsis AKINβ3 (At2g28060; 61% identity, 68% similarity), lacking the N-terminal region of typical β-subunits (Supplemental Fig. S4). Despite the truncated structure, AKINβ3 can interact with the other subunits of the SnRK1 complex and complement the yeast β-subunit deletion mutant (*sip1Δsip2Δgal83Δ*; Gissot et al., 2005). Consistent with these observations, the β-subunit Os09g20010 has also been identified in the Os05g45420 kinase complex by TAPtag analyses (J.S. Rohila and M.E. Fromm, unpublished data), suggesting that the rice kinases Os03g17980 and Os05g45420 function redundantly as the α-subunits in the conserved SNF1-like complexes.

Os03g17980 and Os05g45420 also interacted with a protein (Os03g63940) homologous to the Arabidopsis AKIN$\beta\gamma$ (At1g09020; 67% identity, 80% similarity) and maize (*Zea mays*) ZmAKIN$\beta\gamma$-1 (AF276085; 85% identity, 93% similarity) proteins that form a plant-specific class of regulator proteins (Fig. 4; Lumbreras et al., 2001). This class of proteins is characterized by the presence of an N-terminal KIS domain (characteristic of β-subunits) and a C-terminal region related to the animal SNF4/AMPKγ protein (Supplemental Fig. S4).

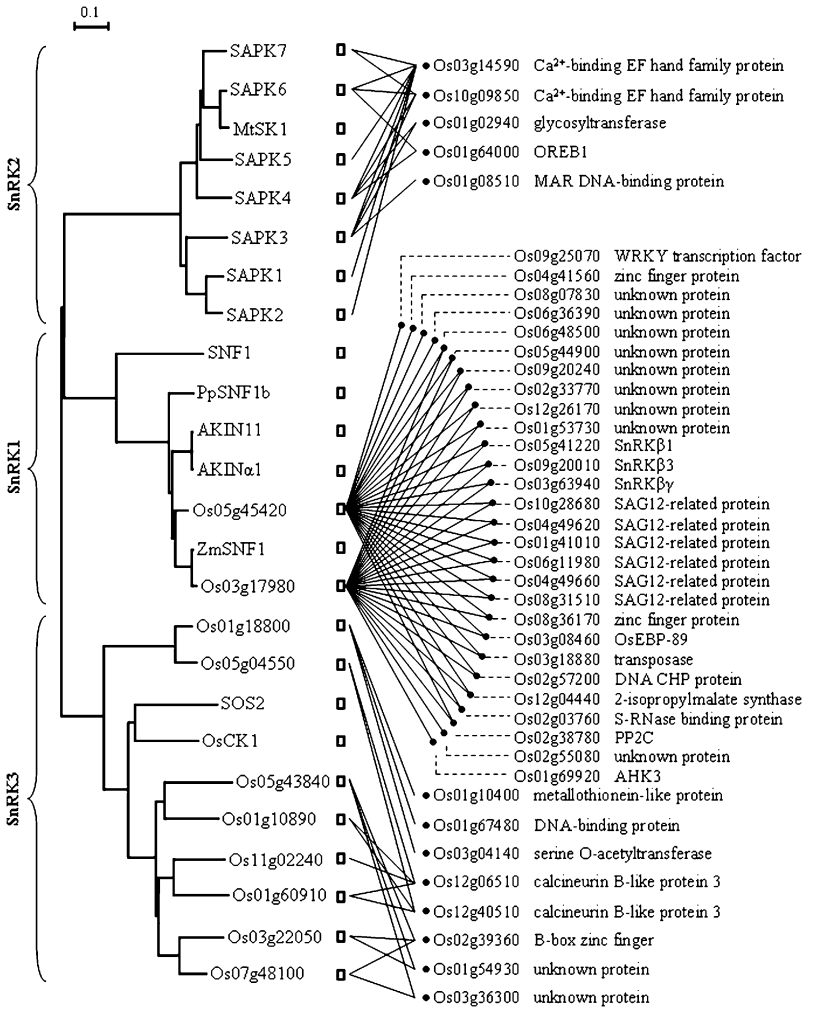


Figure 4. Proteins associated with rice SnRKs. A phylogenetic tree for 17 rice SnRKs and their related kinases (ZmSNF1 from maize; AKIN11 and AKINα1 from Arabidopsis; PpSNF1b from moss; SNF1 from *S. cerevisiae*; MtSK1 from *Medicago truncatula*; SOS2 from Arabidopsis) is shown. Classification of these kinases is indicated on the left. The interacting proteins (black circles) are connected by solid lines with their corresponding kinases (white squares).

The *ZmAKIN$\beta\gamma$-1* gene is capable of complementing the yeast *Δsnf4-2* mutant. It has been proposed that plants may possess two distinct types of SNF1 complexes with two or three subunits (Lumbreras et al., 2001). In line with our observation, the $\beta\gamma$-subunit Os03g63940 has been associated with the Os05g45420 kinase in planta by TAPtag analyses (J.S. Rohila and M.E. Fromm, unpublished data). Additionally, AMPKα can be dephosphorylated by a type 2C protein phosphatase (Davies et al., 1995). The interaction between a putative type 2C protein phosphatase (Os02g38780) and Os03g17980 or Os05g45420 offers the possibility that Os02g38780 is involved in dephosphorylation of these two rice kinases (Fig. 4).

Ten rice SnRK2s are known as osmotic stress/ABA-activated protein kinases (SAPKs) 1 to 10 (Kobayashi et al., 2004). We found that SAPK4 and SAPK6 interacted with OREB1, a protein related to bZIP transcription factors/ABA-responsive element-binding factors, and the SAPK4-OREB1 interactions were confirmed to occur in the cytoplasm of rice mesophyll protoplasts by the BiFC system (Fig. 4; Table II; Supplemental Fig. S2). Moreover, the SAPK6 (also known as OSRK1)-OREB1 interactions have recently been demonstrated in yeast by Chae et al. (2007). They showed that OREB1 can be strongly phosphorylated by SAPK6 and that both proteins can colocalize in nuclear bodies in onion (*Allium cepa*) epidermal cells. This discrepancy in subcellular localizations of SAPK4 and OREB1 may reflect the differences of the cell systems used in the study and the potential subcellular translocations of these proteins under distinct conditions. A similar phenomenon has been observed when the subcellular localization of SAPK4 was determined in onion epidermal cells and Arabidopsis mesophyll protoplasts (Diédhiou et al., 2008). Our results suggest that OREB1 might be regulated by multiple SAPKs.

Nine kinases of the rice SnRK3 subfamily were used as bait to screen for binding proteins. Among them, four (Os05g04550, Os11g02240, Os01g60910, and Os01g10890) interacted with one or both of two closely related rice proteins (Os12g06510 and Os12g40510) that belong to the family of the SOS3-like Ca^{2+} sensor/binding proteins (ScaBPs, also referred to as calcineurin B-like proteins [CBLs]; Fig. 4; Supplemental Fig. S5; Gong et al., 2004). In Arabidopsis, there are 24 SnRK3s

(also known as protein kinases related to SOS2 [PKSs] or CBL-interacting protein kinases) and nine ScaBPs. The interactions between specific PKSs and ScaBPs have been shown to regulate various targets, including potassium transporter, the plasma membrane H^+-ATPase, and those involved in salt, drought, and cold stresses and ABA signaling (Halfter et al., 2000; Liu et al., 2000; Luan et al., 2002; Gong et al., 2004; Li et al., 2006; Fuglsang et al., 2007). Thus, rice might also utilize PKS-ScaBP complexes in stress signaling.

Interactions with a Rice LAMMER Kinase

LAMMER kinases are one of two families of kinases that can phosphorylate and regulate Ser/Arg-rich (SR) proteins in RNA splicing (Reddy, 2004; Isshiki et al., 2006). We found that a rice LAMMER kinase (Os01g62080) interacted with four SR proteins (SCL30a, SCL30b, RSZp23, and Os06g50890) that all possess a RNA recognition motif (RRM) and an Arg/Ser-rich (RS) domain (Fig. 5). SCL30a and SCL30b are members of the SC35-type group, whereas OsRSZp23 belongs to the 9G8-type family containing a single CCHC-type zinc knuckle motif between the RRM and RS domains (Isshiki et al., 2006). Differing from many other SR proteins, the RS domain of Os06g50890 is located at the N terminus (Fig. 5). The rice LAMMER kinase Os01g62080 is similar to the tobacco (*Nicotiana tabacum*) ethylene-induced LAMMER kinase PK12 (AAC04324; 56% identity, 65% similarity). It has been reported that PK12 and its Arabidopsis homolog, AFC2, can interact with and phosphorylate several plant and animal SR proteins (Golovkin and Reddy, 1999; Savaldi-Goldstein et al., 2000, 2003). Therefore, the rice kinase Os01g62080 likely regulates its interacting SR proteins SCL30a, SCL30b, RSZp23, and Os06g50890 in RNA splicing.

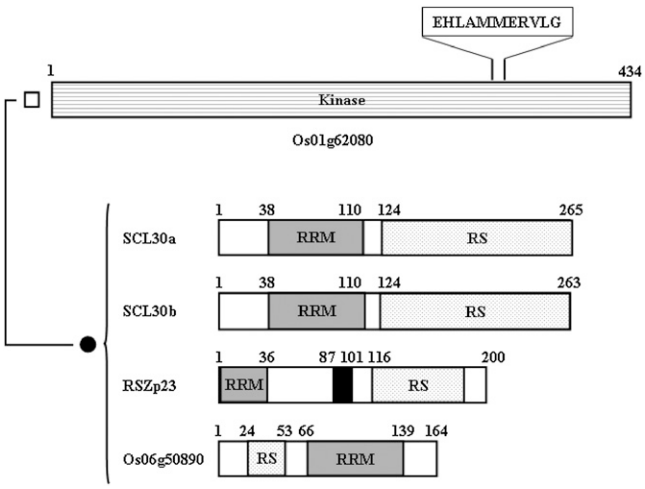


Figure 5. Protein interactions with the rice LAMMER-type kinase Os01g62080. Schematic representation of Os01g62080 (top) and comparison of four interacting proteins (bottom). In Os01g62080, the characteristic sequence motif (boxed) for the LAMMER-type kinase is indicated. In the Os01g62080-binding proteins, the RRM and the RS regions are shown. The black box in RSZp23 denotes a zinc finger motif. Numbers above domains indicate amino acid positions in the full-length proteins.

The CK2 Protein Complex

CK2 (formerly known as casein kinase II), a kinase essential for cell viability, forms a tetrameric complex with two catalytic (α and α') subunits and a dimeric β-subunit (Meggio and Pinna, 2003). The rice CK2 kinase II α-subunit-like protein Os07g02350 contains the conserved HIKE domain (Olsten et al., 2005). As expected, a CK2 β-subunit (Os07g31280) was identified as an interactor of Os07g02350 (Supplemental Table S2). This observation has been supported in a TAPtag study in planta (Rohila et al., 2006).

Kinases Associated with Putative Transcription Regulators

We have previously shown that the XA21 RLK binds to a WRKY-type transcription factor, suggesting that XA21 may be directly involved in regulating gene expression (Peng et al., 2008). Interestingly, among the 254 identified kinase interactors, 15% appear to be putative DNA binding/transcription factors, further supporting a role for kinase regulation of transcription factors (Supplemental Fig. S1B). Examples from our data set include the OREB1-SAPK interactions described above and the interactions between the rice TOUSLED-like kinase Os03g53880 and the protein Os01g08680 (Supplemental Table S2). The Arabidopsis TOUSLED kinase, required for leaf and flower development, is capable of oligomerizing and interacting with the C-terminal portion of the SANT/myb domain protein TKI1 (Roe et al., 1997; Ehsan et al., 2004). Os03g53880 exhibits 62% identity (74% similarity) to TOUSLED, whereas Os01g08680 shares homology to the C-terminal half (amino acids 210–741) of TKI1 (AF530160; 28% identity, 40% similarity). Our interaction results support that Os03g53880 may be orthologous to the Arabidopsis TOUSLED kinase.

Comparison of the Yeast Two-Hybrid and TAPtag Approaches

We used both yeast two-hybrid and TAPtag methods to identify proteins associated with the same set of rice kinases (Rohila et al., 2006; this study). In addition to the interactions described above, the resulting information obtained also allows assessment of these two methods to study protein interactions in plants. TAPtag identified a greater number of proteins when a protein complex was relatively abundant and stable. For example, in the broadly conserved CDK (Os03g02680) and CAK (Os05g32600) complexes, 17 components were found by TAPtag analyses (Rohila et al., 2006). In contrast, the yeast two-hybrid approach identified only three interactors for these two kinases (Fig. 3). Another drawback to the yeast two-hybrid approach is

that further experiments are required to confirm that interactions take place in planta. However, the yeast two-hybrid assay is a more practical technique for use in high-throughput studies. It can also detect both stable and transient interactions often missed by TAPtag. This aspect is particularly important for those kinase-protein interactions in which the kinases form weak and/or transient contacts with their substrates, or when the kinase complexes are sensitive to the purification procedure. In support of this reasoning, a number of interactions involved in the MAPK cascade were identified, supporting a greater degree of sensitivity of yeast two-hybrid assays. Moreover, no interactors were found when the RLK protein XA21 was subjected to TAPtag analyses (W.-H. Xu, M.E. Fromm, and W.-Y. Song, unpublished data). In contrast, more than 20 XA21-binding proteins were identified by yeast two-hybrid screens (L.-Y. Pi, X.-D. Ding, and W.-Y. Song, unpublished data; T. Richter and P.C. Ronald, unpublished data), and at least four of them have been confirmed to be associated with XA21 in planta or in vivo (Wang et al., 2006; Park et al., 2008; Peng et al., 2008; Y.-N. Jiang and W.-Y. Song, unpublished data).

Comparative analysis reveals that there is little overlap between the two protein-kinase interaction data sets generated by the yeast two-hybrid and TAPtag assays. Among the 93 kinases that have been analyzed, only four common kinase-binding proteins (Os07g12780, Os09g20010, Os03g63940, and Os07g31280) were identified by these two methods (Rohila et al., 2006). This could reflect the differences between the rice tissues used for these two assays and the fact that whereas TAPtag analyses identify proteins that indirectly associate with a kinase, the yeast two-hybrid analysis detects only those proteins that interact directly (see ''Materials and Methods''; Rohila et al., 2006). This could also be attributed to the fact that many protein-kinase interactions in vivo rely on the phosphorylation status of the proteins and/or the kinases, which may be regulated by developmental program and environmental stimuli. Another reason is that both assays likely miss many interactors (also known as false negatives). Similar situations could occur when BiFC assays are used to verify the interactions identified by yeast two-hybrid analyses.

There has also been little overlap in the data sets in large scale protein-protein interaction studies in yeast. For instance, of the 80,000 yeast protein associations detected or predicted by various approaches, including yeast two-hybrid and TAPtag, only approximately 2,400 were supported by more than one method (von Mering et al., 2002). In two genome-wide yeast two-hybrid screens using the same 6,000 yeast open reading frames, only about 10% of the interactions were identified as having common characteristics (Ito et al., 2002). When the two-hybrid data sets were compared with the protein complex data sets generated by TAPtag, only 7% of the interactions were identified by both methods (Gavin et al., 2002). Likewise, the protein complex data sets obtained from two distinct purification techniques (TAPtag versus high-throughput mass spectrometric protein complex identification) shared only 10% of the common proteins detected (Gavin et al., 2002; Ho et al., 2002; Ito et al., 2002). It has been estimated that the false-negative rate for high-throughput screens could be more than 80% (Stanyon et al., 2004). In our system, only two MAPKK-MAPK interactions were identified when eight out of 15 rice MAPKs were subjected to library screenings. If a given MAPK interacts with at least one MAPKK, the false-negative rate for the system could be more than 75%. These assessments indicate the limitation of each method used for studying protein-protein interactions, thus supporting the idea that distinct techniques are required to obtain the information necessary to unravel the complex interactome in a cell (von Mering et al., 2002; Zhu et al., 2003).

Protein kinases are involved in crucial steps along numerous cell signaling pathways, ranging from the proximal receptors that perceive external signals to the distal kinases that directly regulate transcription factors. The identification of the proteins associated with 116 kinases using yeast two-hybrid analyses is an important step toward understanding the global signaling network regulated by rice kinases. Because of the presence of false positives and negatives, and the inability to calculate reliable positive and negative discovery rates, caution is needed when using some of the data. However, the large-scale nature of the protein interaction data set allows for exploration of the patterns of kinase-protein interactions in rice and provides a basis for future functional characterization of these interactions. This can be particularly significant because rice has a much larger kinome size and most plant RLKs have Ser/Thr specificity that differs from the receptor Tyr kinases predominantly present in animals. Finally, our comparison of the overlap of the TAPtag and yeast two-hybrid methods supports the strategy of using complementary screening methods to find greater numbers of interacting proteins to more fully develop interaction networks. This strategy and the tools developed from this and previous studies (Rohila et al., 2006) should facilitate large-scale proteomics work in rice and in other plants.

MATERIALS AND METHODS

Cloning of Rice Kinase Genes

Total RNA was isolated from rice (*Oryza sativa* 'Nipponbare') leaves using Trizol reagent (Invitrogen). First-strand cDNA was synthesized using RETROscript reverse transcriptase (Ambion). PCR was performed to quantify the amount of cDNA synthesized. The cDNA products were amplified using *LA Taq* DNA polymerase (LA PCR Kit, version 2.1; Takara Mirus Bio). The PCR primers contained the *att*B1 and *att*B2 sequences for cloning PCR products into pDONR207 by BP reaction (Gateway; Invitrogen). Primers were designed using Primer3 Input software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi). The forward primer contained an ATG starting codon, and the reverse primer had a termination codon to synthesize a cDNA clone without a 5′ untranslated region and a 3′ untranslated region. The 5′ open reading frame was in frame with the upstream Gateway reading frame (AttB1 forward:

5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTCAACCATG, where ATG is the translation initiation codon). For the RLKs, extracellular and transmembrane domains were excluded and only the cytoplasmic kinase region was amplified. All amplified kinase clones were verified by sequencing.

Construction of Rice cDNA Libraries

Three rice cDNA libraries were prepared. The first was constructed from the *japonica* cv Taipei309. Two- to 3-week-old rice seedlings were treated by cold (4°C for 24 h), heat (42°C for 6 h), benzothiadiazole (3 mM three times per 48 h), salt (100, 200, and 300 mM NaCl for 12 h), drought (air dried for 2 h), or the plant hormone ABA (0.1 mM three times per 24 h). Equal amounts of tissue from entire plants were harvested and combined to isolate RNA. Up to 50 μg of mRNA was purified using the Poly(A)Purist mRNA purification kit (Ambion). A rice cDNA library was constructed using the mRNA and the HybriZAP-2.1 XR library construction kit (Stratagene). The titer of the library is approximately 2.8 million plaque-forming units, with an average insert size of 1.6 kb. The second library was made from 3- to 6-week-old *indica* plants, cv IRBB21, carrying the resistance gene *Xa21* (Chern et al., 2001). RNA used for making the library was isolated from leaf tissue harvested after inoculation with *Xanthomonas oryzae* pv *oryzae* Philippine Race 6. The third library was generated from 3-week-old leaves of *japonica* cv Moroberekan.

Yeast Two-Hybrid Screens

The detailed procedure for screening the libraries is described by Ding et al. (2007). Briefly, equal amounts of plasmid DNA recovered from the three cDNA libraries were combined and transformed into the yeast strain Y187. Transformants were harvested and aliquots were stored in a −80°C freezer. Rice kinases in pDONOR207 were converted into both pXDGATcy86 and pXDGATU86 vectors (Ding et al., 2007) using the Gateway LR Clonase Enzyme Mix (Invitrogen) according to the manufacturer's instructions. The resulting bait constructs were transformed into the yeast strain HF7c and subjected to testing for bait autoactivation. The kinases that did not autoactivate the *His3* reporter were then used to screen the cDNA libraries. HF7c cells containing a pXDGATcy86-derived rice kinase were mated with the Y187 cells transformed with the cDNA libraries. Ten to 20 million zygotes were typically obtained and selected for their interacting proteins on a SD/-Leu-Trp-His + 2 mM 3-amino-1,2,4-triazole medium. After 10 d, the plasmids were rescued by RCA from the yeast colonies grown on selective medium. One portion of the RCA-amplified plasmids was used for prey sequencing, and the other portion was transformed into the yeast strain MaV203. After removing the pXDGATcy86-derived kinase bait by counterselection (Ding et al., 2007), the yeast cells were transformed with the same kinase cloned into the pXDGATU86 vector or with the pXDGATU86 empty vector. The transformants were then selected on a SD/-Leu-Trp-His + 50 mM 3-amino-1,2,4-triazole medium. Only a prey that showed a specific interaction with the bait, not with the activation domain, was scored as a positive interactor.

DNA Sequencing and Analysis

Sequencing templates were prepared directly from yeast or bacterial cultures using the TempliPhi DNA Sequencing Template Amplification method as specified by the manufacturer (GE Healthcare). Dye terminator DNA sequencing reactions utilized the primer SSO20 (5′-AGGGATGTTTAATACCACTAC-3′) and ET-Terminators from Amersham Biosciences. Sequence reactions were desalted by ethanol precipitation, dried, and resuspended in 10 μL of a 0.06% aqueous solution of Seakem Gold agarose (Cambrex) prior to electrokinetic injection. All DNA sequencing was performed on capillary array DNA sequencing units (MegaBACE 1000; GE Healthcare).

To specify the identity of each prey, the Michigan State University Rice Genome Annotation Project Database and Resource (http://rice.plantbiology.msu.edu/; formerly hosted by The Institute for Genomic Research, Rockville, MD) was searched using the BLASTP algorithm. The European Molecular Biology Open Software Suite program "needle" was used to perform sequence alignment and to calculate the percentage of sequence identity and similarity.

The PAUP (version 4.0b10) software package (Swofford, 2002) was used to conduct phylogenetic analyses. Neighbor joining was used to reconstruct the phylogenetic relationships of these amino acids. Bootstrap values were derived from 1,000 replicates to quantify the relative support for branches of the inferred phylogenetic tree.

Split-YFP Assays

We modified the split-YFP vectors developed by Bracha-Drori and colleagues (2004) to make them compatible with the Gateway system of Invitrogen. Simultaneously, we introduced a more typical flexible linker (G-G-G-G-S-G-G-G-G-S) and, for the YFPN-term vector (p736GC), a c-myc tag, since the original poly-Glu tag was reported to be ineffective (Bracha-Drori et al., 2004). First, pSY vectors were digested with *Sal*I and *Bam*HI and ligated with a synthetic DNA segment, introducing a blunt-ended restriction site unique to the vector (*Pme*I) and the flexible linker and tag. The resulting vectors were then cut and ligated with Gateway cassette reading frame C.1 to create p735GC [YFPC-term-HA tag-2X(G4S)-Gateway Cassette] and p736GC [YFPN-term-myc tag-2X(G4S)-Gateway Cassette]. Each bait and prey was previously cloned into pENTR and were recombined into the split-YFP vectors following the manufacturer's protocol (Invitrogen). Unless otherwise stated, baits were recombined into p735GC and preys into p736GC. After recombination, the junction sites and inserts were sequenced.

We conducted split-YFP assays through transient transformation of protoplasts prepared from etiolated aboveground tissues of 7- to 14-d-old Kitaake rice plants, prepared essentially as described (Bart et al., 2006). To promote consistent seedling growth, rice seeds were surface sterilized for 25 min in 40% bleach prior to germination on medium containing 0.8% agar, 1× Murashige and Skoog medium, and 30% Suc. We found that transformation of protoplasts with the Gateway-compatible vectors containing the chloramphenicol resistance and *ccdB* genes supported reconstitution of YFP in the absence of interactors. Therefore, we used the original vectors (pSY736 and pSY735) developed by Bracha-Drori et al. (2004) as the empty, unfused vectors for negative control experiments.

All microscopic images were generated using a Zeiss Axiovert 25 fluorescence microscope and taken with a Nikon D70s digital camera with the Nikon Capture 4 software. YFP fluorescence was visualized under the Zeiss YFP filter cube 46HE (excitation, BP500/25; beamsplitter, FT515; emission, BP535/30). YFP expression was monitored between 18 and 48 h after rice protoplast transformation.

The accession numbers for the proteins from the other species are as follows: ZmSNF1, AAS59400; AKIN11, NP_974375; AKINα1, P92958; PpSNF1b, AAR03831; SNF1, A26030; MtSK1, AAV41843; SOS2, AAF62923; SOS3, AF060553; AtFHA1, AAF20220; AtFHA2, AAF20224; NtFHA1, AAL05884; FHL1, P39521; Cks-1, P61025; SUC1, CAA21308; and AHK3, NP_564276.

Supplemental Data

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

Supplemental Figure S1. A rice kinase-protein interaction map.

Supplemental Figure S2. BiFC assay to visualize protein-protein interaction in rice protoplasts.

Supplemental Figure S3. Components in the rice CDK signaling pathway.

Supplemental Figure S4. Structure comparison of the β- and $\beta\gamma$-subunits of rice SNF1-like complexes.

Supplemental Figure S5. Sequence alignment of Os12g06510, Os12g40510, and the Arabidopsis SOS3 protein.

Supplemental Table S1. List of the 22 kinases that can autoactivate the *His3* reporter.

Supplemental Table S2. List of the 378 rice kinase-protein interactions identified by the yeast two-hybrid screen.

Supplemental Table S3. List of the 66 kinases that yielded no interactors.

Supplemental Table S4. Amino acid sequence comparisons between the kinase domains of the 12 rice RLK/Pelles described in Figure 1A.

ACKNOWLEDGMENTS

We thank Margaret Joyner and Terry Davoli for critical reading of the manuscript, Drs. Heidi Zhang and Kyle Gardenour for technical assistance, and Dr. Marc Vidal for providing the yeast strain MaV203.

Received August 20, 2008; accepted December 18, 2008; published December 24, 2008.

LITERATURE CITED

Afzal AJ, Wood AJ, Lightfoot DA (2008) Plant receptor-like serine threonine kinases: roles in signaling and plant defense. Mol Plant Microbe Interact **21:** 507–517

Andrade MA, Petosa C, O'Donoghue SI, Muller CW, Bork P (2001) Comparison of ARM and HEAT protein repeats. J Mol Biol **309:** 1–18

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signaling cascade in Arabidopsis innate immunity. Nature **415:** 977–983

Bart R, Chern M, Park CJ, Bartley L, Ronald PC (2006) A novel system for gene silencing using siRNAs in rice leaf and stem-derived protoplasts. Plant Methods **2:** 13

Bhat RA, Lahaye T, Panstruga R (2006) The visible touch: in planta visualization of protein-protein interactions by fluorophore-based methods. Plant Methods **2:** 12

Bieri S, Mauch S, Shen QH, Peart J, Devoto A, Casais C, Ceron F, Schulze S, Steinbiss HH, Shirasu K, et al (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

Bracha-Drori K, Shichrur K, Katz A, Oliva M, Angelovici R, Yalovsky S, Ohad N (2004) Detection of protein-protein interactions in plants using bimolecular fluorescence complementation. Plant J **40:** 419–427

Chae MJ, Lee JS, Nam MH, Cho K, Hong JY, Yi SA, Suh SC, Yoon IS (2007) A rice dehydration-inducible SNF1-related protein kinase 2 phosphorylates an abscisic acid responsive element-binding factor and associates with ABA signaling. Plant Mol Biol **63:** 151–163

Chen X, Shang J, Chen D, Lei C, Zou Y, Zhai W, Liu G, Xu J, Ling Z, Cao G, et al (2006) A B-lectin receptor kinase gene conferring rice blast resistance. Plant J **46:** 794–804

Chern MS, Fitzgerald H, Yadav RC, Canlas P, Dong X, Ronald PC (2001) Evidence for a resistance signaling pathway in rice similar to the NPR1-mediated pathway in Arabidopsis. Plant J **27:** 101–113

Chinchilla D, Boller T, Robatzek S (2007a) Flagellin signalling in plant immunity. Adv Exp Med Biol **598:** 358–371

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

Colcombet J, Hirt H (2008) Arabidopsis MAPKs: a complex signalling network involved in multiple biological processes. Biochem J **413:** 217–226

Dardick C, Ronald P (2006) Plant and animal pathogen recognition receptors signal through non-RD kinases. PLoS Pathog **2:** e2

Davies SP, Helps NR, Cohen PTW, Hardie DG (1995) 5′-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase: studies using bacterially expressed human protein phosphatase-2Cα and native bovine protein phosphatase-2Ac. FEBS Lett **377:** 421–425

Diédhiou CJ, Popova OV, Dietz KJ, Golldack D (2008) The SNF1-type serine-threonine protein kinase SAPK4 regulates stress-responsive gene expression in rice. BMC Plant Biol **8:** 49

Diener AC, Ausubel FM (2005) *RESISTANCE TO FUSARIUM OXYSPORUM 1*, a dominant Arabidopsis resistance gene, is not race specific. Genetics **171:** 305–321

Ding X, Cory G, Song WY (2004) A high-throughput system to verify candidate interactors from yeast two-hybrid screening using rolling circle amplification. Anal Biochem **331:** 195–197

Ding X, Snyder AK, Shaw R, Farmerie WG, Song WY (2003) Direct retransformation of yeast with plasmid DNA isolated from single yeast colonies using rolling circle amplification. Biotechniques **35:** 774–779

Ding X, Zhang Y, Song WY (2007) Use of rolling-circle amplification for large-scale yeast two-hybrid analyses. Methods Mol Biol **354:** 85–98

Ehsan H, Reichheld JP, Durfee T, Roe JL (2004) TOUSLED kinase activity oscillates during the cell cycle and interacts with chromatin regulators. Plant Physiol **134:** 1488–1499

Fuglsang AT, Guo Y, Cuin TA, Qiu Q, Song C, Kristiansen KA, Bych K, Schulz A, Shabala S, Schumaker KS, et al (2007) *Arabidopsis* protein kinase PKS5 inhibits the plasma membrane H^+-ATPase by preventing interaction with 14-3-3 protein. Plant Cell **19:** 1617–1634

Furukawa M, He YJ, Borchers C, Xiong Y (2003) Targeting of protein ubiquitination by BTB-Cullin 3-Roc1 ubiquitin ligases. Nat Cell Biol **5:** 1001–1007

Ganoth D, Bornstein G, Ko TK, Larsen B, Tyers M, Pagano M, Hershko A (2001) The cell-cycle regulatory protein Cks1 is required for SCF(Skp2)-mediated ubiquitinylation of p27. Nat Cell Biol **3:** 321–324

Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, et al (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature **415:** 141–147

Gingerich DJ, Hanada K, Shiu SH, Vierstra RD (2007) Large-scale, lineage-specific expansion of a bric-a-brac/tramtrack/broad complex ubiquitin-ligase gene family in rice. Plant Cell **19:** 2329–2348

Giot L, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Ooi CE, Godwin B, Vitols E, et al (2003) A protein interaction map of *Drosophila melanogaster*. Science **302:** 1727–1736

Gissot L, Polge C, Bouly JP, Lemaitre T, Kreis M, Thomas M (2005) AKINβ3, a plant specific SnRK1 protein, is lacking domains present in yeast and mammals non-catalytic β-subunits. Plant Mol Biol **56:** 747–759

Godiard L, Sauviac L, Torii KU, Grenon O, Mangin B, Grimsley NH, Marco Y (2003) ERECTA, an LRR receptor-like kinase protein controlling development pleiotropically affects resistance to bacterial wilt. Plant J **36:** 353–365

Golovkin M, Reddy AS (1999) An SC35-like protein and a novel serine/arginine-rich protein interact with Arabidopsis U1-70K protein. J Biol Chem **274:** 36428–36438

Gong D, Guo Y, Schumaker KS, Zhu JK (2004) The SOS3 family of calcium sensors and SOS2 family of protein kinases in Arabidopsis. Plant Physiol **134:** 919–926

Gu T, Mazzurco M, Sulaman W, Matias DD, Goring DR (1998) Binding of an ARM repeat protein to the kinase domain of the S-locus receptor kinase. Proc Natl Acad Sci USA **95:** 382–387

Halfter U, Ishitani M, Zhu JK (2000) The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. Proc Natl Acad Sci USA **97:** 3735–3740

Hamel LP, Nicole MC, Sritubtim S, Morency MJ, Ellis M, Ehlting J, Beaudoin N, Barbazuk B, Klessig D, Lee J, et al (2006) Ancient signals: comparative genomics of plant MAPK and MAPKK gene families. Trends Plant Sci **11:** 192–198

Hata S (1991) cDNA cloning of a novel cdc2+/CDC28-related protein kinase from rice. FEBS Lett **279:** 149–152

Hazbun T, Fields S (2001) Networking proteins in yeast. Proc Natl Acad Sci USA **98:** 4277–4278

Heese A, Hann DR, Gimenez-Ibanez S, Jones AM, He K, Li J, Schroeder JI, Peck SC, Rathjen JP (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc Natl Acad Sci USA **104:** 12217–12222

Ho Y, Gruhler A, Heilbut A, Bader GD, Moore L, Adams SL, Millar A, Taylor P, Bennett K, Boutilier K, et al (2002) Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. Nature **415:** 180–183

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

Hrabak EM, Chan CW, Gribskov M, Harper JF, Choi JH, Halford N, Kudla J, Luan S, Nimmo HG, Sussman MR, et al (2003) The Arabidopsis CDPK-SnRK superfamily of protein kinases. Plant Physiol **132:** 666–680

Hu CD, Kerppola TK (2003) Simultaneous visualization of multiple protein interactions in living cells using multicolor fluorescence complementation analysis. Nat Biotechnol **21:** 539–545

Hu H, Xiong L, Yang Y (2005) Rice SERK1 gene positively regulates somatic embryogenesis of cultured cell and host defense response against fungal infection. Planta **222:** 107–117

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

Isshiki M, Tsumoto A, Shimamoto K (2006) The serine/arginine-rich protein family in rice plays important roles in constitutive and alternative splicing of pre-mRNA. Plant Cell **18:** 146–158

Ito T, Chiba T, Ozawa R, Yoshida M, Hattori M, Sakaki Y (2001) A comprehensive two-hybrid analysis to explore the yeast protein interactome. Proc Natl Acad Sci USA **98:** 4569–4574

Ito T, Ota K, Kubota H, Yamaguchi Y, Chiba T, Sakuraba K, Yoshida M (2002) Roles for the two-hybrid system in exploration of the yeast protein interactome. Mol Cell Proteomics **1:** 561–566

Jonak C, Okresz L, Bogre L, Hirt H (2002) Complexity, cross talk and integration of plant MAP kinase signaling. Curr Opin Plant Biol **5:** 415–424

Kobayashi Y, Yamamoto S, Minami H, Kagaya Y, Hattori T (2004) Differential activation of the rice sucrose nonfermenting1-related protein kinase2 family by hyperosmotic stress and abscisic acid. Plant Cell **16:** 1163–1177

LaCount DJ, Vignali M, Chettier R, Phansalkar A, Bell R, Hesselberth JR, Schoenfeld LW, Ota I, Sahasrabudhe S, Kurschner C, et al (2005) A protein interaction network of the malaria parasite *Plasmodium falciparum.* Nature **438:** 103–107

Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC (2002) BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell **110:** 213–222

Li L, Kim BG, Cheong YH, Pandey GK, Luan S (2006) A Ca(2)+ signaling pathway regulates a K(+) channel for low-K response in Arabidopsis. Proc Natl Acad Sci USA **103:** 12625–12630

Li S, Armstrong CM, Bertin N, Ge H, Milstein S, Boxem M, Vidalain PO, Han JD, Chesneau A, Hao T, et al (2004) A map of the interactome network of the metazoan *C. elegans.* Science **303:** 540–543

Lieberherr D, Thao NP, Nakashima A, Umemura K, Kawasaki T, 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

Lim J, Hao T, Shaw C, Patel AJ, Szabó G, Rual JF, Fisk CJ, Li N, Smolyar A, Hill DE, et al (2006) A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration. Cell **125:** 801–814

Liu J, Ishitani M, Halfter U, Kim CS, Zhu JK (2000) The *Arabidopsis thaliana SOS2* gene encodes a protein kinase that is required for salt tolerance. Proc Natl Acad Sci USA **97:** 3730–3734

Llorente F, Alonso-Blanco C, Sanchez-Rodriguez C, Jorda L, Molina A (2005) ERECTA receptor-like kinase and heterotrimeric G protein from Arabidopsis are required for resistance to the necrotrophic fungus *Plectosphaerella cucumerina.* Plant J **43:** 165–180

Lu CA, Lin CC, Lee KW, Chen JL, Huang LF, Ho SL, Liu HJ, Hsing YI, Yu SM (2007) The SnRK1A protein kinase plays a key role in sugar signaling during germination and seedling growth of rice. Plant Cell **19:** 2484–2499

Luan S, Kudla J, Rodriguez-Concepcion M, Yalovsky S, Gruissem W (2002) Calmodulins and calcineurin B-like proteins: calcium sensors for specific signal response coupling in plants. Plant Cell (Suppl) **14:** S389–S400

Lumbreras V, Alba MM, Kleinow T, Koncz C, Pages M (2001) Domain fusion between SNF1-related kinase subunits during plant evolution. EMBO Rep **2:** 55–60

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

Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) The protein kinase complement of the human genome. Science **298:** 1912–1934

Meggio F, Pinna LA (2003) One-thousand-and-one substrates of protein kinase CK2? FASEB J **17:** 349–368

Menke FL, van Pelt JA, Pieterse CM, Klessig DF (2004) Silencing of the mitogen-activated protein kinase MPK6 compromises disease resistance in *Arabidopsis.* Plant Cell **16:** 897–907

Morillo SA, Tax FE (2006) Functional analysis of receptor-like kinases in monocots and dicots. Curr Opin Plant Biol **9:** 460–469

Morris ER, Walker JC (2003) Receptor-like protein kinases: the keys to response. Curr Opin Plant Biol **6:** 339–342

Mudgil Y, Shiu SH, Stone SL, Salt JN, Goring DR (2004) A large complement of the predicted Arabidopsis ARM repeat proteins are members of the U-box E3 ubiquitin ligase family. Plant Physiol **134:** 59–66

Nam KH, Li J (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell **110:** 203–212

Nodzon L, Song WY (2004) Yeast two-hybrid technology. *In* RM Goodman, ed, Encyclopedia of Plant and Crop Science. Marcel Dekker, New York, pp 1302–1304

Olsten ME, Weber JE, Litchfield DW (2005) CK2 interacting proteins: emerging paradigms for CK2 regulation? Mol Cell Biochem **274:** 115–124

Park CJ, Peng Y, Chen X, Dardick C, Ruan D, Bart R, Canlas PE, Ronald PC (2008) Rice XB15, a protein phosphatase 2C, negatively regulates cell death and XA21-mediated innate immunity. PLoS Biol **6:** e231

Parrish JR, Gulyas KD, Finley RL Jr (2006) Yeast two-hybrid contributions to interactome mapping. Curr Opin Biotechnol **7:** 387–393

Parrish JR, Yu J, Liu G, Hines JA, Chan JE, Mangiola BA, Zhang H, Pacifico S, Fotouhi F, DiRita VJ, et al (2007) A proteome-wide protein interaction map for *Campylobacter jejuni.* Genome Biol **8:** R130

Peng Y, Bartley LE, Chen X, Dardick C, Chern M, Ruan DL, Canlas PE, Ronald PC (2008) OsWRKY62 is a negative regulator of basal and Xa21-mediated defense against *Xanthomonas oryzae* pv. *oryzae* in rice. Molecular Plant **1:** 446–458

Phizicky E, Bastiaens PI, Zhu H, Snyder M, Fields S (2003) Protein analysis on a proteomic scale. Nature **422:** 208–215

Pintard L, Willis JH, Willems A, Johnson JL, Srayko M, Kurz T, Glaser S, Mains PE, Tyers M, Bowerman B, et al (2003) The BTB protein MEL-26 is a substrate-specific adaptor of the CUL-3 ubiquitin-ligase. Nature **425:** 311–316

Polge C, Thomas M (2007) SNF1/AMPK/SnRK1 kinases, global regulators at the heart of energy control? Trends Plant Sci **12:** 20–28

Potuschak T, Doerner P (2001) Cell cycle controls: genome-wide analysis in Arabidopsis. Curr Opin Plant Biol **4:** 501–506

Rain JC, Selig L, De Reuse H, Battaglia V, Reverdy C, Simon S, Lenzen G, Petel F, Wojcik J, Schachter V, et al (2001) The protein-protein interaction map of *Helicobacter pylori.* Nature **409:** 211–215

Reddy AS (2004) Plant serine/arginine-rich proteins and their role in pre-mRNA splicing. Trends Plant Sci **9:** 541–547

Roe JL, Durfee T, Zupan JR, Repetti PP, McLean BG, Zambryski PC (1997) TOUSLED is a nuclear serine/threonine protein kinase that requires a coiled-coil region for oligomerization and catalytic activity. J Biol Chem **272:** 5838–5845

Rohila JS, Chen M, Chen S, Chen J, Cerny R, Dardick C, Canlas P, Xu X, Gribskov M, Kanrar S, et al (2006) Protein-protein interactions of TAP-tagged protein kinases in rice. Plant J **46:** 1–13

Rual JF, Venkatesan K, Hao T, Hirozane-Kishikawa T, Dricot A, Li N, Berriz GF, Gibbons FD, Dreze M, Ayivi-Guedehoussou N, et al (2005) Towards a proteome-scale map of the human protein-protein interaction network. Nature **437:** 1173–1178

Samuel MA, Mudgil Y, Salt JN, Delmas F, Ramachandran S, Chilelli A, Goring DR (2008) Interactions between the S-domain receptor kinases and AtPUB-ARM E3 ubiquitin ligases suggest a conserved signaling pathway in Arabidopsis. Plant Physiol **147:** 2084–2095

Samuel MA, Salt JN, Shiu SH, Goring DR (2006) Multifunctional arm repeat domains in plants. Int Rev Cytol **253:** 1–26

Sato S, Shimoda Y, Muraki A, Kohara M, Nakamura Y, Tabata S (2008) A large-scale protein protein interaction analysis in *Synechocystis* sp. PCC6803. DNA Res **14:** 207–216

Savaldi-Goldstein S, Aviv D, Davydov O, Fluhr R (2003) Alternative splicing modulation by a LAMMER kinase impinges on developmental and transcriptome expression. Plant Cell **15:** 926–938

Savaldi-Goldstein S, Sessa G, Fluhr R (2000) The ethylene-inducible PK12 kinase mediates the phosphorylation of SR splicing factors. Plant J **21:** 91–96

Scheer JM, Ryan CA Jr (2002) The systemin receptor SR160 from *Lycopersicon peruvianum* is a member of the LRR receptor kinase family. Proc Natl Acad Sci USA **99:** 9585–9590

Shimoda Y, Shinpo S, Kohara M, Nakamura Y, Tabata S, Sato S (2008) A large scale analysis of protein-protein interactions in the nitrogen-fixing bacterium *Mesorhizobium loti.* DNA Res **15:** 13–23

Shimotohno A, Umeda-Hara C, Bisova K, Uchimiya H, Umeda M (2004) The plant-specific kinase CDKF;1 is involved in activating phosphorylation of cyclin-dependent kinase-activating kinases in *Arabidopsis.* Plant Cell **16:** 2954–2966

Shiu SH, Karlowski WM, Pan R, Tzeng YH, Mayer KF, Li WH (2004) Comparative analysis of the receptor-like kinase family in *Arabidopsis* and rice. Plant Cell **16:** 1220–1234

Song WY, Wang G, Chen L, Kim H, Pi LY, Gardner J, Wang B, Holsten T, Zhai W, Zhu L, et al (1995) A receptor kinase-like protein encoded by the rice disease resistance gene *Xa21.* Science **270:** 661–667

Spruck C, Strohmaier H, Watson M, Smith AP, Ryan A, Krek TW, Reed SI (2001) A CDK-independent function of mammalian Cks1: targeting of SCF(Skp2) to the CDK inhibitor p27Kip1. Mol Cell **7:** 639–650

Stanyon CA, Liu G, Mangiola BA, Patel N, Giot L, Kuang B, Zhang H, Zhong J, Finley RL Jr (2004) A Drosophila protein-interaction map centered on cell-cycle regulators. Genome Biol **5:** R96

Sun X, Cao Y, Yang Z, Xu C, Li X, Wang S, Zhang Q (2004) *Xa26*, a gene

conferring resistance to *Xanthomonas oryzae* pv *oryzae* in rice, encodes an LRR receptor kinase-like protein. Plant J **37:** 517–527

Swofford DL (2002) PAUP: Phylogenetic Analysis Using Parsimony, Version 40b10. Sinauer Associates, Sunderland, MA

Tornero P, Merritt P, Sadanandom A, Shirasu K, Innes RW, Dangl JL (2002) RAR1 and NDR1 contribute quantitatively to disease resistance in *Arabidopsis*, and their relative contributions are dependent on the R gene assayed. Plant Cell **14:** 1005–1015

Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P, et al (2000) A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae.* Nature **403:** 623–627

Umeda M, Bhalerao RP, Schell J, Uchimiya H, Koncz C (1998) A distinct cyclin-dependent kinase-activating kinase of *Arabidopsis thaliana.* Proc Natl Acad Sci USA **95:** 5021–5026

van den Heuvel S (2004) Protein degradation: CUL-3 and BTB—partners in proteolysis. Curr Biol **14:** R59–R61

Vandepoele K, Raes J, De Veylder L, Rouze P, Rombauts S, Inze D (2002) Genome-wide analysis of core cell cycle genes in *Arabidopsis.* Plant Cell **14:** 903–916

Vidalain PO, Boxem M, Ge H, Li S, Vidal M (2004) Increasing specificity in high-throughput yeast two-hybrid experiments. Methods **32:** 363–370

von Mering C, Krause R, Snel B, Cornell M, Oliver SG, Fields S, Bork P (2002) Comparative assessment of large-scale data sets of protein-protein interactions. Nature **417:** 399–403

Wang YS, Pi LY, Chen X, Chakrabarty PK, Jiang J, Lopez De Leon A, Liu GL, Li L, Benny U, Oard J, et al (2006) The rice XA21 binding protein 3 is a ubiquitin ligase and required for full *Xa21*-mediated disease resistance. Plant Cell **18:** 3635–3646

Wu H, Arron JR (2003) TRAF6, a molecular bridge spanning adaptive immunity, innate immunity and osteoimmunology. Bioessays **25:** 1096–1105

Xenarios I, Salwinski L, Duan XJ, Higney P, Kim SM, Eisenberg D (2002) DIP, the Database of Interacting Proteins: a research tool for studying cellular networks of protein interactions. Nucleic Acids Res **30:** 303–305

Xiang Y, Cao Y, Xu C, Li X, Wang S (2006) *Xa3*, conferring resistance for rice bacterial blight and encoding a receptor kinase-like protein, is the same as *Xa26.* Theor Appl Genet **113:** 1347–1355

Xiong L, Yang Y (2003) Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. Plant Cell **15:** 745–759

Xu L, Wei Y, Reboul J, Vaglio P, Shin TH, Vidal M, Elledge SJ, Harper JW (2003) BTB proteins are substrate-specific adaptors in an SCF-like modular ubiquitin ligase containing CUL-3. Nature **425:** 316–321

Xu WH, Wang Y, Liu G, Chen X, Tinjuangjun P, Pi LY, Song WY (2006) The autophosphorylated Ser686, Thr688 and Ser689 residues within a putative protease cleavage motif in the juxtamembrane domain of XA21 are implicated in the stability control of the rice receptor-like kinase. Plant J **45:** 740–751

Yamaguchi M, Umeda M, Uchimiya H (1998) A rice homolog of Cdk7/MO15 phosphorylates both cyclin-dependent protein kinases and the carboxy-terminal domain of RNA polymerase II. Plant J **16:** 613–619

Zhu H, Bilgin M, Snyder M (2003) Proteomics. Annu Rev Biochem **72:** 783–812