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Crosstalk of Signaling Mechanisms Involved in Host Defense and Symbiosis Against Microorganisms in Rice

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Abstract: Rice is one of the most important food crops, feeding about half population in the world. Rice pathogens cause enormous damage to rice production worldwide. In plant immunity research, considerable progress has recently been made in our understanding of the molecular mechanisms underlying microbe-associated molecular pattern (MAMP)-triggered immunity. Using genome sequencing and molecular techniques, a number of new MAMPs and their receptors have been identified in the past two decades. Notably, the mechanisms for chitin perception via the lysine motif (LysM) domain-containing receptor OsCERK1, as well as the mechanisms for bacterial MAMP (e.g. flg22, elf18) perception via the leucine-rich repeat (LRR) domain-containing receptors FLS2 and EFR, have been clarified in rice and *Arabidopsis*, respectively. In chitin signaling in rice, two direct substrates of OsCERK1, Rac/ROP GTPase guanine nucleotide exchange factor OsRacGEF1 and receptor-like cytoplasmic kinase OsRLCK185, have been identified as components of the OsCERK1 complex and are rapidly phosphorylated by OsCERK1 in response to chitin. Interestingly, OsCERK1 also participates in symbiosis with arbuscular mycorrhizal fungi (AMF) in rice and plays a role in the recognition of short-chitin molecules (CO4/5), which are symbiotic signatures included in AMF germinated spore exudates and induced by synthetic strigolactone. Thus, OsCERK1 contributes to both immunity and symbiotic responses. In this review, we describe recent studies on pathways involved in rice immunity and symbiotic signaling triggered by interactions with microorganisms. In addition, we describe recent advances in genetic engineering by using plant immune receptors and symbiotic microorganisms to enhance disease resistance of rice.

Keywords: Immunity, Symbiosis, PRRs, RK, MAMPs, Chitin, Myc factor.

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1. INTRODUCTION

The global population is growing rapidly; it now exceeds 7 billion and is predicted to rise to over 9.5 billion by 2050 [1]. Significant improvement of crop yield in modern agriculture is necessary to support this population growth. Rice is one of the most important food crops, feeding more than 3.5 billion people. The International Rice Research Institute (IRRI) estimates that in each of the next 20 years, the world will need an additional 8–10 million tons of rice [2]. A large number of pathogenic microorganisms cause important diseases in rice. IRRI showed that, on average, farmers lose 37% of their rice yield due to diseases and pests [3]. Rice research, therefore, can have a major beneficial impact on human wellbeing, and the improvement of disease resistance in rice is a key research goal.

Plants are static organisms and thus are constantly challenged with harmful pathogens and associated with beneficial symbiotic microorganisms. Plants employ pattern recognition receptors (PRRs), which can perceive microbe-associated molecular patterns (MAMPs). Host plant perceptions of MAMPs induce a defense response called MAMP-triggered immunity (MTI), which includes Ca^{2+} influx to the cytoplasm, the production of reactive oxygen species (ROS), and the induction of defense-related genes [4]. The downstream components of PRR remained elusive until recently, but some key partners are becoming clear. Virulent pathogens are able to suppress MTI by secretion of their effectors into plant cells. To counter this activity of pathogen effectors, resistance proteins act as intracellular receptors that recognize pathogen effectors and activate effector-triggered immunity (ETI) [5, 6]. These two layers of defense constitute plant immunity against pathogenic microorganisms.

Plant PRRs typically possess a leucine-rich repeat (LRR) or lysin motif (LysM) within ectodomains that recognize conserved MAMPs, such as bacterial flg22 and elf18 and fungal chitin. Most PRRs are either receptor kinases (RKs), which consist of a ligand-binding ectodomain, a transmembrane domain, and a cytoplasmic kinase domain, or receptor-like proteins (RLPs), which lack a cytoplasmic kinase domain [6]. Rice and *Arabidopsis* encode more than 1,100 and 600 RKs/RLPs in their genomes, respectively, and these molecules are involved in numerous cellular signaling path-

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ways [7]. Recent studies have shown that RKs associate with RLPs to recognize MAMPs and transmit signals from the cell surface to intracellular downstream proteins [8, 9].

Mutualistic symbioses provide benefits to both plants and microorganisms, and such associations have significant benefits for global agriculture. For example, symbiotic nitrogen fixation provides 40–60 million tons of nitrogen for global agricultural systems per year [10]. The best studied symbiotic microbial symbionts are rhizobial bacteria and arbuscular mycorrhizal fungi (AMF). Legume roots respond to rhizobial bacteria-derived signals named Nod factors, which are necessary for nodule formation on roots. Nitrogen-fixing bacteria reside in these nodules as organelle-like structures. Rhizobial bacteria are released into a plant-derived membrane-bound compartments inside the cells of the nodule [11]. Within these structures, bacteria convert atmospheric nitrogen to ammonia, a form of nitrogen that is readily available to plants. This makes legumes important crops that can enrich soil nitrogen and valuable sources of plant and/or animal protein. However, non-legume plant species generally do not associate with nitrogen-fixing rhizobial bacteria. By contrast, AMF have symbiotic relationships with almost all land plants, except species in *Brassicaceae* and *Chenopodiaceae*. AMF produce mycorrhizal factors (Myc factors) that activate symbiosis pathways of host plants to promote AMF root invasion [11]. In many habitats, nutrient resources in the soil are limited. AMF can supply nutrients, such as phosphate, zinc and nitrates, to plants, and this has a significant impact on plant growth and health [12].

This review summarizes our current knowledge on interactions between rice and microorganisms that lead to plant immunity and symbiosis, and highlights the mechanisms by which rice plants perceive and respond to microorganism signatures, such as MAMPs and Myc factors (Fig. **1**).

2. MOLECULAR SIGNATURES OF PATHOGENIC MICROBES AND SIGNALING PATHWAYS

2.1. Chitin/PGN-induced Rice Immunity

Chitin is an oligomer of β-1, 4-linked *N*-acetyl-glucosamine (GlcNAc) and a cell wall component that provide structural rigidity to most fungi, insects, and crustaceans (crabs, shrimp, etc.) [13]. They are molecular signatures that are recognized by surveillance systems in mammals, insects, and plants [14, 15]. Three decades ago, studies in mice showed that chitin and its derivatives induce the production of H_2O_2 and cytokines, resulting in nonspecific immune responses [16-18]. The silkworm *Bombyx mori* perceives chitin as well as bacterial lipopolysaccharides, triggering the expression of insect antibacterial proteins [14]. In plants, chitin is one of the best-studied MAMPs, along with flagellin (flg22) and elongation factor-Tu (EF-Tu). The first chitin receptor in plants, OsCEBiP, was identified in rice. It binds directly to chitin with high affinity and is essential for chitin response in rice suspension cells [8, 19]. OsCEBiP family proteins possess three LysMs in their ectodomain, which directly bind to not only chitin, but also peptidoglycans (PGNs) to detect various fungi and bacteria [20]. Interestingly, in rice, two distinct OsCEBiP molecules mediate the recognition of a single chitin molecule, resulting in the formation of sandwich-type OsCEBiP dimerization [8]. OsCEBiP is an RLP with no intracellular kinase domain and may be tethered by a glycosylphosphatidylinositol (GPI) to the cell surface [21]. It requires an RK to transmit extracellular chitin signals to cells. In fact, OsCEBiP cooperates with the RK OsCERK1, which also has three LysMs in its ectodomain [22]. Coimmunoprecipitation analyses indicate that a portion of OsCEBiP and OsCERK proteins interact with each other without chitin stimulation, and chitin stimulation enhances complex formation between OsCEBiP and OsCERK1 [22, 23]. This OsCERK1-OsCEBiP complex on

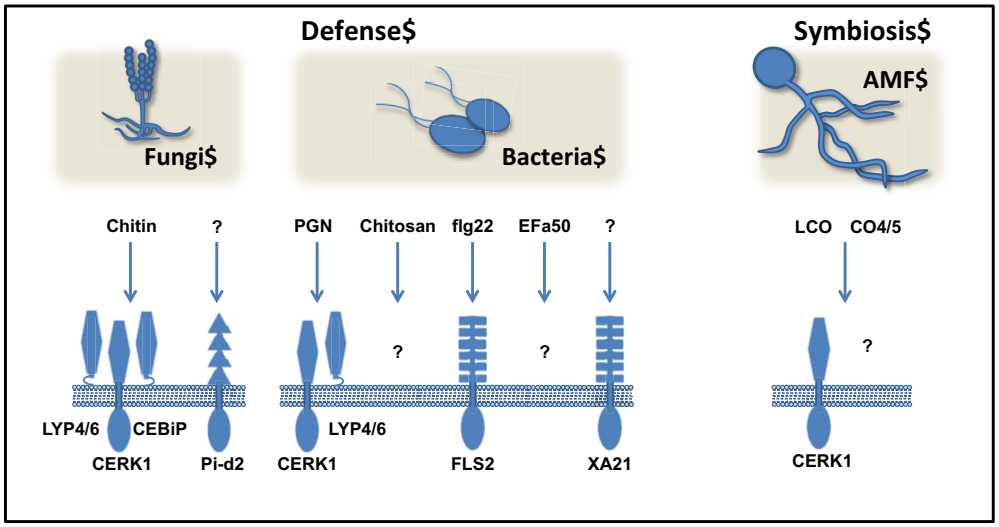

Fig. (1). Schematic representation of rice receptor kinases (RKs) and receptor-like kinases (RLKs) in the immune and symbiosis signaling pathways. Fungi-derived chitin binds to homodimers of the lysine motif (LysM)-RLP CEBiP, which induces the recruitment of the LysM-RK OsCERK1 on the cell surface to transduce chitin signals to downstream components. The LysM RLPs OsLYP4 and OsLYP6 also form a complex with OsCERK1 and participate in chitin and bacterial-derived peptidoglycan (PGN) signaling. OsFLS2 directly binds the flagellin-derived epitope flg22 and transduces flagellin signals. The B-lectin RK Pi-d2 and the LRR-RK XA21 participate in immune signaling against bacteria or fungi, respectably; however, their ligands are unknown. The fungi-derived molecule chitosan and bacterial EF-Tu-derived EFa50 can induce immune responses in rice cells; however, the receptors remain unidentified.

the cell surface efficiently transduces chitin signals to downstream components for immune responses. In wheat, since the silencing of homologs of either CERK1 or CEBiP is sufficient to increase colonization by the normally nonpathogenic *Mycosphaerella graminicola* Mag3LysM deletion mutant, CERK1 and CEBiP homologbs likely work together, like in the rice system [24]. Interestingly, in *Arabidopsis*, AtCERK1 alone directly binds chitin and sufficiently induces chitin signaling, and AtCEBiP (also known as LYM2) is not involved in these events [25-28]. Cao *et al.* recently showed that two LysM-RKs, AtLYK4 and AtLYK5, are required for chitin signaling, and AtLYK5 interacts with AtCERK1 in a chitin-dependent manner [29]. It is likely that, in *Arabidopsis*, AtCERK1 has a role in the chitin perception together with AtLYK4/5. Thus, it appears that *Arabidopsis* does not employ the CERK1-CEBiP *system*, at least in the perception of chitin at the plasma membrane. AtCEBiP, which is localized at the plasmodesmata, functions in intracellular flux and confers resistance against the necrotrophic fungus *Botrytis cinerea*, but not against the hemibiotrophic fungus *Colletotrichum higginsianum* or the bacteria *Pseudomonas syringae pv. tomato* DC3000 [30]. Therefore, it is possible that AtCEBiP employs other unknown RK(s) in the AtCERK1-independent signaling pathways, and the CEBiP-RK system is a conserved feature of monocot and dicot plants.

PGNs, which are a major component of gram-positive and gram-negative bacterial cell walls and are structurally similar to chitin, act as MAMPs in some plant and animal species. In *Arabidopsis*, genetic analyses suggest that AtCERK1, together with two LysM proteins, LYM1 and LYM3, can recognize structurally different PGNs from gram-negative and gram-positive bacteria as well as fungal chitin [31]. Likewise, in rice, OsLYP4 and OsLYP6, LysM-containing proteins, and the rice homologs of the *Arabidopsis* LYM1 and LYM3, play key roles in PGN-triggered immunity [9, 20]. An *in vitro* ligand-binding assay has shown that OsLYP4 and OsLYP6 can bind not only to PGN, but also to chitin [20]. Moreover, OsCERK1 interacts with OsLYP4 or OsLYP6 in a PGN-dependent manner [9]. Using the knock out plants of OsCERK1, Kouzai *et al.* showed that OsCERK1 plays a role in the perception of PGN, probably by interacting with either or both LYP4 and LYP6 [32]. Thus, in rice, OsCERK1/OsLYP4 or OsLYP6 complexes may recognize both PGN and chitin and transmit those signals to intracellular interacting partners.

Fungal pathogens disturb the chitin perception system of plants to enhance their virulence. *Cladosporium fulvum*, which is a fungal pathogen that causes tomato leaf mold, and the wheat pathogen *Mycosphaerella graminicola* secrete the LysM-containing effector proteins Ecp6 and Mg3LysM, which suppress plant immunity by binding chitin oligomers to prevent their recognition by plant receptors [33, 34].

The kinase domain of OsCERK1 transduces chitin signals to downstream molecules (Fig. **2**). Two major down-

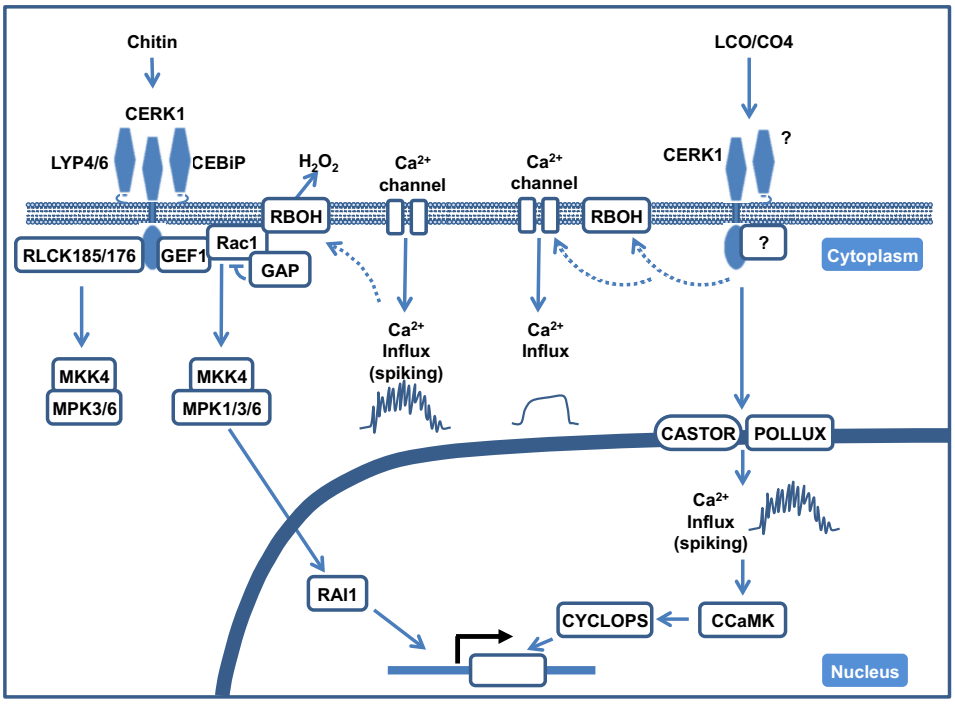

Fig. (2). Rice immunity and symbiotic signaling pathways triggered by microorganism signatures. In immune signaling, OsCEBiP directly binds to chitin and forms a receptor complex with OsCERK1 to transduce an immune response. OsCERK1-dependent phosphorylation of OsRacGEF1 leads to the activation of the small GTPase OsRac1 at the plasma membrane. OsRac1 activates the transcription factor RAI1 via the MAPK pathway, including OsMPK3/4 and MKK4. In addition, OsRac1 directly binds to the N-terminus of OsRbohB to regulate reactive oxygen species (ROS) production. The kinase domain of OsCERK1 also phosphorylates the cytoplasmic receptor-like kinase OsRLCK185/176 and phosphorylated RLCK185/176 activates the MAPK cascade. In symbiosis signaling, unknown receptors recognize Myc-LCO or CO4/5. OsCERK1 may transduce the extracellular signals to the cytoplasmic region. In the rice genome, CASTOR, POLLUX, CYCLOPS, and CCaMK are highly conserved. All four proteins in rice may contribute to the symbiosis pathway.

stream molecules of OsCERK1 are known to participate in this process. One is a guanine nucleotide exchange factor (GEF) for the Rac/ROP family small GTPase OsRac1, termed OsRacGEF1 [35]. The Rac/ROP family small GTPases constitute a plant-specific Rho subfamily and undergo exchange reactions between an inactive GDP-bound form and an active GTP-bound form to actuate functional switches [36]. The Rac/ROP family contributes to diverse signal transduction processes, such as those governing disease resistance, pollen tube growth, root hair development, ROS production, cell wall patterning, and hormone responses [37-41]. OsRac1 is one of the master regulators in rice innate immunity and positively regulate MAMP-induced ROS production and resistance to *Magnaporthe oryzae* [37, 38, 40, 42]. Chitin-induced phosphorylation of OsRacGEF1 at S549 by OsCERK1 activates GEF activity and facilitates the exchange of GDP for GTP on OsRac1, leading to OsRac1 activation. This OsRac1 activation plays important roles in MTI, as descried below. Rice respiratory burst oxidase homolog B (OsRbohB) is an enzyme responsible for ROS production in rice immunity via direct interactions between the N-terminal region of OsRbohB and OsRac1 [43]. Structural studies have provided insight into the mode of interaction between OsRbohB and OsRac1; the switch I region of OsRac1, particularly at Tyr39 and Asp45, is important for the direct interaction with OsRbohB [44]. In addition, the expression of *Metallothionein2b* (*MT2b*), an ROS scavenging gene, is downregulated by OsRac1 [45]; thus, OsRac1 can regulate ROS production via NADPH oxidase and ROS scavengers. However, the detailed regulatory mechanisms of ROS production are still unclear in rice MTI.

Another protein that interacts with the kinase domain of OsCERK1 is receptor-like cytoplasmic kinase 185 (RLCK185). OsRLCK185 is a target protein of *Xoo*1488, an effector of the rice pathogen *Xanthomonas oryzae* [46]. It belongs to RLCK subfamily VII. OsCERK1 is associated with and phosphorylates OsRLCK185 after chitin treatment [46]. The phosphorylation of OsRLCK185 by OsCERK1 activates the MAPK cascade, including OsMPK3 and OsMPK6, in response to chitin and PGN [46]. Similarly, another RLCK subfamily VII member, RLCK176, functions downstream of OsCERK1 in the PGN and chitin signaling pathways [9]. In *Arabidopsis*, BIK1, a member of the RLCK subfamily VII, also works together with various RKs, such as the flagellin receptor FLAGELLIN SENSING 2 (FLS2) and EF-Tu receptor (EFR), similar to OsCERK1 and OsRLCK185/176. It appears that combinations of RKs and RLCKs are common modules for MTI signaling in plants. Ca^{2+} influx acts as a second messenger for plant immunity and plays important roles in regulating various defense responses [47, 48]. Interestingly, immunity and symbiotic signaling trigger Ca^{2+} spiking in the cytoplasm and the nucleus, respectively, and the different Ca^{2+} compartments may contribute to distinct behaviors after the perception of pathogens and symbionts (see below). Although *Arabidopsis* BIK1 directly phosphorylates and activates RBOH [49], it is still not known how rice OsRLCK185/176 regulates RBOH. Taken together, two signaling complexes, OsRacGEF1-OsRac1 and OsRLCK-OsMAPKs, function downstream of OsCERK1 in rice chitin-induced immunity.

2.2. Chitosan-induced Rice Immunity

Chitosan is widely distributed in nature, mainly as the structural component of fungal cell walls and arthropod exoskeletons [50]. Fungal pathogens change their cell wall components to avoid degradation by lytic enzymes when they invade host plant cells, and deacetylation of cell wall chitin to chitosan (poly-*N*-acetylglucosamine) is a likely pathogen infection strategy. Chitosan is also a MAMP in plants, inducing MTI and priming systemic acquired resistance (SAR) [51, 52]. In the soybean, chitosan is able to induce Ca^{2+} influx in the cytoplasm and ROS production within a few minutes [53]. In *Cocos nucifera* calli, chitosan activates MAPK-like proteins and induces the expression of defense-related genes [52]. In rice, it is unclear whether deacetylated chitosan oligomers (chitooligosaccharides) act as MAMPs. Chitin (*N*-acetylchitoheptaose) at a concentration of 1 $\mu g \cdot ml^{-1}$ (873 nM) is able to trigger ion influxes in rice cells, but chitoheptaose at 1 $\mu g \cdot ml^{-1}$ (873 nM) is not [54]. However, high concentrations of chitosan (>15 $\mu g \cdot ml^{-1}$) induce ROS production and defense-related gene expression in rice cells [55].

As previously described, *Arabidopsis* CERK1 can directly bind to chitin, but not to chitosan [25]. To date, it is still unclear whether chitosan-triggered immunity employs RKs, similar to other MTI. Some studies have identified potential chitosan receptors, including a putative receptor for chitosan in Chinese cabbage [50, 56], and chitosan-induced expression of an RK in *Cocos nucifera* calli [52]. Alternatively, it is possible that chitosan triggers host immunity by destabilizing pores in the plasma membrane because it is a polycationic molecule that can bind the negatively charged hydrophilic portion of phospholipid bilayers [57].

2.3. Flagellin-induced Rice Immunity

A well-characterized bacterial signature is flg22, an N-terminal peptide of bacterial flagellin that is recognized via the direct binding of an LRR containing RK FLS2 in *Arabidopsis* [58]. This flg22-FLS2 signaling mechanism is conserved not only in dicots but also in monocots; for example, rice possesses a conserved flagellin recognition system. Flg22 treatment induces ROS production and defense-related gene expression in rice leaves [59], and rice OsFLS2 is an *Arabidopsis* FLS2 ortholog [60]. An affinity cross-linking analysis using biotinylated flg22 showed direct binding between OsFLS2 and flg22 [61], and overexpression of OsFLS2 in rice cells increases flg22-induced gene expression and ROS production [60]. The rice OsFLS2 may possess different flagellin perception specificity than that of *Arabidopsis* FLS2 and less flexibility to respond to flagellins derived from different kinds of bacteria than that of *Arabidopsis* FLS2 [62]. Indeed, OsFLS2 responds to flagellin in *Acidovorax avenae*, but not in *Xanthomonas oryzae* pvs. *oryzae* (*Xoo*) or *oryzicola* (*Xoc*), which are devastating rice bacterial pathogens [62]. In *Arabidopsis*, recent biochemical and structural studies have revealed the interaction between FLS2 and BRASSINOSTEROID INSENSITIVE 1 (BRI1)-associated receptor kinase 1 (AtBAK1). AtBAK1 is an LRR receptor kinase and acts as a co-receptor of various RKs, including EFR, BRI1, etc. [4]. In addition to the direct interaction between AtFLS2 and flg22, the ectodomain of AtBAK1 also binds to the C-terminus of flg22 [63]. Although

OsBAK1 is involved in brassinosteroid signaling, there are no reports of OsBAK1 involvement in OsFLS2 signaling [64]. It appears that the main epitope regions in flagellins are different between AtFLS2 and OsFLS2 because rice suspension cells are weakly sensitive to flg22, but respond strongly to the C-terminal domain of flagellin derived from incompatible strains of *A. avenae*, implying that rice recognizes *A. avenae* flagellin via different (or additional) mechanisms [65]. Further studies are necessary to clarify the perception mechanisms of flagellin by OsFLS2 [65].

2.4. EF-Tu-induced Rice Immunity

EF-Tu is an abundant, highly conserved protein that comprises as much as 5% to 10% of the cytoplasmic proteins in bacteria [66]. Its *N*-acetylated peptide comprising the first 18 amino acids, called elf18, is a MAMP in *Arabidopsis* and other *Brassicaceae* species, but not in other plant families (e.g., *Cyperus papyrus*, *Nicotiana tabacum*, and *Medicago sativa*) [67]. The *Arabidopsis* RK EFR directly recognizes elf18 [68], resulting in dimerization with AtBAK1 to form a receptor complex [4]. However, rice has no EFR orthologs and elf18 does not cause an immune response, such as ROS production, defense-related gene expression, or callose deposition in rice leaves [69]. Interestingly, Furukawa *et al.* recently showed that rice recognize EF-Tu from *A. avenae* via a different epitope called EFa50 [69]. EFa50 is a 50-amino acid peptide derived from the central region of EF-Tu; it induces immune responses (e.g., ROS production, callose deposition, and defense-related gene expression), and confers resistance against infection with *A. avenae* in rice [69]. The difference in epitope regions within EF-Tu between rice and *Brassicaceae* species implies that plants established EFR systems after monocots diverged from dicots.

2.5. XA21-mediated Immunity to *Xanthomonas oryzae* pv. *oryzae*

Bacterial blight caused by *Xoo* is one of the most notorious bacterial diseases affecting rice. In 1995, rice XA21 was identified as the first RK in plant immunity and shown to confer resistance to *Xoo* [70]. Although XA21 was long considered to be a resistance protein, it may act as a PRR for an unknown molecular signature of *Xoo*. Rice somatic embryogenesis receptor kinase 2 (OsSERK2), which is a co-receptor for XA21, positively regulates rice immunity [71]. OsSERK2 and XA21 form a heteromeric complex through their kinase domains in the absence of ligand treatment. It is likely that OsSERK2 has a similar role to that of AtBAK1 because it participates in OsFLS2 signaling, but not in chitin signaling.

The intracellular signaling network that includes XA21 has been reported in rice. The phosphorylation state of XA21 is closely associated with its function because XA21 binding protein 15 (OsXB15), a PP2C phosphatase, dephosphorylates XA21 to negatively regulate XA21-mediated immune responses [72]. XA21 binding protein 24 (OsXB24), one of a large class of broadly conserved ATPases of unknown function, binds to XA21 [73]. Silencing of OsXB24 enhances XA21-mediated immunity, and OsXB24 dissociates from XA21 in response to *Xoo* [73]. Thus, OsXB24 negatively regulates XA21 PRR function in the absence of *Xoo*, and releases from XA21 in the presence of *Xoo* signals. OsXB3, an E3 ubiquitin ligase and substrate for XA21 Ser and Thr kinases, is necessary for the accumulation of the XA21 protein and for XA21-mediated resistance [74]. It is hypothesized that XA21 activates OsXB3 via phosphorylation, and the activated OsXB3 may induce the degradation of negative regulators of XA21-mediated defense signaling. OsXB25, which belongs to the plant-specific ankyrin repeat (PANK) family, promotes weak phosphorylation by XA21 [75]. Since the downregulation of OsXB25 results in a reduction in XA21 in adult rice plants, OsXB25 appears to play a role in the stability of XA21 [75]. Activated and accumulated XA21 is cleaved to release the intracellular kinase domain that possesses a nuclear localization sequence [76]. The cleaved intracellular domain binds to the Class IIA-type transcription factor OsWRKY62, a negative regulator of XA21-mediated immunity [77].

2.6. Pi-d2-mediated Immunity

Pi-d2 (previously named Pi-d(t)2) is a plant R protein involved in antifungal immunity [78]. *Pi-d2* encodes an RK protein with a predicted extracellular domain of mannose-specific binding lectin (B-lectin) and an intracellular kinase domain. Although the corresponding ligand is still unknown, transgenic rice expressing the Pi-d2 transgene confer race-specific resistance against the rice fungus *Magnaporthe oryzae* strain ZB15. Chen *et al.* have shown that the intracellular kinase domain phosphorylates OsPUB15, which possesses E3 ubiquitin ligase activity [79]. The phosphorylated OsPUB15 acts as a functional E3 ubiquitin ligase to mediate the degradation of unknown substrate(s) and may regulate cell death and disease resistance to rice blast fungus [79].

3. GENETIC ENGINEERING USING PRRS

One of the goals of genetic engineering is improving plant resistance to pathogenic bacteria and fungi. In several crops, the transfer of PRRs between different plants is a promising approach for engineering broad-spectrum and durable disease resistance [80, 81]. EFR is effective in various plants for strengthening broad-spectrum bacterial resistance [80]. Lu *et al.* recently generated transgenic rice plants and cultured cells expressing *Arabidopsis* EFR driven by the CaMV 35S promoter [80]. These transgenic plants and cultured cells show increased ROS production and expression of defense-related genes, such as OsPBZ1, in response to elf18 [80]. Moreover, they exhibit significantly enhanced resistance to *Xoo* after pretreatment with elf18, and the level of resistance to *Xoo* clearly depends on the amount of EFR protein [80]. Interestingly, XA21 is phylogenetically closely related to EFR and is in the same sub-family XII of LRR-RKs, the most expanded sub-family in rice [82]. Indeed, the ectodomain of EFR is functional for sensing elf18 response in rice because rice plants expressing a chimeric receptor between the ectodomain of EFR and the transmembrane and intracellular domains of XA21 successfully respond to elf18 treatment [83]. This receptor can bind to AtBAK1 and AtBIK1, which are major downstream molecules of EFR, to trigger MTI against the bacterium *Pseudomonas syringae* pv. *tomato* DC3000 [82]. On the other hand, *Arabidopsis* PLL4/PLL5 and AtXB24, which are orthologs of OsXB15 and OsXB24, respectively, are required for EFR-mediated

immunity in *Arabidopsis* [82]. Thus, immune signaling underlying RK is conserved between rice and *Arabidopsis*. The approach used in rice is useful in other plants. Schoonbeek *et al.* generated transgenic wheat plants expressing *Arabidopsis* EFR driven by the rice actin promoter, and these plants demonstrate enhanced induction of defense-related genes, callose deposition, and resistance against the cereal bacterial pathogen *Pseudomonas syringae* pv. *oryzae* [84]. Banana is an important staple food for 100 million people in east Africa [81]. Banana *Xanthomonas* wilt (BXW), caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (*Xcm*), is the most devastating disease for banana in east and central Africa [81]. Tripathi *et al.* constitutively expressed the rice Xa21 gene driven by the maize Ubiquitin promoter in banana, resulting in enhanced resistance against *Xcm* [81]. Because the immune components acting downstream of distinct LRR-RK-type PRRs may be highly conserved between monocots and dicots, the genetic engineering of plants using PRRs is a useful strategy to enhance resistance to pathogens in crops.

4. SYMBIOTIC ASSOCIATIONS INVOLVING RICE

4.1. Arbuscular Mycorrhizal Fungi Signal to Host Plants

Many plant species have developed mutually beneficial interactions with diverse microorganisms (called symbioses). Microorganisms capture nutrients that are necessary for plant growth and trade these nutrients with the plant in exchange for photosynthetically fixed carbon sources that are required for survival and propagation [11]. Legume roots respond to rhizobial bacteria-secreted Nod factor, which is necessary for the formation of root nodules, within which nitrogen-fixing bacteria reside [11]. Rhizobacteria-derived lipochitooligosaccharides (LCO; i.e., chitins in which the non-reducing end is *N*-acylated) act as Nod factors in establishing legumes-rhizobia symbioses [85]. However, non-legume plant species generally do not associate with nitrogen-fixing rhizobial bacteria. AMF have associated with plants for more than 400 million years and have symbiotic relationships with 70–90% of land plant species, including rice, but not with species of *Brassicaceae* or *Chenopodiaceae*, among others [12]. As AMF are obligate biotrophs, they depend on living host plants to complete their life cycle including generating of spores [12]. AMF produce Myc factors that are also LCOs and comprise chitin modified by N-acylation and sulfation [11, 85]. The perception of AMF- or rhizobia-derived signals triggers early signal transduction, mediated by a common symbiosis pathway, which contains common symbiosis genes that are required for plant symbiosis with both AMF and rhizobia (e.g. *DMI1*, *DMI2* and *DMI3* (does not make infections 1, 2 and 3)) and Ca^{2+} spiking in the nucleus [11, 12]. This pathway then promotes AMF root invasion.

4.2. Lipochitooligosaccharides

Associations between symbiotic fungi and host plants are achieved via chemical communication in the rhizosphere. Strigolactones, i.e., terpenoid lactones derived from carotenoid metabolism, are released from host plant roots and promote the germination of AMF spores [86]. Although the genome of the AMF species *Rhizophagus irregularis* has been investigated, a fungal homolog of known strigolactone receptors in plants has not been detected [87]. Therefore, the mechanism of strigolactone perception by AMF is still unknown. In turn, AMF produces and secretes Myc-LCO signaling molecules, which contain a chitin backbone with an acyl group at the non-reducing end [88]. The perception of Myc-LCO induces the activation of a common symbiosis signaling pathway, including Ca^{2+} spiking in rhizodermal cells and the expression of host plant genes in *M. truncatula* [11, 85, 86, 88], resulting in starch accumulation in roots and lateral root formation prior to colonization [89].

In legumes, Nod factor receptors have been identified in the last decade. NFR1 and NFR5 act as Nod factor receptors in *Lotus japonicus* [90-92]. Recent studies have shown that NFR1 is required for the establishment of Myc-LCO in *L. japonicus* [93]. Interestingly, rice OsCERK1 has the highest homology to NFR1 and the AMF *R. irregularis* cannot form internal hyphae or arbuscules in the roots of oscerk1 mutants [94]. Thus, rice OsCERK1 is required not only for chitin-triggered immune responses, but also for AM symbiosis, indicating that OsCERK1 contributes to opposing biological processes—defense and symbiosis [93, 94].

4.3. Short-chain Chitin Oligomers (CO4/5)

In addition to Myc-LCO, AMF also utilize short-chain chitooligosaccharides (COs). Short-chain COs are included in AMF germinated spore exudates, and treatment with the synthetic strigolactone analogue GR24 induces CO4 and CO5 production in *R. irregularis* spores [95]. In *M. truncatula,* although both short-chain COs and long-chain COs have similar chitooligosaccharide backbones, host plants exhibit different responses. Short-chain COs can trigger Ca^{2+} spiking in the nucleus, with CO4 and CO5 exhibiting the maximum activity, and not with the long-chain CO8, a known MAMP [95]. An interesting question is how host plants use different receptors to recognize symbiotic fungi-derived short-chain and pathogenic fungi-derived long-chain COs and activate different pathways. Ca^{2+} spiking triggered by CO4 is dependent on proteins in the common symbiosis signaling pathway that includes does not make infections 1 and 2 (DMI1/DMI2), but not Nod factor perception (NFP), the putative *Sinorhizobium meliloti* Nod factor receptor [95]. CO4 can induce Ca^{2+} spiking in the nucleus of rice as well as legumes, but Myc-LCO cannot [96]. AMF and GSEs induce changes in root structure; in particular, they promote lateral root outgrowth [97]. CO4 and Myc-LCO-dependent lateral root outgrowth is independent of the common symbiosis pathway [12].

5. CALCIUM SIGNALING IN RICE IMMUNITY AND SYMBIOSIS

MAMP perception by PRRs induces a calcium flux across the plasma membrane and concomitant membrane depolarization [98]. This response is one of the earliest signaling events that occurs after MAMP perception [4], and is required for the full activation of NADPH oxidase-mediated ROS production [49]. Using the calcium reporter Yellow Cameleon 3.6, it was reported that, in a single guard cell of *Arabidopsis*, flg22 induces a dynamic increase of cytosolic free calcium within two minutes, which consists of multiple short spikes (sharp periodic increases in the calcium concen-

tration) [98]. Each response has an average of 33.6 ± 1.6 peaks for up to 45 min [98]. Therefore, the prolonged single oscillation observed in aequorin reflects unsynchronized oscillations in surrounding cells [98]. In addition, a pharmacological approach hints at calcium stores and channel types involved in Ca^{2+} spiking in the cytosol. Treatment with EGTA and La^{3+}, a Ca^{2+} chelator and a plasma membrane Ca^{2+} channel blocker, respectively, completely abolished the flg22-induced oscillations [98], demonstrating the requirement of Ca^{2+} influx from extracellular sources. U73122 (an inhibitor of phospholipase C) and neomycin (an inhibitor of PLC and translation) block the synthesis of inositol trisphosphate (IP3), which triggers the release of Ca^{2+} from the ER and vacuoles. Since these two inhibitors completely abolish flg22-induced Ca^{2+} oscillations [98], it is likely that phospholipid signaling plays a key role in flg22-induced Ca^{2+} oscillations. Moreover, the NADPH oxidase inhibitor DPI partially reduces this response, indicating that NADPH oxidase-dependent ROS production also contributes to Ca^{2+} influx. As described previously, BIK1 directly phosphorylates RBOHD after MAMP recognition [49]. This phosphorylation may induce the activation of RBOHD and calcium-dependent protein kinase (CDPK)-mediated phosphorylation. At the same time, unknown Ca^{2+} channel(s) is/are activated at the plasma membrane and induce Ca^{2+} influx to the cytoplasm [49].

Symbiosis-induced Ca^{2+} spiking differs from MAMP-induced Ca^{2+} spiking in *Arabidopsis*. The spiking during symbiosis occurs in the nucleus and around the nucleus from about 10 minutes until 4 hours after Nod factor treatment [99, 100]. Although the mechanisms that link the perception of symbiotic signatures by receptors to Ca^{2+} spiking in the nucleus remain unclear, unidentified secondary messengers are predicted to regulate calcium channels on the nuclear membrane. Two cation channels, CASTOR and POLLUX, have been identified as essential factors for symbiotic Ca^{2+} spiking in the nucleus during rhizobial infection and nodule organogenesis or arbuscule formation in *L. japonicus* [11, 12]. Moreover, this nuclear Ca^{2+} spiking decoded by a calcium and calmodulin-binding kinase (CCaMK), and gain-of-function CCaMK mutations activate nodule development without rhizobia inoculation and Nod factor treatment. CCaMK interacts with CYCLOPS in the nucleus and is essential for nodule organogenesis or arbuscule formation [11]. Singh *et al.* showed that CYCLOPS, a direct substrate of CCaMK, is a DNA-binding transcriptional activator that binds DNA in a sequence-specific and phosphorylation-dependent manner and transactivates the NODULE INCEPTION (NIN) gene, an essential factor that positively regulates nodulation processes [101]. In the rice genome, CASTOR, POLLUX, CYCLOPS, and CCaMK are highly conserved. All four mutants display impaired AMF interactions and alter AMF-specific gene expression patterns [102], implying functional conservation of symbiotic signaling mechanisms between legumes and rice. However, little is known about the commonality of the common symbiosis pathway between rice and legumes. In addition to Ca^{2+} spiking in the nucleus, Nod factor can also trigger a transient influx of calcium in the plasma membrane, but it is different from flg22-induced cytoplasmic Ca^{2+} spiking [99]. The calcium channels for Nod factor-induced calcium influx on the plasma membrane remain unknown. Thus, Nod factor perception differentially activates the two signaling pathways, i.e., Ca^{2+} spiking in the nucleus and Ca^{2+} influx in the cytoplasm, and both are important for efficient symbiosis.

6. MICROORGANISMS THAT PROMOTE PLANT GROWTH

All plants are thought to exhibit symbiotic relationships with microorganisms (called endophytes), and these relationships have profound effects on nitrogen fixation, plant immunity, and growth [103, 104]. *Azospirillum*, a well-characterized genus of plant growth-promoting rhizobacteria, are nitrogen-fixing bacteria found in close association with host roots and stems. *Azospirillum* strains as well as other plant growth-promoting rhizobacteria have been identified in several plant species. *Zemrany et al.* reported that the inoculation of seeds with *Azospirillum lipoferum* CRT1 induces beneficial changes in maize root morphology [105]. Inoculation with a strain of *A. lipoferum* in the roots of rice plants promotes early tillering and reproductive growth, although the total dry weight and nitrogen content of the rice plants are not affected [104]. Similarly, another strain, *Azospirillum* sp. B510, has a beneficial effect on rice production [106]. Investigations under field and greenhouse conditions have shown that rice plants inoculated with *Azospirillum* sp. B510 have increased tiller numbers, shoot biomass, and growth of newly generated leaves. Even in paddy fields, OsCCaMK, a component of the common symbiosis pathway, plays a key role in symbiosis and N_2 fixation in rice. *Alphaproteobacteria* are remarkably decreased in a recessive homozygous mutant of *OsCCaMK* compared with a dominant homozygous mutant of *OsCCaMK* in paddy fields. Moreover, the gene dosage of *OsCCaMK* is related to shoot length, tiller number, and plant weight in upland conditions [107]. In a knockout mutant of *CCaMK*, plant growth and N2 fixation activity in root microbiomes are decreased [108]. The application of knowledge acquired from basic research on symbiosis will potentially facilitate the identification of genetic traits that improve yield and reduce pathogen invasion, and will thus bring considerable innovations to agriculture.

7. CONCLUDING REMARKS

Detailed studies of rice PRRs and MAMPs over the past two decades have improved our understanding of rice immune responses to pathogens (Fig. **1**). Notably, fungal chitin-triggered immunity via LysM receptors is well characterized (Fig. **2**). However, there are still large gaps in our knowledge of the signaling mechanisms that mediate the perception of symbiosis signatures and subsequent activation of Ca^{2+} spiking in the nucleus. Of particular interest is the evolutionary process that gave rise to symbiosis from plant immunity. Both pathways employ the same receptor kinase, OsCERK1, to transmit signals to intercellular proteins. Future challenges include demonstrating direct substrates of OsCERK1 in symbiosis signaling, and determining the distribution of distinct signals from the same receptor. It is also necessary to define both calcium and ROS signaling pathways to fully understand the molecular basis of immune and symbiosis pathways. This basic knowledge will promote genetic engineering of crops to feed the expanding global population.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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