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MINI-REVIEW

Molecular biology of disease resistance in rice

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Rice is one of the most important staple foods for the increasing world population, especially in Asia. Diseases are among the most important limiting factors that affect rice production, causing annual yield loss conservatively estimated at 5 %. More than 70 diseases caused by fungi, bacteria, viruses or nematodes have been recorded on rice [*68*], among which rice blast (*Magnaporthe grisea*), bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) and sheath blight (*Rhizoctonia solani*) are the most serious constraints on high productivity [*68*]. Resistant cultivars and application of pesticides have been used for disease control. However, the useful life-span of many resistant cultivars is only a few years, due to the breakdown of the resistance in the face of high pathogenic variability of the pathogen population. Use of pesticides is costly as well as environmentally undesirable. Thus, there is a need to develop strategies providing durable resistance, giving protection for a long time and over a broad geographic area. Among such new strategies, systemic acquired resistance (SAR) is an example of a defense mechanism offering long-lasting disease resistance against a broad-spectrum of pathogens, and is promising for sustainable rice production in the future. New information and knowledge gained from research on the molecular biology of SAR as well as disease resistance gene-mediated defense responses will undoubtedly provide new insights into the nature of rice disease resistance, which in turn offers opportunities for creating new rice varieties with high resistance against multiple diseases. To this end, understanding the molecular biology of disease resistance in rice is a prerequisite.

In recent years, rice has been recognized as a genetic model for molecular biology research aimed toward understanding mechanisms for growth, development and stress tolerance as well as disease resistance [*34*]. Rice as a model crop is a fortuitous situation since it is also a crop of world significance. Rice is an attractive model for plant genetics and genomics because it has a relatively small genome. Considerable progress has been made in rice towards cloning and identification of disease resistance genes, characterization of defense responses, and elucidation of signal transduction leading to activation of defense responses [*93*]. The results of this work provide new insights into the molecular basis of disease resistance in rice. In this review, the focus is on the progress towards the understanding of the molecular biology of disease resistance in rice against blast, bacterial leaf blight and sheath blight.

DISEASE RESISTANCE GENES IN RICE

The inheritance of resistance against blast and bacterial leaf blight diseases of rice has been extensively studied. Major genes from various resistant donors have been identified and to date more than 30 loci conditioning resistance against blast and 25 against bacterial leaf blight have been found [*33*, *43*]. Some of these resistance genes or loci have been widely used in rice breeding programs, and some of them have been mapped to their chromosomal locations and are subject to cloning efforts. To date, four resistance genes, *Xa21* and *Xa1* to bacterial leaf blight, and *Pib* and *Pi-ta* to blast, have been cloned and studied in detail at the molecular level [*7*, *86*, *97*, *108*]. Genetic variability for high levels of resistance against sheath blight is lacking in both cultivated rice and its wild relatives [*6*]; therefore cloning of genes for sheath blight resistance is lagging.

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Abbreviations used in the text: AFLP, amplified fragment length polymorphism; BTH, ben zothiadiazole; INA, dichloroisonicotinic acid; LOX, lipoxygenase; LRD, leucine-rich domain; LRR, leucine rich repeats; MAP kinase, mitogen-activated protein kinase; PLD, Phospholipase D; PR, pathogenesis-related; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; RGA, resistance gene analogues; ROI, reactive oxygen intermediates; SA, salicylic acid; SAR, systemic acquired resistance; *Xoo*, *X.oryzae* pv. oryzae.

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Xa21 was transferred to the rice cultivar IR24 from the wild species *Oryza longistaminata* and was found to confer resistance against many known *X. oryzae* pv. *oryzae (Xoo)* races in India and the Philippines [*39*]. *Xa21* was cloned by a map-based cloning strategy and found to encode a receptor kinase-like protein carrying leucine-rich repeats (LRR) in the putative extracellular domain, a single transmembrane domain and a serine/threonine kinase intracellular domain [*86*]. Compared with the proteins encoded by other cloned plant disease-resistance genes, the structure of Xa21 protein is unique because it contains the extracellular receptor LRR domain and the intracellular kinase domain. *Xa21*-mediated resistance increases progressively from the susceptible juvenile two-leaf stage through later stages, with 100 % resistance at the adult leaf 9/10 stage. Expression of *Xa21* is independent of plant developmental stage, infection with *Xoo*, or wounding. These results suggest that *Xa21*-mediated resistance is controlled by development either post-transcriptionally or by other factors [*11*]. Transgenic rice plants expressing the cloned *Xa21* showed the same spectrum of resistance as did that of the donor line [*88*, *89*, *93*].

Xa21 is a member of a small multigene family containing seven members; these members can be grouped into two classes based on sequence similarity [*85*]. The *Xa21* class contains members *Xa21*, D and F; and class A2 contains A1, A2, C and E. The identity of nucleotide sequence within each class is very high (98 % for *Xa21* class and 95·2 % for A2 class), but only 63·5 % of the identity was observed between the two classes [*85*]. One member of the *Xa21* family, *Xa21D*, encodes a receptor-like protein carrying LRR motifs in the presumed extracellular domain but lacks the transmembrane and cytoplasmic kinase domains [*94*]. In transgenic rice plants, *Xa21D* conferred partial resistance to *Xoo* at an intermediate level compared with that of *Xa21* but showed the same spectrum of resistance as *Xa21*. However, other members (A1, A2, C, E and F) did not confer any observable resistance in transgenic plants [*94*]. These results suggest that the extracellular LRR domain of *Xa21D* is involved in pathogen recognition. Moreover, a highly conserved 233-bp sequence was identified among all seven *Xa21* family members [*85*] and 15 transposon-like elements were found within the gene cluster [*84*], suggesting that these elements may play a role in the diversification of the *Xa21* family members, probably by duplication, recombination and transposition.

Xa1 confers a high level of specific resistance to race 1 strains of *Xoo* in Japan. *Xa1* was mapped on rice chromosome 4 and cloned recently by a map-based cloning strategy [*108*]. The *Xa1* gene encodes a protein containing several regions, with similarity to the deduced polypeptide domains of other disease-resistance genes such as *Arabidopsis RPS2* and *RPM*, tobacco *N* and flax *L6*. The Xa1 protein contains two motifs of NBS in the amino-terminal half and an LRR domain in the carboxyl-terminal half of the molecule. The LRR domain is composed of six almost perfect repeats, each 93 amino acids long, with 62–99 % simple homology to each other at the amino acid level. Expression of *Xa1* is induced upon wounding and infection by both compatible and incompatible strains of *Xoo* [*108*].

The dominant gene *Pib* confers high resistance to most Japanese races of *M. grisea*, the causal agent of blast disease. *Pib* is located at the distal end of the long arm of chromosome 2. The *Pib* gene was cloned recently and found to encode a polypeptide of 1251 amino acids [*97*]. The deduced protein encoded by *Pib* contains an NBS region and C-terminal LRRs but no distinct transmembrane domain; thus, the *Pib* gene belongs to the NBS–LRR class of plant disease resistance genes. Interestingly, the *Pib* protein contains an N-terminal duplication of the conserved kinase 1a (P-loop), 2 and 3a domains of NBS region and eight cysteine residues clustered in LRR 7 and 8. The significance of the duplication and the clustered cysteine is unknown. Expression of *Pib* is induced by infection with both incompatible and compatible strains of *M. grisea* and altered environmental conditions such as temperature and darkness [*97*].

The *Pi-ta* gene sequence predicts a 928 amino acid protein that is cytoplasmic and contains an NBS and a leucine-rich carboxyl terminus [*7*]. The leucine-rich domain (LRD) in the predicted Pi-ta protein contains an irregular highly imperfect repeating structure with the consensus LxxLxxL. Sequence analysis of 11 alleles of *Pi-ta* from resistant, intermediate, and susceptible varieties showed that the Pi-ta proteins in susceptible varieties have one amino acid substitution compared with that in the resistant varieties. More than 40 % of the transgenic plants biolistically transformed with the cloned *Pi-ta* gene or cDNA conferred resistance or intermediate resistance against blast, confirming the identity of the cloned *Pi-ta*.

Interestingly, an *R* gene-like gene, *RPR1*, was cloned by differential display from probenazole-treated rice plants and was found to encode an NBS–LRR protein, sharing structural similarity with the NBS–LRR class of *R* genes [*72*]. *RPR1* was mapped to a region close to the location of *Pi-ta* on chromosome 11. These features suggested that *RPR1* might act as a resistance gene. The expression of *RPR1* in rice can be induced by both treatments with SAR inducers such as salicylic acid (SA) or benzothiadiazole (BTH) and by infection with *M. grisea*.

In addition to these cloned disease resistance genes from rice, more than 15 major genes and dozens of quantitative trait loci (QTL) associated with resistance against blast and bacterial leaf blight diseases have been identified and localized through the use of molecular marker technologies such as restriction fragment length polymorphism (RFLP) and amplified fragment length

polymorphism (AFLP) [*13*, *51*, *60*, *96*, *111*, *112*]. Moreover, some resistance gene analogues (RGA) were also cloned by PCR using degenerate primers that were designed based on the conserved regions of the cloned disease resistance genes [*31*, *49*, *53*]. Results of subsequent studies showed that some of these RGAs are linked tightly to known RFLP or RAPD molecular markers in the genetic maps of the rice chromosome [*31*, *49*, *53*]. Results from the research on these well-mapped resistance genes, QTLs and RGAs, which are currently being studied extensively, will provide a new insight into the molecular biology of disease resistance genes in rice as well as their function and evolution.

DEFENSE RESPONSES AND DEFENSE-RELATED GENES IN RICE

Direct assessment of the biochemical and physiological changes during disease development has identified some putative defense responses in rice disease resistance against blast, bacterial leaf blight and sheath blight diseases. On the other hand, the use of genetic and molecular biology technologies in research on rice disease resistance has led to isolation and identification of a number of defense-related genes that might be involved in disease resistance. However, the molecular biology of disease resistance in rice is not as well defined as in the dicot model plants such as Arabidopsis and tobacco.

Phytoalexins

Phytoalexins are low-molecular weight antimicrobial compounds that are synthesized and accumulated in plant tissues upon pathogen attack. In rice, two different types of phytoalexins, diterpenes and phenolics have been isolated and identified. The diterpene isoprenoid phytoalexins, synthesized via the isoprenoid pathway, include momilactone A and B, and oryzalexin A–F [*10*, *46*, *78*]. Only one phenolic phytoalexin has been found in rice so far, the flavanone sakuranetin, which is formed in rice in response to UV irradiation or blast infection [*44*]. These phytoalexins have been shown to be effective in inhibiting *in vitro* growth by the blast fungus *M. grisea* and they accumulate more rapidly and to larger quantities in the incompatible interaction with the pathogen than in the compatible interaction. They also accumulate in response to treatments with chemicals such as dichlorocyclopropan and elicitors prepared from cell walls of the blast fungus [*8*, *9*, *44*, *104*]. Consistent qualitative and quantitative differences were found among rice cultivars in the phytoalexins produced and there was a strong correlation between the accumulation of the phytoalexins sakuranetin, momilactone and oryzalexin, and resistance to blast disease [*20*].

The rate-limiting enzyme in the isoprenoid pathway is 3-hydroxy-3-methyglutaryl coenzyme A reductase (HMGR); a gene encoding HMGR was cloned from rice and its expression was found to be strongly and rapidly induced in suspension cells by a fungal cell wall elicitor from the blast fungus [*61*]. Momilactone A at elevated levels was also found in lesion mimic mutants that exhibited significant enhanced resistance to blast [*87*]. These results indicated that the induction of phytoalexin biosynthesis upon pathogen attack may play a key role as a component of the inducible defense mechanism in rice.

Pathogenesis-related (PR) proteins

PR proteins have been well studied as a major defense response in several dicot plants, both in *R* gene-mediated resistance and in SAR. The roles of *PR* genes in disease resistance have been suggested by the tight correlation between expression levels of *PR* genes and disease resistance and by the observation of enhanced disease resistance in the transgenic plants overexpressing certain *PR* genes. Several defense-related PR-like genes have been cloned from rice. These include genes encoding for the PR-3 group of chitinases such as *RC24*, *RCH10* and *Rcht2* [*2*, *40*, *103*, *114*], the PR-2 group of glucanases such as *Gns1* [*81*], the PR-5 group of thaumatin-like proteins such as *Pir2* [*70*, *91*], PR-9 group of peroxidases such as *POX8.1* and *POX22.3* [*17*], the acidic PR-1 group gene *OsPR1a* [*1*], the PR-10 group genes *RPR10a*, *RPR10b*, and *RPR10c* [*57*], the phenylalanine ammonia-lyase gene [*113*], the probenazole-induced *PBZ1* [*58*] and the *Pseudomonas syringae* pv. *syringae*-induced *Rir1* and *Pir7b* [*56*, *71*, *100*]. Hundreds of differentially expressed cDNAs associated with BTH-induced SAR have been cloned and identified by PCR-based suppression subtractive hybridization and some of these cDNAs may represent defense-related genes (Song and Goodman, unpublished work). Moreover, several defense-related genes induced by a fungal elicitor from the rice blast fungus [*41*] and a TNP2-like gene *Rim2* induced by infection with *M. grisea* [*29*] were also cloned and identified by differential display-PCR technique.

Chitinases and β-1,3-glucanases can hydrolyze chitin and β-1,3-glucan, respectively, which are major components of fungal cell walls. Hydrolysis of these fungal cell-wall constituents leads to the inhibition of the growth of several fungi *in vitro*. In *Rhizoctonia solani*-infected rice plants, the activities of both chitinase and glucanase were increased and expression of the corresponding genes was also induced [*2*]. Expression of chitinase and glucanase genes was also activated by infection with blast fungus and treatments with elicitors and chemicals [*27*, *40*, *75*]. Most importantly, transgenic rice plants overexpressing either chitinase alone or in combination with glucanase

showed enhanced disease resistance against blast and sheath blight [52, 63]. A thaumatin-like protein from rice shows antifungal activity *in vitro* and expression of its gene was induced by infection by both *M. grisea* and *R. solani* [75, 91] and by jasmonic acid [76]. Overexpression of the thaumatin-like protein gene or a chitinase gene in transgenic rice plants enhanced resistance against sheath blight [18, 19]. These results suggest that chitinase and glucanase as well as thaumatin-like protein may play a role in rice resistance against fungal diseases such as blast and sheath blight.

The defense response in rice against bacterial leaf blight was characterized by an increase in peroxidase activity [109]. Two peroxidase genes, *POX8.1* and *POX22.3*, were expressed during incompatible interactions [17]. Increases in activities of specific extracellular peroxidases were spatially and temporally associated with a decrease in the rate of pathogen multiplication and spread, suggesting an active role for peroxidase in resistance against bacterial leaf blight. Genes for peroxidases were also induced by infection with *M. grisea* [55, 75].

Systemic resistance in rice against blast can be induced both biologically and chemically. Pre-inoculation of lower leaves with avirulent *M. grisea* or the non-pathogens *Bipolaris sorokiniana* and *P. syringae* induced disease resistance, resulting in systemic protection against blast [54, 82]. Likewise, enhanced disease resistance against blast was also induced by treatment with SA, BTH and dichloroisonicotinic acid (INA) [22, 72, 75, 77], which are well-documented SAR activators in dicot plants. Probenazole, known as an effective fungicide in protecting rice plants from blast infection [101], was demonstrated to function through disease resistance induced in the host plants since it shows only a weak direct effect on the fungus [58, 101]. Moreover, wounding of the lower leaf on young rice seedlings also induced systemic resistance against blast, probably through jasmonic acid [74]. BTH and harpin elicitor, the secreted protein encoded by an *hrp* gene of *P. syringae*, have also been shown to induce systemic resistance against bacterial leaf blight [15, 36].

In the case of systemic resistance induced by *P. syringae*, harpin encoded by an *hrp* gene is the main factor for inducing systemic resistance in rice against bacterial leaf blight and the peptide containing the N-terminal 137 amino acids is sufficient for inducing SAR [36]. Since *hrp* gene clusters are widespread in *P. syringae*, enhanced resistance against bacterial leaf blight can be induced by different pathovars of *P. syringae* [36]. Another factor in *P. syringae*, which is responsible for inducing systemic resistance in rice, is syringolin. The syringolins constitute a family of structurally related compounds that are secreted by *P. syringae* pv. *syringae* under certain culture conditions [98, 99]. Exogenous application of syringolins can induce disease resistance and activate defense responses both in intact rice leaves and in suspension cells [25, 98]. This may, therefore, represent one of the molecular determinants of *P. syringae* by which rice plants can perceive and respond. The incompatible strain N1141, but not the compatible strain H8301 of *Pseudomonas avenae*, can induce defense responses in rice. Recently, it was demonstrated that the flagellin existing in the incompatible strain of *P. avenae* but not in the flagellin of the compatible strain is the determinant of the bacteria required for the induction of the defense responses and hypersensitive cell death in rice [12].

Several defense-related genes that are associated with *P. syringae* pv. *syringae*-induced resistance in rice have been cloned and identified. *Pir1a* and *Pir1b* were induced locally but not systemically in rice leaf tissue upon inoculation with *P. syringae* pv. *syringae* or *M. grisea*, and by treatment with INA [56, 73]. *Pir1a* was found to encode a 107 amino acid protein that is relatively rich in glycine and proline. The Pir1a protein is secreted from rice protoplasts transiently and accumulates in the cell wall compartment of rice leaves upon inoculation with *P. syringae* pv. *syringae*, suggesting that it may be a cell wall structural protein, like hydroxyproline-rich glycoproteins, proline-rich proteins and glycine-rich proteins and thus play a role in resistance via reinforcement of the cell walls to invading pathogen [56]. Transgenic rice plants consitutively expressing *Pir1b* gene showed enhanced resistance against blast disease [73]. *Pir7b*, another defense-related gene associated with *P. syringae* pv. *syringae*-induced resistance, encodes an α/β hydrolase fold protein with esterase activity [71, 100]. Expression of the *Pir7b* gene was specifically induced by *P. syringae* pv. *syringae* in rice leaf and by syringolin in suspension cells [25, 71] as it was not induced by treatment with INA and BTH, which induced resistance [75, 77].

The probenazole-induced *PBZ1* encodes a protein with significant homology at the amino acid level to the intracellular PR proteins and is induced sooner by infection with incompatible race of the blast fungus than that with compatible race [58]. Recently, an INA- and BTH-induced gene *RCI1* was isolated and found to be induced by INA or BTH but not by *P. syringae* pv. *syringae* [77]. However, the functions of *PBZ1* and *RCI1* in rice disease resistance remain to be determined.

SIGNAL TRANSDUCTION IN RICE DISEASE RESISTANCE

Recognition between R and Avr proteins

The cloned *Xoo* resistance genes *Xa21* and *Xa1* represent two different types of disease resistance genes but both are classical *R* genes. *Xa1*, a member of the NBS–LRR class, is predicted to encode an NBS–LRR protein residing

within the cytoplasm. *Xa21*, a member of the LRR-kinase class, is likely to be a transmembrane protein with the LRRs exposed extracellularly. Therefore, it is likely that the rice plant can recognize avirulence proteins of *Xoo* both extra- and intracellularly via different types of *R* genes. Transgenic rice plants consitutively expressing *Xa21D* gene, which contains the extracellular LRR domain but lacks the cytoplasmic kinase and the transmembrane domains, only conferred partial race-specific resistance [*94*]. Most recently, it was observed that several defense responses were initiated in transgenic rice cells expressing a fusion gene composed of the extracellular LRR and transmembrane domains of the Arabidopsis receptor kinase *BRI1* and the serine/threonine kinase domain of *Xa21* upon treatment with brassinosteroids, which is the ligand for the *BRI1*-encoded protein kinase [*28*]. These results indicated that the extracellular LRR domain of the Xa21 protein functions in recognition of the *Xoo* Avr proteins and its intracellular serine/threonine kinase domain transmits the signal to activate the defense responses.

Two *avr* genes (*avrXa7* and *avrXa10*) have been identified in *Xoo* [*30*] and recently it was demonstrated that AvrXa10 protein contains a conserved transcriptional activation domain in the C terminus and this domain is required for its avirulence function [*116*, *117*]. This result led to a hypothesis that the AvrXa10 might enter the rice nucleus and alter transcriptional activation of host genes, possibly the *R* gene directly. This idea is consistent with the observation that *Xa1* was induced by inoculation with the incompatible bacterial strain [*108*]. Significantly, it was demonstrated that the virulence factor AvrXa7 of *Xoo* is a type III secretion pathway-dependent nuclear-localized double-stranded DNA-binding protein [*106*]. Recently, the *avr* genes were found to contribute differently and specifically to *Xoo* aggressiveness on rice [*3*, *92*]. How the Avr protein is delivered into rice cells and whether it is delivered through the *hrp*-encoded type III secretory system as that in *P. syringae* and *X. campestris* are not yet clear. Several *hrp* genes have been cloned and identified in *Xoo* [*115*]; further study of these genes will help to address these questions.

The predicted proteins of the cloned blast-resistance genes *Pib* and *Pi-ta* belong to the NBS–LRR class of disease-resistance genes, and are most likely to localize in cytoplasm [*7*, *97*]. In the yeast two-hybrid assay, the product of the *avr* gene *avr-Pita*, AVR-Pita$_{176}$, directly interacted with the 341 amino acid C-terminal LRD polypeptide but not the full length 928 amino acid form of Pi-ta. Interactions were not observed between the 341-amino acid LRD encoded by the susceptible allele and AVR-Pita$_{176}$, and between Pi-ta LRD and the *avr-pita* product. Moreover, transient expression of *Pi-ta* and *AVR-Pita* within rice cells induces resistance. These results suggested that the direct interaction between *Pi-ta* and *avr-Pita* products is most likely to occur inside the rice cell and the recognition domain of Pi-ta resides within the LRD. It was proposed that the AVR-Pita protein is secreted into the rice cell during penetration and interacts with a cytoplasmic Pi-ta receptor initiating a defense response [*7*, *35*, *67*].

Early events in signal transduction after recognition

After initial recognition of the Avr proteins or elicitors, plant cells activate a variety of early signal events, including rapid and transient depolarization of the plasma membrane, alteration of ion channel activities, change in calcium homeostasis, and occurrence of oxidative burst, which occur prior to the transcriptional activation of defense-related genes and appear to be mediated through the regulation of plasma membrane-bound enzymes or proteins [*4*]. Among these early signal events, an oxidative burst, which consists of an increase in reactive oxygen intermediates (ROIs) such as superoxide and hydrogen peroxide (H_2O_2), has been demonstrated to play an important role in signal transduction leading to activation of defense responses [*5*]. Analysis of the oxidative burst, by measuring the generation rate of superoxide and the H_2O_2 level in the incompatible and compatible interactions between rice seedlings and *M. grisea*, showed that an obvious and strong oxidative burst occurred in the incompatible interaction [*21*, *23*]. A rice cDNA clone whose product interacted with the *Xa21* cytoplasmic kinase domain in the yeast two-hybrid system was found to encode catalase B [*15*]. In the transgenic rice cells expressing the fusion gene comprising the extracellular LRR and transmembrane domains of the Arabidopsis receptor kinase *BRI1* and the serine/threonine kinase domain of the *Xa21* gene, treatment with brassinosteroids resulted in accumulation of H_2O_2 [*28*]. These results implied the involvement of the oxidative burst, probably H_2O_2 itself, in the *Xa21*-mediated signal transduction pathway. The rice lesion mimic mutants cdr1 and cdr2 (cell death and resistance) exhibited enhanced disease resistance against blast and also showed elevated levels of H_2O_2 [*87*]. Taken together, it is likely that the oxidative burst might be an important early signal leading to defense responses in rice.

The plasma membrane-bound NADPH oxidase has been demonstrated to play a key role in regulating ROIs production. *N*-acetylchitoheptaose, an oligosaccharide elicitor, induced expression of defense-related genes [*27*, *59*, *62*, *105*]. In rice suspension-cultured cells, *N*-acetylchitoheptaose induced a biphasic oxidative burst, probably through regulation of plasma membrane-bound NADPH oxidase after binding its receptor on the plasma membrane [*32*, *47*, *79*]. Several rice homologues of NADPH oxidase were cloned and identified [*24*, *38*]. However, no direct evidence exists currently to associate

NADPH oxidase with the induction of defense responses. Moreover, it was also demonstrated that small GTP-binding protein and GTPase Rac might be involved in regulation of ROIs production [37, 66]. Several homologues of the rab-specific GDP-dissociation inhibitor were recently identified in rice and found to interact with the small GTP-binding proteins [42].

Protein phosphorylation/dephosphorylation, catalysed by protein kinases and phosphatases, is an important step in signal transduction pathways in plants. Expression of two defense-related genes, *Pir7b* and a class II chitinase *Rcht2*, induced by syringolin and fungal elicitor, respectively, was suppressed by phosphatase inhibitors [1, 25, 40]. However, treatment of rice suspension cells with Ser/Thr protein kinase inhibitor, K-252a, strongly inhibited expression of *EL2*, *EL3* and *PAL* induced by oligosaccharide elicitor, *N*-acetylchitoheptaose, but not chitinase and glucanase [27]. These results suggest that protein phosphorylation and/or dephosphorylation are required for activation of defense responses and that they play a role in the different signal transduction pathways or at the different points of the signal transduction pathway. A mitogen-activated protein (MAP) kinase, *BWMK1*, was recently cloned from rice [26]. Expression of *BWMK1* was induced as early as 4 h after infection with blast fungus and also induced by wounding, suggesting a role in both defense and wound signaling in rice. *OsBIMK1*, another MAP kinase gene in rice, has also been found to be specifically induced in BTH-induced SAR and *R* gene-mediated defense responses (Song and Goodman, unpublished work).

Phospholipase D (PLD), which is involved in the phospholipid signaling, might play a role in the signaling pathways leading to defense responses in plants. In rice cells undergoing resistant response against *Xoo*, PLD was found to distribute on the plasma membranes at regions where bacteria are immediately adjacent to the cell wall [110]. It was suggested that PLD might be involved in the modulation of the magnitude of the defense response by regulating increased localized secretion of defense response compounds.

To elucidate the molecular biology of disease resistance in rice, Song and Goodman (unpublished work) and Xiong *et al*. [102] employed the PCR-based suppression subtractive hybridization method and identified a large number of differentially expressed cDNAs that may be associated with disease resistance responses. Some of these cDNAs may encode MAP kinases, phospholipases C and D, zinc finger proteins, ankyrin-containing proteins, 14-3-3 proteins, calmodulin, G-protein and histone deacetylase, all of which may be involved in the signaling pathways and/or regulation of gene expression in defense responses. However, the precise roles and functions of these genes in disease resistance still remain to be studied in detail.

Sphingolipids, ubiquitous components of the membranes of all eukaryotic cells, and their metabolic products, the ceramides, play important roles in the signal transduction pathway in animals and fungi. Recently, it was demonstrated that cerebrosides, sphingolipid-derived compounds prepared from diverse pathogens including *M. grisea*, induced the accumulation of phytoalexins, cell death, and increased resistance to subsequent infection by compatible pathogens [45, 90]. Since in animal cells sphingolipids modulate various protein kinases, the sphingolipid-mediated signaling pathway may function in plant disease resistance by modulating various protein kinases in a manner similar to that observed in animal systems.

Signaling pathways leading to activation of defense responses in rice

It is well established that SA and the SA-mediated signaling pathway play pivotal roles in *R* gene-mediated disease resistance and SAR in Arabidopsis and tobacco, however, this does not seem to be true in rice. Rice plants normally contain high levels of SA in leaves and shoots but low levels in roots and suspension cells [14]. Correlation between SA level in leaves and the generalized blast resistance among varieties was observed and enhanced resistance against blast was also reported [72, 80]. However, the SA level did not increase in rice plants in response to infection with the non-pathogen *P. syringae* or with the pathogens *M. grisea* and *Xoo* [80]. It is most likely that SA is not a limiting factor in the signaling pathway leading to activation of defense response in rice as it is in other plants such as *Arabidopsis* and tobacco. It is known that *NPR1* is a key regulator in SA-mediated signaling pathway leading to SAR and also involved in signaling pathway leading to induced systemic resistance in Arabidopsis. Transgenic rice plants expressing Arabidopsis *NPR1* showed enhanced disease resistance against *Xoo* [15]. Two *NPR1* rice homologues, sharing 49 and 38 % identities with Arabidopsis *NPR1*, respectively, and two types of *NPR1* interactors, including a proline-rice *NPR1* interactor and four bZIP transcription factors rTGA2.1, rTGA2.2, rTGA2.3 and rLG2, have been isolated [15, 16]. Among the four transcription factors, rTGA2.1 was found to bind to the rice *RCH10* gene promoter and to a *cis*-element required sequence-specifically for SA responsiveness, indicating that these transcription factors are involved in activation of defense-related gene expression in rice [16]. SA was shown to activate expression of defense-related genes by transcriptional activation through the *as*-1 element [21]. Therefore, it is most likely that an SA-mediated signaling-like signaling pathway may exist in rice and that this signaling pathway shares some common components downstream of SA with the SA-mediated signaling pathway. However,

the outline of the pathway remains to be explored in detail.

The activity of lipoxygenase (LOX), an important enzyme in the octadecanoid pathway leading to the synthesis of jasmonic acid, was increased in rice leaves after infection with blast fungus and the increases were significantly higher in the incompatible interaction than in the compatible interaction [65]. The expression of a LOX-encoding gene was induced in the early stage of infection with an incompatible strain of *M. grisea* but only induced to a relatively low level in the late stage of infection with a compatible strain [69]. Treatment with several metabolic precursors and oxygenated fatty acids was shown to induce phytoalexin production in suspension cells and disease resistance against blast [50, 83]. Increased levels of jasmonic acid were observed in rice leaves exogenously treated with INA or wounding, followed by induction of defense-related genes and enhanced disease resistance [74, 75, 76]. In suspension-cultured rice cells, jasmonic acid was required for the elicitor-induced phytoalexin production [64]. These results indicated that the octadecanoid pathway might be activated in rice–*M. grisea* interactions and therefore imply that jasmonic acid signaling pathway may play a role in the activation of defense responses in rice against blast. However, the jasmonate-inducible defense-related genes were activated in rice by infection with *M. grisea* without a concomitant increase in endogenous jasmonic acid levels [76], implying that the involvement of jasmonic acid signaling in rice disease resistance might be complex. Recently, a gene *JAmyb* encoding a Myb transcription factor was isolated from rice [48]. Expression of the *JAmyb* gene was induced after infection with *M. grisea* in resistant and susceptible interactions and the expression level was much higher in susceptible interactions. *JAmyb* was activated rapidly by jasmonic acid or wounding, but not by SA and BTH [48]. Thus, this *JAmyb* gene may be involved in the jasmonic acid-mediated signaling pathways in rice.

PERSPECTIVES

As a result of its importance as a staple food in the world, rice is getting much attention in studies of the molecular biology of disease resistance, which will be helpful in improving rice varieties for high production for our increasing population. Although considerable progress has been made towards understanding the nature of disease resistance genes, defense responses, and the signal transduction leading to activation of defense responses in rice, the whole story is still far from clear. Information is limited compared with the large body of references in other model plant species such as Arabidopsis and tobacco. The completion of the rice genome project, which will result in massive structural genomic data available to the public, and development of new methodologies such as functional geonomics and DNA microarrays that allow global analysis of gene expression will undoubtedly accelerate research on the molecular biology of rice disease resistance.

Although the *Xa21* confers resistance against several races of *Xoo*, most cloned *R* genes only offer resistance to one or few related race(s) or strain(s) of the pathogens. This restricts the use of *R* genes in improvement of rice disease resistance by the means of genetic engineering since the resistance conferred by cloned *R* genes in the genetic engineered rice will be easily overcome due to changes in the pathogen population. With the cloning of several *R* genes from rice [7, 86, 97, 108] and screening of mutants that are altered in disease resistance [87, 107], signaling pathways leading to disease resistance will be elucidated in more detail and genes involved in the pathways will be identified and cloned. These genes will be very useful in the generation of new rice varieties with high resistance (probably durable resistance) against multiple diseases caused by different types of pathogens.

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