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REVIEW

Recent advances in plant immunity: recognition, signaling, response, and evolution

S. HOU*, C. ZHANG, Y. YANG, and D. WU

School of Municipal and Environmental Engineering, Shandong Jianzhu University, Fengming Road 1000, Jinan 250101, P.R. China

Abstract

Innate immune system is employed by plants to defend against phytopathogenic microbes through specific perception of non-self molecules and subsequent initiation of resistance responses. Current researches elucidate that plants mostly rely on cell surface-located pattern recognition receptors (PRRs) and intracellular nucleotide-binding leucine-rich repeat proteins (NB-LRRs) to recognize pathogen-associated molecular patterns (PAMPs) and effector proteins from microbial pathogens, initiating PAMP- and effector-triggered immunity (PTI and ETI), respectively. Some pathogenic bacterial effector proteins are usually secreted into plant cells and play a virulence function by suppressing plant PTI, implying an evolutionary process of plant immunity from PTI to ETI. In the past several years, a great progress has been achieved to reveal fascinating molecular mechanisms underlying the pathogenic recognition, resistance signaling transduction, and plant immunity evolution. Here, we summarized the latest breakthroughs about these topics and offered an integral understanding of plant molecular immunity.

Additional key words: ETI, hypersensitive response, NB-LRR, PTI, receptor-like cytoplasmic kinases, RIN4.

Introduction

A great progress has been achieved in the field of plant immunity during the past few decades. In 1940s, plant pathologist Harold Flor found that plant race-specific resistance against pathogens is dominated by a single gene, and thus introduced the famous "gene-for-gene" hypothesis. Based on his theory, resistance is initiated by specific recognition of phytopathogenic avirulence (*Avr*) gene products by matching plant resistance (*R*) gene products (Flor 1971). Due to the technology innovation in gene cloning, numerous *Avr* genes in phytopathogenic viruses, bacteria, fungi and oomycetes and their cognate *R* genes in varieties of plants were indentified in the following years. The overwhelming majority of *R* genes encode nucleotide-binding leucine-rich repeat (NB-LRR)

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Abbreviations: Adi3 - AvrPto-dependent Pto-interacting protein 3; AGO - argonaute; ATG - autophagy-related protein; ATR13 - *Arabidopsis thaliana* recognized 13; BAK1 - BRI1-associated kinase; BIK1 - *Botrytis*-induced kinase 1; BRI1 - brassinosteroid intensitive 1; CBL - calcineurin B-like protein; CEBiP - chitin elicitor binding protein; CERK1 - chitin elicitor receptor kinase; CDPK - calcium-dependent protein kinase; DAMP - damage-associated molecular pattern; EFR - EF-Tu receptor; EDS1 - enhanced disease susceptibility 1; ETI - effector-triggered immunity; FLS2 - flagellin sensing 2; FRK1 - flg22-induced receptor-like kinase 1; HGA - homogalacturonan; HSP90 - heat shock protein 90; KAPP - kinase-associated protein phosphatase; LRR-RLK - leucine-rich repeats-receptor-like kinase; MAPK - mitogen activated protein kinase; MPK - MAP kinase phosphatase; NB-LRR - nucleotide-binding leucine-rich repeat proteins; NLS - nuclear localization signal; NRIP1 - nuclear-receptor-interacting protein 1; OGs - oligogalacturonides; PAMP - pathogen-associated molecular pattern; PBS1 - avrPphB susceptible 1; PCD - programmed cell death; PEPR1 - PEP receptor 1; PP2C - protein phosphatase 2C; PopP2 - *Pseudomonas* outer protein P2; PPR - pattern recognition receptor; PTI - PAMP-triggered immunity; RAR1 - required for Mla12 resistance protein; RD19 - responsive to dehydration 19; RIN4 - RPM1-interacting protein 4; RIPK - RIN4-interacting receptor-like protein kinase; RLCK - receptor-like cytoplasmic kinase; RLF - receptor like kinase; RLP - receptor like protein; ROS - reactive oxygen species; RPG1-b - resistance to *Pseudomonas syringae* pv. *glycinea* 1b; RPM1 - resistance to *P. syringae pv. maculicola*; RPP13 - *Arabidopsis* resistance to *Peronospora parasitica* 13; RPS2 - resistance to *Pseudomonas syringae* 2; RPS4 - resistance to *Pseudomonas syringae* 4; RRS1-R - resistance to *Ralstonia solanacerum* 1-R; SGT1 - suppressor of the G allele of skp1; T3SEs - type III secreted effectors; TAO1 - target of AvrB operation 1; WAK1 - wall-associated kinase 1.

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* Corresponding author; fax: (+86) 0531 88872717, e-mail: biohou@sdjzu.edu.cn

proteins. Besides, some *R* genes also encode transmembrane receptor-like protein (RLP), transmembrane receptor-like kinase (RLK), cytoplasmic kinase (CK), and proteins with atypical molecular motifs (Martin *et al.* 1993, Jones *et al.* 1994, Song *et al.* 1995, Chu *et al.* 2006). *Avr* gene products are usually delivered into plant cells by a device termed the type III secretion system (T3SS), and thus were named as the type III secreted effectors (T3SEs) (Hueck 1998, Galán and Collmer 1999). Therefore, the classical gene-for-gene resistance based on R protein-dependent T3SEs recognition is also called effector-triggered immunity (ETI) (Chisholm *et al.* 2006).

A few early studies indicated that pretreated bacterial cells devoid of pathogenic activity or purified cell components as elicitors also induced broad-spectrum resistance in plants (Lovrekovich and Farkas 1965, Burgyán and Klement 1979). These general elicitors, such as bacterial oligosaccharides (Stefani *et al.* 1994), lipopolysaccharides (Newman *et al.* 1997), and flagellin (Gómez-Gómez *et al.* 1999), as well as fungal chitin, glycoproteins, glucan, *etc* (Zhang *et al.* 1998, Wan *et al.* 2004, Daxberger *et al.* 2006), are non-specific for particular pathogens but highly conserved for whole classes of microbes. These molecules were named pathogen/microbe-associated molecular patterns (PAMPs/MAMPs). Thus, the general elicitor-induced resistance was termed as the PAMP-triggered immunity (PTI) (Chisholm *et al.* 2006). PTI responses usually include activation of mitogen-activated protein kinase (MAPK) cascades, transcription of resistance-related genes, reactive oxygen species (ROS) production, callose deposition, and stomatal closure (Ligterink *et al.* 1997, Asai *et al.* 2002, Navarro *et al.* 2004, Melotto *et al.* 2006). PAMPs are specifically recognized by plant cell-surface pattern-recognition receptors (PRRs). *Arabidopsis* flagellin sensitive 2 (FLS2) represents one of the most characteristic PRRs, which can specifically perceive bacterial flagellin and trigger downstream resistance signaling (Gómez-Gómez *et al.* 1999). Recently, it was also found that plants can initiate resistance responses similar to PTI through perceiving damage-associated molecular patterns (DAMPs), secondary signal molecules derived from plant cell components after pathogenic attack (Yamaguchi *et al.* 2006).

T3SEs showed avirulence functions in plants expressing the corresponding R proteins but showed virulence functions in plants lacking the corresponding R proteins. It was found that the virulence function of pathogenic T3SEs usually means blocking a plant PTI signaling pathway. The most typical examples come from *Pseudomonas syringae* T3SEs AvrPto, AvrPtoB, HopF2, and HopAI1, which all can suppress the FLS2-mediated PTI through directly targeting different sites in this pathway (Zhang *et al.* 2007, Rosebrock *et al.* 2007, Göhre *et al.* 2008, Xiang *et al.* 2008, Wang *et al.* 2010). Moreover, many T3SEs have also been indentified to inhibit an ETI response (Guo *et al.* 2009). A "zigzag" model was brought forward to elucidate the evolution of plant immunity. The model explained that PTI was primarily evolved to recognize general feature of pathogenic and nonpathogenic microbes, but it was subsequently suppressed by pathogenic evolved T3SEs; R proteins were then evolved by plants to recognize pathogen T3SEs to initiate the advanced ETI response (Jones and Dangl 2006, Chisholm *et al.* 2006).

Here, we highlight the most recent progress of the molecular mechanisms underlying in plant immunity which mainly includes pathogen perception, initiation and transduction of resistance signaling, as well as the evolutionary principles from PTI to ETI.

PAMP-triggered immunity (PTI)

PAMP recognition: The perception of PAMPs/MAMPs by specific PRRs is the first step of plant PTI. A growing amount of PAMPs/MAMPs and their corresponding PRRs have been identified (Table 1). Except for FLS2, the elongation factor EF-Tu receptor (EFR) in *Arabidopsis*, and the Xa21 in rice are two other best-characterized PRRs (Zipfel *et al.* 2006, Lee *et al.* 2009). FLS2, EFR, and Xa21 all belong to the same class of leucine-rich repeat receptor-like kinase (LRR-RLK), encoding a membrane resident RLK with an extracellular LRR domain and a cytoplasmic Ser/Thr kinase domain. Structural and biochemical studies revealed the specific sites of PAMPs which are perceived by the extracellular LRR domain of these PRRs. FLS2 directly interacts with flg22, a 22-amino acid peptide derived from the amino terminus of flagellin (Felix *et al.* 1999, Gómez-Gómez *et al.* 1999), EFR specially perceives the first 18 amino acids of the N-terminus of *Agrobacterium* EF-Tu (Zipfel *et al.* 2006), the elf18 peptide, and Xa21 was identified to specially recognize a sulfated 17-amino acid peptide derived from the amino terminus of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) type I secreted protein Ax21 (Lee *et al.* 2009) (Table 1).

Lysin motif (LysM) receptor kinases with extracellular LysM and intracellular Ser/Thr kinase domain represent another kind of PRRs which are required for the perception of fungal cell wall component chitin. Chitin elicitor receptor kinase (CERK1), identified from *Arabidopsis*is, is one typical representative of this kind of PRRs (Miya *et al.* 2007) (Table 1). *Arabidopsis* CERK1 contains three extracellular LysMs which recently were reported to directly bind with the chitin oligosaccharide elicitor (Iizasa *et al.* 2010, Petutschnig *et al.* 2010). It was also reported that *Arabidopsis* CERK1 homologue, OsCERK1, is essential for chitin signaling in rice (Shimizu *et al.* 2010) (Table 1). Another protein isolated from rice, chitin elicitor binding protein (CEBiP), contains two LysMs in its extracellular portion but has no intracellular kinase domain and was also reported to be involved in perceiving chitin (Kaku *et al.* 2006) (Table 1).

Table 1. Representative MAMPs/PAMPs/DAMPs and corresponding receptors in bacteria, fungi, oomycetes, and plants.

Source	MAMPs/PAMPs/ DAMPs	Active epitope	Responsive plants	PRR	PRR structure	References
Bacteria	flagellin	flg22	most plants	FLS2	LRR-RLK	Felix *et al.* 1999, Gómez-Gómez and Boller 2000
	EF-Tu	elf18	*Brassicaceae*	EFR	LRR-RLK	Kunze *et al.* 2004, Zipfel *et al.* 2006
	Ax21	axY^{S}22	rice	Xa21	LRR-RLK	Song *et al.* 1995, Lee *et al.* 2009
	lipopolysaccharides	undefined	*Arabidopsis*, tobacco, pepper	unknown	unknown	Newman *et al.* 1997, Meyer *et al.* 2001, Zeidler *et al.* 2004
	peptidoglycan		*Arabidopsis*	unknown	unknown	Felix and Boller 2003
	harpin	undefined	various plants	unknown	unknown	He *et al.* 1993, Lee *et al.* 2001
	cold-shock protein	csp15	*Solanaceae*	unknown	unknown	Felix and Boller 2003
Fungi	chitin	chitin	*Arabidopsis,* rice	CERK1	LysM-RLK	Miya *et al.* 2007, Shimizu *et al.* 2010
		oligosaccharides	*Arabidopsis*, rice, barley	CEBiP	LysM-RLP	Kaku *et al.* 2006, Shimizu *et al.* 2010, Tanaka *et al.* 2010
	β-glucan	β-heptaglucan	rice	Unknown	unknown	Sharp *et al.* 1984, Yamaguchi *et al.* 2000
	xylanase	TKLGE pentapeptide	tobacco, tomato	LeEIX2	LRR-RLP	Hanania and Avni 1997, Ron and Avni 2004
	elicitin	undefined	tobacco	unknown	unknown	Ricci *et al*. 1989
Oomycetes	transglutaminase	Pep13	parsley, potato	unknown	unknown	Nürnberger *et al.* 1994, Brunner *et al.* 2002
	β-glucans	hepta-β-glucoside	legumes	GnGBP		Umemoto *et al.* 1997, Fliegmann *et al.* 2004
		oligo-β-glucosides	tobacco	unknown	unknown	Klarzynski *et al.* 2000
Plant	PEPR1	Pep1	*Arabidopsis*, maize	PEPR1	LRR-RLK	Krol *et al.* 2010
	prosystemin	systemin	*Solanaceae*	SR160	LRR-RLK	Pearce *et al.* 1991, Scheer and Ryan 2002
	homogalacturonan	oligogalacturonides	*Arabidopsis*	WAK1	EGF-RLK	Brutus *et al.* 2010

Mechanical injury and insect herbivore damage release specific signals, which are known as DAMPs. Plant PRRs can also recognize DAMPs in a fashion similar to PAMPs and activate the defense signaling cascade. *Arabidopsis* PEP receptor 1 (PEPR1), the first receptor for DAMPs perception, was indentified recently. AtPEPR1 also belongs to the LRR-RLK family which can specifically recognize *Arabidopsis* Pep1, a 23-amino-acid peptide derived from the C-terminus of a pathogen/wound-induced gene product, PROPEP1 (Krol *et al.* 2010) (Table 1). Oligogalacturonides (OGAs), fragments of the plant cell wall component homogalacturonan (HGA), are regarded as other kinds of DAMPs, which can induce the expression of defense genes, protecting plants against fungal diseases (Denoux *et al.* 2008). Recent reports also revealed that wall-associated kinase 1 (WAK1) is a receptor of OGAs (Brutus *et al.* 2010) (Table 1). WAK1 represents as a typical receptor like kinase but with an extracytoplasmic domain containing several epidermal growth factor (EGF)-like repeats.

Signaling activation: PAMPs-induced rapid heterodimerization of PRRs is an important event for switching on the PTI signaling downstream. FLS2 instantaneously dimerizes with brassinosteroid intensitive 1 (BRI1)-associated kinase (BAK1) upon elicitor stimulation (Chinchilla *et al.* 2007, Heese *et al.* 2007, Schulze *et al.* 2010). BAK1 is an LRR-RLK which previously was known as a co-receptor of the plant hormone brassinosteroid receptor BRI1. BAK1 only participates in the signaling initiation but not in the recognition of flg22, because the protein itself does not bind flg22, and FLS2 binding with flg22 is independent of its association with BAK1. Besides, BAK1 is also required for PTI responses induced by other PAMPs, such as EF-Tu (Chinchilla *et al.* 2007), suggesting that BAK1 is a central player for the mediation of PTI signaling.

Some cell surface receptors, such as Cf-2/9, Xa21D, and CEBiP, do not carry the transmembrane-spanning or kinase domains, but confer resistance to pathogens (Dixon *et al.* 1996, Wang *et al.* 1998, Kaku *et al.* 2006). It was suggested that they may interact with transmembrane protein kinase upon ligand binding and the heterodimerization will activate an intracellular kinase enzyme

which triggers downstream events and results in resistance (Wang *et al.* 1998, Kaku *et al.* 2006). This assumption was recently verified for CEBiP. As introduced above, both OsCERK1 and CEBiP are functional for chitin recognition and signaling in rice. OsCERK1 has a classical RLK structure, but CEBiP contains only two LysMs in its extracellular portion without any functional intracellular domains for signaling. Knockdown of OsCERK1 resulted in a marked suppression of the defense responses in rice cells induced by chitin oligosaccharides, indicating a crucial role of OsCERK1 in chitin signaling in rice. Results of a yeast two-hybrid and immunoprecipitation studies indicated that both CEBiP and OsCERK1 form a heterooligomer receptor complex in a ligand dependent manner (Shimizu *et al.* 2010). Thus, heteromerization with OsCERK1 is suggested to be essential for CEBiP-mediated signaling.

Since the PRRs represent functional kinases, phosphorylation events are certainly regarded as important regulatory mechanisms for PTI signaling. The kinase domain of FLS2 is rapidly phosphorylated by stimulation with flg22 peptide and the phosphorylation of FLS2 is required for both flg22 interaction and signaling (Gómez-Gómez *et al.* 2001). Additionally, flg22 treatment also induces phosphorylation of BAK1 and a receptor-like cytoplasmic kinase (RLCK) *Botrytis*-induced kinase 1 (BIK1) (Lu *et al.* 2010, Schulze *et al.* 2010). BIK1 appears to function downstream of FLS2/BAK1 because BIK1 phosphorylation requires not only the presence of both FLS2 and BAK1 but also their kinase activity. *In vitro* assay showed that BIK1 not only can be phosphorylated by BAK1, but also can trans-phosphorylate BAK1 and FLS2. In addition, the association of BIK1 with FLS2 and BAK1 appears to be reduced upon flagellin perception (Lu *et al.* 2010). Based the data described, the whole phosphorylation process might be envisaged in four steps. First, flagellin stimulates FLS2 and BAK1 association and phosphorylation, second, phosphorylated BAK1 phosphorylates BIK1, third, phosphorylated BIK1 and FLS2/BAK1 phosphorylates each other to amplify the signaling, and finally, phosphorylated BIK1 is released to activate signaling component downstream.

Dephosphorylation of the phosphorylated PRRs by protein phosphatases is a major way for negative regulation of activated PTI signaling. The serine/threonine specific protein phosphatases 2C (PP2Cs) were reported to regulate a subset of LRR-RLKs in plant immunity. One of the characterized plant PP2Cs is the *Arabidopsis* kinase-associated protein phosphatase (KAPP) which was proved to negatively regulate FLS2 activation because FLS2 exists in an inactive state and is insensitive to flg22 stimulation in KAPP-overexpressed plants (Gómez-Gómez *et al.* 2001). In rice, another novel PP2C, XA21 binding protein 15 (XB15), was also confirmed to dephosphorylate autophosphorylated XA21 to abolish XA21-mediated immunity in rice (Park *et al.* 2008).

Flg22-induced endocytosis of FLS2 was detected by using GFP tagging and confocal microscopy imaging. FLS2 endocytosis requires a PEST motif present in the juxtamembrane domain. Once stimulated by flg22, FLS2 tends to accumulate in intracellular vesicles and then is degraded in a proteasome-dependent manner (Robatzek *et al.* 2006). Ligand-induced internalization is also likely to happen to the rice Xa21 because Xa21 not only contains a PEST motif in its intracellular juxtamembrane domain but can also directly interact with a nuclear located WRKY transcriptional factor (Ding *et al.* 2009).

Downstream responses: Conserved MAPK signaling cascades are key mediators of PTI signaling. In *Arabidopsis*, two kinds of MAPK cascades, MKK4/ MKK5-MPK3/MPK6 and MEKK1/MKK1/ MKK2-MPK4, were identified to play downstream of the FLS2 receptor complex (Asai *et al.* 2002, Suarez-Rodriguez *et al.* 2007, Gao *et al.* 2008). Once activated by PAMP/PRR specific interaction, MAPKs will lead to the transcription of some early defense-related genes. For example, activated MPK3 and MPK6 strongly induce transcription of WRKY transcription factors WRKY22 and WRKY29, and flg22-induced receptor-like kinase 1 (FRK1) (Asai *et al.* 2002). A transcription factor of the ethylene response factor (ERF) family, ERF104, was also recently indentified to be a substrate of MPK6. MPK6 normally interacts with ERF104 in the nucleus. Once activated by PAMPs, MPK6 will phosphorylate and disjoin ERF104 which is released to regulate transcription activation of other resistance-related genes (Bethke *et al.* 2009). A similar regulation mechanism is also employed by MPK4. MPK4 is constitutively in combination with WRKY33 transcription factor and a MAP kinase substrate 1 (MKS1) in the nucleus. Once activated, MPK4 will phosphorylate MKS1 to release WRKY33 to regulate the expression of phytoalexin deficient 3 (PAD3), a resistance-related gene involved in camalexin biosynthesis (Qiu *et al.* 2008).

More and more data show that MAP kinase phosphatase (MKP) also plays important roles in the negative regulation of immune responses. It is thought that MKP specially dephosphorylates activated MAPK molecules to determine the magnitude and duration of MAPK signaling. One of MKPs, MKP1, was recently indentified to play as a negative regulator of MPK6-mediated PTI signaling pathway in *Arabidopsis* (Anderson *et al.* 2011).

Calcium ion rapid influx into the cytosol of plant cells from the apoplast is one of the earliest PTI events. The activation of a plasma membrane Ca^{2+}-conducting channel might be due to PRR phosphorylation, G-protein molecular switch, and the activation of the cyclic nucleotide gated channels (Qi *et al.* 2010). Ca^{2+} elevation in cytosol plays a pivotal role in mediating other plant immune processes, including ROS production, stomatal closure, *etc*. (Nomura *et al.* 2008, Ogasawara *et al.* 2008). Recently, calcium-dependent protein kinases (CDPKs), acting as Ca^{2+} sensor protein kinases, were reported to be major mediators of the early PTI immune signaling (Boudsocq *et al.* 2010). Although the direct targets of CDPKs were not identified, the gene expression analysis in *Arabidopsis* protoplasts transformed with constitutively

active CDPK representatives suggested that CDPKs work through mediating transcriptional reprogramming in plant immune signaling.

Besides, calcineurin B-like proteins (CBLs) were also indentified to be Ca^{2+} sensors in rice (Kurusu *et al.* 2010). In response to calcium ion, CBLs activate the kinase activity of CBL-interacting protein kinases (CIPKs), and the activated CIPKs next mediate some early PTI responses.

PAMPs-induced production of ROS is another early event attributing to plant defense. The ROS are considered to exert antimicrobial action through strengthening host cell walls *via* cross-linking of glycoproteinsand mediating other defense signaling (Lamb and Dixon 1997). Plant respiratory burst oxidase homologue D (RbohD), an NADPH oxidase, was thought to play a central role in mediating the ROS production during the early interaction between plants and pathogens (Torres and Dangl 2005). The upstream signals mediating oxidative burst is still unclear. It was suggested that RbohD-dependent ROS production seems to be downstream of MAPK activation (Zhang *et al.* 2007). Recent studies also revealed that cytosolic free Ca^{2+} can bind with the EF-hand motifs of AtrbohD and activate the AtRbohD through inducing conformational change of the enzyme (Ogasawara *et al.* 2008).

The glucan polymer callose usually deposits between the cell wall and the plasma membrane of the plant sites suffering from pathogen attacks. Callose deposition is a common PTI response which mainly leads to plant cell wall thickenings to limit the penetration of pathogens. Previous studies revealed that callose synthesis in response to flg22 might be triggered by ROS, because AtrbohD mutants exhibit fewer callose deposits after flg22 treatment (Zhang *et al.* 2007). Recent studies uncovered that glucosinolate metabolites, previously identified as important in avoiding damage by herbivores, are also involved in the PAMP-induced synthesis of callose deposition in *Arabidopsis* (Clay *et al.* 2009). In the process, ethylene (ET) signaling is required for the full induction of MYB51 in response to flg22, and MYB51 transcription factor activates the transcription of indole glucosinolate (IGS) biosynthetic genes. The putative myrosinase presenilin enhancer 2 (PEN2), phytochelatin synthase 1 (PCS1), and phytochelatin synthase PCS1-dependent and salicylic acid (SA)-mediated pathways participate in hydrolysis of 4-methoxy-I3G. 4-methoxy-I3G hydrolytic products might be released to the plasma membrane/cell wall where the final synthesis of callose components is catalyzed by the callose synthase PMR4. The abscisic acid (ABA) signaling and methyl jasmonate (MeJA) signaling can negatively regulate the callose formation by antagonizing ET signaling and IGS hydrolysis, respectively.

Stomata are major channels for entry of bacterial pathogens into the plant tissue. Thus, controlling closure of stomata in response to bacteria is employed by plant to defense against infection of bacterial pathogens (Melotto *et al.* 2006). Investigations demonstrated that recognition of flagellin by FLS2 is a key or the only cause for stomatal closure in *Arabidopsis* during plant immunity because stomata of *fls2* plants are completely unresponsive to *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 bacteria (Zeng *et al.* 2010). Signaling events downstream of FLS2 for stomatal closure still remain largely unexplored. It was revealed that flg22-triggered stomatal response involves K^+ channel regulation, and this regulation is dependent on FLS2 and a heterotrimeric G-protein- mediated signaling (Zhang *et al.* 2008). A transient increase in cytosolic free Ca^{2+} concentration in the guard cells also has a tight association with the stomata closure. Recently, it was found that flg22 can also induce Ca^{2+} concentration increase in guard cell chloroplasts, and the increased Ca^{2+} is next sensed by the thylakoid-localized Ca^{2+}-sensing receptor (CaSR) to regulate stomatal closure (Uemura *et al.* unpublished data). Chloroplasts are the places for the synthesis of salicylic acid (SA) and abscisic acid (ABA), and SA and ABA signaling also participate in the bacteria-triggered stomatal closure in *Arabidopsis* (Melotto *et al.* 2006). However, correlations between Ca^{2+} signaling and phytohormone signaling involved in PAMP-induced stomatal closure are unclear.

Small RNA biogenesis and RNA silencing process are also involved in plant defense against pathogens. MiR393 is the first flg22-induced miRNA identified in *Arabidopsis*. It plays an important role in growth restriction of the bacterium *P. syringae* through negatively regulating messenger RNAs of the F-box auxin receptors transport inhibitor response 1 (TIR1), auxin signaling F-box proteins 2 and 3 (AFB2 and AFB3; Navarro *et al.* 2006). Furthermore, miRNA167 and miRNA160, which target auxin response factors/receptors to negatively regulate auxin signaling, are also induced after infection with *Pst* DC3000 *HrcC*$^-$ (Fahlgren *et al.* 2007).

Argonaute (AGO) subfamily proteins are involved in biogenesis and foundational performance of miRNAs in plants. Recently, it was found that *Arabidopsis* AGO1 is required for PTI responses. And, a number of AGO1-bound miRNAs were also indentified to be up- or down-regulated by flg22 inoculation. Among these AGO1-bound miRNAs, miR160a positively regulates PAMP-induced callose deposition whereas miR398b and miR773 negatively regulate PAMP-induced callose deposition and disease resistance to bacteria (Li *et al.* 2010).

Effector-triggered immunity (ETI)

Effector recognition: Specific recognition of pathogen Avr effectors by plant R proteins is crucial for the onset of the ETI responses. The classical “gene-for-gene” theory emphasizes that the resistance is determined by the direct interaction between plant R protein and Avr effector with a one-to-one manner. Direct interaction between R and Avr

proteins has been well-exemplified in the flax-flax rust system. Flax rust fungus effector AvrL567 can be recognized by the L5, L6, and L7 R proteins of flax. The physical interactions also conferred a co-evolutionary arms race between the interaction pair (Dodds *et al.* 2006). Furthermore, direct interactions were also confirmed between R rice proteins Pi-ta, *Arabidopsis* resistance to *Peronospora parasitica* 13 (RPP13) and resistance to *Ralstonia solanacerum* 1-R (RRS1-R), and their cognate Avr proteins AvrPi-ta, *Arabidopsis thaliana* recognized 13 (ATR13), and *Pseudomonas* outer protein P2 (PopP2) from *Magnaporthe grisea*, *Hyaloperonospora parasitica*, and *Ralstonia solanacearum* (Jia *et al.* 2000, Deslandes *et al.* 2003, Rentel *et al.* 2008) (Table 2).

An indirect recognition mechanism has been shown for multiple coiled coil (CC)-type NB-LRR R proteins and their cognate pathogen effectors. RPM1-interacting protein 4 (RIN4) is the classic example of a host target that is guarded by NB-LRR R proteins and is modified by pathogen effectors (Mackey *et al.* 2002). The immune receptors resistance to *P. syringae* pv. *maculicola* (RPM1) and resistance to *Pseudomonas syringae* 2 (RPS2) recognize modifications to RIN4 induced by three different pathogen effectors (Axtell and Staskawicz 2003, Mackey *et al.* 2003). Similarly, RPS5 recognizes the cleavage of the plant RLCK, avrPphB susceptible 1 (PBS1), by the pathogen effector AvrPphB (Ade *et al.* 2007, Shao *et al.* 2003). Pto is also a RLCK. It was originally identified as an immune receptor, but is actually a host factor guarded by the NB-LRR protein Prf in response to AvrPto (Mucyn *et al.* 2006) (Table 2).

An increasing number of researches also indicated R/Avr interactions with "one-to-many", "many-to-one", and "many-to-many" modes. That is to say, different kinds of R proteins function cooperatively or separately in response to a single or a series of effectors. For example, both tomato Prf and *Arabidopsis* RPM1 were found to recognize two groups of sequence-unrelated Avr effectors, AvrPto, AvrPtoB, and AvrRpm1, AvrB, respectively, and three R proteins, RPM1 and target of AvrB operation 1 (TAO1) in *Arabidopsis*, and resistance to *Pseudomonas syringae* pv. *glycinea* 1b (RPG1-b) in soybean, can recognize AvrB (Mackey *et al.* 2002, Ashfield *et al.* 2003, Pedley and Martin 2003, Eitas *et al.* 2008). It is not difficult to understand the first mode (one-to-many) because all the different effectors usually have the same host target which is associated with the R protein. For the later two modes, a plausible explanation is that *R* genes locating adjacently in genome tend to co-express and form heteromultimeric proteins to recognize effector (Eitas and Dangl 2010).

R protein activation: R proteins are proposed to act as molecular switches that control the ETI signaling downstream. Most R proteins are multidomain NB-LRRs which are usually composed of the central NB and C-terminal LRRs domain, N-terminal CC domain, or a domain that has homology to the toll/interleukin-1

Table 2. Best-characterized NB-LRR/Avr pairs.

Interaction	Pathogen	Avr effector	Plant	R protein	R protein structure	Reference
Direct	*Magnaporthe grisea*	AvrPi-ta	rice	Pi-ta	CC-NB-LRR	Jia *et al.* 2000
	Melampsora lini	AvrL567	flax	L5/L6/L7	TIR-NB-LRR	Dodds *et al.* 2006
	Blumeria graminis	Avr10	barley	MLA10	CC-NB-LRR	Ridout *et al.* 2006
	Hyaloperonospora parasitica	ATR13	*Arabidopsis*	RPP13	CC-NB-LRR	Allen *et al.* 2004
	Hyaloperonospora parasitica	ATR1	*Arabidopsis*	RPP1	TIR-NB-LRR	Rehmany *et al.* 2005
	Potato virus X	Coat protein	potato	Rx	CC-NB-LRR	Rairdan and Moffett 2006
	Ralstonia solanacearum	PopP2	*Arabidopsis*	RRS1-R	TIR-NB-LRR-WRKY	Deslandes *et al.* 2003
	Tobacco mosaic virus	P50	tobacco	N	TIR-NB-LRR	Burch-Smith *et al.* 2007
Indirect	*Pseudomonas syringae*	AvrPto/AvrPtoB	tomato	Prf	CC-NB-LRR	Salmeron *et al.* 1996, Kim *et al.* 2002, Mucyn *et al.* 2006
	Pseudomonas syringae	AvrPphB	*Arabidopsis*	RPS5	CC-NB-LRR	Shao *et al.* 2003, Ade *et al.* 2007
	Pseudomonas syringae	AvrRpt2	*Arabidopsis*	RPS2	CC-NB-LRR	Axtell and Staskawicz 2003
	Pseudomonas syringae	AvrRpm1/AvrB	*Arabidopsis*	RPM1	CC-NB-LRR	Mackey *et al.* 2002
	Pseudomonas syringae	AvrB	soybean	RPG1-B	TIR-NB-LRR	Ashfield *et al.* 2004, Selote and Kachroo 2010
Unknown	*Pseudomonas syringae*	AvrB	*Arabidopsis*	TAO1	TIR-NB-LRR	Eitas *et al.* 2008
	Pseudomonas syringae	AvrRps4	*Arabidopsis*	RPS4	TIR-NB-LRR	Hinsch *et al.* 1996, Gassmann *et al.* 1999
	Tobacco mosaic virus	P50	tobacco	NRG1	TIR-NB-LRR	Peart *et al.* 2005
	Xanthomonas oryzae	AvrRxo1	maize	Rxo1	TIR-NB-LRR	Zhao *et al.* 2005
	Xanthomonas campestris	AvrBs2	pepper	Bs2	NB-LRR	Tai *et al.* 1999, Mudgett *et al.* 2005

receptor (TIR) domain (Takken *et al.* 2006). The C-terminal LRRs and N-terminal CC/TIR domains are thought as the regulatory domains which appear to interact with upstream and downstream signaling partners and to modulate the activation of the R protein. The three domains synergistically ensure a complete function of the full-length R protein. In absence of Avr effectors, R proteins keep an ADP-binding inactive state through intra-molecular interactions between their different domains and/or extra-molecular interaction with other host protein(s). Pathogen secreted effectors can induce conformational changes of R protein, which is prone to ADP/ATP exchange. Nucleotide exchange then triggers a second conformational change that enables the R protein N-terminus (TIR, CC) to interact with and activate downstream targets (Takken and Tameling 2009).

Some host proteins participate in the Avr effector-triggered activation of R proteins. The most elaborately illuminated proteins are heat shock protein 90 (HSP90), required for Mla12 resistance protein (RAR1), and suppressor of the G allele of *skp1* (SGT1). The three host proteins interact *in vivo* and compose a ternary complex and some reports have confirmed that the complex plays an important role in ETI by regulating the stability of the NB-LRR protein. HSP90, a molecular chaperone, is considered as a determinant of steady-state NB-LRR protein accumulation and it is involved in all examined R protein-mediated disease resistance pathways (Takahashi *et al.* 2003, Hubert *et al.* 2003, Botër *et al.* 2007). The precise roles of RAR1 and SGT1 for stability of the nucleotide-binding site leucine-rich repeat (NB- LRR) proteins have remained elusive. Current researches showed that the two proteins function additively for some R proteins but antagonistically for other R proteins (Takahashi *et al.* 2003, Holt *et al.* 2005, Botër *et al.* 2007). It was postulated that they may determine whether NB-LRR proteins are functional in disease resistance or marked for signaling termination through degradation (Holt *et al.* 2005).

Plant NBS-LRR R proteins are similar to the mammalian nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) protein family. Therefore, it was postulated that protein activity regulated by oligomerization may also occur in plant NB-LRRs. This speculation was verified by the tobacco TIR-NB-LRR N protein whose oligomerization occurs in the presence of the elicitor p50, the 50-kD helicase domain of the *Tobacco mosaic virus* (TMV) replicase (Mestre and Baulcombe 2006). But it is unclear if the oligomerization is a common phenomenon for other NB-LRR R proteins.

ETI signaling

Signaling for HR: In contrast to the well-established models about R/Avr interaction and R protein activation, understandings about the ETI regulatory mechanisms for downstream signaling are relatively poor. Plant ETI is often accompanied by rapid cell death in and around the initial infection site, a reaction known as hypersensitive reaction (HR; Klement 1963). Some evidence showed that plant HR shares similar molecular basis with animal programmed cell death (PCD). Autophagy-related proteins (ATGs) are major regulators of autophagic cell death in animal. Analysis of genomic sequence showed that *Arabidopsis* also contains autophagy-related genes. Moreover, Hofius *et al.* (2009) found that TIR type NB-LRR R protein resistance to *Pseudomonas syringae* 4 (RPS4) can activate autophagic cell death during HR. However, RPS2, a CC-type NB-LRR R protein, activate HR in an autophagy independent manner. These data suggested that more than one signaling pathways are employed by plant to execute the HR. Meanwhile, they also found that AvrRpm1 can trigger both autophagy-dependent and autophagy-independent cell death. It was speculated that recognition of AvrRpm1 by CC-NB-LRR R protein RPM1 mediates autophagy-independent cell death, and a Toll/IL-1R type R protein is possibly present in *Arabidopsis* to perceive AvrRpm1 and trigger autophagy-dependent cell death HR.

Although plants apparently lack apoptotic regulatory caspases, caspase-like activities are required for HR in plants (Hatsugai *et al.* 2004, Woltering 2004). Using caspase-3 inhibitor in *Arabidopsis*, Hatsugai *et al.* (2009) found that avirulent bacteria-induced HR was effectively abolished suggesting that caspase-3-like activity is crucial for proper execution of disease resistance-associated PCD upon avirulent bacterial pathogen infection. Meanwhile, they proved that proteasome β1 subunit PBA1 executes the caspase-3-like DEVDase (an enzyme that cleaves aspartate-glutamate-valine-aspartate target sequences) activity and established a direct relationship between HR and the proteasome. PBA1 mediates membrane fusion of central vacuole with the plasma membranes. The membrane fusion leads to the release of vacuolar anti-microbial contents into the apoplast and consequently causes bacterial proliferation inhibition and host cell PCD.

In tomato, AvrPto-dependent Pto-interacting protein 3 (Adi3), an AGC kinase, was found to negatively regulate AvrPto-Pto mediated cell death (Devarenne *et al.* 2006). Adi3 is a nuclear localized protein. Nuclear localization of Adi3 is required for cell death suppression (CDS) activity, and loss of nuclear localization causes elimination of Adi3 CDS activity and constitutive induction of cell death. The loss of Adi3 function is associated with the hypersensitive response cell death initiated by the Pto/AvrPto interaction. Thus, disruption of Adi3 nuclear localization may be a mechanism for induction of cell death during the Pto/AvrPto interaction (Ek-Ramos *et al.* 2010).

Nucleocytoplasmic trafficking of plant R protein: Emerging findings showed a dynamic nuclear import of

plant R protein complex after effector recognition. Nuclear pores are the unique channels for nucleo-cytoplasmic trafficking of plant R proteins. Classical nuclear import involves binding the nuclear localization signal (NLS) by importin-α which together with other factors mediates active transport of the protein through the nuclear pores into the nucleus (Lange *et al.* 2007). Although some R proteins are predicted to own NLSs (Shen and Schulze-Lefert 2007, Caplan *et al.* 2008b), most NB-LRR R proteins are located in cytoplasm, and it is not easy for them to diffuse through nuclear pores for their large molecular sizes. Thus, the relocation into the nucleus of R proteins is predicted to be an important way for resistance activation. This kind of immunity mechanism was firstly revealed in barley. Barley MLA10 recognizes Avr_{A10} effector of the powdery mildew fungus *Blumeria graminis* in the cytoplasm. After recognition of Avr_{A10}, MLA10 moves into nucleus and interacts with HvWRKY2 transcription factors. The interaction between MLA10 and HvWRKY2 subsequently blocks the transcription repressor activity of HvWRKY1/2 and thus enables rapid defense gene activation (Shen *et al.* 2007). *Arabidopsis* TIR-NB-LRR R protein SNC1 (suppressor of *npr1-1* constitutive 1) was recently shown to be relocated into the nucleus to activate downstream defense responses by modulating the transcriptional repression activity of TPR1 which targets some negative regulators of immune responses (Zhu *et al.* 2010).

Direct interaction with transcription factors or a transcription repressor seems to be a feasible mechanism required for the ETI activation. However, in most cases, the nuclear imported R proteins do not operate the transcriptional reprogramming directly but act together with some other host proteins. For example, *Arabidopsis* RRS1-R encodes a TIR-NBS-LRR type of R proteins but possesses a C-terminal WRKY transcriptional factor domain and a putative bipartite NLS (Deslandes *et al.* 2003). Although the tripartite structure implies that R proteins interact with transcription factors to regulate gene expression, the recruitment of responsive to dehydration 19 (RD19), a cysteine protease, to the nucleus by *Ralstonia solanacearum* effector PopP2 is essential for RRS1-R-mediated resistance (Bernoux *et al.* 2008). TIR-NB-LRR type R protein N confers resistance to TMV through specific recognition of p50 (Whitham *et al.* 1994, Erickson *et al.* 1999, Burch-Smith *et al.* 2007). Like MLA, nuclear localization of N is required for its function. A tobacco host protein, nuclear-receptor-interacting protein 1 (NRIP1), is also involved in N-mediated resistance (Caplan *et al.* 2008a). It was reported that NRIP1 normally localizes to the chloroplast but is partially recruited to the nucleus by the p50 effector, and this recruitment probably leads to the formation of mature p50-NRIP1 complex which is recognized by nuclear protein to activate defense signaling. *Arabidopsis* TIR-NB-LRR type R protein RPS4 specially recognizes the *P. syringae* type III effector AvrRps4 and triggers an enhanced disease susceptibility 1 (EDS1)-dependent resistance (Gassmann *et al.* 1999). AvrRps4-induced accumulation RPS4 and EDS1 in the nucleus is necessary for the immunity initiation (Wirthmueller *et al.* 2007). Recent researches demonstrated that a change in EDS1 nucleo-cytoplasmic status is the direct cause of transcriptional reprogramming triggered by RPS4 recognition of AvrRps4 (García *et al.* 2010).

Evolution from PTI to ETI

Despite the "zigzag" evolutionary model was firmly confirmed by identifications and functional interpretations of more and more effectors which can block PTI, little is known about the detailed relationships or residual evolutionary footprints between PTI and ETI. As we know, Avr effectors also show virulence functions in plants in absence of their corresponding R protein by suppressing PTI. Therefore, the virulence targets of these Avr effectors are important regulators of PTI signaling pathway. For the same effector, is the virulence target identical to the avirulence target? If not, what is the relationship between the two kinds of host proteins? Recently, several studies on Pto, PBS1, and RIN4 answered these questions and revealed how plant immunity might have evolved by using comparative biological and biochemical methods.

PTO: As mentioned above, Pto is the avirulence target of AvrPto. AvrPto is able to induce Prf/Pto-mediated ETI in resistant tomato but strongly inhibits PTI in *Arabidopsis* and susceptible tomato lacking Pto suggesting that Pto is not the virulence target of AvrPto. AvrPto can block flg22-induced MAPK activation upstream of MAPK cascade implying a very early signal component(s) in PTI signaling pathway is targeted by the effector (He *et al.* 2006). Subsequently, the targets were proved to be multiple PRRs, including FLS2, EFR, and a tomato FLS2 homologue (Xiang *et al.* 2008). FLS2 is evolutionarily conserved in monocot and dicot plants and AvrPto is present in a number of *P. syringae* isolates infecting a wide range of host plants including cucurbit, mulberry, bean, tomato, and tobacco, suggesting that AvrPto as an ancient effector was originally evolved to inhibit receptor kinases in these plants (Lin and Martin 2007). In contrast, Pto is present only in a few wild tomato species and Pto family proteins appear to be limited to *Solanaceae* plants suggesting recent evolution of this family (Chang *et al.* 2002). Despite its emergence later than FLS2, Pto is similar to the kinase domain of FLS2 in amino acid sequence. The FLS2-AvrPto interaction and Pto-AvrPto interaction share similar sequence requirements. Moreover, Pto can compete with FLS2 for binding AvrPto. Together, it was deduced that Pto might be evolved as a molecular mimic of receptor kinases targeted by AvrPto to mediate Prf-dependent ETI (Xiang *et al.* 2008).

P. syringae effector AvrPtoB can also suppress FLS2-mediated PTI in *Arabidopsis* and elicit

Pto/Prf-mediated ETI in tomato (Kim *et al.* 2005, He *et al.* 2006, Xiao *et al.* 2007). AvrPtoB is structurally distinct from AvrPto. It is a bipartite protein with an N-terminal region and a C-terminal E3 ligase domain (Janjusevic *et al.* 2006). The AvrPtoB-induced ETI is triggered by the N-terminal region of AvrPtoB ($AvrPtoB_{1\text{-}307aa}$) but independent of the E3 ligase domain in its C-terminus (Xiao *et al.* 2007). It was also revealed that a Pto-independent HR, known as resistance suppressed by AvrPtoB C terminus (Rsb) immunity, can be induced by $AvrPtoB_{1\text{-}387}$ but not by $AvrPtoB_{1\text{-}307}$ and the full-length AvrPtoB in tomato (Rosebrock *et al.* 2007). Fen, a kinase encoded by *Pto* family gene, is responsible for this immunity. Interestingly, AvrPtoB C-terminual E3 ligase domain ($AvrPtoB_{388\text{-}553}$) can inhibit the Rsb immunity through mediating ubiquitination and elimination of Fen. But the C-terminal E3 ubiqutin ligase cannot inhibit the Pto/Prf dependent ETI for the reason that Pto is invulnerable to the degradation mediated by the E3 ubiqutin ligase (Ntoukakis *et al.* 2009). Moreover, a screen of 37 accessions of wild species of tomato revealed that 21 express Rsb immunity but only five seem to have Pto-mediated immunity. The wide occurrence of Rsb is consistent with phylogenetic analyses indicating that the *Fen* gene arose before the *Pto* gene in the *Solanum* species (Rosebrock *et al.* 2007). Based on all the cases above, a four-step co-evolutionary process between Pto family and AvrPtoB is put forward here. Step 1, $AvrPtoB_{1\text{-}387}$, as an original form of AvrPtoB, inhibited PTI signaling through targeting FLS2 kinase domain. Step 2, susceptible tomato subsequently evolved some FLS2 kinase domain-like proteins and one of the evolved proteins, Fen, can recognize the $AvrPtoB_{308\text{-}387}$ region to trigger Prf-mediated ETI. Step 3, an E3 ubiquitin ligase integrated with the $AvrPtoB_{1\text{-}387}$ and abolished Fen/Prf dependent ETI through ubiquitinating and inducing elimination of Fen, and FLS2 can also be degraded because its kinase domain is structurally similar to Fen. Step 4, an E3 ubiquitin ligase insensitive protein, Pto, was evolved to recognize some sites in $AvrPtoB_{1\text{-}307}$ and restore the Prf-activated ETI.

In a resistant tomato cultivar, *Pto* and *Fen* are located within a cluster of five *Pto* homologues. Another Pto homologue in this cluster (Martin *et al.* 1994), PtoC, can interact with $AvrPtoB_{1\text{–}307}$ similarly to Pto and is involved in Pto/Prf complex but it does not induce HR. Moreover, PtoC is insensitive to the E3 ubiqutin ligase activity of AvrPtoB (Mucyn *et al.* 2009). Thus, it was suggested that PtoC was probably evolved as an intermediate between Fen and Pto.

PBS1: *P. syringae* effector AvrPphB encodes a functional cysteine protease. It triggers a HR in *Arabidopsis* expressing the RPS5 R protein by a specific cleavage of PBS1 (Shao *et al.* 2003, Ade *et al.* 2007). In *Arabidopsis* lacking RPS5, AvrPphB inhibits PTI responses, and its protease activity is also required for the PTI inhibition but the cleavage of PBS1 does not appear to account for the PTI-inhibition activity of AvrPphB because *pbs1* mutants showed only minimal defects in PTI defenses (Zhang *et al.* 2010). Therefore, some other substrates of AvrPphB cysteine protease were suggested to be the genuine virulence targets of this effector. AvrPphB cleaves PBS1 after the sequence 'GDK', a motif that defines AvrPphB substrate specificity (Shao *et al.* 2003). PBS1 belongs to RLCK subfamily VII. In *Arabidopsis*, more than twenty RLCK VII numbers (named PBS1-like proteins, PBLs) fit this feature in *Arabidopsis* and at least eight of them were identified to be cleaved by AvrPphB. Of these, BIK1 was confirmed to play an important role in mediating PTI and PBS1, and some other PBLs display relatively minor but functionally additive effects in PTI defense (Lu *et al.* 2010, Zhang *et al.* 2010). These studies suggested that an ancestral protein, maybe BIK1, was targeted by AvrPphB to inhibit PTI, then some PBS1-like kinases were generally evolved from the original virulence target to relieve the competitive pressure from AvrPphB to PTI inhibition, until the final generation of PBS1 with an ability on interaction with the NB-LRR R protein RPS5. The association of PBS1 with RPS5 allows the latter to trigger ETI upon sensing the cleavage-induced conformational change in PBS1 (Ade *et al.* 2007, Zhang *et al.* 2010).

RIN4: RIN4 was indentified as the avirulence target of AvrRpt2, AvrRpm1, and AvrB in *Arabidopsis*. AvrRpm1 and AvrB induce phosphorylation of RIN4, and AvrRpt2, a cysteine protease, can cleave RIN4 and induce RIN4 degradation. The phosphorylation and proteolysis of RIN4 then activate RPM1- and RPS2-mediated ETI. The sites of RIN4 binding with and modified by AvrRpt2, AvrRpm1, and AvrB overlap in a short C-terminal nitrate-induced (NOI) domain including amino acids 142-176. Recently, it was reported that the phospho- rylation of RIN4 at a position of threonine 166 is necessary for AvrB-triggered RPM1 activation (Chung *et al.* 2011, Liu *et al.* 2011). Meanwhile, an *Arabidopsis* RLCK, RIN4-interacting receptor-like protein kinase (RIPK), was confirmed to phosphorylate RIN4 at threonine 21, threonine 166, and serine 160, and *RIPK* knockout (KO) lines exhibit reduced RIN4 phosphorylation and blunted RPM1-mediated defense responses, suggesting RIPK is directly involved in RPM1 activation (Liu *et al.* 2011). Although only one RIN4 homologue is present in *Arabidopsis*, *Arabidopsis* contains about 15 paralogous NOI domain-containing proteins (Kim *et al.* 2005, Chisholm *et al.* 2006) and all these proteins own a conserved F/YT××F×K motif surrounding the three residues phosphorylated by RIPK (Liu *et al.* 2011).

RIN4 is also a negative regulator of PTI because RIN4 overexpression lines exhibit increased flg22-triggered cell wall thickening and *rin4 rps2* double mutant lines shows slightly enhanced PTI response (Kim *et al.* 2005). Like *rin4*, *ripk* KO exhibits enhanced resistance and *RIPK* overexpression exhibits enhanced disease susceptibility to virulent *Pst* DC3000 (Liu *et al.* 2011). However, the expression of phosphomimetic mutant of RIN4 (pRIN4) under control of the *RIN4* native promoter in *rpm1*/*rps2*/*rin4* background exhibited similar bacteria

growth as *rpm1*/*rps2*/*rin4*, suggesting phosphorylation of other NOI domain-containing proteins may be responsible for the suppression of PTI responses by RIPK. AvrRpt2, AvrRpm1, and AvrB suppress PTI in *Arabidopsis* lacking their matching R proteins. Although RIN4 is targeted by the three effectors, RIN4 is not required for their virulence functions in *Arabidopsis* and on the contrary, the lack of RIN4 increases the virulence of AvrRpt2 to a certain extent indicating the existence of additional proteins targeted by these effectors for their virulence functions (Belkhadir *et al.* 2004, Lim *et al.* 2004). According to these scenarios described above, NOI domain-containing proteins are possibly the virulence targets of AvrB, AvrRpm1, and AvrRpt2 which play a role in their virulence strategy by promoting the phosphorylation of NOI domain-containing proteins by RIPK (Liu *et al.* 2011).

Overall, NOI domain-containing proteins may be the genuine target(s) of the RIN4-interacted effectors, and different NOI domain-containing proteins perhaps represent products of plant immunity during different evolutionary stages. RIN4 was possibly evolved from NOI domain-containing proteins to play as a decoy protein to structurally mimic the genuine virulence target(s) of RIN4-interacted effectors (Van der Hoorn and Kamoun 2008). The mimicry leads to activation of NB-LRR R proteins as well as partially relieves the virulence effects acting on the genuine targets in absence of R proteins.

Perspectives

Although much consensus has been achieved in the field of plant immunity, many potential problems still need to be resolved in future. PRRs have high specificity for PAMP recognition. They are required for most defense responses activated. How many PRRs are required for pathogens recognition and what are their corresponding PAMPs? How do the PRRs initiate the early resistance signaling including Ca^{2+} influx, oxidative burst, MAPK activation, and so on? The activation of ETI involves an indirect-recognition mechanism, and it is elaborately regulated by shared and special host proteins. How does the chaperone protein complex HSP90-SGT1-RAR1 participate in the dynamic shift of NB-LRR protein activity? Whether oligomerization is a universal phenomenon for NB-LRR-mediated resistance and how it leads to the downstream activation of hypersensitive cell death? Both PTI and ETI are integrally and differently involved in plant defense. How many similar mechanisms or identical signaling components are deployed by the two kinds of immunities? What is the potential crosstalk between the two different resistances? Despite several candidate theories about the evolution of plant immunity have been established, there are also some questions difficult to understand. For example, whether RIN4 indeed structurally mimics the virulence targets of AvrRpt2 and AvrRpm1. If RIN4 was evolved for interaction with pathogen effectors and ETI activation and why it is so conserved in plants. Except for AvrRpt2, AvrRpm1, and AvrB at least five pathogen effectors can also target RIN4 (Mackey *et al.* 2002, 2003, Luo *et al.* 2009, Wilton *et al.* 2010). Why is it targeted by so many effectors? Therefore, great efforts should be exerted to resolve these puzzles and unraveling the detailed molecular mechanisms of plant immunity will attribute to the application of these discoveries to modify plants in control of a wide spectrum of pathogens.

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