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REVIEW

Role of Ubiquitination in Plant Innate Immunity and Pathogen Virulence

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Abstract Plant diseases are a major constraint for stable crop production in the world. Plants are constantly threatened by different pathogens and have developed an array of mechanisms to defend themselves. A growing body of evidence indicates that ubiquitination, which is one of the most important cellular processes for protein modification in eukaryotic organisms, is involved in the regulation of host defense signaling. Pathogens also exploit ubiquitination to block or interfere with plant defenses. Recent studies in a few model plants have demonstrated that ubiquitination plays a critical role in plant–pathogen interactions that lead either to plant resistance or to successful pathogen invasion of the plant host. This review discusses recent findings about the functions of ubiquitination in host defense and pathogen invasion.

Keywords Ubiquitination · Disease resistance · Pathogen effector · Innate immunity

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Introduction

Plants are constantly exposed to pathogenic microorganisms such as bacteria, viruses, fungi, and oomycetes. To detect these potentially dangerous microbes and to defend against infection, plants have developed an effective innate immune system that minimizes the impact of pathogens on plant growth and development. The first layer of the innate immune system is the perception of pathogen microbe-associated molecular patterns (PAMPs) by pattern recognition receptors (PRR) in plant cell membranes (Schwessinger and Zipfel 2008). PAMPs are conserved molecules common to a wide array of pathogenic microorganisms. Examples of PAMPs include flagellin from bacteria and chitin from fungi. The PAMP-triggered immunity (PTI) prevents invasion of plants by most pathogens. The second layer of the innate immune system is the so-called gene-for-gene interaction in which a resistance (R) protein in the host recognizes a cognate pathogen-secreted effector, historically called an avirulence (Avr) protein. Such recognition triggers a complex signal transduction cascade, which results in both local defense responses (e.g., the hypersensitive response or HR) and global defense responses (systemic acquired resistance or SAR; Heath 2000). This second layer of the host defense is now called effector-triggered immunity (ETI) (Chisholm et al. 2006; Jones and Dangl 2006). Both PTI- and ETI-mediated defense responses represent significant metabolic changes in the infected plants, which should be modulated rapidly to maintain a proper function and regulation of biological processes in the cell. One of the most important mechanisms for modulating PTI and ETI is ubiquitination-mediated protein degradation via 26S proteasome. Here, we review how plants use ubiquitination to prevent or reduce pathogen infection and how pathogens manipulate this vital

process to achieve infection. We will first briefly introduce the essential components, i.e., E1, E2, and E3, in the ubiquitination machinery and then discuss the functions of recently identified ubiquitination-related genes in both plants and pathogens. A comprehensive list of those genes to be discussed in this review is presented in Table 1.

Ubiquitination Machinery

Ubiquitin is a highly conserved, 76-amino acid protein found exclusively in eukaryotes. Ubiquitin has a C-terminal glycine, which forms an isopeptide linkage with the ε-NH_2 group of the internal lysine residue in the substrate protein. Ubiquitin also has internal lysine residues that can serve as internal acceptors for binding to C-terminal glycine of other ubiquitin molecules to form an ubiquitin chain (Glickman and Ciechanover 2002).

Ubiquitination is a sequential reaction involving three major classes of enzymes: E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and E3 ubiquitin ligase enzyme. Ubiquitin activation by E1 enzyme is ATP dependent and occurs through a two-step reaction in which a high-energy E1-thiol-ester–ubiquitin intermediate is generated. The activated ubiquitin molecule is then transferred to an internal cysteine residue of E2. E2s catalyze

Table 1 Host and pathogen genes involved in ubiquitination during interactions between plant and pathogen

No.	Protein	Organism	Role in ubiquitination	Proposed function	Reference
1	UBA-1	*At*	E1	Involvement in activation or downstream signaling of certain R genes	Goritschnig et al. 2007
2	RIN2/RIN3	*At*	E3	Positive regulators of RPM1-mediated HR	Kawasaki et al. 2005
3	SGT1b	*At*	Component of SCF E3 ligases	Negative regulation of R protein accumulation before infection and positive regulation of HR during infection	Azevedo et al. 2002; Holt et al. 2005
4	ACRE74/ CMPG1	*Nb*	E3	Positive regulator of plant defense and disease resistance	Gonzalez-Lamothe et al. 2006
5	ACIF1	*Nb* and *Le*	E3	Positive regulator of plant defenses	van den Burg et al. 2008
6	EL5	*Os*	E3	Elicitor responsive, acts in basal defense	Takai et al. 2002
7	OsUbc5b	*Os*	E2	Elicitor responsive, acts in basal defense	Takai et al. 2002
8	PUB22/ PUB23/ PUB24	*At*	E3	Regulation of PAMP response	Trujillo et al. 2008
9	SON1	*At*	E3	Regulation of defense responses independent of SA and SAR	Kim and Delaney 2002
10	SIZ1	*At*	SUMO E3	Regulation of SAR	Lee et al. 2007
11	BAH1/NLA	*At*	E3	Negative regulation of Pst DC3000-induced accumulation of SA	Yaeno and Iba 2008
12	COI1	*At*	Component of SCF E3 ligase	Degradation of JAZ repressors to activate transcription of early JA responsive genes	Xie et al. 1998; Thines et al. 2007; Chini et al. 2007
13	SPL11	*Os*	E3	Negative regulation of cell death	Zeng et al. 2004
14	RING1	*At*	E3	Positive regulator of PCD induced by toxin FB1 and infection by *Pst* DC3000	Lin et al. 2008
15	AvrPtoB	*Ps*	E3	Suppression of *Fen*-mediated resistance in tomato, degradation of FLS2 andvacuolar degradation of ubiquitinated CERK1 in Arabidopsis to suppress PAMP signaling	Rosebrock et al., 2007; Gohre et al. 2008; Gimenez-Ibanez et al. 2009
16	GALA	*Rs*	Component of SCF E3 ligases?	Degradation of host protein to promote virulence, acts as specificity factor in infection of *Medicago truncatula*	Angot et al. 2006
17	HopM1	*Ps*	–	Degradation of *At*MIN7 by proteasomal pathway to block host vesicular transport during infection	Nomura et al. 2006
18	P0	*Polerovirus*	Component of SCF E3 ligase	Acts as silencing suppressor by degradation of proteins involved in host silencing machinery	Pazhouhandeh et al. 2006
19	SylA	*Ps*	–	Irreversibly inhibits catalytic sites of eukaryotic proteasome	Groll et al. 2008

Abbreviations: *At Arabidopsis thaliana*, *Nb Nicotiana benthamiana*, *Le Lycopersicum esculentum*, *Os Oryza sativa*, *Ps Pseudomonas syringae*, *Rs Ralstonia solanacearum*

conjugation of ubiquitins to protein substrates, which is normally mediated by E3 ligase enzymes. This conjugation is brought about by covalent attachment of ubiquitin to a target protein directly, or in the case of E3s with the homology to E6 carboxyl terminus (HECT) domain, the ubiquitin is first transferred to E3 ligase, and then the E3 ligase transfers that moiety to target proteins. E3 ligases are generally considered to impart the specificity and selectivity to the ubiquitination process.

Polyubiquitination is brought about by addition of more ubiquitin residues on the lysine residue of previously attached ubiquitin in the given substrate protein. Such a polyubiquitinated substrate is then targeted to the 26S proteasome complex for further degradation if the poly-ubiquitin chain is Lys-48 linked. However, proteins can also be monoubiquitinated or polyubiquitinated through alternative linkages, i.e., K6, K11, K27, K29, K33, and K63. Ubiquitination by these noncanonical linkages does not lead to subsequent 26S proteasome-mediated degradation, but rather, it serves a role in various processes such as endocytosis of plasma membrane proteins (Hicke and Dunn 2003), DNA repair (Spence et al. 1995), and signal transduction (Deng et al. 2000). More recently, research has demonstrated that certain pairs of E2s and E3s synthesize nonaegradable ubiquitin chains involving K6+K11, K27+K29, or K29+K33 isopeptide linkages; these linkages form forked ubiquitin chains (Kim et al. 2007). Also, monoubiquitination, which was not previously thought to be involved in protein degradation, has been reported to be involved in proteasomal degradation of Pax3 protein in mice (Boutet et al. 2007).

Ubiquitination requires a high level of specificity to maintain functional relevance in the organism. This specificity is rendered by E3 ligase enzymes, which are involved in the final step of the ubiquitination cascade. E3s are classified into groups based on their subunit composition. The HECT domain-containing E3s consist of a single subunit, and during ubiquitination, these E3s first transfer the ubiquitin moiety from E2 to themselves before transferring it to the substrate protein. A relatively small number of HECT E3 ligases have been found in the plants (Sullivan et al. 2003, Vierstra 2003). Really interesting new gene (RING) and U-box family of E3 ligases contain a motif called RING finger. The RING finger motif is maintained by a specific arrangement of eight cysteine and histidine residues that chelate two zinc ions in RING E3 ligases. In U-box E3 ligases, the RING motif is maintained by intra-molecular forces among the residues. RING E3s and U-box E3s are abundant in the model plants *Arabidopsis* and rice (Vierstra 2003; Azevedo et al. 2001).

Apart from these single polypeptide E3 ligases, there are other classes of E3 ligases that are protein complexes. SCF-type E3s consist of S-phase kinase-associated protein 1 (SKP-1), Cullin, F-box protein, and ring-box 1 (RBX-1). In this complex Cullin and SKP-1 provide the main scaffold, and F-box protein binds to SKP-1 whereas RBX-1 binds to Cullin. Similar to RING E3s and U-box E3s, SCF E3s act as a scaffold between E2s and the substrate proteins. SCF complexes are unique because the F-box protein binds to the substrate and brings specificity to the ubiquitination, whereas RBX-1 containing RING finger motif binds to the E2 (Devoto et al. 2003).

Functions of Ubiquitination-Related Genes in Host Defense

Role of E1 in Host Defense

E1 ubiquitin-activating enzymes act in the first step of the ubiquitination and, thus, are vital in any ubiquitination reaction. *Arabidopsis* has two E1 enzymes, UBA1 and UBA2. Both of these enzymes can bind to ubiquitin and transfer it to E2 ubiquitin-conjugating enzymes (Hatfield et al. 1997). Using a suppressor screen for the *snc1 npr1-1* double mutant with enhanced resistance to an oomycete and bacterial pathogen in *Arabidopsis*, Goritschnig and coworkers identified a mutation in UBA-1 (*mos5* allele of *Uba1*) that affects the resistance conferred by the *snc1 npr1-1* double mutant. Interestingly, the *mos5* single mutant has enhanced susceptibility to the virulent bacterial pathogen *Pseudomonas syringae* pv. *maculicola* ES4326 but has differential susceptibility to avirulent strains of the pathogen. This suggests the requirement of ubiquitination machinery in activation or downstream signaling of certain R proteins and the involvement of an E1 enzyme in defense responses. UBA1 seems to have a role in either the degradation of regulatory proteins in the *snc-1*-mediated resistance pathway or in the activation of positive regulators of the pathway by ubiquitination (Goritschnig et al. 2007).

Role of E2 in Host Defense

The human ubiquitin-conjugating E2 enzymes Ubc13 and UEV1a and the *Drosophila* homologs of Ubc13 and UEV1a are required for activation of the innate immunity pathway in their respective organisms (Deng et al. 2000; Zhou et al. 2005). In contrast, the direct role of E2 enzymes in plant immunity has not been elucidated. Takai et al. (2002) found that two rice E2s (OsUBC5a, OsUBC5b) of the Ubc4/5 subfamily function as E2 to catalyze EL5-mediated ubiquitination, and both *OsUBC5b* and *El5* were induced by an elicitor, suggesting that EL5 and OsUBC5b have roles in the rice defense response through the turnover of protein(s) via the ubiquitin/proteasome system.

Function of Plant E3 Ligases in Host Defense

E3 ligases have been the most extensively studied component of the ubiquitination pathway in plant host defense. In the following sections, we will discuss the functions of recently discovered E3 ligases in either R gene-mediated or basal defense.

Plant E3 Ligases in R Gene-Mediated Resistance

Although over 70 R genes in plants have been cloned, only a few of their defense pathways are related to ubiquitination (Liu et al. 2007). The *RPM1* gene in *Arabidopsis* encodes an LZ-NBS-LRR protein that imparts resistance to the bacterial pathogen *P. syringae* pv. *tomato* DC3000 carrying either *avrRpm1* or *avrB*. (Grant et al. 1995). When *Arabidopsis* is challenged with DC3000 (*avrRpm1* or *avrB*), the RPM1 protein localizes to the plasma membrane and disappears rapidly at initiation of the HR (Boyes et al. 1998). In yeast two-hybrid screens, RIN2 and RIN3 were found to interact with RPM1 (Kawasaki et al. 2005). RIN2 and RIN3 are both RING finger E3 ligases. Although RIN2 and RIN3 are E3 ligases and interact strongly with RPM1, time of disappearance of RPM1 in wild-type Col-0 plants and in *rin2rin3* double-mutant plants inoculated with DC3000 (*avrRpm1*) was almost identical, indicating that RIN2 and RIN3 are not required for degradation of RPM1 but rather function as positive regulators of RPM1-mediated HR. Because *rin2 rin3* plants showed a weaker HR than Col-0 but did not alter pathogen growth when inoculated with DC3000, the authors suspect that the RIN2/RIN3 RING E3 ligases might act on a substrate that regulates RPM1-dependent HR.

Barley *RAR1* (*HvRAR1*) was identified as a gene required for several cases of R gene-mediated resistance involving R genes *Mla6* and *Mla12*, which confer resistance to *Blumeria graminis* f.sp. *hordei* (Shirasu et al. 1999). SGT1 was identified as interacting with RAR1. It also associates with SKP1 and CUL1, subunits of the SCF ubiquitin ligase complex. Also, COP9 signalosome (CSN) complex, a multiprotein complex associated with protein degradation by the 26S proteasome, interacts with the RAR1–SGT1 complex (Azevedo et al. 2002). Interactions among all these components suggest a connection between disease resistance and ubiquitination. Because loss of function of *SGT1, SKP1*, or CSN compromises resistance, it is tempting to speculate that these components target negative regulators of the defense response toward SCF-mediated protein degradation. Interestingly, in another genetic analysis experiment, SGT1b seemed to have two distinct functions: antagonizing RAR1 to negatively regulate R protein accumulation before infection as well as acting as a positive regulator of HR during infection (Holt et al. 2005).

Many *Avr9/Cf-9 rapidly elicited* (ACRE) genes show rapid expression changes after Avr9 treatment in Cf-9 tobacco cells (Gonzalez-Lamothe et al. 2006). Among them, a few encode ubiquitin E3 ligases such as the U-box gene ACRE74/CMPG1. In the Avr9/Cf-9 interaction, silencing of *CMPG1* leads to a reduction in HR, whereas overexpression of *CMPG1* leads to an increase in HR. CMPG1 also participates in the *Pto/avrPto* and *Inf1* responses. Thus, CMPG1 acts as a positive regulator of disease resistance (Gonzalez-Lamothe et al. 2006). Another ACRE gene involved in R gene-mediated resistance is *ACRE189/ACIF-1*, which encodes an F-box protein. ACIF1 is necessary for the HR elicited by Avr9, Avr4, AvrPto, Inf1, and P50 helicase of tobacco mosaic virus. Thus, this F-box protein acts as positive regulator of plant defenses in several examples of R gene-mediated resistance (van den Burg et al. 2008).

Plant E3 Ligases in Basal Defense

As mentioned above, PTI forms the first layer of defense for plants after preformed defenses, such as physical barriers, are overcome by the pathogen. EL5 is a RING-H2 finger protein identified as an elicitor-responsive gene in rice (Takai et al. 2002). *EL5* is rapidly and transiently induced when suspension-cultured rice cells are treated with the elicitor *N*-acetylchitooligosaccharide. In *Arabidopsis*, three plant U-box (PUB) E3 ubiquitin ligase encoding genes, i.e., *PUB22*, *PUB23*, and *PUB24*, were highly induced after treatment with the PAMP factor flg22 (Trujillo et al. 2008). When inoculated with *P. syringae* pv. *tomato*, reactive oxygen species (ROS) production was significantly higher in the triple mutant (*pub22 pub23 pub24*) than in the wild type, and the triple-mutant also significantly restricted pathogen growth. In accord with these observations, the expression of genes involved in the production of ROS (such as *RbohD*) was elevated in the triple mutant. Oxidative burst was also induced in the triple-mutant plants when treated with other PAMPs such as chitin and elf18. These results provided evidence that ubiquitination can negatively regulate PTI in plants. Further, the triple-mutant plants were as fertile as the wild-type plants and did not have any apparent morphological alternations, suggesting that these genes are unlikely to play roles in normal plant growth and development (Trujillo et al. 2008).

Plant E3 Ligases in Systemic Immunity

Once a plant is attacked by pathogens, the cells distant from the primary and secondary infection sites are protected by SAR. SAR generally imparts broad-spectrum resistance and is dependent on plant hormones like salicylic acid (SA), jasmonic acid (JA), ethylene, and abscisic acid (de Wit

2007). The following paragraphs describe the role of different E3 ubiquitin ligases like F-box and RING-containing proteins in SAR and in other kinds of induced defense responses.

The F-box protein SON1 was the first E3 ubiquitin ligase shown to be involved in induced defense responses in *Arabidopsis* (Kim and Delaney 2002), but those defense responses were found to be independent of both SA and SAR, thus confirming the novelty of the induced defense response. SAR in *Arabidopsis* is regulated by NPR1 and SA. *npr1* plants showed hyper-susceptibility to the downy mildew pathogen, *Peronospora parasitica. SON1* was identified from mutational screens designed to identify mutants in the *npr1* background that restore resistance to *P. parasitica*. This restoration in resistance was without the induction of SAR-associated genes, and the resistance was expressed even in *npr1 son1 NahG* plants. Because NahG degrades SA, SA accumulation clearly was not required for *son1*-mediated resistance (Kim and Delaney 2002). It would be interesting to know the targets of SON1 because if SON1 is a part of the SCF ubiquitin ligase complex that degrades specific substrate proteins, then those substrate proteins must be playing a role in either SAR or induced systemic resistance. However, Spoel et al. (2009) recently demonstrated that a Cullin3-based ubiquitin ligase is required for the NPR1-mediated SAR in *Arabidopsis*. They found that the inducers of SAR promote NPR1 phosphorylation at residues Ser11/Ser15 and then facilitate its recruitment to a Cullin3-based ubiquitin ligase, providing a link between SAR and ubiquitination in plants.

The RING E3 ligase BAH1/NLA was shown to be involved in the SA synthesis in *Arabidopsis*. SA is synthesized by two pathways, one involving isochorismate synthase1 (ICS1) and the other involving benzoic acid (BA). The *Arabidopsis sid2* mutant is defective in the ICS1 pathway. *bah1-D* mutant plants accumulate higher levels of SA when inoculated with *P. syringae* pv. *tomato* DC3000, and the mutation also causes SA-dependent pathogen-induced localized cell death. Thus, BAH1/NLA negatively regulates DC3000-induced accumulation of SA (Yaeno and Iba 2008). Because the ICS1 pathway of SA synthesis is considered a major pathway, it warrants further study to determine why and how the BA pathway of SA synthesis is negatively regulated in an ubiquitination-dependent manner.

The F-box E3 ligase COI-1 was found to be involved in JA signaling over 10 years ago (Xie et al. 1998). JA and its derivatives mediate plant responses to attacks by pathogens and insects and to wounding. A crucial component in JA signaling is the protein COI-1, which is a component of an SCF type E3 ubiquitin ligase SCF^{COI1}. The importance of COI-1 to JA signaling and its being part of the SCF complex indicated that activation of JA-dependent responses could involve proteasome-dependent protein degradation (Xie et al. 1998). JA-dependent gene expression is dependent on transcriptional activators that are regulated by jasmonate ZIM domain (JAZ)-containing proteins. In *Arabidopsis*, there are 12 members of the JAZ protein family. The JAZ1 protein is the target for degradation by SCF^{COI1}-mediated ubiquitination. When the jasmonoyl–isoleucine conjugate signal is perceived by the SCF^{COI1} complex, the complex polyubiquitinates the JAZ1 repressor protein for 26S proteasome-mediated degradation. Such degradation releases those transcriptional activators suppressed by JAZ1 resulting in the transcription of early JA-responsive genes, which also include JAZ genes as the feedback loop (Thines et al. 2007; Chini et al. 2007).

Plant E3 Ligases in Programmed Cell Death

Evidence is accumulating that E3 ligases are important players for the control of plant E3 ligases in programmed cell death (PCD) in plants. Dead cells inhibit the pathogen's growth by limiting access to the nutrition in living cells, and dead cells also generate signals that are carried to distant living plant cells and cause the distant cells to prepare for pathogen attack. A role of E3 ligases in PCD was first demonstrated by the lesion mimic mutant s*pl11*. This mutant generates spontaneous lesions on leaves and sheaths and confers enhanced nonrace-specific resistance to both the rice blast pathogen, *Magnaporthe oryzae*, and the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae*. Following lesion formation, various defense-related genes are activated that correlate with the resistance observed against these pathogens (Zeng et al. 2004). SPL11, a U-box E3 ubiquitin ligase, serves as a negative regulator of cell death and was the first example from the U-box E3 ubiquitin ligase family to be associated with PCD. Functional characterization of the SPL11 interactors should generate more information on the contribution of the ubiquitination system to plant defense signaling and to the regulation of cell death at the molecular level. Also, research that reveals the components of the *spl11*-mediated cell death pathway and its correlation with other defense pathways would help clarify the overall role of SPL11 in plant host defense.

RING1 is a positive regulator of PCD and is localized to plasma membrane lipid rafts in *Arabidopsis* (Lin et al. 2008). It is induced by a PCD-inducing fungal toxin fumonisin B1 (FB1) and other biotic stresses such as infection by *P. syringae.* Knock-down of RING1 transcripts leads to FB1 hyposensitivity, but overexpression of RING1 confers hypersensitivity. Researchers have speculated that RING1 acts as a signal from the plasma membrane lipid rafts to trigger the FB1-induced PCD pathway by ubiquitinating an unknown substrate protein, which can be a negative regulator of PCD. This speculation awaits addi-

tional proof by the identification of the RING1 substrates and characterization of their role in PCD (Lin et al. 2008).

Role of Small Ubiquitin-Like Modifier Proteins in Defense Response

Sumoylation is directed by an enzymatic cascade analogous to that involved in ubiquitination. Small ubiquitin-like modifiers (SUMOs) are slightly larger than ubiquitin, can be attached to a substrate in a fashion similar to ubiquitination, and require E1 activating enzyme, E2 conjugating enzyme, and E3 ligase enzymes. The reversible modifications brought about by sumoylation to the substrate can play different roles in cellular processes. For example, SIZ1 is an *Arabidopsis* SUMO E3 ligase, and its mutant *siz1* shows a high level of SAR, increased levels of SA, increased expression of PR genes, and increased resistance to *P. syringae* pv. *tomato* DC3000. *siz1 NahG* plants show a reversal of the phenotype to the wild type. Genetic analyses revealed that *SIZ1* and *PAD4* interact epistatically to regulate PR gene expression and disease resistance (Lee et al. 2007). It is likely that SIZ1 targets some components of SA signaling for sumoylation. SUMO modification and SA-mediated innate immunity are important new areas of research in defense signaling.

Use of Pathogen Effector Proteins to Interfere with the Host Ubiquitination Machinery for Pathogenic Advantage

Surprisingly, pathogens also use ubiquitination to counter host defenses in plants. In *P. syringae,* AvrPtoB is the cognate avirulence protein of the tomato R protein Pto, and its N-terminal region is sufficient to elicit Pto/Prf-mediated HR (Abramovitch et al. 2003). The C-terminal region of AvrPtoB contains a domain that shows structural homology to RING finger- and U-box-type of E3 ubiquitin ligases and has E3 ubiquitin ligase activity (Janjusevic et al. 2006). In tomato varieties lacking the Pto kinase, truncated AvrPtoB lacking the C-terminal E3 ligase domain is recognized by Fen kinase, triggering a novel resistance referred to as Rsb (Abramovitch et al. 2003). Rsb resistance is suppressed when full-length AvrPtoB is presented. A recent study demonstrated that AvrPtoB ubiquitinates Fen (a highly related kinase to Pto) and targets it for degradation in a 26S proteasome-dependent manner (Rosebrock et al. 2007). AvrPtoB also targets PAMP signaling by eliminating FLS2 (de Torres et al. 2006). FLS2, which is one of the PRRs and is a member of receptor-like kinases, recognizes the bacterial flagellin flg22. Through its N-terminal domain, AvrPtoB associates with FLS2, and its C terminus domain carrying E3 ubiquitin ligase activity causes the polyubiquitination of the kinase domain of FLS2 that leads to the elimination of FLS2 from the cell periphery (Gohre et al. 2008). CERK1 is another PRR that is targeted by AvrPtoB for degradation via the ubiquitination pathway, and the degradation of ubiquitinated CERK1 takes place in vacuoles rather than in the 26S proteasome (Gimenez-Ibanez et al. 2009). Interestingly, AvrPtoB functions as an inhibitor of kinases such as FLS2, BAK1, CERK1, Fen, and Pto, and AvrPtoB's interaction with these proteins in plants leads to kinase deactivation. Thus, AvrPtoB is a very versatile bacterial effector protein that can inactivate the R gene-mediated resistance pathway as well as PAMP-dependent basal defenses.

HopM1 is another effector produced by *P. syringae* that might be involved in defense-related protein degradation in *Arabidopsis*. Although it is unrelated to any kind of ubiquitination proteins, HopM1 was found to degrade the *Arabidopsis* protein AtMIN7 via the 26S proteasomal pathway (Nomura et al. 2006). AtMIN7 is member of the guanine-nucleotide exchange factors–ADP-ribosylation factor (ARF–GEF) family of proteins in *Arabidopsis*. ARF–GEF proteins are major components in vesicular transport in eukaryotic cells. Extracellular secretion of certain compounds via vesicular transport has been shown to play an important role in plant immunity (Wang et al. 2005). Researchers speculated that HopM1 may work as an adaptor protein that interacts with an unknown E3 ligase to facilitate the degradation of AtMIN7 in the infected cells.

The GALA proteins in *Ralstonia solanacearum*, a soil-borne bacterial pathogen, contain an F-box-like domain, similar to that of eukaryotic F-box proteins (Cunnac et al. 2004). The functioning of GALA7 in the interaction between *R. solanacearum* and *Medicago truncatula* requires the functional F-box domain, indicating that the virulence function of GALA7 could be associated with ubiquitination and possibly with degradation of a target plant protein (Angot et al. 2006). Identification of those targeted plant proteins remains the next challenge in elucidating the role of GALA7 in the interaction between *R. solanacearum* and its host.

The silencing suppressor protein P0 in Poleroviruses contains an F-box domain (Mayo and Ziegler-Graff 1996). Through its F-box motif, P0 interacts with *Arabidopsis thaliana* orthologs of the SKP1, a component of the SCF family of ubiquitin E3 ligases. The F-box-like motif in P0 is essential for the P0–SKP1 ortholog interaction, virus pathogenicity, and the silencing suppressor activity of P0. In addition, silencing of a SKP1 ortholog in *Nicotiana benthamiana* compromised plant resistance to polerovirus infection. These results suggest that P0 acts as an E3 ligase that targets an essential component of the host ubiquitination machinery.

When infecting *Phaseolus vulgaris*, *P. syringae* pv. *syringae* (Pss) secrets a virulence factor called syringolin A (SylA) that irreversibly inhibits all three catalytic activities of eukaryotic proteasomes. Thus, it promotes virulence of the bacteria by interfering with the host's proteasomal degradation machinery (Groll et al. 2008). Because ubiquitination and 26S proteasomal degradation are involved in host defense against pathogen attack, this specific property of SylA represents another effector that pathogens use to manipulate the entire ubiquitination machinery in the host.

Evidence for the role of eukaryotic effectors in fungi and oomycetes is emerging. The effector protein Avr3a of the oomycete *Phytophthora infestans* encodes a protein that is recognized in the host cytoplasm, where it triggers R3a-dependent cell death in potato (Armstrong et al. 2005). Recently, research revealed that Avr3a interacts with the U-box E3-ubiquitin ligase CMPG1 in yeast and suppresses INF1-mediated cell death in potato plants (Birch et al. 2009). CMPG1 is required for the activation of the defense response mediated by multiple resistance genes in tobacco and tomato (Gonzalez-Lamothe et al. 2006). Another example concerns the rice blast fungus *M. oryzae,* the causal agent of the devastating rice blast disease. We recently cloned *AvrPiz-t*, the cognate *Avr* gene for the blast *R* gene *Piz-t,* which encodes a predicted 108-amino acid polypeptide with an 18-aa secretion signal at the N terminus in *M. oryzae* (Li et al. 2009). In the yeast two-hybrid screens using AvrPiz-t as the bait, we identified 12 AvrPiz-t interacting proteins (APIPs). Interestingly, three *APIP* genes encode RING E3 ligases, and another one encodes a protein homologous to the yeast UFD1 protein, which is responsible for the retrotranslocation of ubiquitinated proteins with their partners (Ye et al. 2003). The findings in the Avr3a and Piz-t studies suggest that like bacterial effectors, fungal and oomycete effectors may also target the host ubiquitination system to interfere with the defense responses. Further experiments are required to elucidate the function of the identified E3 ligases in the two pathosystems.

Concluding Remarks and Future Perspectives

As more food will be needed to feed the growing world population, plant diseases will continue to be one of the major constraints for stable crop production and food security. Designing novel strategies to combat plant diseases will require a deeper understanding of the molecular basis underlying disease resistance in general and ubiquitination in particular. Recent studies have revealed that ubiquitination is a critical cellular event in plant–pathogen interactions and that ubiquitination is intimately involved in R gene-mediated resistance, basal defense, induced defense responses, and PCD. One major research challenge will be identifying the targets of the plant E3 ligases and their functions in defense signaling transduction. In addition, by mimicking or manipulating certain ubiquitination-related components in the host immunity signaling pathways, plant pathogens can interfere the ubiquitination process to enhance their own growth and survival. Thus, another major challenge will be elucidating the function of pathogen effector proteins that mimic or manipulate host ubiquitination machinery. Understanding how these effectors interfere the host ubiquitination machinery will increase our knowledge about the molecular basis for host and microbe interactions. Finally, the functions of proteasome-independent ubiquitination and sumoylation remain largely unclear and also require study.

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