Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2014) 15(7), 737–746 DOI: 10.1111/mpp.12128

Review

Ubiquitination of pattern recognition receptors in plant innate immunity

BO LI[1,2,3,†], DONGPING LU[4,†] AND LIBO SHAN[1,2,*]

[1]*Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA*

[2]*Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843, USA*

[3]*The Provincial Key Laboratory of Plant Pathology of Hubei Province, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China*

[4]*Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang, Hebei 050021, China*

SUMMARY

Lacking an adaptive immune system, plants largely rely on plasma membrane-resident pattern recognition receptors (PRRs) to sense pathogen invasion. The activation of PRRs leads to the profound immune responses that coordinately contribute to the restriction of pathogen multiplication. Protein post-translational modifications dynamically shape the intensity and duration of the signalling pathways. In this review, we discuss the specific regulation of PRR activation and signalling by protein ubiquitination, endocytosis and degradation, with a particular focus on the bacterial flagellin receptor FLS2 (flagellin sensing 2) in *Arabidopsis*.

Keywords: endocytosis, pattern recognition receptors (PRRs), plant innate immunity, protein degradation, ubiquitination.

INTRODUCTION

Plants and animals are exposed to an environment full of microorganisms and have to contend with the risk of infections. The first line of immune signalling is activated via sensing of the conserved signatures among different microbial species, termed pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs), by plasma membrane (PM)-resident pattern recognition receptors (PRRs) (Boller and Felix, 2009; Schwessinger and Ronald, 2012). Recent evidence has also indicated that PRRs detect endogenous molecules derived from damaged cells, termed damage-associated molecular patterns (DAMPs) (Albert, 2013; Yamaguchi and Huffaker, 2011). Plant PRRs are often members of receptor-like kinases (RLKs) and receptor-like proteins (RLPs), which mediate PAMP- or MAMP-triggered immunity (PTI or MTI), contributing to host resistance against a broad spectrum of microbial infections (Antolin-Llovera *et al*., 2012; Monaghan and Zipfel, 2012). To overcome PTI, adapted pathogens have acquired virulence mechanisms, including the delivery of a cocktail of effectors by a bacterial type III secretion system into the host cells (Lindeberg *et al*., 2006). Various effectors have been shown to target components of the immune system and interfere with PTI or host physiological responses, termed effector-triggered susceptibility (ETS) (Jones and Dangl, 2006). To confine pathogens, plants have further evolved disease resistance (R) proteins that directly or indirectly recognize effectors to elicit effector-triggered immunity (ETI) (Chisholm *et al*., 2006; DeYoung and Innes, 2006; Jones and Dangl, 2006). The dynamic co-evolution of plant–microbe interactions is depicted as a zig-zag model (Bent and Mackey, 2007; Dodds and Rathjen, 2010; Jones and Dangl, 2006). Accumulating evidence also supports the likely continuum and intimate cross-talks between PTI and ETI (Thomma *et al*., 2011).

The correct activation of PRRs ensures rapid defence responses to fend off potential infections. However, the excessive activation of defence responses can be detrimental, even fatal, to hosts. For instance, uncontrolled cytokine production in animals often leads to autoimmune or immune-mediated inflammatory diseases, such as rheumatoid arthritis and Crohn's disease (O'Shea *et al*., 2002). In plants, various lesion-mimic or dwarf mutants have been indicated to be associated with elevated or constitutive activation of defence responses (Lenk and Thordal-Christensen, 2009; Lorrain *et al*., 2003). Thus, the activated immune responses must be kept in check to avoid defence from running amok.

Emerging evidence suggests that endocytosis and degradation of receptors serve as one of the common mechanisms to modulate signalling outputs (Altenbach and Robatzek, 2007; Sorkin and von Zastrow, 2009). In metazoans, on growth factor ligand activation, receptor tyrosine kinases (RTKs) undergo endocytosis and subsequent intracellular degradation of both ligands and receptors (Lemmon and Schlessinger, 2010). The ligand-induced internalization and intracellular trafficking of PRRs have been reported in both plant and animal innate immunity (Kagan *et al*., 2008; Robatzek *et al*., 2006). One of the major mechanisms to trigger receptor endocytosis and lysosomal targeting is ubiquitination of the cytosolic domain of membrane receptors (Raiborg *et al*., 2003; Strous and Gent, 2002).

*Correspondence: Email: lshan@tamu.edu

†These authors contributed equally to this work.

Ubiquitination is a protein post-translational modification in which various numbers of ubiquitin moieties are covalently attached to the substrates (Kerscher *et al*., 2006). The ubiquitination process consists of a stepwise reaction catalysed by a series of enzymes, including ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin–protein ligase (E3) (Smalle and Vierstra, 2004). The substrate specificity is largely determined by E3 ligases, which are broadly classified into four groups: HECT (homologous to E6-AP C-terminus), RING finger type, U-box and Cullin–RING ligases (CRLs) (Vierstra, 2009). The consequences of ubiquitination vary with the modes of ubiquitination (mono-ubiquitination versus poly-ubiquitination), type of ubiquitin chain linkages and the length of the ubiquitin chain (Komander and Rape, 2012; Tanno and Komada, 2013). An increasing body of evidence suggests the importance of ubiquitination in the fine-tuning of two major types of plant immune response mediated by cell surface PRRs and intracellular R proteins (Cheng and Li, 2012; Dielen *et al*., 2010; Marino *et al*., 2012). Here, we focus on the recent advances in ubiquitination-mediated PRR degradation and endocytosis, and their roles in the fine-tuning of plant PTI responses.

PLANT PRR UBIQUITINATION AND SIGNALLING

The sessile plants appear to encode much expanded members of gene families potentially involved in the ubiquitination process, with about 1415 putative E3s in *Arabidopsis* (Mazzucotelli *et al*., 2006). Interestingly, many of these genes exhibit transcriptional changes during various biotic and abiotic stress responses (Ramonell *et al*., 2005; Salinas-Mondragon *et al*., 1999).

Ubiquitination in FLS2 (flagellin sensing 2) signalling

Arabidopsis FLS2, a leucine-rich repeat receptor-like kinase (LRR-RLK), functions as a PRR for bacterial flagellin or its active peptide derivative flg22 (Gomez-Gomez and Boller, 2000). On flg22 perception, FLS2 instantaneously complexes with another LRR-RLK, BAK1 (brassinosteroid-insensitive 1-associated kinase 1) (Chinchilla *et al*., 2007; Heese *et al*., 2007; Schulze *et al*., 2010). BAK1 can directly phosphorylate BIK1 (Botrytis-induced kinase 1), a PM-localized receptor-like cytoplasmic kinase (RLCK), to transduce flagellin signalling. BIK1 forms a complex with FLS2/BAK1 and is released from the FLS2/BAK1 complex on flg22 perception (Lu *et al*., 2010; Zhang *et al*., 2010). The activation of mitogen-activated protein kinases (MAPKs) and calcium-dependent protein kinases (CDPKs) functions independently or synergistically downstream of the FLS2/BAK1 receptor complex to activate the expression of flg22-responsive genes (Asai *et al*., 2002; Boudsocq *et al*., 2010). In addition, flg22 perception leads to Ca^{2+} ion fluxes, the production of reactive oxygen species (ROS) and ethylene, the deposition of callose and stomatal closure to prevent pathogen entry (Fig. 1). The reader is directed to many excellent and comprehensive reviews that cover the flg22 and other MAMP perception and signalling events (Boller and Felix, 2009; Dodds and Rathjen, 2010; Nicaise *et al*., 2009; Schwessinger and Ronald, 2012). Plant innate immune signalling also appears to be under the tight control of negative regulation. A protein phosphatase functions as a negative regulator of FLS2 signalling by interacting with FLS2 (Gomez-Gomez *et al*., 2001). *Arabidopsis* MAPK phosphatase 1, MKP1, negatively regulates flg22 and other MAMP responses and plant immunity, probably through dephosphorylation of MAPKs (Anderson *et al*., 2011). FLS2 is ubiquitinated by two closely related plant U-box E3 ubiquitin ligases, PUB12 and PUB13, and subjected to degradation (Lu *et al*., 2011). PUB12 and PUB13 interact with BAK1, and are recruited to FLS2 on flg22 perception. BAK1 phosphorylates PUB12 and PUB13 directly, and is required for FLS2 and PUB12/13 association. PUB12/13 can directly poly-ubiquitinate FLS2, but not BAK1 or BIK1, suggesting the specificity of substrate ubiquitination of the receptor complex (Fig. 1) (Lu *et al*., 2011).

Protein phosphorylation and ubiquitination are two intertwined post-translational modifications playing essential roles in diverse intracellular signal transduction pathways and physiological responses (Hunter, 2007). Multiple connections between phosphorylation and ubiquitination, which act either positively or negatively in both directions, have been established. For instance, ligand-induced trans-autophosphorylation of mammalian RTKs could lead to ubiquitination of receptor kinases for degradation (Lu and Hunter, 2009). The mechanism of activation of FLS2 ubiquitination appears to be unique and distinct from RTK signalling. PUB12 and PUB13 phosphorylation by BAK1 did not enhance its ubiquitination ability on FLS2 (Lu *et al*., 2011). Instead, phosphorylation seems to be required for flg22-induced FLS2–PUB12/13 association, as a kinase inhibitor dramatically suppressed this association. The identification and characterization of PUB12 and PUB13 phosphorylation sites by BAK1 will facilitate the further elucidation of the detailed mechanisms of BAK1-mediated PUB12/13 phosphorylation on FLS2 ubiquitination. PUB22, PUB23 and PUB24, another subgroup of *Arabidopsis* U-box E3 ligases, function redundantly and negatively regulate flagellin-mediated signalling (Trujillo *et al*., 2008). Interestingly, PUB22 interacts with and ubiquitinates Exo70B2, a subunit of the exocyst complex that mediates vesicle tethering during exocytosis (Stegmann *et al*., 2012). In addition to its role in the secretion of toxic compounds and cell wall reinforcement, exocytosis in vesicle trafficking contributes to maintain membrane integrity and remodelling in response to environmental cues (Ding *et al*., 2011). Exo70B2 is required for full activation of multiple MAMP-triggered responses and resistance against different pathogen infections. The perception of flg22 stabilizes PUB22 and promotes PUB22-mediated ubiquitination and degradation of Exo70B2 via the

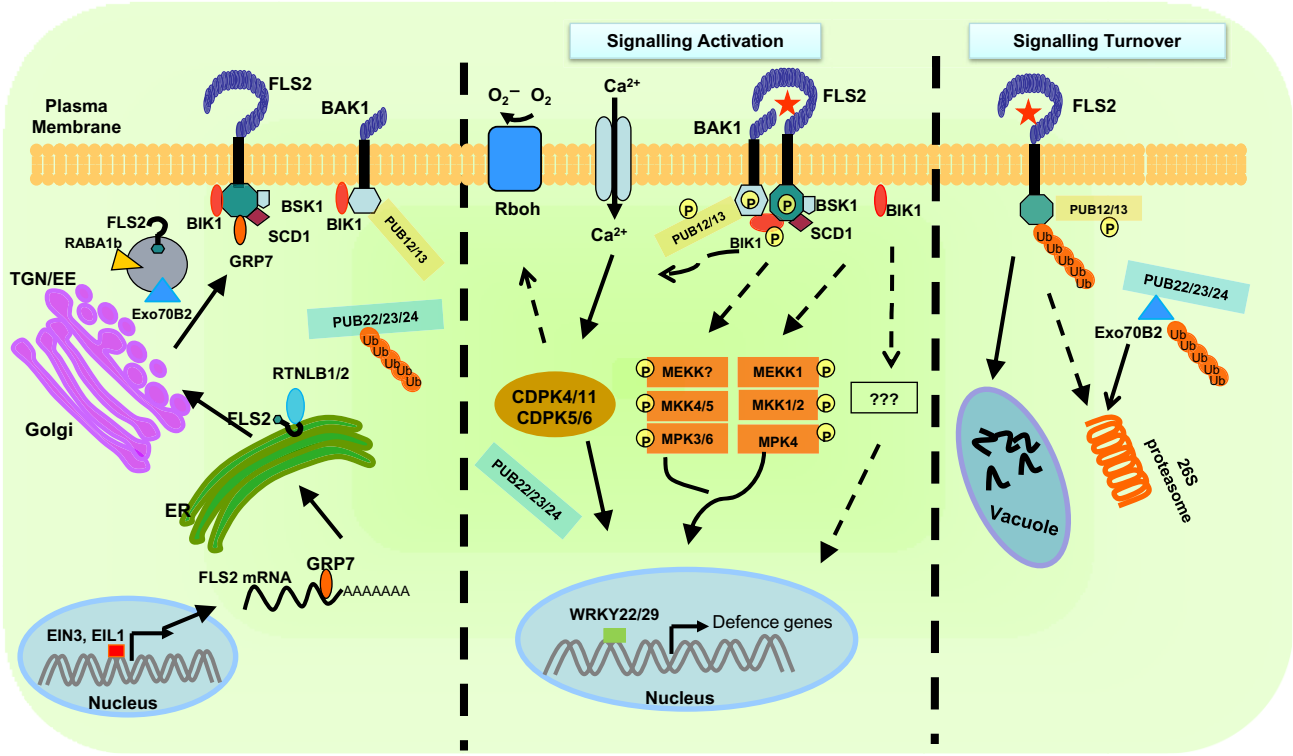

Fig. 1 FLS2 (flagellin sensing 2) signalling pathway in *Arabidopsis*. The transcription, translation and maturation of FLS2, the *Arabidopsis* receptor of bacterial flagellin (flg22), require EIN3 (ethylene insensitive 3)/EIL1 (ethylene insensitive 3-like 1) (Boutrot *et al*., 2010; Mersmann *et al*., 2010), GRP7 (glycine-rich protein 7) (Nicaise *et al*., 2013), endoplasmic reticulum (ER)-resident reticulon-like proteins RTNLB1/RTNLB2 (Lee *et al*., 2011) and RABA1b (Ras genes from rat brain a1b) (Choi *et al*., 2013). FLS2 constitutively interacts with BIK1 (Botrytis-induced kinase 1) and BSK1 (BR-signalling kinase 1) (Shi *et al*., 2013), two receptor-like cytoplasmic kinases (RLCKs) that positively regulate FLS2 signalling, and SCD1 (stomatal cytokinesis-defective 1) required for certain FLS2 responses (Korasick *et al*., 2010). BAK1 (brassinosteroid-insensitive 1-associated kinase 1) constitutively interacts with BIK1 and the E3 ubiquitin ligases PUB12/13. Binding of flg22 probably causes conformational change of FLS2, which further recruits BAK1 to the complex. The dimerization of FLS2/BAK1 leads to the phosphorylation of the FLS2/BAK1/BIK1 complex, and subsequent release of BIK1. Rapid Ca^{2+} influx, an oxidative burst mediated by plasma membrane (PM)-resident NADPH-oxidase Rboh, and activation of mitogen-activated protein kinase (MAPK) and calcium-dependent protein kinase (CDPK) cascades collectively activate and amplify the defence gene reprogramming and other defence responses. PUB12/13 are phosphorylated by BAK1 and interact with FLS2 on flg22 perception, thereby promoting poly-ubiquitination of FLS2 to tune down the signalling. PUB22/23/24 poly-ubiquitinate Exo70B, a subunit of the exocyst complex, for 26S proteasome degradation to down-regulate defence signalling. EE, early endosome; MEKK, MAPK kinase kinase; MKK, MAPK kinase; MPK, MAP kinase (MAPK); TGN, trans-Golgi network; Ub, ubiquitin.

26S proteasome, thereby attenuating flg22-mediated signalling (Fig. 1) (Stegmann *et al*., 2012). It is possible that the Exo70B2-associated exocyst complex contributes to the recycling of certain important components in PTI signalling.

Ubiquitination in XA21 and Cf-9 signalling

XA21 is an LRR-RLK PRR from rice (*Oryza sativa*) that confers resistance to specific races of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Song *et al*., 1995). In the absence of pathogen infection, XA21 interacts with XB24 (XA21 binding protein 24), an ATPase, which keeps XA21 in an inactive state (Chen X *et al*., 2010). On pathogen infection, XB24 is disassociated from XA21, probably through the perception of certain MAMPs from *Xoo*, which has been proposed to activate XA21 signalling (Chen X *et al*., 2010). An E3 ubiquitin ligase, XB3, was identified as an interacting protein of XA21 and serves as a kinase substrate of XA21 (Wang *et al*., 2006). XB3 contains an ankyrin repeat domain mediating its interaction with XA21 and a RING finger motif carrying auto-ubiquitination activity. Unlike PUB12/13, XB3 positively regulates XA21 signalling, and silencing of *XB3* increases rice susceptibility to *Xoo* infection. In addition, XB3 is required for XA21 protein abundance (Wang *et al*., 2006). However, it remains unknown whether XB3 can ubiquitinate XA21 directly or ubiquitinate other components in XA21 signalling. In addition, the attenuation of XA21 signalling is, in part, achieved by dephosphorylation of XA21 by the protein phosphatase 2C XB15 (Park *et al*., 2008).

Tomato Cf-9 confers resistance to races of the leaf mould fungus *Cladosporium fulvum* expressing the corresponding avirulence gene *Avr9* (Jones *et al*., 1994). Although classified as a

plant *R* gene, *Cf-9* encodes a PM-resident LRR-RLP. Some members of LRR-RLPs have been shown or have been proposed to function as MAMP receptors (Bar and Avni, 2009; Thomma *et al*., 2011). Among the Avr9/Cf-9 rapidly elicited (ACRE) genes in tomato, there are at least three genes encoding E3 ubiquitin ligases, and two of these, *ACRE276* and *ACRE74*, encode U-box E3 ubiquitin ligases. Silencing of tomato *ACRE276* leads to breakdown of Cf-9-specified resistance against *C. fulvum* leaf mould. Both ACRE276 and ACRE74 are positive regulators of cell death and disease resistance (Gonzalez-Lamothe *et al*., 2006; Yang *et al*., 2006). ACRE189, also referred to as ACIF1 (Avr9/Cf-9-induced F-box 1), is an F-box protein with an LRR domain. Silencing of tobacco *ACRE189* suppresses the hypersensitive responses (HRs) triggered by various elicitors, including Avr9, Avr4, AvrPto and the P50 helicase of *Tobacco mosaic virus* (TMV) (van den Burg *et al*., 2008). It is likely that these ACRE proteins may function downstream of the Cf-9 receptor. The identification of their substrates and activation mechanisms will shed light on their biochemical and physiological involvement in the modulation of defence responses.

Ubiquitination of PRRs and other host proteins by pathogen effectors

To launch a successful infection, adapted pathogens deploy various virulence strategies to interfere with plant immune responses. Interestingly, certain bacterial type III effectors possess E3 ligase activity and ubiquitinate PRRs or other host proteins directly in the suppression of plant immunity. AvrPtoB was originally identified as an avirulence protein from *Pseudomonas syringae* pv. *tomato* that recognizes tomato protein kinase Pto to trigger ETI responses (Kim *et al*., 2002). In *Arabidopsis*, AvrPtoB suppresses flg22 and many other MAMP responses by targeting BAK1 as one of the virulence mechanisms (Cheng *et al*., 2011; He *et al*., 2006; Shan *et al*., 2008; Zhou *et al*., 2013). AvrPtoB is a modular protein with a carboxy-terminal domain that is an E3 ubiquitin ligase (Janjusevic *et al*., 2006). It has been shown that AvrPtoB is able to ubiquitinate several PRRs, including FLS2 and CERK1, a receptor for fungal chitin (Gimenez-Ibanez *et al*., 2009; Gohre *et al*., 2008). AvrPtoB preferentially ubiquitinates the kinase domain of FLS2 *in vitro*, and promotes FLS2 degradation *in vivo* (Gohre *et al*., 2008). The E3 ligase activity of AvrPtoB is required for its full virulence in *P. syringae* pv. *tomato* and its suppression of host programmed cell death defences (Gohre *et al*., 2008; Janjusevic *et al*., 2006). Similarly, AvrPtoB ubiquitinates the kinase domain of CERK1 *in vitro*, and directs CERK1 degradation *in vivo* (Gimenez-Ibanez *et al*., 2009). Interestingly, CERK1, a PRR of fungal chitin that mediates plant resistance to fungal pathogens, was found to be an important determinant of plant immunity to bacterial infection, which provides a rationale for CERK1 as a target of bacterial effector AvrPtoB (Gimenez-Ibanez *et al*., 2009). Indeed, CERK1 is required for peptidoglycan (PGN)-mediated responses and immunity to bacterial infections, and has been proposed to be a part of the plant PGN receptor complex (Willmann *et al*., 2011).

As a modular protein, the N-terminal domain ($AvrPtoB_{1–387}$) of AvrPtoB lacking E3 ligase activity elicits a Pto-independent plant immunity, termed Rsb (resistance suppressed by AvrPtoB C-terminus), in tomato varieties lacking Pto and in *Nicotiana benthamiana* (Abramovitch *et al*., 2003). Fen, a homologue of Pto, interacts with $AvrPtoB_{1–387}$ and mediates the Rsb phenotype in tomato, but does not recognize full-length AvrPtoB (Rosebrock *et al*., 2007). Interestingly, the C-terminal E3 ligase domain of AvrPtoB specifically ubiquitinates Fen, but not Pto or other Pto homologues, and promotes its degradation, thereby leading to the lack of recognition of Fen by AvrPtoB (Rosebrock *et al*., 2007). How does Pto activate immunity without being ubiquitinated by AvrPtoB? An elegant study has shown that Pto phosphorylates AvrPtoB at threonine-450 (T450) accompanied by the inactivation of AvrPtoB E3 ligase activity (Ntoukakis *et al*., 2009). Importantly, $AvrPtoB^{T450D}$, a phospho-mimic mutant, lost E3 ligase activity and was able to trigger the Fen-mediated Rsb phenotype, just like $AvrPtoB_{1–387}$ and its E3 ligase mutants. AvrPtoB ubiquitinates Fen at lysine-164 (K164), an invariant residue in protein kinase that often mediates phosphotransfer during the phosphorylation reaction. The study suggests a model in which AvrPtoB poly-ubiquitinates Fen within the catalytic cleft of the kinase for proteasome-mediated degradation, whereas Pto avoids degradation by phosphorylating and inhibiting AvrPtoB E3 ligase activity (Ntoukakis *et al*., 2009). This research provides an example of the intertwined relationship between protein phosphorylation and ubiquitination in the regulation of plant immune responses.

CONSEQUENCES OF PRR UBIQUITINATION

Different types of ubiquitin chains generated by E3 ubiquitin ligases provide versatility of target proteins to distinct fates. One major consequence of protein ubiquitination is the subsequent targeting of substrates to the 26S proteasome for degradation, which can be experimentally inhibited by various proteasome inhibitors, such as MG132. In addition, ubiquitination can modulate the activity or localization of a target protein. For integral membrane proteins, ubiquitination often serves as one of the major triggers for protein internalization through endocytic pathways to the early endosome (EE) for signalling activation or further to the late endosome (LE)/multivesicular body (MVB), and finally to fuse with vacuoles/lysosomes for degradation (Haglund and Dikic, 2012; Komander and Rape, 2012; Vierstra, 2009). Evidence also indicates that the 26S proteasome-mediated protein degradation pathway may be associated with endocytic pathways, and it is possible that they are not two completely independent path-

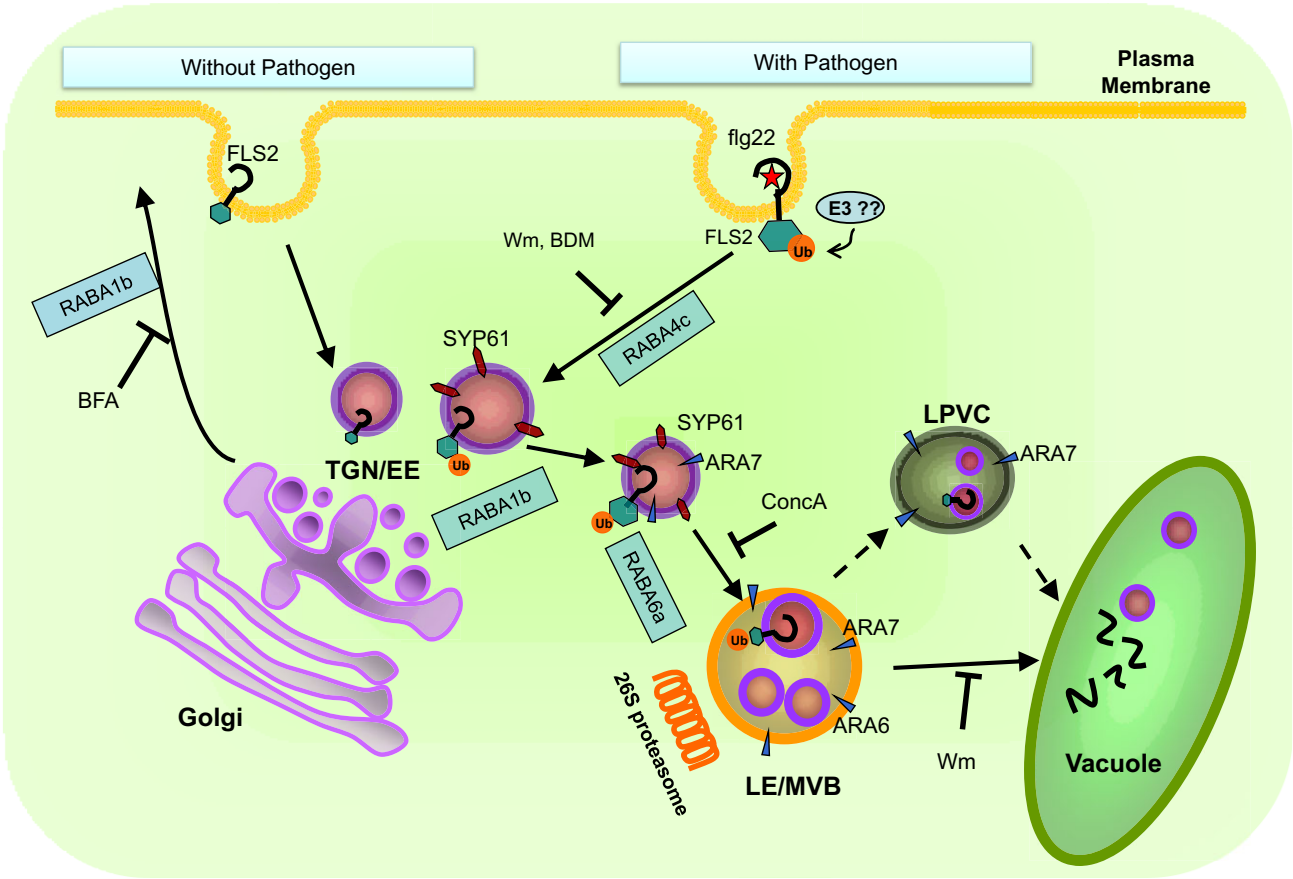

Fig. 2 Endocytic pathways involved in FLS2 (flagellin sensing 2) internalization. The nonactivated FLS2 undergoes a constitutive recycling between plasma membrane (PM) and trans-Golgi network (TGN)/early endosome (EE) compartments via a brefeldin A (BFA)-sensitive endosomal pathway. The flg22-activated FLS2 receptor traffics via a Wortmannin (Wm) and Concanamycin A (ConcA)-sensitive pathway and is further sorted into the vacuole for degradation. Mono-ubiquitination mediated by the PEST motif of FLS2 might be involved in FLS2 endocytosis initiation or protein sorting steps. The route of activated FLS2 endocytosis includes the SYP61-labelled TGN/EE compartment, SYP61- and ARA7-labelled intermediate compartment with properties between TGN/EE and late endosome (LE)/multivesicular body (MVB), ARA6-labelled LE/MVB compartment and, finally, the vacuole for degradation. MVB containing FLS2 may also traffic into the late prevacuolar compartment (LPVC) before fusion with the vacuole. RABA (Ras genes from rat brain a) family proteins RABA6a and RABA4c play roles in distinct steps of FLS2 endocytosis, and RABA1b is required for normal morphology of TGN/EE and transport of newly synthesized FLS2 to PM. BDM, 2,3-butanedione monoxime; Ub, ubiquitin.

ways in mediating protein degradation (Abas and Wisniewska, 2006; Clague and Urbe, 2010; van Kerkhof *et al*., 2000).

PRR endocytosis

In mammals, lipopolysaccharide (LPS) and its receptor TLR4 are endocytosed, trafficked to the EE/sorting endosome, and then the LE/lysosome for degradation. Endosomal trafficking of the LPS receptor complex is essential for signal termination (Husebye *et al*., 2006). It has been shown that FLS2 translocates into intracellular vesicles on flg22 perception, followed by degradation (Robatzek *et al*., 2006). Recently, evidence has suggested that internalized FLS2 proteins enter endocytic pathways with two distinct trafficking routes depending on their activation status (Beck *et al*., 2012). In the absence of flg22, nonactivated FLS2 constitutively recycles between PM and EE independent of its signalling partner BAK1 (Fig. 2). This endocytic recycling is likely to regulate the abundance of receptors at PM and maintain a constant pool of signalling receptors (Beck *et al*., 2012). On flg22 perception, FLS2 enters a distinct endocytic trafficking pathway in which it transiently localizes at the trans-Golgi network (TGN)/EE in the early stage, and transports to intermediate compartments with features between the TGN/EE and LE/MVB, followed by sorting into the LE/MVB and vacuole for degradation (Fig. 2) (Beck *et al*., 2012; Choi *et al*., 2013). In *Arabidopsis* seedlings, the FLS2-green fluorescent protein (GFP) fluorescence signal disappeared from PM after approximately 20–40 min on flg22 treatment and subsequently fluorescence-labelled vesicles appeared in the cytoplasm. These vesicles probably arise from a Wortmannin-sensitive endocytic process and can be blocked by actin inhibitors (Robatzek

et al., 2006). In plants, endocytosis is mainly mediated by the vesicle coat protein clathrin, called clathrin-mediated endocytosis (CME) (Chen *et al*., 2011). Recently, the plant defence hormone salicylic acid (SA) has been reported to affect CME of several PM-resident proteins, but not FLS2 (Du *et al*., 2013). These results suggest that various pathways may be involved in the endocytosis of plant PM proteins.

The mechanisms of activation of FLS2 internalization and differential cargo sorting of endocytosis remain largely unknown. Phosphorylation and ubiquitination have been shown to play essential roles in the activation of endocytosis and cargo sorting for various proteins (Goh *et al*., 2010). The kinase inhibitor K252a completely abolishes flg22-mediated FLS2 endocytosis. A mutation in a potential phosphorylation site, $FLS2^{T867V}$, also compromises its endocytosis (Robatzek *et al*., 2006). Consistent with the potential transphosphorylation between BAK1 and FLS2, BAK1 is required for ligand-induced FLS2 endocytosis. It remains elusive whether phosphorylation serves as a trigger for FLS2 internalization. The involvement of ubiquitination in endocytosis could occur either in endocytosis initiation or the cargo sorting step. For mammalian PM-resident RTKs, TLRs and G-protein-coupled receptors (GPCRs), ubiquitination does not appear to be required for efficient endocytosis initiation, as prevention of receptor ubiquitination, in many cases, has been shown to have little effect on endocytosis (Clague *et al*., 2012; Haglund and Dikic, 2012; Hislop and von Zastrow, 2011). However, ubiquitination is an important signal to direct the sorting of receptors into the MVB for lysosomal degradation (Tanno and Komada, 2013). Mutation of the ubiquitination sites often blocks the degradation of internalized mammalian receptors (Haglund and Dikic, 2012; MacGurn *et al*., 2012). *Arabidopsis* BOR1 (Requires High Boron 1) is mono- or di-ubiquitinated on boron application. Boron-induced ubiquitination of BOR1 is not required for endocytosis from PM, but is crucial for further sorting to MVB and subsequent degradation in vacuoles (Kasai *et al*., 2011). In another case, *Arabidopsis* IRT1 (iron-regulated transporter 1) is found in TGN/EE and probably undergoes endocytosis and subsequent degradation in vacuoles (Barberon *et al*., 2011). IRT1 is mono-ubiquitinated via unknown E3 ubiquitin ligase(s) on several cytosol-exposed residues *in vivo*. Mutations of two putative mono-ubiquitination sites stabilize IRT1 at PM, leading to extreme lethality by metal overload (Barberon *et al*., 2011). Recently, a RING-type E3 ubiquitin ligase IDF1 (IRT1 degradation factors 1) has been found to be required for IRT1 protein stability (Shin *et al*., 2013). It remains unknown whether IDF1 or other E3 ubiquitin ligases mediate the IRT1 endocytosis process.

FLS2 possesses a PEST-like motif at its C-terminus, which is often associated with mono-ubiquitination in yeasts and mammals (Roth and Davis, 2000). Mutation in the PEST motif impairs FLS2 endocytosis, suggesting that modification of the PEST motif, probably through mono-ubiquitination, may be involved in the initiation of ligand-induced FLS2 endocytosis (Robatzek *et al*., 2006). It remains an open question whether PUB12 and PUB13 are involved in FLS2 internalization. Evidence suggests that PUB12- and PUB13-mediated FLS2 ubiquitination and flg22-induced FLS2 internalization are probably uncoupled. The FLS2 PEST motif and kinase inactive mutants are compromised in FLS2 endocytosis, but do not affect FLS2 ubiquitination by PUB12 and PUB13 *in vitro* (Lu *et al*., 2011). This finding is not surprising as FLS2 ubiquitination by PUB12/13 is mainly poly-ubiquitination, which often leads to different substrate fates from mono-ubiquitination. The intracellular juxtamembrane domain of FLS2 is required for PUB12/13-mediated ubiquitination (D. Lu and L. Shan, unpublished data). This is consistent with the role of the juxtamembrane domain of receptor kinases in creating docking sites to recruit components into the fine-tuning of the signalling output (Lemmon and Schlessinger, 2010). The likely uncoupling of ligand-induced FLS2 endocytosis and degradation suggests distinct ubiquitination mechanisms operating these two linked processes. It is possible that a distinct E3 ligase mediates the initiation of FLS2 endocytosis through the PEST domain.

The rice RLK XA21 is internalized and probably transported via the TGN/EE compartment (Chen F *et al*., 2010). Similar to the ligand-independent constitutive endocytosis trafficking of FLS2, XA21 is internalized via brefeldin A (BFA)-sensitive vesicles in rice protoplasts. It would be of interest to investigate the endocytosis and recycling of XA21 during pathogen infection. Nevertheless, whether XA21-associated E3 ligase XB3 or other E3 ligase-mediated ubiquitination serves as one of the triggers for this endocytosis process remains unknown.

The tomato RLP receptor LeEix2 initiates defence responses on perception of fungal protein EIX (Ethylene-Inducing Xylanase). EIX triggers the internalization of LeEix2 from PM to EE compartments labelled by the FYVE (Fab-1, YGL023, Vps27 and EEA1) domain (Bar and Avni, 2009). Inhibition of internalization by chemical treatments results in a complete arrest of EIX-induced signalling. Furthermore, some EE compartments undergo a directional movement to a greater distance at an elevated speed on EIX application. The data suggest that internalization of the LeEix2 receptor is required for LeEix2-mediated signalling (Bar and Avni, 2009; Sharfman *et al*., 2011). It remains to be determined whether LeEix2 undergoes ubiquitination on EIX treatment. LeEix2 endocytosis requires the tyrosine-based motif YXXΦ, a putative internalization signal that binds to clathrin-associated proteins. Mutation of this motif inhibits LeEix2 internalization and abolishes its ability to induce HR in tomato (Ron and Avni, 2004). A similar motif is present in the cytoplasmic C-terminus of tomato RLP Cf-4 and is required for Cf-4 function (Vossen *et al*., MPMI congress abstracts, 2009). Moreover, the analysis of LRR-RLPs from *Arabidopsis* and rice revealed that nine of the 56 *Arabidopsis* proteins and 20 of the 90 rice proteins contain the YXXΦ motif (Fritz-Laylin *et al*., 2005). In addition, some LRR-RLKs, including

EFR, XA21 and BAK1, also possess this motif (Geldner and Robatzek, 2008). Although the biological function of this motif is not clear, this suggests the existence of a common mechanism of endocytosis in the mediation of RLK and RLP signalling.

PRR degradation

In mammals, a RING-type E3 ubiquitin ligase, Triad3A, ubiquitinates the Toll-like receptors TLR4 and TLR9 and promotes their proteolytic degradation (Chuang and Ulevitch, 2004). The extent of Triad3A-dependent TLR9 ubiquitination is increased in the presence of the proteasome inhibitor MG132. Consistently, the degradation of TLR9 is blocked by treatment with the irreversible proteasome inhibitor lactacystin, but not by the lysosomotropic agent NH_4Cl or the lysosomal protease inhibitor E64. Together, the data indicate that Triad3A-mediated ubiquitination promotes 26S proteasome-mediated TLR degradation. Genetic analysis with overexpression and loss of function of Triad3A suggests that it negatively regulates TLR activation and controls the intensity and duration of TLR signalling (Chuang and Ulevitch, 2004). Evidence also exists for the 26S proteasome-mediated degradation of plant PRRs. The degradation and internalization of FLS2-GFP are substantially compromised by the treatment of MG132 (Robatzek *et al*., 2006). PUB12/13-mediated ubiquitination and degradation of FLS2 also involve the 26S proteasome, as MG132 blocks flg22-induced degradation. Similar to Triad3A, PUB12/13 negatively regulate plant PTI signalling, as the *pub12/13* mutant exhibits enhanced immune responses to bacterial infection (Lu *et al*., 2011). The control of the duration and intensity of immunity by E3 ubiquitin ligase-mediated degradation is important for plant normal growth and development. Mutation of the rice *PUB13* orthologue *SPL11* causes plants with spontaneous cell death, a probably uncontrolled immune response (Zeng *et al*., 2004). A similar phenotype was observed in the *Arabidopsis pub13* mutant under excessive light conditions and high humidity (Li *et al*., 2012).

The *Lotus japonicus* SYMRK (Symbiosis RLK) is required for signal transduction in root symbiosis (Stracke *et al*., 2002). SINA4, a SYMRK-interacting protein, belongs to the SINA (Seven in Absentia) E3 ubiquitin ligase family. Overexpression of SINA4 in both *N. benthamiana* and *L. japonicus* nodulated roots induces SYMRK degradation and re-localization (Den Herder *et al*., 2012). However, it is not clear whether SINA4 mediates SYMRK ubiquitination. It is also possible that SINA4 ubiquitinates other components that regulate SYMRK protein stability.

Ubiquitin-tagged proteins can be degraded via three major pathways: proteasome, lysosome/vacuole and autophagosome (Clague and Urbe, 2010). The internalized membrane-located receptors are often degraded in the vacuole or the lysosome. For instance, AvrPtoB-mediated CERK1 degradation is probably vacuolar dependent as it is blocked by Bafilomycin A1, a vacuolar-type H^+-ATPase inhibitor, not by MG132 (Gimenez-Ibanez *et al*., 2009). However, a considerable number of studies have also indicated that the proteasome inhibitor MG132 can block membrane protein endocytosis and degradation. In addition to the above-mentioned FLS2 and TLR9 (Chuang and Ulevitch, 2004; Gohre *et al*., 2008; Lu *et al*., 2011; Robatzek *et al*., 2006), the auxin efflux carrier PIN2 and water channel aquaporin PIP2;1 can be stabilized with MG132 treatment (Abas and Wisniewska, 2006; Lee *et al*., 2009). The ligand-induced degradation of mammalian growth hormone receptor (GHR) and epidermal growth factor receptor (EGFR) can be blocked by various proteasome-specific inhibitors (van Kerkhof *et al*., 2000; Longva *et al*., 2002). It is likely that the effects of proteasome inhibitors on the lysosome degradation of integral membrane proteins might be indirect. These inhibitors, such as MG132, may affect the activity of lysosomal enzymes, or reduce the ubiquitin pool in the cell (Gorbea *et al*., 2010; Melikova *et al*., 2006). Existing evidence from mammalian studies also suggests the direct involvement of the proteasome activity in certain steps of membrane protein endosomal sorting processes. Translocation of the activated EGFR from the outer membrane to inner membrane of MVBs could be blocked by various proteasome inhibitors (Longva *et al*., 2002). Ligand-induced lysosomal EGFR degradation is preceded by EGFR de-ubiquitination, which requires 26S proteasome activity (Alwan *et al*., 2003). Similarly, the lysosomal degradation of human TrkA (neurotrophic tyrosine kinase receptor type I) also requires proteasome-dependent de-ubiquitination. Ubiquitinated TrkA employs the endosomal–lysosomal pathway for degradation and a proteasome-dependent de-ubiquitination step precedes its delivery to lysosomes (Geetha and Wooten, 2008). In addition, evidence also suggests the existence of a proteasome pool that associates with endosomes and influences receptor endosomal sorting. Human Ecm29-associated 26S proteasomes are present on flotillin-positive endosomes and Ecm29 functions as an adaptor in the localization of the 26S proteasome on endosomes, endoplasmic reticulum membrane and centrosome (Gorbea *et al*., 2010). Thus, it is possible that the proteasome pathway is involved in an endosomal sorting step of ubiquitinated proteins to lysosomes, thereby providing a mechanism for regulated degradation.

CONCLUSION AND PERSPECTIVE

The PM-resident PRRs serve as an array of surveillance radar antennas, which promptly detect the microbial and danger signals and launch robust defence responses. Precise and efficient activation and attenuation of PRR signalling are crucial for any organism survival. It has become an emerging theme that ubiquitination and endocytosis play important roles in the fine-tuning of PRR signalling. Despite the fact that a mechanistic understanding is still missing, various components involved in ubiquitination and endocytosis processes, for instance, distinct families of E3

ubiquitin ligases, have been found to be involved in PRR signalling. Certain E3 ubiquitin ligases are able to ubiquitinate PRR receptors directly and mediate their degradation, whereas others seem to ubiquitinate the components associated with PRR signalling. However, the connection between ubiquitination and endocytosis has not been established. In addition, it remains largely unknown what are the mechanisms underlying ligand-induced PRR endocytosis activation and whether endocytosis is linked with signalling activation. Ubiquitination, as one of the most prevalent post-translational modifications, is probably involved in the regulation of various signalling components in plant innate immunity. Genome-wide characterization of ubiquitination dynamics in plant immune signalling will provide a global view of the role of protein ubiquitination in the fine-tuning of a variety of signalling outputs. The development of versatile and sensitive *in vivo* and *in vitro* ubiquitination assays, in combination with label-free quantitative proteomics, will lead to the identification of novel components in ubiquitination-mediated plant PRR signalling.

ACKNOWLEDGEMENTS

We thank Dr Ping He for insightful discussions and critical reading of the manuscript, and two anonymous reviewers for their constructive comments and suggestions to improve the manuscript. This work was supported by funds from the National Institutes of Health (NIH) (R01GM097247) and the Robert A. Welch Foundation (A-1795) to LS. BL was partially supported by Dr Daohong Jiang's laboratory from Huazhong Agricultural University, China.

REFERENCES

Abas, L. and Wisniewska, J. (2006) Intracellular trafficking and proteolysis of the Arabidopsis auxin-efflux facilitator PIN2 are involved in root gravitropism. *Nat. Cell Biol.* **8**, 249–256.

Abramovitch, R.B., Kim, Y.J., Chen, S., Dickman, M.B. and Martin, G.B. (2003) Pseudomonas type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. *EMBO J.* **22**, 60–69.

Albert, M. (2013) Peptides as triggers of plant defence. *J. Exp. Bot.* **64**, 5269–5279.

Altenbach, D. and Robatzek, S. (2007) Pattern recognition receptors: from the cell surface to intracellular dynamics. *Mol. Plant–Microbe Interact.* **20**, 1031–1039.

Alwan, H.A.J., van Zoelen, E.J.J. and van Leeuwen, J.E.M. (2003) Ligand-induced lysosomal epidermal growth factor receptor (EGFR) degradation is preceded by proteasome-dependent EGFR de-ubiquitination. *J. Biol. Chem.* **278**, 35 781–35 790.

Anderson, J.C., Bartels, S., Gonzalez Besteiro, M.A., Shahollari, B., Ulm, R. and Peck, S.C. (2011) Arabidopsis MAP Kinase Phosphatase 1 (AtMKP1) negatively regulates MPK6-mediated PAMP responses and resistance against bacteria. *Plant J.* **67**, 258–268.

Antolin-Llovera, M., Ried, M.K., Binder, A. and Parniske, M. (2012) Receptor kinase signaling pathways in plant–microbe interactions. *Annu. Rev. Phytopathol.* **50**, 451–473.

Asai, T., Tena, G., Plotnikova, J., Willmann, M.R., Chiu, W.L., Gomez-Gomez, L., Boller, T., Ausubel, F.M. and Sheen, J. (2002) MAP kinase signalling cascade in Arabidopsis innate immunity. *Nature*, **415**, 977–983.

Bar, M. and Avni, A. (2009) EHD2 inhibits ligand-induced endocytosis and signaling of the leucine-rich repeat receptor-like protein LeEix2. *Plant J.* **59**, 600–611.

Barberon, M., Zelazny, E., Robert, S., Conejero, G., Curie, C., Friml, J. and Vert, G. (2011) Monoubiquitin-dependent endocytosis of the IRON-REGULATED TRANSPORTER 1 (IRT1) transporter controls iron uptake in plants. *Proc. Natl. Acad. Sci. USA*, **108**, E450–E458.

Beck, M., Zhou, J., Faulkner, C., MacLean, D. and Robatzek, S. (2012) Spatio-temporal cellular dynamics of the Arabidopsis flagellin receptor reveal activation status-dependent endosomal sorting. *Plant Cell*, **24**, 4205–4219.

Bent, A.F. and Mackey, D. (2007) Elicitors, effectors, and R genes: the new paradigm and a lifetime supply of questions. *Annu. Rev. Phytopathol.* **45**, 399–436.

Boller, T. and Felix, G. (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* **60**, 379–406.

Boudsocq, M., Willmann, M.R., McCormack, M., Lee, H., Shan, L.B., He, P., Bush, J., Cheng, S.H. and Sheen, J. (2010) Differential innate immune signalling via Ca^{2+} sensor protein kinases. *Nature*, **464**, 418–422.

Boutrot, F., Segonzac, C., Chang, K.N., Qiao, H., Ecker, J.R., Zipfel, C. and Rathjen, J.P. (2010) Direct transcriptional control of the Arabidopsis immune receptor FLS2 by the ethylene-dependent transcription factors EIN3 and EIL1. *Proc. Natl. Acad. Sci. USA*, **107**, 14 502–14 507.

van den Burg, H.A., Tsitsigiannis, D.I., Rowland, O., Lo, J., Rallapalli, G., MacLean, D., Takken, F.L. and Jones, J.D. (2008) The F-box protein ACRE189/ACIF1 regulates cell death and defense responses activated during pathogen recognition in tobacco and tomato. *Plant Cell*, **20**, 697–719.

Chen, F., Gao, M.J., Miao, Y.S., Yuan, Y.X., Wang, M.Y., Li, Q., Mao, B.Z., Jiang, L.W. and He, Z.H. (2010) Plasma membrane localization and potential endocytosis of constitutively expressed XA21 proteins in transgenic rice. *Mol. Plant*, **3**, 917–926.

Chen, X., Chern, M., Canlas, P.E., Ruan, D., Jiang, C. and Ronald, P.C. (2010) An ATPase promotes autophosphorylation of the pattern recognition receptor XA21 and inhibits XA21-mediated immunity. *Proc. Natl. Acad. Sci. USA*, **107**, 8029–8034.

Chen, X., Irani, N.G. and Friml, J. (2011) Clathrin-mediated endocytosis: the gateway into plant cells. *Curr. Opin. Plant Biol.* **14**, 674–682.

Cheng, W., Munkvold, K.R., Gao, H., Mathieu, J., Schwizer, S., Wang, S., Yan, Y.B., Wang, J., Martin, G.B. and Chai, J. (2011) Structural analysis of *Pseudomonas syringae* AvrPtoB bound to host BAK1 reveals two similar kinase-interacting domains in a type III effector. *Cell Host Microbe*, **10**, 616–626.

Cheng, Y.T. and Li, X. (2012) Ubiquitination in NB-LRR-mediated immunity. *Curr. Opin. Plant Biol.* **15**, 392–399.

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

Chisholm, S.T., Coaker, G., Day, B. and Staskawicz, B.J. (2006) Host–microbe interactions: shaping the evolution of the plant immune response. *Cell*, **124**, 803–814.

Choi, S.W., Tamaki, T., Ebine, K., Uemura, T., Ueda, T. and Nakano, A. (2013) RABA members act in distinct steps of subcellular trafficking of the FLAGELLIN SENSING2 receptor. *Plant Cell*, **25**, 1174–1187.

Chuang, T.H. and Ulevitch, R.J. (2004) Triad3A, an E3 ubiquitin-protein ligase regulating Toll-like receptors. *Nat. Immunol.* **5**, 495–502.

Clague, M.J. and Urbe, S. (2010) Ubiquitin: same molecule, different degradation pathways. *Cell*, **143**, 682–685.

Clague, M.J., Liu, H. and Urbe, S. (2012) Governance of endocytic trafficking and signaling by reversible ubiquitylation. *Dev. Cell*, **23**, 457–467.

Den Herder, G., Yoshida, S., Antolin-Llovera, M., Ried, M.K. and Parniske, M. (2012) *Lotus japonicus* E3 ligase SEVEN IN ABSENTIA4 destabilizes the symbiosis receptor-like kinase SYMRK and negatively regulates rhizobial infection. *Plant Cell*, **24**, 1691–1707.

DeYoung, B.J. and Innes, R.W. (2006) Plant NBS-LRR proteins in pathogen sensing and host defense. *Nat. Immunol.* **7**, 1243–1249.

Dielen, A.S., Badaoui, S., Candresse, T. and German-Retana, S. (2010) The ubiquitin/26S proteasome system in plant–pathogen interactions: a never-ending hide-and-seek game. *Mol. Plant Pathol.* **11**, 293–308.

Ding, Z.J., Galvan-Ampudia, C.S., Demarsy, E., Langowski, L., Kleine-Vehn, J., Fan, Y.W., Morita, M.T., Tasaka, M., Fankhauser, C., Offringa, R. and Friml, J. (2011) Light-mediated polarization of the PIN3 auxin transporter for the phototropic response in Arabidopsis. *Nat. Cell Biol.* **13**, 447–452.

Dodds, P.N. and Rathjen, J.P. (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. *Nat. Rev. Genet.* **11**, 539–548.

Du, Y.L., Tejos, R., Beck, M., Himschoot, E., Li, H.J., Robatzek, S., Vanneste, S. and Friml, J. (2013) Salicylic acid interferes with clathrin-mediated endocytic protein trafficking. *Proc. Natl. Acad. Sci. USA*, **110**, 7946–7951.

Fritz-Laylin, L.K., Krishnamurthy, N., Tor, M., Sjolander, K.V. and Jones, J.D.G. (2005) Phylogenomic analysis of the receptor-like proteins of rice and Arabidopsis. *Plant Physiol.* **138**, 611–623.

Geetha, T. and Wooten, M.W. (2008) TrkA receptor endolysosomal degradation is both ubiquitin and proteasome dependent. *Traffic*, **9**, 1146–1156.
Geldner, N. and Robatzek, S. (2008) Plant receptors go endosomal: a moving view on signal transduction. *Plant Physiol.* **147**, 1565–1574.
Gimenez-Ibanez, S., Hann, D.R., Ntoukakis, V., Petutschnig, E., Lipka, V. and Rathjen, J.P. (2009) AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. *Curr. Biol.* **19**, 423–429.
Goh, L.K., Huang, F., Kim, W., Gygi, S. and Sorkin, A. (2010) Multiple mechanisms collectively regulate clathrin-mediated endocytosis of the epidermal growth factor receptor. *J. Cell Biol.* **189**, 871–883.
Gohre, V., Spallek, T., Haweker, H., Mersmann, S., Mentzel, T., Boller, T., de Torres, M., Mansfield, J.W. and Robatzek, S. (2008) Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. *Curr. Biol.* **18**, 1824–1832.
Gomez-Gomez, L. and Boller, T. (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. *Mol. Cell*, **5**, 1003–1011.
Gomez-Gomez, L., Bauer, Z. and Boller, T. (2001) Both the extracellular leucine-rich repeat domain and the kinase activity of FSL2 are required for flagellin binding and signaling in Arabidopsis. *Plant Cell*, **13**, 1155–1163.
Gonzalez-Lamothe, R., Tsitsigiannis, D.I., Ludwig, A.A., Panicot, M., Shirasu, K. and Jones, J.D.G. (2006) The U-Box protein CMPG1 is required for efficient activation of defense mechanisms triggered by multiple resistance genes in tobacco and tomato. *Plant Cell*, **18**, 1067–1083.
Gorbea, C., Pratt, G., Ustrell, V., Bell, R., Sahasrabudhe, S., Hughes, R.E. and Rechsteiner, M. (2010) A protein interaction network for Ecm29 links the 26 S proteasome to molecular motors and endosomal components. *J. Biol. Chem.* **285**, 31 616–31 633.
Haglund, K. and Dikic, I. (2012) The role of ubiquitylation in receptor endocytosis and endosomal sorting. *J. Cell Sci.* **125**, 265–275.
He, P., Shan, L., Lin, N.C., Martin, G.B., Kemmerling, B., Nurnberger, T. and Sheen, J. (2006) Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in Arabidopsis innate immunity. *Cell*, **125**, 563–575.
Heese, A., Hann, D.R., Gimenez-Ibanez, S., Jones, A.M.E., He, K., Li, J., Schroeder, J.I., Peck, S.C. and Rathjen, J.P. (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proc. Natl. Acad. Sci. USA*, **104**, 12 217–12 222.
Hislop, J.N. and von Zastrow, M. (2011) Role of ubiquitination in endocytic trafficking of G-protein-coupled receptors. *Traffic*, **12**, 137–148.
Hunter, T. (2007) The age of crosstalk: phosphorylation, ubiquitination, and beyond. *Mol. Cell*, **28**, 730–738.
Husebye, H., Halaas, O., Stenmark, H., Tunheim, G., Sandanger, O., Bogen, B., Brech, A., Latz, E. and Espevik, T. (2006) Endocytic pathways regulate Toll-like receptor 4 signaling and link innate and adaptive immunity. *EMBO J.* **25**, 683–692.
Janjusevic, R., Abramovitch, R.B., Martin, G.B. and Stebbins, C.E. (2006) A bacterial inhibitor of host programmed cell death defenses is an E3 ubiquitin ligase. *Science*, **311**, 222–226.
Jones, D.A., Thomas, C.M., Hammondkosack, K.E., Balintkurti, P.J. and Jones, J.D.G. (1994) Isolation of the tomato Cf-9 gene for resistance to *Cladosporium-fulvum* by transposon tagging. *Science*, **266**, 789–793.
Jones, J.D.G. and Dangl, J.L. (2006) The plant immune system. *Nature*, **444**, 323–329.
Kagan, J.C., Su, T., Horng, T., Chow, A., Akira, S. and Medzhitov, R. (2008) TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. *Nat. Immunol.* **9**, 361–368.
Kasai, K., Takano, J., Miwa, K., Toyoda, A. and Fujiwara, T. (2011) High boron-induced ubiquitination regulates vacuolar sorting of the BOR1 borate transporter in *Arabidopsis thaliana*. *J. Biol. Chem.* **286**, 6175–6183.
van Kerkhof, P., Govers, R., Alves dos Santos, C.M. and Strous, G.J. (2000) Endocytosis and degradation of the growth hormone receptor are proteasome-dependent. *J. Biol. Chem.* **275**, 1575–1580.
Kerscher, O., Felberbaum, R. and Hochstrasser, M. (2006) Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu. Rev. Cell Dev. Biol.* **22**, 159–180.
Kim, Y.J., Lin, N.C. and Martin, G.B. (2002) Two distinct *Pseudomonas* effector proteins interact with the Pto kinase and activate plant immunity. *Cell*, **109**, 589–598.
Komander, D. and Rape, M. (2012) The ubiquitin code. *Annu. Rev. Biochem.* **81**, 203–229.
Korasick, D.A., McMichael, C., Walker, K.A., Anderson, J.C., Bednarek, S.Y. and Heese, A. (2010) Novel functions of stomatal cytokinesis-defective 1 (SCD1) in innate immune responses against bacteria. *J. Biol. Chem.* **285**, 23 340–23 348.
Lee, H.K., Cho, S.K., Son, O., Xu, Z.Y., Hwang, I. and Kim, W.T. (2009) Drought stress-induced Rma1H1, a RING membrane-anchor E3 ubiquitin ligase homolog, regulates aquaporin levels via ubiquitination in transgenic Arabidopsis plants. *Plant Cell*, **21**, 622–641.
Lee, H.Y., Bowen, C.H., Popescu, G.V., Kang, H.G., Kato, N., Ma, S.S., Dinesh-Kumar, S., Snyder, M. and Popescu, S.C. (2011) Arabidopsis RTNLB1 and RTNLB2 reticulon-like proteins regulate intracellular trafficking and activity of the FLS2 immune receptor. *Plant Cell*, **23**, 3374–3391.
Lemmon, M.A. and Schlessinger, J. (2010) Cell signaling by receptor tyrosine kinases. *Cell*, **141**, 1117–1134.
Lenk, A. and Thordal-Christensen, H. (2009) From nonhost resistance to lesion-mimic mutants: useful for studies of defense signaling. *Adv. Bot. Res.* **51**, 91–121.
Li, W., Ahn, I.P., Ning, Y.S., Park, C.H., Zeng, L.R., Whitehill, J.G.A., Lu, H., Zhao, Q., Ding, B., Xie, Q., Zhou, J.M., Dai, L. and Wang, G.L. (2012) The U-Box/ARM E3 ligase PUB13 regulates cell death, defense, and flowering time in Arabidopsis. *Plant Physiol.* **159**, 239–250.
Lindeberg, M., Cartinhour, S., Myers, C.R., Schechter, L.M., Schneider, D.J. and Collmer, A. (2006) Closing the circle on the discovery of genes encoding Hrp regulon members and type III secretion system effectors in the genomes of three model *Pseudomonas syringae* strains. *Mol. Plant–Microbe Interact.* **19**, 1151–1158.
Longva, K.E., Blystad, F.D., Stang, E., Larsen, A.M., Johannessen, L.E. and Madshus, I.H. (2002) Ubiquitination and proteasomal activity is required for transport of the EGF receptor to inner membranes of multivesicular bodies. *J. Cell Biol.* **156**, 843–854.
Lorrain, S., Vailleau, F., Balaque, C. and Roby, D. (2003) Lesion mimic mutants: keys for deciphering cell death and defense pathways in plants? *Trends Plant Sci.* **8**, 263–271.
Lu, D., Wu, S., Gao, X., Zhang, Y., Shan, L. and He, P. (2010) A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proc. Natl. Acad. Sci. USA*, **107**, 496–501.
Lu, D.P., Lin, W.W., Gao, X.Q., Wu, S.J., Cheng, C., Avila, J., Heese, A., Devarenne, T.P., He, P. and Shan, L. (2011) Direct ubiquitination of pattern recognition receptor FLS2 attenuates plant innate immunity. *Science*, **332**, 1439–1442.
Lu, Z. and Hunter, T. (2009) Degradation of activated protein kinases by ubiquitination. *Annu. Rev. Biochem.* **78**, 435–475.
MacGurn, J.A., Hsu, P.C. and Emr, S.D. (2012) Ubiquitin and membrane protein turnover: from cradle to grave. *Annu. Rev. Biochem.* **81**, 231–259.
Marino, D., Peeters, N. and Rivas, S. (2012) Ubiquitination during plant immune signaling. *Plant Physiol.* **160**, 15–27.
Mazzucotelli, E., Belloni, S., Marone, D., De Leonardis, A.M., Guerra, D., Fonzo, N., Cattivelli, L. and Mastrangelo, A.M. (2006) The E3 ubiquitin ligase gene family in plants: regulation by degradation. *Curr. Genomics*, **7**, 509–522.
Melikova, M.S., Kondratov, K.A. and Kornilova, E.S. (2006) Two different stages of epidermal growth factor (EGF) receptor endocytosis are sensitive to free ubiquitin depletion produced by proteasome inhibitor MG132. *Cell Biol. Int.* **30**, 31–43.
Mersmann, S., Bourdais, G., Rietz, S. and Robatzek, S. (2010) Ethylene signaling regulates accumulation of the FLS2 receptor and is required for the oxidative burst contributing to plant immunity. *Plant Physiol.* **154**, 391–400.
Monaghan, J. and Zipfel, C. (2012) Plant pattern recognition receptor complexes at the plasma membrane. *Curr. Opin. Plant Biol.* **15**, 349–357.
Nicaise, V., Roux, M. and Zipfel, C. (2009) Recent advances in PAMP-triggered immunity against bacteria: pattern recognition receptors watch over and raise the alarm. *Plant Physiol.* **150**, 1638–1647.
Nicaise, V., Joe, A., Jeong, B.R., Korneli, C., Boutrot, F., Westedt, I., Staiger, D., Alfano, J.R. and Zipfel, C. (2013) *Pseudomonas* HopU1 modulates plant immune receptor levels by blocking the interaction of their mRNAs with GRP7. *EMBO J.* **32**, 701–712.
Ntoukakis, V., Mucyn, T.S., Gimenez-Ibanez, S., Chapman, H.C., Gutierrez, J.R., Balmuth, A.L., Jones, A.M. and Rathjen, J.P. (2009) Host inhibition of a bacterial virulence effector triggers immunity to infection. *Science*, **324**, 784–787.
O'Shea, J.J., Ma, A. and Lipsky, P. (2002) Cytokines and autoimmunity. *Nat. Rev. Immunol.* **2**, 37–45.
Park, C.J., Peng, Y., Chen, X.W., Dardick, C., Ruan, D.L., Bart, R., Canlas, P.E. and Ronald, P.C. (2008) Rice XB15, a protein phosphatase 2C, negatively regulates cell death and XA21-mediated innate immunity. *PLoS Biol.* **6**, e231.
Raiborg, C., Rusten, T.E. and Stenmark, H. (2003) Protein sorting into multivesicular endosomes. *Curr. Opin. Cell Biol.* **15**, 446–455.
Ramonell, K., Berrocal-Lobo, M., Koh, S., Wan, J.R., Edwards, H., Stacey, G. and Somerville, S. (2005) Loss-of-function mutations in chitin responsive genes show

increased susceptibility to the powdery mildew pathogen *Erysiphe cichoracearum*. *Plant Physiol.* **138**, 1027–1036.

Robatzek, S., Chinchilla, D. and Boller, T. (2006) Ligand-induced endocytosis of the pattern recognition receptor FLS2 in Arabidopsis. *Gene Dev.* **20**, 537–542.

Ron, M. and Avni, A. (2004) The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato. *Plant Cell*, **16**, 1604–1615.

Rosebrock, T.R., Zeng, L., Brady, J.J., Abramovitch, R.B., Xiao, F. and Martin, G.B. (2007) A bacterial E3 ubiquitin ligase targets a host protein kinase to disrupt plant immunity. *Nature*, **448**, 370–374.

Roth, A.F. and Davis, N.G. (2000) Ubiquitination of the PEST-like endocytosis signal of the yeast a-factor receptor. *J. Biol. Chem.* **275**, 8143–8153.

Salinas-Mondragon, R.E., Garciduenas-Pina, C. and Guzman, P. (1999) Early elicitor induction in members of a novel multigene family coding for highly related RING-H2 proteins in *Arabidopsis thaliana*. *Plant Mol. Biol.* **40**, 579–590.

Schulze, B., Mentzel, T., Jehle, A.K., Mueller, K., Beeler, S., Boller, T., Felix, G. and Chinchilla, D. (2010) Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1. *J. Biol. Chem.* **285**, 9444–9451.

Schwessinger, B. and Ronald, P.C. (2012) Plant innate immunity: perception of conserved microbial signatures. *Annu. Rev. Plant Biol.* **63**, 451–482.

Shan, L., He, P., Li, J., Heese, A., Peck, S.C., Nurnberger, T., Martin, G.B. and Sheen, J. (2008) Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. *Cell Host Microbe*, **4**, 17–27.

Sharfman, M., Bar, M., Ehrlich, M., Schuster, S., Melech-Bonfil, S., Ezer, R., Sessa, G. and Avni, A. (2011) Endosomal signaling of the tomato leucine-rich repeat receptor-like protein LeEix2. *Plant J.* **68**, 413–423.

Shi, H., Shen, Q.J., Qi, Y.P., Yan, H.J., Nie, H.Z., Chen, Y.F., Zhao, T., Katagiri, F. and Tang, D. (2013) BR-SIGNALING KINASE1 physically associates with FLAGELLIN SENSING2 and regulates plant innate immunity in Arabidopsis. *Plant Cell*, **25**, 1143–1157.

Shin, L.J., Lo, J.C., Chen, G.H., Callis, J., Fu, H. and Yeh, K.C. (2013) IRT1 DEGRADATION FACTOR1, a RING E3 ubiquitin ligase, regulates the degradation of IRON-REGULATED TRANSPORTER1 in Arabidopsis. *Plant Cell*, **25**, 3039–3051.

Smalle, J. and Vierstra, R.D. (2004) The ubiquitin 26S proteasome proteolytic pathway. *Annu. Rev. Plant Biol.* **55**, 555–590.

Song, W.Y., Wang, G.L., Chen, L.L., Kim, H.S., Pi, L.Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.X., Zhu, L.H., Fauquet, C. and Ronald, P. (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. *Science*, **270**, 1804–1806.

Sorkin, A. and von Zastrow, M. (2009) Endocytosis and signalling: intertwining molecular networks. *Nat. Rev. Mol. Cell Biol.* **10**, 609–622.

Stegmann, M., Anderson, R.G., Ichimura, K., Pecenkova, T., Reuter, P., Zarsky, V., McDowell, J.M., Shirasu, K. and Trujillo, M. (2012) The ubiquitin ligase PUB22 targets a subunit of the exocyst complex required for PAMP-triggered responses in Arabidopsis. *Plant Cell*, **24**, 4703–4716.

Stracke, S., Kistner, C., Yoshida, S., Mulder, L., Sato, S., Kaneko, T., Tabata, S., Sandal, N., Stougaard, J., Szczyglowski, K. and Parniske, M. (2002) A plant receptor-like kinase required for both bacterial and fungal symbiosis. *Nature*, **417**, 959–962.

Strous, G.J. and Gent, J. (2002) Dimerization, ubiquitylation and endocytosis go together in growth hormone receptor function. *FEBS Lett.* **529**, 102–109.

Tanno, H. and Komada, M. (2013) The ubiquitin code and its decoding machinery in the endocytic pathway. *J. Biochem.* **153**, 497–504.

Thomma, B., Nurnberger, T. and Joosten, M. (2011) Of PAMPs and effectors: the blurred PTI–ETI dichotomy. *Plant Cell*, **23**, 4–15.

Trujillo, M., Ichimura, K., Casais, C. and Shirasu, K. (2008) Negative regulation of PAMP-triggered immunity by an E3 ubiquitin ligase triplet in Arabidopsis. *Curr. Biol.* **18**, 1396–1401.

Vierstra, R.D. (2009) The ubiquitin-26S proteasome system at the nexus of plant biology. *Nat. Rev. Mol. Cell Biol.* **10**, 385–397.

Vossen, J.H., Liebrand, T.W.H., Tameling, W.I.L. and Joosten, M.H.A.J. (2009) Endocytosis plays an important role in Cf-4-mediated resistance of tomato to *Cladosporium fulvum*. ISMPMI International Congress abstracts, p. 151.

Wang, Y.S., Pi, L.Y., Chen, X.H., Chakrabarty, P.K., Jiang, J., De Leon, A.L., Liu, G.Z., Li, L., Benny, U., Oard, J., Ronald, P.C. and Song, W.Y. (2006) Rice XA21 binding protein 3 is a ubiquitin ligase required for full Xa21-mediated disease resistance. *Plant Cell*, **18**, 3635–3646.

Willmann, R., Lajunen, H.M., Erbs, G., Newman, M.A., Kolb, D., Tsuda, K., Katagiri, F., Fliegmann, J., Bono, J.J., Cullimore, J.V., Jehle, A.K., Götz, F., Kulik, A., Molinaro, A., Lipka, V., Gust, A.A. and Nürnberger, T. (2011) Arabidopsis lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. *Proc. Natl. Acad. Sci. USA*, **108**, 19 824–19 829.

Yamaguchi, Y. and Huffaker, A. (2011) Endogenous peptide elicitors in higher plants. *Curr. Opin. Plant Biol.* **14**, 351–357.

Yang, C.W., Gonzalez-Lamothe, R., Ewan, R.A., Rowland, O., Yoshioka, H., Shenton, M., Ye, H., O'Donnell, E., Jones, J.D. and Sadanandom, A. (2006) The E3 ubiquitin ligase activity of Arabidopsis PLANT U-BOX17 and its functional tobacco homolog ACRE276 are required for cell death and defense. *Plant Cell*, **18**, 1084–1098.

Zeng, L.R., Qu, S.H., Bordeos, A., Yang, C.W., Baraoidan, M., Yan, H.Y., Xie, Q., Nahm, B.H., Leung, H. and Wang, G.L. (2004) Spotted leaf11, a negative regulator of plant cell death and defense, encodes a U-box/armadillo repeat protein endowed with E3 ubiquitin ligase activity. *Plant Cell*, **16**, 2795–2808.

Zhang, J., Li, W., Xiang, T., Liu, Z., Laluk, K., Ding, X., Zou, Y., Gao, M., Zhang, X., Chen, S., Mengiste, T., Zhang, Y. and Zhou, J.M. (2010) Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. *Cell Host Microbe* **7**, 290–301.

Zhou, J., Wu, S., Chen, X., Liu, C., Sheen, J., Shan, L. and He, P. (2013) *Pseudomonas syringae* effector HopF2 suppresses Arabidopsis immunity by targeting BAK1. *Plant J.* **77**, 235–245.