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The Plant Ubiquitin–Proteasome System as a Target for Microbial Manipulation

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Abstract

The plant immune system perceives pathogens to trigger defense responses. In turn, pathogens secrete effector molecules to subvert these defense responses. The initiation and maintenance of defense responses involve not only de novo synthesis of regulatory proteins and enzymes but also their regulated degradation. The latter is achieved through protein degradation pathways such as the ubiquitin–proteasome system (UPS). The UPS regulates all stages of immunity, from the perception of the pathogen to the execution of the response, and, therefore, constitutes an ideal candidate for microbial manipulation of the host. Pathogen effector molecules interfere with the plant UPS through several mechanisms. This includes hijacking general UPS functions or perturbing its ability to degrade specific targets. In this review, we describe how the UPS regulates different immunity-related processes and how pathogens subvert this to promote disease.

1. INTRODUCTION

To prevent outbreaks of diseases caused by phytopathogens, plants have developed a complex evolutionarily driven multilayered immune system (56). The first layer of this immune system depends on the recognition of conserved microbial molecules, termed pathogen-associated molecular patterns (PAMPs), by cell surface pattern-recognition receptors (PRRs) (24). This first layer of defense is known as PAMP-triggered immunity (PTI) (56). Adapted plant pathogens are able to overcome these PTI responses by delivering effector proteins into the host cells, where they manipulate the host cell machinery for the benefit of the pathogen (60). In turn, plants have evolved a second potentiated layer of defense that is driven by the recognition of these effectors by intracellular receptors (93). This second layer is referred to as effector-triggered immunity (ETI) (56). Recognition of microbial molecules by immune receptors activates downstream defense reactions via hormone signaling and transcriptional reprogramming, which lead to the secretion of antimicrobial compounds and/or programmed cell death (PCD) to counteract the invasion (8, 21). This so-called zig-zag model involves a complex interplay among many cellular processes and for years served as a framework to study plant–microbe interactions at the molecular level (92). This requires a high degree of proteomic plasticity involving both the synthesis and turnover of regulatory proteins (98). Therefore, it is not surprising that protein degradation pathways such as the ubiquitin–proteasome system (UPS) have been identified as major coordinators of plant immunity and determine the outcome of plant–microbe interactions (66).

The UPS is a highly conserved pathway involved in the degradation of up to 80% of eukaryotic proteins (22). Poly-ubiquitination of target proteins is a prerequisite for recycling proteins through the UPS (22). To this end, an enzymatic cascade involving ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligase (E3) enzymes is required (135). In the first step, activated ubiquitin binds to an E1 and is then transferred to an E2. This E2 carries the activated ubiquitin to the E3, which in turn facilitates the transfer of the ubiquitin from the E2 to a lysine residue of the target protein. After several rounds of the E1→E2→E3 cascade, the substrate protein bears one or multiple ubiquitin chains that are then recognized by the 26S proteasome for its subsequent degradation (135). The 26S proteasome is a 2.5-MDa ATP-dependent protease complex composed of a 20S core protease (CP) and one or two 19S regulatory particles (RPs), each of which contains a lid and a base subunit (83). The CP is a broad-spectrum ATP- and ubiquitin-independent protease complex harboring peptidase activity (83). The assembly of these two subcomplexes leads to the formation of a capped cylindric megacomplex that is able to recognize, unfold, and degrade ubiquitinated proteins. Substrate recognition is followed by deubiquitination and unfolding by lid RP subunits (83). The linearized target protein is processed through the base RP subunits and delivered to the CP core particles. Here, the CP peptidases cleave a broad array of polypeptides, with the β1, β2, and β5 active sites providing trypsin-like, chymotrypsin-like, and caspase-like cleavage properties, respectively. The RP subcomplex is responsible for recognizing ubiquitinated target proteins and opening the channel of the CP to later insert the unfolded substrate into the CP chamber for degradation (83). Since the discovery of ubiquitin more than 50 years ago, the UPS has gained prominence as one of the most ubiquitous, versatile, and efficient regulators of many different cellular processes in eukaryotes (135, 144). For instance, the *Arabidopsis* genome encodes more than 1,600 genes (>6% of the total genome) involved in UPS-related functions. Most of these genes (>1,400) encode putative E3s (25). The diversification of E3s in plants is essential to ensure substrate specificity and provides the UPS with huge flexibility in reacting to different environmental changes. In plants, E3s can be classified into four main types: really interesting new gene (RING); Cullin–RING ligases (CRLs); homologous to the E6-AP carboxyl terminus (HECT); and plant U-box (PUB) proteins. RING,

HECT, and PUB E3s act as monomeric proteins, whereas CRLs form multimeric complexes (12). In plants, the UPS pathway, and particularly E3s, has been shown to be involved in responses to many internal and external stimuli (25, 112). Characterization of E3s involved in plant immunity has led to historical milestones in the understanding of defense mechanisms. However, recent studies highlighted that other UPS components such as E1, E2, and the 26S proteasome complex itself are also required to mount effective plant defense reactions. Owing to this prominent role in plant immunity, pathogens have evolved strategies to manipulate the UPS to their own advantage. In this review, we first describe the different ways in which the UPS regulates plant immunity. We then detail the different mechanisms that plant pathogens have evolved to interfere with the UPS in their effort to subvert plant defenses and trigger disease.

2. THE UBIQUITIN–PROTEASOME SYSTEM REGULATES ALL STAGES OF PLANT IMMUNITY

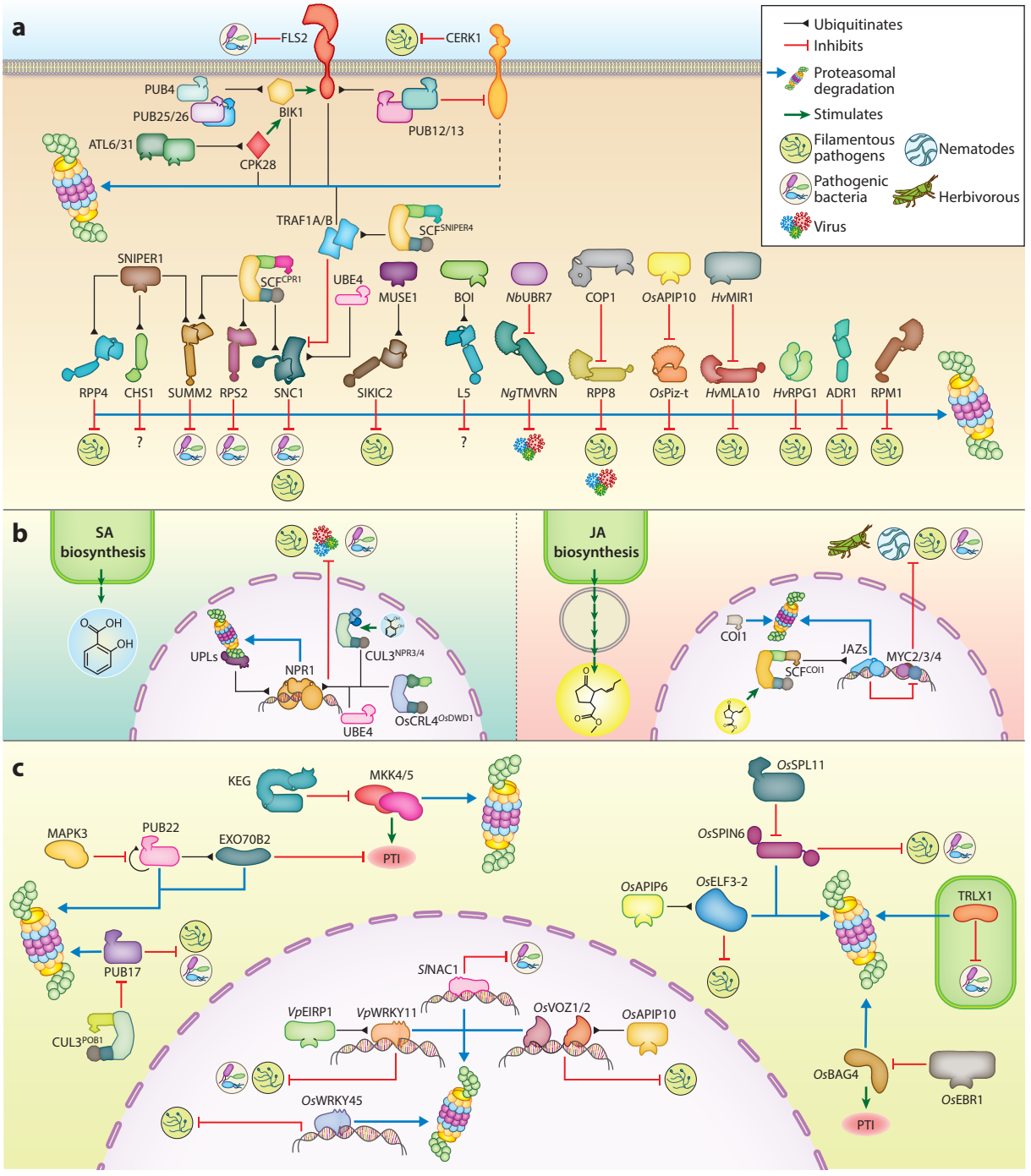
The UPS is involved in the regulation of many developmental and stress response processes, including plant immunity. UPS regulation of plant immunity affects all stages, from pathogen perception to execution of the different defense responses.

2.1. The Ubiquitin–Proteasome System Degrades Key Immune Components

Upon pathogen perception, plant immune components undergo degradation by the UPS. In the following sections, we summarize key findings about how immune receptors and other defense components are degraded by the UPS.

2.1.1. Immune receptors. Pathogen recognition is the first step in plant immune responses. This recognition occurs at two levels: PAMP perception by cell-surface PRRs and effector recognition by intracellular receptors. Examples of both types of immune receptors have been reported as targets of UPS-mediated degradation (**Figure 1*a***).

Pattern-recognition receptors and associated proteins. PRRs encode receptor kinases and receptor-like proteins located mostly at the plasma membrane, where they recognize conserved PAMPs (161). Given their importance in initiating plant defense responses, their controlled turnover is a key regulatory process in which the UPS is heavily involved. *Arabidopsis* flagellin-sensitive 2 (FLS2), probably the best-characterized PRR, recognizes flg22, a peptide derived from the bacterial flagellin. Upon flg22 recognition, FLS2 associates with its coreceptor BRI1-associated receptor kinase 1 (BAK1) following multiple phosphorylation events mediated by their respective kinase domains (24). After this recognition, FLS2 undergoes a rapid turnover that has been associated with the UPS, as chemical inhibition of proteasomal degradation with MG132 stabilizes FLS2 protein levels (77). Additionally, FLS2 also undergoes ubiquitination by the E3 pair PUB12 and 13 (77, 89). Although ubiquitination of FLS2 has not yet been clearly associated with proteasomal degradation, it cannot be ruled out that it does play a role through yet unknown mechanisms. Among the first downstream components of the phosphorylation signaling cascade activated by FLS2 we find *Botrytis*-induced kinase 1 (BIK1). BIK1 directly associates with the FLS2/BAK1 complex, becomes phosphorylated by them, and transduces the signal to additional intracellular downstream components (89). BIK1 is also targeted for proteasomal degradation upon ubiquitination mediated by yet another PUB pair, PUB25 and 26 (136). A more recent report also identified PUB4 as responsible for ubiquitin-mediated proteasomal degradation of BIK1 (153). Interestingly, when it comes to BIK1 phosphorylation, contrasting roles have been described. Although BIK1 phosphorylation by the FLS2–BAK1 complex positively regulates BIK1 activity, calcium-dependent protein kinase 28 (CPK28)-mediated phosphorylation of BIK1



(Caption appears on following page)

Figure 1 (*Figure appears on preceding page*)

The 26S proteasome is a master regulator of immune component turnover from early to later immune signaling. (*a*) Proteasomal degradation mediates regulation of multiple immune receptors, including cell surface pattern recognition receptors and intracellular nucleotide-binding leucine-rich repeat receptors in a wide range of species. (*b*) Defense hormone-dependent transcriptional reprogramming against pathogens is fine-tuned in the nucleus by the 26S proteasome. (*c*) The 26S proteasome coordinates later immune signals through regulation of a broad range of proteins involved in signal transduction and transcriptional response. Abbreviations: JA, jasmonic acid; JAZs, jasmonate ZIM domain proteins; PTI, PAMP-triggered immunity; SA, salicylic acid; UPLs, ubiquitin-protein ligases.

negatively impacts its activity (89). CPK28 itself is another target of proteasomal degradation mediated by ubiquitination via the RING domain E3 pair *Arabidopsis* toxicos en levadura 6/31 (ATL6 and 31) (75).

Another well-known PRR is *Arabidopsis* chitin elicitor receptor kinase 1 (CERK1). CERK1 recognizes chitin, a conserved PAMP from fungal cell walls, to induce the correspondent downstream immune signaling via phosphorylation of PBL27 (24). Similar to FLS2, CERK1 protein levels are also stabilized upon chemical inhibition of proteasomal degradation (38). PUB12 regulates the CERK1–PBL27 complex. Both PUB12 and PUB13 are able to interact with CERK1, and, additionally, PUB12 negatively regulates CERK1 stability (145). Intriguingly, CERK1 has also been shown to be subjected to ectodomain shedding (101). This is a mechanism better characterized in animals, in which upon proteolytical cleavage, the extracellular domain of a receptor is released into the extracellular matrix, whereas the intracellular domain is internalized and ultimately undergoes proteasomal degradation (62). Monocot PRRs such as rice SPL11 cell-death suppressor 2 (OsSDS2) have also been found to be targeted by the UPS (32). Altogether, these reports demonstrate how the UPS acts as a central regulator of early immune signaling pathways and highlight the eminent role of plant E3s in this process.

Intracellular immune receptors. Intracellular immune receptors play key roles in resistance to adapted pathogens. Most of them encode nucleotide-binding leucine-rich repeat (NLR) proteins. NLR-mediated immunity usually leads to PCD, also called hypersensitive response (HR) in these cases (21). For this reason, it is vital for the survival of the plant to control the basal activity of these proteins and avoid autoimmunity. This is mediated by the UPS, as evidenced by multiple reports identifying NLR proteins from *Arabidopsis* (17, 30, 42, 48, 71, 73, 80, 109, 141), *Nicotiana benthamiana* (156), rice (100), and barley (95, 139) targeted for proteasomal degradation (**Figure 1*a***).

Altogether, these reports highlight the importance of the UPS in the regulation of both PTI and ETI responses in multiple plant species against a wide variety of pathogens. Interestingly, the UPS can act as both a positive and negative regulator of immune responses, evidencing the complexity and versatility of the system.

2.1.2. Defense-related hormone signaling components. Perception of plant pathogens results in a massive reprogramming of various cellular processes to orchestrate appropriate defense responses. An important part of this reprogramming occurs at the transcriptional level and involves hormone signaling. Interestingly, hormone-induced transcriptional reprogramming in plants is tightly linked to proteasomal degradation (**Figure 1*b***). This applies to the two main hormones involved in immunity: salicylic acid (SA) and jasmonic acid (JA).

Salicylic acid. SA is the key hormone implicated in defense responses against biotrophic pathogens. SA-mediated defense responses are regulated by the master transcriptional regulator non-expresser of PR genes 1 (NPR1) (8). NPR1 protein levels are tightly controlled by SA perception via the $CRL3^{NPR3/NRP4}$ receptor complex (34, 117). Under basal conditions, the UPS targets NPR1 for degradation via $CRL3^{NPR3}$-mediated ubiquitination. Upon early SA

accumulation, NPR3 is inhibited, leading to NPR1 stabilization. In turn, NPR4 acts as a sort of guardrail by being activated to induce NPR1 degradation upon SA overaccumulation (34). A recent study has expanded the importance of NPR1 ubiquitination for its activity. It has been shown that primary CRL3 ubiquitination of NPR1 increases its activity, but it is also the first step for the subsequent UBE4-mediated ubiquitin chain elongation, ultimately leading to NPR1 degradation (115). Moreover, the recent discovery of two HECT E3s, ubiquitin-protein ligase 3/4 (UPL3 and 4), as modifiers of NPR1 adds an extra layer of complexity to the ubiquitin-mediated control of NPR1 turnover (140). Rice OsNPR1 is also targeted for degradation by the UPS via the CRL4 complex (18), indicating either an alternative degradation mechanism for NPR1 or evolutionary divergence between different plant lineages.

Jasmonic acid. JA mediates defense responses against necrotrophic pathogens. JA signaling relies on transcriptional repression that is mainly mediated by the jasmonate ZIM domain protein (JAZ) family. JAZ proteins are negative regulators of the transcription factors (TFs) MYC2, 3, and 4 (33). Upon pathogen perception, JA accumulation is perceived in the nucleus by the SCF^{COI1} complex, which triggers the ubiquitination/degradation of the JAZ repressor and ultimately derepresses the abovementioned MYCs (125, 147). In turn, COI1 is also a target of proteasomal degradation, evidencing contrasting roles of the UPS in JA signaling (146).

2.1.3. Downstream immune components. Plant immunity relies on a complex molecular network that integrates the abovementioned external (e.g., pathogen perception) and internal signals (e.g., hormones) to orchestrate the correspondent appropriate defense responses. For this, it is essential to regulate the abundance and activity of the proteins involved in these processes. This regulation occurs largely thanks to post-translational modifications (PTMs) such as phosphorylation, acetylation, SUMOylation, and ubiquitination. Ubiquitination is a way for the UPS to monitor the stability of the targets (**Figure 1*c***).

E3 ubiquitin ligases. Based on the previously mentioned reports, the importance of E3s in regulating plant immunity is evident. Interestingly, some of the E3s involved in immunity also undergo proteasomal degradation. This applies mostly to E3s from the PUB family. PUB22 dimerization and autoubiquitination lead to PUB22's degradation in basal conditions and are prevented upon infection through phosphorylation by mitogen-activated protein kinase 3 (MAPK3) (35). This stabilization allows PUB22 to ubiquitinate its substrate, the exocyst subunit EXO70B2, which is required for the attenuation of PAMP-induced signaling (118). PUB17 regulates HR and is involved in resistance against the oomycete *Phytophthora infestans* and RPM1- and RPS4-mediated resistance against *Pseudomonas syringae* (46, 149). PUB17 is targeted to the UPS upon ubiquitination by the POZ–BTB-containing protein 1 (POB1) component of the CRL3 E3 complex (97). The involvement in immunity of PUB17 orthologs has been reported for many other plant species such as tobacco, cotton, and potato, evidencing PUB17 as an important conserved immune hub in many plant lineages against a wide variety of pathogens (85, 103).

Signaling and other immune-related proteins. Phosphorylation is a key PTM ruling signaling pathways, including defense-related pathways. For instance, MAPK cascades are core components of PTI signaling (24). A recent study showed that the E3 KEEP ON GOING (KEG) mediates ubiquitination and subsequent degradation of MAPK kinase 4 and 5 (MKK4 and 5) (37). Interestingly, KEG seems to be degraded upon fungal infection, supporting its role as the negative regulator of immunity (45). Other important signaling proteins are GTPases and associated proteins. For instance, rice Rho GTPase-activating protein SPL11-interacting protein 6 (*Os*SPIN6) is a key regulator of antifungal and bacterial HR (74). *Os*SPIN6 turnover is controlled by ubiquitination/proteasomal degradation mediated by the rice PUB13 ortholog *Os*SPL11 (74). Also

involved in rice responses against fungi, the circadian clock regulator *Os*ELF3–2 is a UPS target upon *Os*APIP6-mediated ubiquitination (94). Rice cell death regulator BCL2-associated anthogen 4 (*Os*BAG4) confers broad-spectrum resistance and is targeted to the proteasome upon ubiquitination by enhanced blight and blast resistance 1 (*Os*EBR1) in the basal condition to avoid autoimmunity (152). Organelle signaling is also involved in immunity. For example, the homeostasis of chloroplastic TRX-like 1 (TRXL1) mediated by both the UPS and the intrachloroplastic degradation machinery is involved in defense against *P. syringae* (99).

Transcription factors. An important part of the plant defense responses is controlled at the transcriptional level by several TFs. Therefore, TFs are also targets of UPS during regulation of immunity. A prominent family of such TFs is the plant-specific WRKY family. Many different WRKY family members have been reported as targets of proteasomal degradation in several plant species. For instance, rice WRKY45, involved in the defense against *Magnaporthe grisae*, is subjected to proteasomal degradation in basal conditions (84, 110, 111). In wild grape, the E3 *Erysiphe necator*-induced RING finger protein 1 (*Vp*EIRP1) confers increased resistance against bacterial and fungal pathogens mediating the proteasomal degradation of *Vp*WRKY11 (154). Pepper WRKY40, an ortholog of *Arabidopsis* WRKY40 known to be a negative regulator of PTI, is also degraded by the proteasome to regulate stomatal immunity against the bacterium *Xanthomonas euvesicatoria* (104). Another important family of plant-specific transcription factors is the NAC family. For instance, tomato NAC1, a positive regulator of defense against *P. syringae*, is also targeted by the UPS (86). Recently, rice vascular plant one-zinc-finger 1 and 2 (VOZ1 and 2) have also been found to be degraded upon ubiquitination by the E3 *Os*APIP10 to regulate Piz-t-mediated immunity. Interestingly, *Os*APIP10 also mediates the ubiquitination/degradation of Piz-t itself, making it a major coordinator of rice PTI/ETI responses (100, 137).

Altogether, this evidences the importance of proteasomal degradation of immune-related proteins as a mechanism of regulation in plant immunity. This regulation occurs at all stages, from perception to execution, and allows finely tuned effective defense responses. It is noteworthy that many E3s such as the abovementioned PUB13 or *Os*APIP10 present multiple targets. This guarantees a huge degree of versatility to react to changing environmental conditions, which could explain their evolutionary expansion in the plant lineage. However, it also shows how vulnerable the UPS and its components are toward perturbations by factors that can manipulate these processes, making the UPS an ideal target for plant pathogens.

2.2. The Ubiquitin–Proteasome System Is Also Directly Involved in Plant Immunity

The UPS's ability to regulate plant immunity goes beyond the degradation of immune-related proteins as components of the UPS itself and upstream regulators of its assembly and/or activity are also involved in regulating defense responses (**Figure 2*a***).

2.2.1. Roles of the 26S proteasome complex in immunity. The 26S proteasome complex is the heart of the UPS. Although proteasome subunits have been historically considered housekeeping genes, mounting evidence supports a very dynamic regulation at the transcription, translation, and post-translational levels (83). How the proteasome assembly and functioning are regulated and what additional proteasome-independent functions the individual subunits might have remain elusive.

Proteasome subunits and their function in plant immunity. A general trend among plants upon pathogen perception is the induction of transcription and/or translation of proteasomal subunits to increase their abundance and accessibility; e.g., fungal elicitation induces *Arabidopsis* PBB1

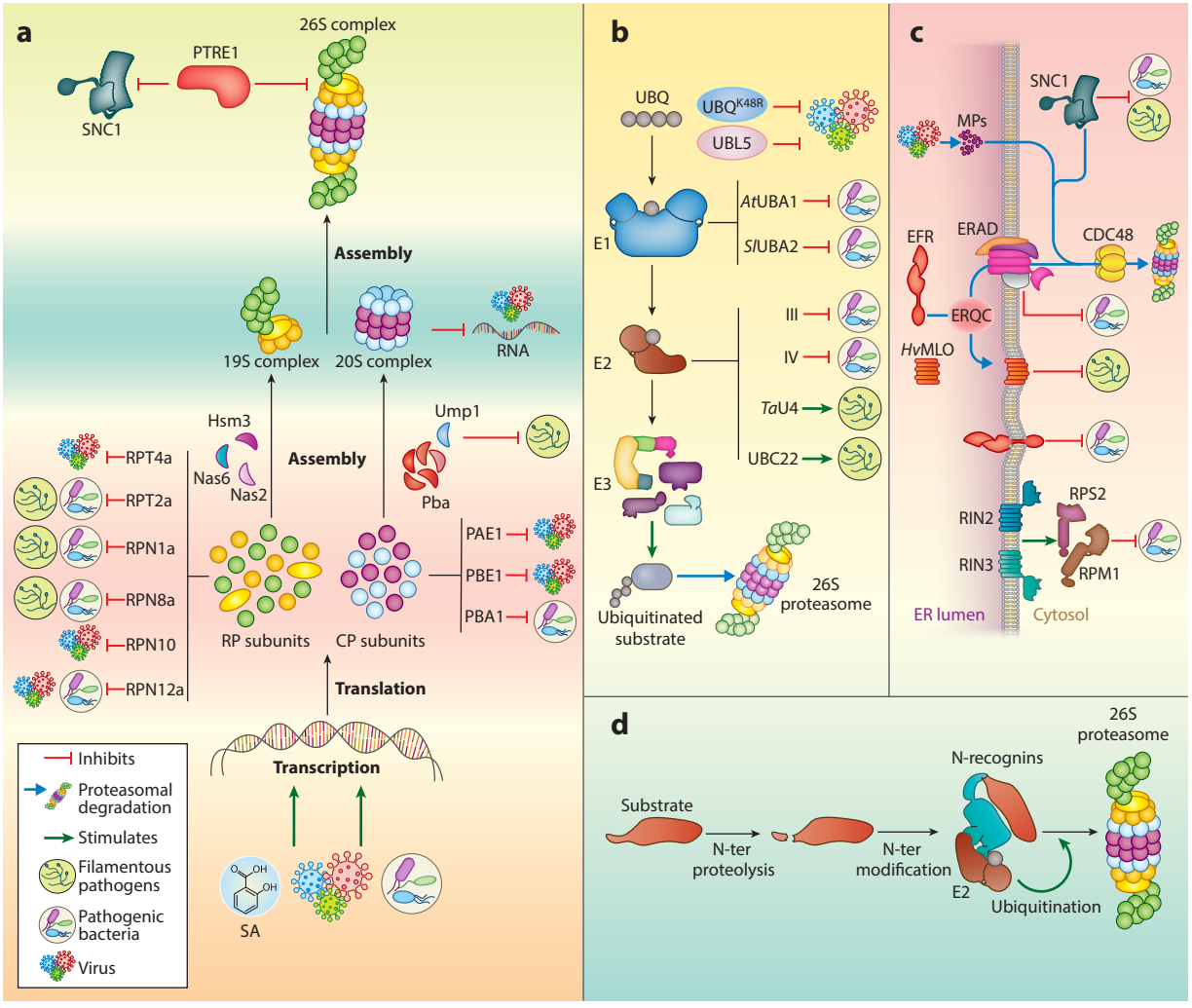


Figure 2

Different ubiquitin–proteasome system (UPS) components are directly involved in plant immunity. (*a*) The 26S proteasome formation requires successive steps of transcription/translation of individual subunits and generation of the 19S regulatory particle (RP) and 20S core particle (CP) complexes and their assembly. All these steps are involved in plant immunity. (*b*) Early components of the ubiquitination machinery are involved in plant immunity. Ubiquitin moieties, E1 activating enzyme, and E2 conjugating enzyme are all involved in plant defense with contrasting roles at several levels. (*c*) The UPS branch endoplasmic reticulum quality control (ERQC) involves ER-resident components to monitor multiple immune-related proteins. ERQC mediates protein subcellular sorting through ER-associated degradation (ERAD). Other ERAD-related components are also involved in cytosolic protein turnover and immune signaling. (*d*) The N-degron pathway, another branch of the UPS, depends on specific post-translational modification of the N-termini of substrate proteins. This modification is recognized by specific E3 ligases for subsequent ubiquitination and proteasomal degradation. Abbreviations: EFR, EF-Tu receptor; MPs, movement proteins; SA, salicylic acid.

accumulation (69), viral infection increases pepper *RPN7* transcription (67), *P. syringae* induces accumulation of PBA1 and RPT2, and SA induces tobacco *RPT6* gene expression (128, 131). Over the years, many studies have genetically investigated the role of proteasomal subunits in immunity. For instance, it was shown that mutating subunits *PBA1*, *RPN1a*, and *RPN8* in *Arabidopsis* result

in increased susceptibility to *P. syringae* and powdery mildew (151). Additionally, silencing *RPT6* abolishes XopJ–SA-induced cell death (129). Proteasome mutants deficient in *RPT2a* display altered NLR stability, reduced systemic acquired resistance (SAR), and increased susceptibility to *P. syringae* (20, 133). Similarly, several mutants of proteasomal subunits also show increased susceptibility to *cauliflower mosaic virus* (CaMV) infection (113). Interestingly, the tomato *RPT4a* is involved in resistance against *tomato leaf curl New Delhi virus* in a proteolytic-independent manner but through viral DNA binding and host RNA polymerase II inhibition (108).

The holoenzyme and its regulation. The assembly of the different proteasomal subunits and subcomplexes requires the coordinated action of several players, some of which have been shown to be directly involved in immunity. For instance, the rice regulators of the 26S proteasome assembly OsUMP1 mediate non-race-specific resistance against *Magnaporthe oryzae* by modulating the H_2O_2 levels (47). Moreover, proteasome regulator 1 (PTRE1) is involved in the degradation of the NLR suppressor of npr1–1 constitutive 1 (SNC1) (126). The proteasomal subcomplexes themselves can also affect immunity prior to assembly. Extracted sunflower 20S complexes were shown to possess endonuclease activity and degrade viral RNA from *tobacco mosaic virus* and *lettuce mosaic virus* (LMV) (3).

2.2.2. Roles of other components and branches of the ubiquitin–proteasome system in immunity. The prominent role of E3s as drivers of target specificity has already been discussed in previous sections. However, ubiquitin-mediated degradation also requires upstream E1 and E2 enzymes to activate and transfer the ubiquitin moiety (**Figure 2*b***). A mutation in one of the two *Arabidopsis* E1 enzymes, ubiquitin-activating enzyme 1 (UBA1), results in increased susceptibility to *P. syringae* (40). In contrast, in tomato it is only UBA2 and not UBA1 that, upon silencing, causes increased susceptibility against the same pathogen, evidencing species-specific differences (159). In the same study, several E2s were found to also contribute to resistance against *P. syringae* (159). Several E2s from *N. benthamiana* have also been described as positive regulators of immunity. In contrast, other E2 enzymes have been described as negative regulators of immunity (158). This is the case of wheat TaU4 or *Arabidopsis* ubiquitin-conjugating enzyme 22 (UBC22) (87, 138). The ubiquitin molecule itself is also important, as overexpression of a variant unable to make K48 linkages results in enhanced tolerance to a viral infection and overaccumulation of the canonical defense marker protein pathogenesis-related 1 (PR1) (5). Furthermore, ubiquitin-like peptide 5 (UBL5) is also a positive regulator of defense against viruses in *N. benthamiana* and rice (10). Finally, the UPL E3 ligases have been identified as novel upstream regulators of the proteasome. The UPLs have been described to be associated with the proteasome to promote its processivity and involved in plant immune responses (36). This is confirmed by a recent study that identified a ubiquitin-ligase relay that involves the UPL3 and UPL4 E3 ligases to promote processive degradation of SA master regulator NPR1 (140).

A specific branch of the UPS occurs at the endoplasmic reticulum (ER), where protein translation and subcellular sorting occur. This UPS branch is called ER quality control (ERQC) and relies on specific ER-associated E2–E3 enzymes that mediate proteasomal degradation of ER-resident proteins through ER-associated degradation (ERAD) (13) (**Figure 2*c***). In plants, ERAD has been widely studied in the context of the brassinosteroid (BR) receptor BR-insensitive 1 (BRI1) quality control (119). However, it has been proposed that ERAD is also involved in degradation of immune receptors such as the *Arabidopsis* EF-Tu receptor (EFR) or barley *Mildew Locus O* (MLO) (70, 90). Additional ERQC components such as *Arabidopsis* E3s RING2 and 3 and the CDC48 retro-translocase complex, as well as several tomato ER-resident E2s, are also involved in different immune responses (23, 59, 159).

Another UPS branch involved in immunity is the N-degron pathway. This pathway relies on the proteolytic cleavage and modification of the N-termini of substrate proteins that make them accessible to specific E3 ligases for subsequent degradation (29) (**Figure 2*d***). The involvement of this pathway in immunity is evidenced by SNC1, whose N-terminal methionine can be targeted by two acetyl transferases antagonistically regulating its stability (143). Additionally, mutation on certain components of the N-degron pathway such as *arginine transferase 1* and *2* (*ATE1* and *2*) or the E3 *proteolysis 1* and *6* (*PRT1* and *6*) show contrasting roles in tolerance/susceptibility to different pathogens (27, 43, 127, 134).

In this section, we have reviewed the complex and multifaceted involvement of the UPS in the regulation of plant immunity. Over the years, it has become quite clear that the UPS is a major immune hub, involved in all stages of plant defenses against all kinds of pathogens across most plant species. This involvement is, however, complex, as different reports show contrasting contributions to defenses depending on the path system, condition, or component studied. This implies an underlying yet unknown regulatory network that determines the outcome of plant–pathogen interactions.

3. PLANT PATHOGENS TARGET THE UBIQUITIN–PROTEASOME SYSTEM FOR THEIR OWN BENEFIT

Given the conservation and importance of the UPS in fine-tuning the plant defense response, it constitutes an ideal target for microbial manipulation. Targeting the proteasome and its components is a very efficient way to subvert cellular processes, as pathogens only need to hit one particular pathway to manipulate and disrupt the entire host system. Creating a chaotic environment in the host benefits the pathogen, as the host cannot react to invading intruders anymore. Thus, during the course of evolution, pathogens gained sophisticated strategies to indirectly and directly target the host UPS. To date, various plant pathogens, from viruses and bacteria to oomycetes, fungi, and nematodes, use different means to manipulate the UPS for their own benefit. In this section, we discuss various examples of how diverse plant pathogens employ different mechanisms to exploit the host UPS to dampen plant immune responses and create a favorable environment for their multiplication.

3.1. Bacteria

To overcome plant defense responses, plant-pathogenic bacteria have acquired a highly conserved type III secretion system (T3SS) to inject so-called type III effectors (T3Es) into the host cell. These T3Es are targeted to many cellular compartments and act as important determinants to promote disease progression by dampening various plant immune reactions (60). Although for many years research was focused on their function to suppress PTI and ETI responses, growing evidence suggests that T3Es target more central processes such as the UPS to subvert cellular processes in the host system (66, 105) (**Figure 3*a***). Initial findings in the effector biology field have been focused on effectors mimicking host eukaryotic proteins. Among the most studied effector proteins is AvrPtoB from *P. syringae*, which displays E3 ligase activity in planta (1). Its E3 ligase function is essential to ubiquitinate and degrade host targets via the 26S proteasome, making AvrPtoB the first effector to hijack the UPS to facilitate its virulence function during bacterial infection. The bacterial effector AvrPtoB was shown to interact with several E2 ligases in the host cell to enable the degradation of various PRRs such as CERK1, FLS2, and other PRR-associated proteins such as BAK1 and BIK1 (16, 38, 39). It is a primary example of how an effector hijacks and tricks host cellular systems by targeting the housekeeping recycling machinery of PRRs to subvert plant defense responses. Many other host targets have been identified

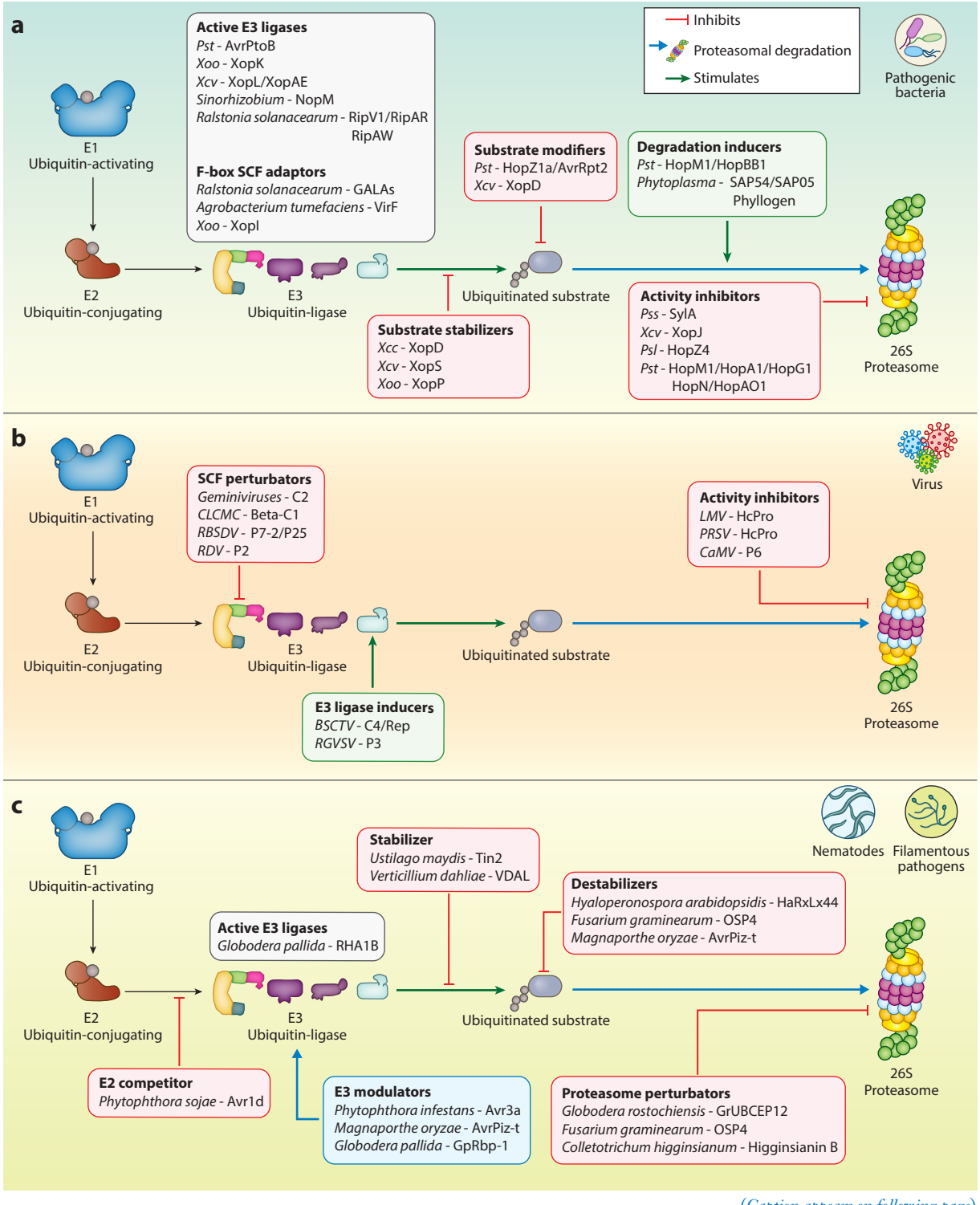


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Figure 3 (*Figure appears on preceding page*)

Plant pathogens deploy diverse mechanisms to exploit the ubiquitin–proteasome system (UPS) to promote disease. Each pathogenic molecule and the species of origin are grouped in boxes based on their modes of action. (*a*) Bacteria use different effector proteins to either degrade or stabilize host targets through different mechanisms. (*b*) Viruses target Skp1-cullin 1-F-box (SCF) complexes, E3 ligases, and the 26S proteasome to modulate the degradation of substrate proteins. (*c*) Eukaryotic pathogens alter the UPS at various steps, including competition with host E2s, modulation of E3 activities, destabilization of effector targets, and perturbation of the 26S proteasome.

that are targeted by AvrPtoB for degradation, of which SA master regulator NPR1 plays one of the most central roles in plant immunity (11). To guarantee a functional SA signaling, NPR1 is constitutively degraded in the nucleus by the 26S proteasome. The T3E AvrPtoB exploits this feature by targeting NPR1 in an SA-dependent manner. Association of both proteins results in a degradation of NPR1, which most likely takes place in the cytoplasm before its delivery into the nucleus. As such, AvrPtoB is not only able to dampen SA-mediated defense responses but also attenuate PTI and SAR reactions. The T3E AvrPtoB is not the only bacterial E3 ligase targeting PTI by degrading PRRs or master regulators of plant immunity. Another T3E that targets a PRR is the E3 ligase XopK from *Xanthomonas oryzae*. XopK interacts and ubiquitinates rice *Os*SERK2 to degrade it via the 26S proteasome (102). Removal of *Os*SERK2 enables the efficient inhibition of multiple PRR signaling pathways, thus providing an attractive target to promote virulence.

Although AvrPtoB is structurally homologous to RING E3s, other identified T3Es lack similarities to known eukaryotic E3 enzymes. Among them there is a family of bacterial E3s containing a novel E3 ligase (NEL) domain. At the structural level, most proteins belonging to the NEL family have an N-terminal domain with leucine-rich repeats (LRRs). The C-terminal region harbors the NEL domain with a catalytic cysteine residue required for the formation of E3–ubiquitin intermediates and E2–ubiquitin complex formation. Although T3Es belonging to the NEL family were first discovered in animal pathogens, later studies revealed that they are also present in plant-associated *Sinorhizobium*, *Ralstonia*, and *Xanthomonas* (7). This is the case of *X. euvesicatoria* XopL. The crystal structure of its C-terminal domain revealed a novel fold, termed XL-box, displaying E3 ligase activity in vitro, whereas the N-terminal region harbors an LRR domain that is necessary to suppress PTI in plants (114). However, its C-terminal domain lacks cysteine residues that are present in other NEL family members. Further studies revealed that XopL associates with the autophagy component SH3P2 to dampen host autophagy (68). Specifically, XopL ubiquitinates SH3P2 in vitro and in planta, which results in proteasome-dependent degradation of SH3P2. The elimination of SH3P2 perturbs autophagic degradation, which is beneficial for the proliferation of *Xanthomonas* in plants. Interestingly, XopL also undergoes autoubiquitination in planta, which may result in its autophagic degradation, termed effectorphagy. This constitutes another example of the evolutionary arms race between plants and pathogens, in which XopL may have acquired its function to degrade SH3P2 to prevent its own removal to exhibit its virulence function in the host system. Alongside XopL, XopAE from the same *Xanthomonas* species has been identified to share structural and functional similarities with XopL harboring an XL-box with E3 ligase activity and dampening PTI responses (68). XopAE plant targets, however, remain elusive. In addition, the E3 ligase function of XopL seems to be conserved across different *Xanthomonas* species. Rice pathogen *X. oryzae* pv. *oryzae* injects XopL to degrade ferredoxin in a proteasome-dependent manner to cause cell death (79). Other *Xanthomonas* species such as *Xanthomonas axonopodis* pv. *punicae* or *Xanthomonas campestris* pv. *campestris* possess XopL homologs that display major virulence function, although their targets remain to be identified (116, 148). NopM from *Sinorhizobium* exhibits E3 ligase activity and is phosphorylated by MAPKs (142). Further studies are required to unveil its targets in the host system as well as how its E3 ligase activity acts during the interaction with host plants. Other NEL members, including RipV1, RipAR, and RipAW, are described in

Ralstonia solanacearum. All these T3Es have been shown to suppress plant PTI reactions in an E3-activity-dependent manner, although their targets remain unknown (15, 91).

Although mimicking E3 ligase activity is a very efficient way for a T3E to degrade host proteins, it is not the only way to achieve the removal of immune-related components. Several plant-pathogenic bacteria have evolved F-box effector proteins that can act as adaptors in the Skp1-cullin 1-F-box (SCF) E3 ligase complex. The RipG T3E family from *R. solanacearum* associates with the host SCF complex and is hypothesized to act as E3s (2). In a similar way, VirF from *Agrobacterium tumefaciens* and, more recently, XopI from *X. oryzae* were shown to act in the SCF complex to mediate the degradation of the transcription factor VIRE2-interacting protein 1 (VIP1) and the rice thioredoxin OsTrxh2, respectively, to subvert plant resistance (51, 64).

Although this seems to be another direct way to target host proteins to the proteasome, some T3Es have been suggested to serve as adaptors between E3 ligases without displaying any known features of F-box proteins or other UPS components. One of the first examples of how an effector leads to the destabilization and degradation of its target is HopM1 from *P. syringae*. HopM1 is able to promote disease by inducing the proteasomal degradation of HopM1 interactor 7 (AtMIN7), a host ADP ribosylation factor guanine nucleotide exchange factor (96). As *At*MIN7 was previously shown to be involved in vesicle trafficking and recycling of auxin efflux transporters (121), it was suggested that HopM1-mediated degradation of *At*MIN7 is suppressing secretion to the benefit of the bacteria. It has now been revealed that HopM1 and AvrE establish an aqueous environment by hijacking abscisic acid signaling and are largely independent of *At*MIN7 (106). Future studies may reveal the immunity-related function of *At*MIN7 degradation. Another example of a T3E promoting degradation of its host target is HopBB1 from *P. syringae*. HopBB1 was identified to promote the degradation of the TF TCP14 by targeting it to the SCF^{COI} complex. With this, HopBB1 modulates phytohormone responses and virulence (150). In a similar manner, SAP54 from phytoplasmas, which are phloem-inhabiting bacteria, hijacks RAD23, a ubiquitin-binding protein and proteasome-shuttle factor, to degrade MADS-box TFs from *Arabidopsis* via the 26S proteasome to promote insect colonization (81). Another study identified that phyllogen, an effector from phytoplasmas, induces the degradation of floral MADS-box TFs (MTFs) via RAD23 shuttle proteins. Interestingly, these findings suggest that phyllogen is functionally mimicking ubiquitin, as MTF–phyllogen–RAD23 complex formation was ubiquitin independent (61, 82). Along this line, another phytoplasma effector, SAP05, was recently described to mediate ubiquitin-independent degradation of *Arabidopsis* TFs SQUAMOSA promoter-binding protein-like (SPL) and GATA to induce symptoms. Degradation of both TFs was achieved by direct interaction of SAP05 with the ubiquitin receptor RPN10, a subunit of the 26S proteasome (49). Most of the mentioned examples involve the proteasome shuttle factor RAD23 and how effectors hijack its function to degrade their targets. Although not shown, HopM1 could also deploy a similar strategy to induce the proteasome-dependent removal of AtMIN7, as it was initially identified to interact with RAD23.

Other examples of ubiquitin-independent degradation of host target proteins involve T3Es with specific enzymatic functions that destabilize their substrates by PTMs. For instance, *Pseudomonas* HopZ1a acts as an acetyltransferase on JAZ repressors to activate JA signaling, which in turn suppresses SA defense responses. Acetylation of JAZ proteins leads to their destabilization and degradation by the UPS (53). Apart from acetylation, SUMOylation also plays a major role in destabilization or stabilization. XopD from *Xanthomonas* exhibits SUMO-protease activity on tomato ethylene responsive factor 4 (ERF4), resulting in its proteasomal degradation. Destabilization of proteins not only includes the modulation of PTMs but can also appear via the N-degron pathway. The first T3E described to hijack this pathway is AvrRpt2 from *P. syringae*, a cysteine protease that degrades host proteins containing a nitrate-induced domain (41).

The manipulation mechanisms we have described so far rely on the degradation of host targets. However, sometimes the opposite is required, as stabilization of negative regulators of immunity could ultimately end in subversion of plant defenses as well. For instance, *Xanthomonas* XopD is able to inhibit the degradation of the DELLA protein repressor of GA (RGA) to promote disease (120). In a similar way, a recent study characterized the role of *X. euvesicatoria* XopS in preventing the proteasomal degradation of pepper TF WRKY40 (104). XopS is a major virulence factor of *X. euvesicatoria* that prevents stomatal closure to promote disease progression. This is dependent on the stabilization of WRKY40, a negative regulator of PTI. XopS interacts with and inhibits the degradation of WRKY40 via a yet unknown mechanism to reduce downstream defense gene expression and alter phytohormone cross talk. Although in the case of XopS the mode of action is yet not understood, another *Xanthomonas* effector, XopP from *X. oryzae* pv. *oryzae*, has been identified to specifically inhibit the E3 ligase activity of *Os*PUB44, a positive regulator of immune responses, to suppress PTI in rice (50).

Although targeting E3 ubiquitin activity or PTMs indirectly affects the proteasomal degradation, there is also a very direct way to inhibit the proteasome: by targeting the proteasome itself. The bacterial toxin syringolin A (SylA) was the first virulence factor described to directly bind to subunits of the 20S proteasome and, thereby, act as an irreversible proteasome inhibitor (44). Direct inhibition of the proteasome by SylA has many positive effects on *P. syringae*: It (*a*) results in a block of SA signaling in adjacent tissues, (*b*) increases the bacterial motility, and (*c*) suppresses immune responses at the local infection site (88). Despite its important virulence function, SylA is not widely distributed in *Pseudomonas* or other plant-pathogenic bacteria (4). Nevertheless, plant-pathogenic bacteria have evolved T3Es that can directly target the 26S proteasome to alter its function. A primary example is XopJ from *X. euvesicatoria*. XopJ directly interacts with the proteasomal subunit RPT6 (130). This interaction results in reduced proteasome activity and suppression of SA-mediated plant defenses. Subsequent studies have revealed that XopJ displays protease activity to degrade RPT6, which hampers the proteasome function. A following study revealed that a closely related *P. syringae* ortholog of XopJ, HopZ4, also interacts with RPT6 and suppresses proteasome activity (132).

Although *P. syringae* pv. *tomato* lacks SylA, it still suppresses proteasome activity in a T3E-dependent manner. Several T3Es from *P. syringae* pv. *tomato* such as HopA1, HopG1, HopN, HopAO1, and HopM1 hamper proteasome activity (133). The latter was additionally shown to associate with several proteasome subunits and HECT UPL E3 ligases in planta (133). Given the function of the UPL E3 as regulators of proteasome function, it is possible that HopM1 suppresses proteasome activity in a direct manner by interfering with their function (140). However, subsequent studies revealed that HopM1 removes proteasome subunits by activating a selective autophagy mechanism termed proteaphagy (131).

3.2. Viruses

Viruses are versatile, intracellular, obligate pathogens with a biotrophic lifestyle, as they lack their own replication machinery and thus require host cellular pathways to colonize their hosts. In contrast to bacteria, viruses are intracellular pathogens and associate with various host cellular compartments that they can reprogram for their own benefit. Emerging evidence suggests that the plant UPS plays an important role during plant–virus interactions: On one hand, the UPS can impair viral pathogenesis; on the other hand, viruses evolved strategies to exploit the UPS (31). Although viral genomes are very small and encode only a few proteins, they can act on multiple targets to generate a very flexible and versatile response to the plant defense machinery. Viruses employ different strategies to manipulate the host UPS, including the direct targeting of the

proteasome, influencing the expression of UPS components and their activities, or perturbing the degradation of host proteins (**Figure 3*b***). Viral effector HcPro, a known gene silencing suppressor, from LMV and *papaya ring spot virus* (PRSV) directly interacts with the catalytic subunits of the 20S proteasome to inhibit the 20S endonuclease and proteasome activity, respectively (3, 107). Inhibition of the 20S activity resulted in enhanced viral accumulation and, therefore, can be considered a proviral mechanism. Similar to HcPro from LMV and PRSV, HcPro from *potato virus x* directly associates with subunits of the 20S proteasome, albeit the nature of this interaction has yet to be determined (55).

Although no viral effectors have been identified that directly degrade host proteins, some viral proteins have been shown to induce endogenous E3 gene expression. For instance, the C4 protein from *beet severe curly top virus* (BSCTV) induces the transcription of related to KPC1 (RKP) in *Arabidopsis*. RKP targets the cell-cycle inhibitor kinase inhibitor protein (KIP)-related protein 2, which was shown to be beneficial for viral replication (65). Another viral protein from BSCTV, Rep, was found to transcriptionally induce variant in methylation 5 (*VIM5*) to induce the transcription of the viral proteins C2 and C3 (14). Similarly, the P3 protein from *rice grassy stunt virus* (RGSV) has been described to induce the transcription of *Os*P3IP1 in rice. *Os*P3IP1 ubiquitinates nuclear RNA polymerase D1a (*Os*NRPD1a), the largest subunit of plant-specific RNA polymerase IV, for proteasomal degradation (155). However, not only E3 ligases and their activities are crucial for viral replication and fitness. Recently, it has been shown that CaMV requires the proteasome for robust infection. CaMV induces proteasome activity in an SA-dependent manner to support systemic viral accumulation and plant fitness. Interestingly, the viral protein P6 attenuates proteasome activity induced by SA during CaMV infection, which may act as a counteracting measure in the proteasome–virus interaction (113). These apparently conflicting findings emphasize the complexity of the intimate interplay between viruses and the UPS pathway.

A more direct way for viruses to perturb E3 ligase activity in the host is to target SCF complexes, which seem to be target hubs for viral proteins. Several viral proteins target these complexes to promote viral replication in different ways. For instance, C2 proteins from geminiviruses subvert ubiquitination by compromising CSN activity, which in turn perturbs the function of SCF complexes and inhibits JA signaling (76). Other viral effectors such as βC1 from *cotton leaf curly multan virus* have been described to interfere with SCF complex formation. Here, βC1 inhibits the association between *Nb*SKP1 and *Nb*CUL1 to disturb JA and gibberellin signaling (57). Other viral proteins interacting with the SCF complex are P7-2 and P25 from *rice black-streaked dwarf virus*, which interacts with eight S-phase kinase-associated protein 1 (SKP1) proteins, a core subunit of the SCF complex (123, 124). Exploiting the SCF complex likely affects degradation of host proteins and thus constitutes an attractive target for viral proteins. In support of this, P2 protein from the *rice dwarf virus* blocks association between *Os*IAA10 and the F-box *Os*TIR1 to dampen *Os*IAA10 proteasomal degradation (54). Another viral protein 2b from *cucumber mosaic virus* (CMV) inhibits JA signaling by preventing the degradation of JAZ repressors to enhance aphid-mediated viral transmission (160).

3.3. Oomycetes, Fungi, Nematodes, and Insects

Similar to bacteria, eukaryotic pathogens such as oomycetes, fungi, nematodes, and insects also exploit their effector arsenals to subvert the host defenses. However, owing to their higher complexity, fewer examples have been characterized. Nevertheless, there is enough current evidence supporting the ability of eukaryotic pathogens to infer the proper functioning of the plant UPS (**Figure 3*c***). For instance, there are several effectors from eukaryotic pathogens that target host proteins for proteasomal degradation to carry out their virulence function. This is the case of the effector HaR×L×44 from the biotrophic oomycete *Hyaloperonospora arabidopsidis*, which interacts

and destabilizes mediator complex 19A (MED19A) in a proteasome-dependent manner (9). MED19A is a subunit of the mediator complex, responsible for the interaction between transcriptional regulators and RNA polymerase II. By doing this, *H. arabidopsidis* tilts the SA–JA antagonistic balance toward the latter, making the plant more susceptible to biotrophic pathogens. The fungal effector AvrPiz-t from *M. oryzae* itself is targeted by the plant proteasome upon ubiquitination by the rice E3 ligases *Os*APIP6 and *Os*APIP10 (100). Interestingly, by doing so, AvrPiz-t targets these E3 ligases to proteasomal degradation as well in a sort of kamikaze move, which, in turn, dampens the rice basal defenses. Similarly, *Zymoseptoria tritici* effector *Zt*SSP2 has been recently shown to interact with the E3 ligase *Ta*E3UBQ, a positive regulator of wheat immunity. However, the influence of the *Zt*SSP2–*Ta*E3UBQ interaction on *Zt*SSP2 and *Ta*E3UBQ stability and activity remains to be discovered (58). Fungal effector orphan secreted protein 24 (OSP24) from *Fusarium graminearum* also induces the proteasomal degradation of the wheat protein *Ta*SNRK1a by competing with the host kinase stabilizer *Fusarium* resistance orphan gene (*Ta*FROG) (52). Another mentioned strategy to trigger host protein degradation is mimicking plant E3 ligases. This strategy could potentially be developed more easily in the case of eukaryotic pathogens compared to bacteria considering their closer phylogenetic relationship to plants. This strategy is deployed by the potato cyst nematode *Globodera pallida*. *G. pallida* effector RHA1B is an active RING E3 ligase that can interact with multiple host E2 enzymes (63). RHA1B possesses a double virulence function, inhibiting both PTI and ETI in E3-independent and E3-dependant manners, respectively. It has been hypothesized that this strategy could also be deployed by gall-forming insects, as suggested by the expansion of secreted RING E3 ligases in *Daktulosphaira vitifoliae* (157).

In addition to targeting positive regulators of immunity for degradation, eukaryotic pathogen effectors can exert their virulence functions also by doing the opposite: stabilizing negative regulators of immunity. This is the case of the oomycete effector Avr3a from *P. infestans*. Avr3a inhibits the proteasomal degradation of the PUB E3 ligase CYS, MET, PRO, and GLY protein 1 (CMPG1), perturbing CMPG1-mediated defensive PCD (6). Fungal effector Tin2 from *Ustilago maydis* stabilizes maize TIN2-targeting kinase 1 (ZmTTK1) by masking its ubiquitin–proteasome degradation motif, ultimately rewiring metabolic fluxes toward the synthesis of anthocyanins instead of defensive tissue lignification (122). Another strategy for stabilization of host targets is to outcompete for the interaction with the correspondent E3 ligase(s). This is, for instance, the case of the fungal effector VDAL from *Verticillium dahliae*, whose interaction with the host PUB25 and PUB26 E3 ligases prevents their association with its native target, MYB6, to possibly expand the biotrophic phase of infection (78).

Besides degrading or stabilizing UPS targets, eukaryotic pathogens can also affect the normal functioning of the UPS in a more direct manner by targeting regulatory processes of proteasomal assembly or activity. For example, the effector ubiquitin carboxyl extension protein 12 (GrUBCEP12) from the yellow potato cyst nematode *Globodera rostochiensis* prevents the expression of the proteasomal subunit encoding gene *RPN2* and, upon proteolytic activation, blocks PTI responses (19). The enzymatic activity of the proteasome can also be targeted by pathogen molecules. For instance, the fungal toxin higginsianin B from *Colletotrichum higginsianum* is a potent inhibitor of the chymotrypsin- and caspase-like proteasomal activities. This inhibition ultimately leads to JAZ stabilization and impaired JA signaling to the benefit of the pathogen (26). There is also evidence of a nematode effector, GpRbp-1 from *G. pallida*, that interacts with a nuclear proteasome-associated HECT E3 ligase UPL3 (28). Although the outcome of this interaction remains to be characterized, it has been reported that the HECT-containing ubiquitin-protein ligases (UPLs) are regulators of the proteasome and plant immunity (36, 140). The E1→E2→E3 cascade can also be targeted by pathogen effectors in their effort to subvert plant defenses. This is the case for the oomycete *Phytophthora sojae* effector Avr1d, which acts as

an E2 competitor occupying the E2 binding site of the soy E3 ligase GmPUB13 and ultimately promoting *P. sojae* proliferation (72). Altogether, these data show how general and important is the manipulation of the UPS and UPS-related proteins among all kinds of plant pathogens.

4. CONCLUDING REMARKS AND PERSPECTIVES

Emerging evidence suggests that the UPS is a key pillar of the plant immune system. The UPS governs the turnover of many immune-related components and hence constitutes a vulnerable target for pathogens. As such, diverse plant pathogens employ sophisticated strategies to manipulate the UPS and combat plant immune reactions. Pathogen effectors can induce or block the degradation of target proteins involved in immune reactions. In this context, effectors can serve as tools to dissect how the UPS is involved in plant–pathogen interactions. Current knowledge implies that the UPS acts as a double-edged sword: on one hand, the UPS is required to maintain efficient plant defense responses; on the other hand, pathogens require its proper function to promote pathogenicity. In addition, different effectors from the same pathogen can display contrasting functions, which could be partially explained by their distinct spatiotemporal modes of action. Taking these observations into consideration, it is evident that we are only scratching the surface of this complex interplay. To gain a better understanding, it will be crucial to decipher the fine-tuning of the proteasome and its components on every level: from transcription and translation to the assembly of the proteasome and its associated components. We will thereby decipher the complexity of the interplay between microbes and the UPS.

5. OUTSTANDING QUESTIONS

Recent findings on the plant UPS, its cross talk with plant immunity, and how it is targeted by plant microbes have advanced our understanding in this field. However, many open questions remain unanswered and need to be addressed in the future to fully decipher the complex interplay between the UPS and microbes:

- How do microbial effectors with contrasting or additive functions on the UPS influence each other?
- Do microbes directly target the regulation of the proteasome complex to impact plant immune reactions and host cellular pathways?
- Are there different proteasome complexes with alternative functions induced during microbial infection?
- What role does cell-type specificity play in the interaction of microbes with the UPS?
- How can we strengthen the host UPS to avoid its targeting by microbes?
- How are organelle- or compartment-specific associated degradation systems influenced by microbes?
- How is the UPS altered if we look beyond binary interactions?

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LITERATURE CITED

1. Abramovitch RB, Janjusevic R, Stebbins CE, Martin GB, Lindow SE. 2006. Type III effector AvrPtoB requires intrinsic E3 ubiquitin ligase activity to suppress plant cell death and immunity. *PNAS* 103(8):2851–56
2. Angot A, Peeters N, Lechner E, Vailleau F, Baud C, et al. 2006. *Ralstonia solanacearum* requires F-box-like domain-containing type III effectors to promote disease on several host plants. *PNAS* 103(39):14620–25
3. Ballut L, Petit F, Le Gall O, Candresse T, Schmid P, et al. 2003. Biochemical identification of proteasome-associated endonuclease activity in sunflower. *Biochim. Biophys. Acta* 1645(1):30–39
4. Baltrus DA, Nishimura MT, Romanchuk A, Chang JH, Mukhtar MS, et al. 2011. Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 *Pseudomonas syringae* isolates. *PLOS Pathog.* 7(7):e1002132
5. Becker F, Buschfeld E, Schell J, Bachmair A. 1993. Altered response to viral infection by tobacco plants perturbed in ubiquitin system. *Plant J.* 3(6):875–81
6. Bos JIB, Armstrong MR, Gilroy EM, Boevink PC, Hein I, et al. 2010. *Phytophthora infestans* effector AVR3a is essential for virulence and manipulates plant immunity by stabilizing host E3 ligase CMPG1. *PNAS* 107(21):9909–14
7. Bullones-Bolaños A, Bernal-Bayard J, Ramos-Morales F. 2022. The NEL family of bacterial E3 ubiquitin ligases. *Int. J. Mol. Sci.* 23(14):7725
8. Bürger M, Chory J. 2019. Stressed out about hormones: how plants orchestrate immunity. *Cell Host Microbe* 26(2):163–72
9. Caillaud MC, Asai S, Rallapalli G, Piquerez S, Fabro G, Jones JDG. 2013. A downy mildew effector attenuates salicylic acid-triggered immunity in *Arabidopsis* by interacting with the host mediator complex. *PLOS Biol.* 11(12):e1001732
10. Chen B, Lin L, Lu Y, Peng J, Zheng H, et al. 2020. Ubiquitin-like protein 5 interacts with the silencing suppressor p3 of rice stripe virus and mediates its degradation through the 26S proteasome pathway. *PLOS Pathog.* 16(8):e1008780
11. Chen H, Chen J, Li M, Chang M, Xu K, et al. 2017. A bacterial type III effector targets the master regulator of salicylic acid signaling, NPR1, to subvert plant immunity. *Cell Host Microbe* 22(6):777–88.e7
12. Chen L, Hellmann H. 2013. Plant E3 ligases: flexible enzymes in a sessile world. *Mol. Plant* 6(5):1388–404
13. Chen Q, Zhong Y, Wu Y, Liu L, Wang P, et al. 2016. HRD1-mediated ERAD tuning of ER-bound E2 is conserved between plants and mammals. *Nat. Plants* 2:16094
14. Chen ZQ, Zhao JH, Chen Q, Zhang ZH, Li J, et al. 2020. DNA geminivirus infection induces an imprinted E3 ligase gene to epigenetically activate viral gene transcription. *Plant Cell* 32(10):3256–72
15. Cheng D, Zhou D, Wang Y, Wang B, He Q, et al. 2021. *Ralstonia solanacearum* type III effector RipV2 encoding a novel E3 ubiquitin ligase (NEL) is required for full virulence by suppressing plant PAMP-triggered immunity. *Biochem. Biophys. Res. Commun.* 550:120–26
16. Cheng W, Munkvold KR, Gao H, Mathieu J, Schwizer S, et al. 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(6):616–26
17. Cheng YT, Li Y, Huang S, Huang Y, Dong X, et al. 2011. Stability of plant immune-receptor resistance proteins is controlled by SKP1-CULLIN1-F-box (SCF)-mediated protein degradation. *PNAS* 108(35):14694–99
18. Choi C, Im JH, Lee J, Il Kwon S, Kim W-Y, et al. 2022. OsDWD1 E3 ligase-mediated OsNPR1 degradation suppresses basal defense in rice. *Plant J.* 112(4):966–81
19. Chronis D, Chen S, Lu S, Hewezi T, Carpenter SCD, et al. 2013. A ubiquitin carboxyl extension protein secreted from a plant-parasitic nematode *Globodera rostochiensis* is cleaved in planta to promote plant parasitism. *Plant J.* 74(2):185–96

20. Chung K, Tasaka M. 2011. RPT2a, a 26S proteasome AAA-ATPase, is directly involved in *Arabidopsis* CC-NBS-LRR protein uni-1D-induced signaling pathways. *Plant Cell Physiol.* 52(9):1657–64
21. Coll NS, Epple P, Dangl JL. 2011. Programmed cell death in the plant immune system. *Cell Death Differ.* 18(8):1247–56
22. Collins GA, Goldberg AL. 2017. The logic of the 26S proteasome. *Cell* 169(5):792–806
23. Copeland C, Woloshen V, Huang Y, Li X. 2016. AtCDC48A is involved in the turnover of an NLR immune receptor. *Plant J.* 88(2):294–305
24. Couto D, Zipfel C. 2016. Regulation of pattern recognition receptor signalling in plants. *Nat. Rev. Immunol.* 16(9):537–52
25. Craig A, Ewan R, Mesmar J, Gudipati V, Sadanandom A. 2009. E3 ubiquitin ligases and plant innate immunity. *J. Exp. Bot.* 60(4):1123–32
26. Dallery JF, Zimmer M, Halder V, Suliman M, Pigné S, et al. 2020. Inhibition of jasmonate-mediated plant defences by the fungal metabolite higginsianin B. *J. Exp. Bot.* 71(10):2910–21
27. de Marchi R, Sorel M, Mooney B, Fudal I, Goslin K, et al. 2016. The N-end rule pathway regulates pathogen responses in plants. *Sci. Rep.* 6(1):26020
28. Diaz-Granados A, Sterken MG, Overmars H, Ariaans R, Holterman M, et al. 2020. The effector GpRbp-1 of *Globodera pallida* targets a nuclear HECT E3 ubiquitin ligase to modulate gene expression in the host. *Mol. Plant Pathol.* 21(1):66–82
29. Dissmeyer N. 2019. Conditional protein function via N-degron pathway-mediated proteostasis in stress physiology. *Annu. Rev. Plant. Biol.* 70:83–117
30. Dong OX, Ao K, Xu F, Johnson KCM, Wu Y, et al. 2018. Individual components of paired typical NLR immune receptors are regulated by distinct E3 ligases. *Nat Plants* 4(9):699–710
31. Dubiella U, Serrano I. 2021. The ubiquitin proteasome system as a double agent in plant-virus interactions. *Plants* 10(5):4–7
32. Fan J, Bai P, Ning Y, Wang J, Shi X, et al. 2018. The monocot-specific receptor-like kinase SDS2 controls cell death and immunity in rice. *Cell Host Microbe* 23(4):498–510.e5
33. Fernández-Calvo P, Chini A, Fernández-Barbero G, Chico JM, Gimenez-Ibanez S, et al. 2011. The *Arabidopsis* bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. *Plant Cell* 23(2):701–15
34. Fu ZQ, Yan S, Saleh A, Wang W, Ruble J, et al. 2012. NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. *Nature* 486(7402):228–32
35. Furlan G, Nakagami H, Eschen-Lippold L, Jiang X, Majovsky P, et al. 2017. Changes in PUB22 ubiquitination modes triggered by MITOGEN-ACTIVATED PROTEIN KINASE3 dampen the immune response. *Plant Cell* 29(4):726–45
36. Furniss JJ, Grey H, Wang Z, Nomoto M, Jackson L, et al. 2018. Proteasome-associated HECT-type ubiquitin ligase activity is required for plant immunity. *PLOS Pathog.* 14(11):e1007447
37. Gao C, Sun P, Wang W, Tang D. 2021. *Arabidopsis* E3 ligase KEG associates with and ubiquitinates MKK4 and MKK5 to regulate plant immunity. *J Integr. Plant Biol.* 63(2):327–39
38. Gimenez-Ibanez S, Hann DR, Ntoukakis V, Petutschnig E, Lipka V, Rathjen JP. 2009. AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. *Curr. Biol.* 19(5):423–29
39. Göhre V, Spallek T, Häweker H, Mersmann S, Mentzel T, et al. 2008. Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. *Curr. Biol.* 18(23):1824–32
40. Goritschnig S, Zhang Y, Li X. 2007. The ubiquitin pathway is required for innate immunity in *Arabidopsis*. *Plant J.* 49(3):540–51
41. Goslin K, Eschen-Lippold L, Naumann C, Linster E, Sorel M, et al. 2019. Differential N-end rule degradation of RIN4/NOI fragments generated by the AvrRpt2 effector protease. *Plant Physiol.* 180(4):2272–89
42. Gou M, Shi Z, Zhu Y, Bao Z, Wang G, Hua J. 2012. The F-box protein CPR1/CPR30 negatively regulates R protein SNC1 accumulation. *Plant J.* 69(3):411–20
43. Gravot A, Richard G, Lime T, Lemarié S, Jubault M, et al. 2016. Hypoxia response in *Arabidopsis* roots infected by *Plasmodiophora brassicae* supports the development of clubroot. *BMC Plant Biol.* 16(1):251

44. Groll M, Schellenberg B, Bachmann AS, Archer CR, Huber R, et al. 2008. A plant pathogen virulence factor inhibits the eukaryotic proteasome by a novel mechanism. *Nature* 452(7188):755–58
45. Gu Y, Innes RW. 2012. The KEEP ON GOING protein of *Arabidopsis* regulates intracellular protein trafficking and is degraded during fungal infection. *Plant Cell* 24(11):4717–30
46. He Q, McLellan H, Boevink PC, Sadanandom A, Xie C, et al. 2015. U-box E3 ubiquitin ligase PUB17 acts in the nucleus to promote specific immune pathways triggered by *Phytophthora infestans*. *J. Exp. Bot.* 66(11):3189–99
47. Hu X-H, Fan J, Wu J-L, Shen S, He J-X, et al. 2021. Proteasome maturation factor UMP1 confers broad-spectrum disease resistance by modulating H_2O_2 accumulation in rice. bioRxiv 433750. **https://doi.org/10.1101/2021.03.03.433750**
48. Huang J, Wu X, Gao Z. 2021. The RING-type protein BOI negatively regulates the protein level of a CC-NBS-LRR in *Arabidopsis*. *Biochem. Biophys. Res. Commun.* 578:104–9
49. Huang W, MacLean AM, Sugio A, Maqbool A, Busscher M, et al. 2021. Parasitic modulation of host development by ubiquitin-independent protein degradation. *Cell* 184(20):5201–14.e12
50. Ishikawa K, Yamaguchi K, Sakamoto K, Yoshimura S, Inoue K, et al. 2014. Bacterial effector modulation of host E3 ligase activity suppresses PAMP-triggered immunity in rice. *Nat. Commun.* 5:5430
51. Ji H, Liu D, Zhang Z, Sun J, Han B, Li Z. 2020. A bacterial F-box effector suppresses SAR immunity through mediating the proteasomal degradation of OsTrxh2 in rice. *Plant J.* 104(4):1054–72
52. Jiang C, Hei R, Yang Y, Zhang S, Wang Q, et al. 2020. An orphan protein of *Fusarium graminearum* modulates host immunity by mediating proteasomal degradation of TaSnRK1α. *Nat. Commun.* 11(1):4382
53. Jiang S, Yao J, Ma KW, Zhou H, Song J, et al. 2013. Bacterial effector activates jasmonate signaling by directly targeting JAZ transcriptional repressors. *PLOS Pathog.* 9(10):e1003715
54. Jin L, Qin Q, Wang Y, Pu Y, Liu L, et al. 2016. Rice dwarf virus P2 protein hijacks auxin signaling by directly targeting the rice OsIAA10 protein, enhancing viral infection and disease development. *PLOS Pathog.* 12(9):e1005847
55. Jin Y, Ma D, Dong J, Jin J, Li D, et al. 2007. HC-Pro protein of Potato virus Y can interact with three *Arabidopsis* 20S proteasome subunits in planta. *J. Virol.* 81(23):12881–88
56. Jones JDG, Dangl JL. 2006. The plant immune system. *Nature* 444(7117):323–29
57. Kamal H, Minhas F, Tripathi D, Abbasi WA, Hamza M, et al. 2019. βC1, pathogenicity determinant encoded by Cotton leaf curl Multan betasatellite, interacts with calmodulin-like protein 11 (Gh-CML11) in *Gossypium hirsutum*. *PLOS ONE* 14(12):e0225876
58. Karki SJ, Reilly A, Zhou B, Mascarello M, Burke J, et al. 2021. A small secreted protein from *Zymoseptoria tritici* interacts with a wheat E3 ubiquitin ligase to promote disease. *J. Exp. Bot.* 72(2):733–46
59. Kawasaki T, Nam J, Boyes DC, Holt BF, Hubert DA, et al. 2005. A duplicated pair of *Arabidopsis* RING-finger E3 ligases contribute to the RPM1- and RPS2-mediated hypersensitive response. *Plant J.* 44(2):258–70
60. Khan M, Subramaniam R, Desveaux D. 2016. Of guards, decoys, baits and traps: pathogen perception in plants by type III effector sensors. *Curr. Opin. Microbiol.* 29:49–55
61. Kitazawa Y, Iwabuchi N, Maejima K, Sasano M, Matsumoto O, et al. 2022. A phytoplasma effector acts as a ubiquitin-like mediator between floral MADS-box proteins and proteasome shuttle proteins. *Plant Cell* 34(5):1709–23
62. Kreitman M, Noronha A, Yarden Y. 2018. Irreversible modifications of receptor tyrosine kinases. *FEBS Lett.* 592(13):2199–212
63. Kud J, Wang W, Gross R, Fan Y, Huang L, et al. 2019. The potato cyst nematode effector RHA1B is a ubiquitin ligase and uses two distinct mechanisms to suppress plant immune signaling. *PLOS Pathog.* 15(4):e1007720
64. Lacroix B, Citovsky V. 2015. Nopaline-type Ti plasmid of *Agrobacterium* encodes a VirF-like functional F-box protein. *Sci Rep.* 5:16610
65. Lai J, Chen H, Teng K, Zhao Q, Zhang Z, et al. 2009. RKP, a RING finger E3 ligase induced by BSCTV C4 protein, affects geminivirus infection by regulation of the plant cell cycle. *Plant J.* 57(5):905–17
66. Langin G, Gouguet P, Üstün S. 2020. Microbial effector proteins: a journey through the proteolytic landscape. *Trends Microbiol.* 28(7):523–35

67. Lee BJ, Kwon SJ, Kim SK, Kim KJ, Park CJ, et al. 2006. Functional study of hot pepper 26S proteasome subunit RPN7 induced by Tobacco mosaic virus from nuclear proteome analysis. *Biochem. Biophys. Res. Commun.* 351(2):405–11
68. Leong JX, Raffeiner M, Spinti D, Langin G, Franz-Wachtel M, et al. 2022. A bacterial effector counteracts host autophagy by promoting degradation of an autophagy component. *EMBO J.* 41(13):e110352
69. Lequeu J, Simon-Plas F, Fromentin J, Etienne P, Petitot AS, et al. 2005. Proteasome comprising a β1 inducible subunit acts as a negative regulator of NADPH oxidase during elicitation of plant defense reactions. *FEBS Lett.* 579(21):4879–86
70. Li J, Chu ZH, Batoux M, Nekrasov V, Roux M, et al. 2009. Specific ER quality control components required for biogenesis of the plant innate immune receptor EFR. *PNAS* 106(37):15973–78
71. Lim G-H, Hoey T, Zhu S, Clavel M, Yu K, et al. 2018. COP1, a negative regulator of photomorphogenesis, positively regulates plant disease resistance via double-stranded RNA binding proteins. *PLOS Pathog.* 14(3):e1006894
72. Lin Y, Hu Q, Zhou J, Yin W, Yao D, et al. 2021. *Phytophthora sojae* effector Avr1d functions as an E2 competitor and inhibits ubiquitination activity of GmPUB13 to facilitate infection. *PNAS* 118(10):e2018312118
73. Liu J, Huang Y, Kong L, Yu X, Feng B, et al. 2020. The malectin-like receptor-like kinase LETUM1 modulates NLR protein SUMM2 activation via MEKK2 scaffolding. *Nat. Plants* 6(9):1106–15
74. Liu J, Park CH, He F, Nagano M, Wang M, et al. 2015. The RhoGAP SPIN6 associates with SPL11 and OsRac1 and negatively regulates programmed cell death and innate immunity in rice. *PLOS Pathog.* 11(2):e1004629
75. Liu X, Zhou Y, Du M, Liang X, Fan F, et al. 2022. The calcium-dependent protein kinase CPK28 is targeted by the ubiquitin ligases ATL31 and ATL6 for proteasome-mediated degradation to fine-tune immune signaling in *Arabidopsis*. *Plant Cell* 34(1):679–97
76. Lozano-Durán R, Rosas-Díaz T, Gusmaroli G, Luna AP, Taconnat L, et al. 2011. Geminiviruses subvert ubiquitination by altering CSN-mediated derubylation of SCF E3 ligase complexes and inhibit jasmonate signaling in *Arabidopsis thaliana*. *Plant Cell* 23(3):1014–32
77. Lu D, Lin W, Gao X, Wu S, Cheng C, et al. 2011. Direct ubiquitination of pattern recognition receptor FLS2 attenuates plant innate immunity. *Science* 332(6036):1439–42
78. Ma A, Zhang D, Wang G, Wang K, Li Z, et al. 2021. *Verticillium dahliae* effector VDAL protects MYB6 from degradation by interacting with PUB25 and PUB26 E3 ligases to enhance Verticillium wilt resistance. *Plant Cell* 33(12):3675–99
79. Ma W, Xu X, Cai L, Cao Y, Haq F, et al. 2020. A *Xanthomonas oryzae* type III effector XopL causes cell death through mediating ferredoxin degradation in *Nicotiana benthamiana*. *Phytopathol. Res.* 2:16
80. Mackey D, Yun D, Nam J. 2021. Proteasome-dependent degradation of RPM1 desensitizes the RPM1-mediated hypersensitive response. *J. Plant Biol.* 64(3):217–25
81. MacLean AM, Orlovskis Z, Kowitwanich K, Zdziarska AM, Angenent GC, et al. 2014. Phytoplasma effector SAP54 hijacks plant reproduction by degrading MADS-box proteins and promotes insect colonization in a RAD23-dependent manner. *PLOS Biol.* 12(4):e1001835
82. Maejima K, Kitazawa Y, Tomomitsu T, Yusa A, Neriya Y, et al. 2015. Degradation of class E MADS-domain transcription factors in *Arabidopsis* by a phytoplasmal effector, phyllogen. *Plant Signal. Behav.* 10(8):e1042635
83. Marshall RS, Vierstra RD. 2019. Dynamic regulation of the 26S proteasome: from synthesis to degradation. *Front. Mol. Biosci.* 6:40
84. Matsushita A, Inoue H, Goto S, Nakayama A, Sugano S, et al. 2013. Nuclear ubiquitin proteasome degradation affects WRKY45 function in the rice defense program. *Plant J.* 73(2):302–13
85. McLellan H, Chen K, He Q, Wu X, Boevink PC, et al. 2020. The ubiquitin E3 ligase PUB17 positively regulates immunity by targeting a negative regulator, KH17, for degradation. *Plant Commun.* 1(4):100020
86. Miao M, Niu X, Kud J, Du X, Avila J, et al. 2016. The ubiquitin ligase SEVEN IN ABSENTIA (SINA) ubiquitinates a defense-related NAC transcription factor and is involved in defense signaling. *New Phytol.* 211(1):138–48

87. Millyard L, Lee J, Zhang C, Yates G, Sadanandom A. 2016. The ubiquitin conjugating enzyme, TaU4 regulates wheat defence against the phytopathogen *Zymoseptoria tritici*. *Sci. Rep.* 6:35683
88. Misas-Villamil JC, Kolodziejek I, Crabill E, Kaschani F, Niessen S, et al. 2013. *Pseudomonas syringae* pv. *syringae* uses proteasome inhibitor syringolin A to colonize from wound infection sites. *PLOS Pathog.* 9(3):10–16
89. Mithoe SC, Menke FL. 2018. Regulation of pattern recognition receptor signalling by phosphorylation and ubiquitination. *Curr. Opin. Plant Biol.* 45(Pt. A):162–70
90. Müller J, Piffanelli P, Devoto A, Miklis M, Elliott C, et al. 2005. Conserved ERAD-like quality control of a plant polytopic membrane protein. *Plant Cell* 17(1):149–63
91. Nakano M, Oda K, Mukaihara T. 2017. *Ralstonia solanacearum* novel E3 ubiquitin ligase (NEL) effectors RipAW and RipAR suppress pattern-triggered immunity in plants. *Microbiology* 163(7):992–1002
92. Ngou BPM, Ding P, Jones JDG. 2022. Thirty years of resistance: zig-zag through the plant immune system. *Plant Cell* 34(5):1447–78
93. Ngou BPM, Heal R, Wyler M, Schmid MW, Jones JDG. 2022. Concerted expansion and contraction of immune receptor gene repertoires in plant genomes. *Nat. Plants* 8(10):1146–52
94. Ning Y, Shi X, Wang R, Fan J, Park CH, et al. 2015. OsELF3-2, an ortholog of *Arabidopsis* ELF3, interacts with the E3 ligase APIP6 and negatively regulates immunity against *Magnaporthe oryzae* in rice. *Mol. Plant* 8(11):1679–82
95. Nirmala J, Dahl S, Steffenson BJ, Kannangara CG, von Wettstein D, et al. 2007. Proteolysis of the barley receptor-like protein kinase RPG1 by a proteasome pathway is correlated with *Rpg1*-mediated stem rust resistance. *PNAS* 104(24):10276–81
96. Nomura K, Debroy S, Lee YH, Pumplin N, Jones J, He SY. 2006. A bacterial virulence protein suppresses host innate immunity to cause plant disease. *Science* 313(5784):220–23
97. Orosa B, He Q, Mesmar J, Gilroy EM, McLellan H, et al. 2017. BTB-BACK domain protein POB1 suppresses immune cell death by targeting ubiquitin E3 ligase PUB17 for degradation. *PLOS Genet.* 13(1):e1006540
98. Orosa B, Üstün S, Calderón Villalobos LIA, Genschik P, Gibbs D, et al. 2020. Plant proteostasis—shaping the proteome: a research community aiming to understand molecular mechanisms that control protein abundance. *New Phytol.* 227(4):1028–33
99. Pant BD, Oh S, Lee HK, Nandety RS, Mysore KS. 2020. Antagonistic regulation by CPN60A and CLPC1 of TRXL1 that regulates MDH activity leading to plant disease resistance and thermotolerance. *Cell Rep.* 33(11):108515
100. Park CH, Shirsekar G, Bellizzi M, Chen S, Songkumarn P, et al. 2016. The E3 ligase APIP10 connects the effector AvrPiz-t to the NLR receptor Piz-t in rice. *PLOS Pathog.* 12(3):e1005529
101. Petutschnig EK, Stolze M, Lipka U, Kopischke M, Horlacher J, et al. 2014. A novel *Arabidopsis* CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) mutant with enhanced pathogen-induced cell death and altered receptor processing. *New Phytol.* 204(4):955–67
102. Qin J, Zhou X, Sun L, Wang K, Yang F, et al. 2018. The *Xanthomonas* effector XopK harbours E3 ubiquitin-ligase activity that is required for virulence. *New Phytol.* 220(1):219–31
103. Qin T, Liu S, Zhang Z, Sun L, He X, et al. 2019. GhCyP3 improves the resistance of cotton to *Verticillium dahliae* by inhibiting the E3 ubiquitin ligase activity of GhPUB17. *Plant Mol. Biol.* 99(4–5):379–93
104. Raffeiner M, Üstün S, Guerra T, Spinti D, Fitzner M, et al. 2022. The *Xanthomonas* type-III effector XopS stabilizes CaWRKY40a to regulate defense responses and stomatal immunity in pepper (*Capsicum annuum*). *Plant Cell* 34(5):1684–708
105. Ramachandran P, Joshi JB, Maupin-Furlow JA, Uthandi S. 2021. Bacterial effectors mimicking ubiquitin-proteasome pathway tweak plant immunity. *Microbiol. Res.* 250:126810
106. Roussin-Léveillée C, Lajeunesse G, St-Amand M, Veerapen VP, Silva-Martins G, et al. 2022. Evolutionarily conserved bacterial effectors hijack abscisic acid signaling to induce an aqueous environment in the apoplast. *Cell Host Microbe* 30(4):489–501.e4
107. Sahana N, Kaur H, Basavaraj, Tena F, Jain RK, et al. 2012. Inhibition of the host proteasome facilitates Papaya ringspot virus accumulation and proteosomal catalytic activity is modulated by viral factor HcPro. *PLOS ONE* 7(12):e52546

108. Sahu PP, Sharma N, Puranik S, Chakraborty S, Prasad M. 2016. Tomato 26S proteasome subunit RPT4a regulates ToLCNDV transcription and activates hypersensitive response in tomato. *Sci. Rep.* 6:27078
109. Saile SC, Ackermann FM, Sunil S, Keicher J, Bayless A, et al. 2021. *Arabidopsis* ADR1 helper NLR immune receptors localize and function at the plasma membrane in a phospholipid dependent manner. *New Phytol.* 232(6):2440–56
110. Shimono M, Koga H, Akagi A, Hayashi N, Goto S, et al. 2012. Rice WRKY45 plays important roles in fungal and bacterial disease resistance. *Mol. Plant Pathol.* 13(1):83–94
111. Shimono M, Sugano S, Nakayama A, Jiang CJ, Ono K, et al. 2007. Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. *Plant Cell* 19(6):2064–76
112. Shu K, Yang W. 2017. E3 ubiquitin ligases: ubiquitous actors in plant development and abiotic stress responses. *Plant Cell Physiol.* 58(9):1461–76
113. Shukla A, Üstün S, Hafrén A. 2021. Proteasome homeostasis is essential for a robust cauliflower mosaic virus infection. bioRxiv 436740. **https://doi.org/10.1101/2021.03.24.436740**
114. Singer AU, Schulze S, Skarina T, Xu X, Cui H, et al. 2013. A pathogen type III effector with a novel E3 ubiquitin ligase architecture. *PLOS Pathog.* 9(1):e1003121
115. Skelly MJ, Furniss JJ, Grey H, Wong K-W, Spoel SH. 2019. Dynamic ubiquitination determines transcriptional activity of the plant immune coactivator NPR1. *eLife* 8:e47005
116. Soni M, Mondal KK. 2018. *Xanthomonas axonopodis* pv. *punicae* uses XopL effector to suppress pomegranate immunity. *J. Integr. Plant Biol.* 60(4):341–57
117. Spoel SH, Mou Z, Tada Y, Spivey NW, Genschik P, Dong X. 2009. Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity. *Cell* 137(5):860–72
118. Stegmann M, Anderson RG, Ichimura K, Pecenkova T, Reuter P, et al. 2012. The ubiquitin ligase PUB22 targets a subunit of the exocyst complex required for PAMP-triggered responses in *Arabidopsis*. *Plant Cell* 24(11):4703–16
119. Su W, Liu Y, Xia Y, Hong Z, Li J. 2011. Conserved endoplasmic reticulum-associated degradation system to eliminate mutated receptor-like kinases in *Arabidopsis*. *PNAS* 108(2):870–75
120. Tan L, Rong W, Luo H, Chen Y, He C. 2014. The *Xanthomonas campestris* effector protein XopD Xcc 8004 triggers plant disease tolerance by targeting DELLA proteins. *New Phytol.* 204(3):595–608
121. Tanaka H, Kitakura S, De Rycke R, De Groodt R, Friml J. 2009. Fluorescence imaging-based screen identifies ARF GEF component of early endosomal trafficking. *Curr. Biol.* 19(5):391–97
122. Tanaka S, Brefort T, Neidig N, Djamei A, Kahnt J, et al. 2014. A secreted *Ustilago maydis* effector promotes virulence by targeting anthocyanin biosynthesis in maize. *eLife* 3:e01355
123. Tao T, Zhou CJ, Wang Q, Chen XR, Sun Q, et al. 2017. Rice black streaked dwarf virus P7-2 forms a SCF complex through binding to *Oryza sativa* SKP1-like proteins, and interacts with GID2 involved in the gibberellin pathway. *PLOS ONE* 12(5):e0177518
124. Thiel H, Hleibieh K, Gilmer D, Varrelmann M. 2012. The P25 pathogenicity factor of Beet necrotic yellow vein virus targets the sugar beet 26s proteasome involved in the induction of a hypersensitive resistance response via interaction with an F-box protein. *Mol. Plant-Microbe Interact.* 25(8):1058–72
125. Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, et al. 2007. JAZ repressor proteins are targets of the SCFCOI1 complex during jasmonate signalling. *Nature* 448(7154):661–65
126. Thulasi Devendrakumar K, Copeland C, Li X. 2019. The proteasome regulator PTRE1 contributes to the turnover of SNC1 immune receptor. *Mol. Plant Pathol.* 20(11):1566–73
127. Till CJ, Vicente J, Zhang H, Oszvald M, Deery MJ, et al. 2019. The *Arabidopsis thaliana* N-recognin E3 ligase PROTEOLYSIS1 influences the immune response. *Plant Dir.* 3(12):e00194
128. Üstün S, Bartetzko V, Börnke F. 2013. The *Xanthomonas campestris* type III effector XopJ targets the host cell proteasome to suppress salicylic-acid mediated plant defence. *PLOS Pathog.* 9(6):e1003427
129. Üstün S, Bartetzko V, Börnke F. 2015. The *Xanthomonas* effector XopJ triggers a conditional hypersensitive response upon treatment of *N. benthamiana* leaves with salicylic acid. *Front. Plant Sci.* 6:599
130. Üstün S, Börnke F. 2015. The *Xanthomonas campestris* type III effector XopJ proteolytically degrades proteasome subunit RPT6. *Plant Physiol.* 168(1):107–19
131. Üstün S, Hafrén A, Liu Q, Marshall RS, Minina EA, et al. 2018. Bacteria exploit autophagy for proteasome degradation and enhanced virulence in plants. *Plant Cell* 30(3):668–85

132. Üstün S, König P, Guttman DS, Börnke F. 2014. HopZ4 from *Pseudomonas syringae*, a member of the HopZ Type III effector family from the YopJ superfamily, inhibits the proteasome in plants. *Mol. Plant-Microbe Interact.* 27(7):611–23
133. Üstün S, Sheikh A, Gimenez-Ibanez S, Jones A, Ntoukakis V, Börnke F. 2016. The proteasome acts as a hub for plant immunity and is targeted by *Pseudomonas* type III effectors. *Plant Physiol.* 172(3):1941–58
134. Vicente J, Mendiondo GM, Pauwels J, Pastor V, Izquierdo Y, et al. 2019. Distinct branches of the N-end rule pathway modulate the plant immune response. *New Phytol.* 221(2):988–1000
135. Vierstra RD. 2009. The ubiquitin-26S proteasome system at the nexus of plant biology. *Nat. Rev. Mol. Cell Biol.* 10(6):385–97
136. Wang J, Grubb LE, Wang J, Liang X, Li L, et al. 2018. A regulatory module controlling homeostasis of a plant immune kinase. *Mol. Cell* 69(3):493–504.e6
137. Wang J, Wang R, Fang H, Zhang C, Zhang F, et al. 2021. Two VOZ transcription factors link an E3 ligase and an NLR immune receptor to modulate immunity in rice. *Mol. Plant* 14(2):253–66
138. Wang S, Li Q, Zhao L, Fu S, Qin L, et al. 2020. *Arabidopsis* UBC22, an E2 able to catalyze lysine-11 specific ubiquitin linkage formation, has multiple functions in plant growth and immunity. *Plant Sci.* 297:110520
139. Wang T, Chang C, Gu C, Tang S, Xie Q, Shen Q-H. 2016. An E3 ligase affects the NLR receptor stability and immunity to powdery mildew. *Plant Physiol.* 172(4):2504–15
140. Wang Z, Orosa-Puente B, Nomoto M, Grey H, Potuschak T, et al. 2022. Proteasome-associated ubiquitin ligase relays target plant hormone-specific transcriptional activators. *Sci. Adv.* 8(42):4466
141. Wu Z, Tong M, Tian L, Zhu C, Liu X, et al. 2020. Plant E3 ligases SNIPER 1 and SNIPER 2 broadly regulate the homeostasis of sensor NLR immune receptors. *EMBO J.* 39(15):e104915
142. Xu CC, Zhang D, Hann DR, Xie ZP, Staehelin C. 2018. Biochemical properties and in planta effects of NopM, a rhizobial E3 ubiquitin ligase. *J. Biol. Chem.* 293(39):15304–15
143. Xu F, Huang Y, Li L, Gannon P, Linster E, et al. 2015. Two N-terminal acetyltransferases antagonistically regulate the stability of a nod-like receptor in *Arabidopsis*. *Plant Cell* 27(5):1547–62
144. Xu FQ, Xue HW. 2019. The ubiquitin-proteasome system in plant responses to environments. *Plant Cell Environ.* 42(10):2931–44
145. Yamaguchi K, Mezaki H, Fujiwara M, Hara Y, Kawasaki T. 2017. *Arabidopsis* ubiquitin ligase PUB12 interacts with and negatively regulates chitin elicitor receptor kinase 1 (CERK1). *PLOS ONE* 12(11):e0188886
146. Yan J, Li H, Li S, Yao R, Deng H, et al. 2013. The *Arabidopsis* F-box protein CORONATINE INSENSITIVE1 is stabilized by SCFCOI1 and degraded via the 26S proteasome pathway. *Plant Cell* 25(2):486–98
147. Yan J, Yao R, Chen L, Li S, Gu M, et al. 2018. Dynamic perception of jasmonates by the F-box protein COI1. *Mol. Plant* 11(10):1237–47
148. Yan X, Tao J, Luo HL, Tan LT, Rong W, et al. 2019. A type III effector XopLXcc8004 is vital for *Xanthomonas campestris* pathovar *campestris* to regulate plant immunity. *Res. Microbiol.* 170(3):138–46
149. Yang CW, González-Lamothe R, Ewan RA, Rowland O, Yoshioka H, et al. 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(4):1084–98
150. Yang L, Teixeira PJPL, Biswas S, Finkel OM, He Y, et al. 2017. *Pseudomonas syringae* type III effector HopBB1 promotes host transcriptional repressor degradation to regulate phytohormone responses and virulence. *Cell Host Microbe* 21(2):156–68
151. Yao C, Wu Y, Nie H, Tang D. 2012. RPN1a, a 26S proteasome subunit, is required for innate immunity in *Arabidopsis*. *Plant J.* 71(6):1015–28
152. You Q, Zhai K, Yang D, Yang W, Wu J, et al. 2016. An E3 ubiquitin ligase-BAG protein module controls plant innate immunity and broad-spectrum disease resistance. *Cell Host Microbe* 20(6):758–69
153. Yu G, Derkacheva M, Rufian JS, Brillada C, Kowarschik K, et al. 2022. The *Arabidopsis* E3 ubiquitin ligase PUB4 regulates BIK1 and is targeted by a bacterial type-III effector. *EMBO J.* 41(23):e107257
154. Yu Y, Xu W, Wang J, Wang L, Yao W, et al. 2013. The Chinese wild grapevine (*Vitis pseudoreticulata*) E3 ubiquitin ligase *Erysiphe necator*-induced RING finger protein 1 (EIRP1) activates plant defense responses by inducing proteolysis of the VpWRKY11 transcription factor. *New Phytol.* 200(3):834–46

155. Zhang C, Wei Y, Xu L, Wu KC, Yang L, et al. 2020. A bunyavirus-inducible ubiquitin ligase targets RNA polymerase IV for degradation during viral pathogenesis in rice. *Mol. Plant* 13(6):836–50
156. Zhang Y, Song G, Lal NK, Nagalakshmi U, Li Y, et al. 2019. TurboID-based proximity labeling reveals that UBR7 is a regulator of N NLR immune receptor-mediated immunity. *Nat Commun*. 10(1):3252
157. Zhao C, Rispe C, Nabity PD. 2019. Secretory RING finger proteins function as effectors in a grapevine galling insect. *BMC Genom*. 20(1):923
158. Zhou B, Mural RV, Chen X, Oates ME, Connor RA, et al. 2017. A subset of ubiquitin-conjugating enzymes is essential for plant immunity. *Plant Physiol*. 173(2):1371–90
159. Zhou B, Wang C, Chen X, Zhang Y, Zeng L. 2021. Two ubiquitin-activating systems occur in plants with one playing a major role in plant immunity. bioRxiv 458739. **https://doi.org/10.1101/2021.09.02.458739**
160. Ziebell H, Murphy AM, Groen SC, Tungadi T, Westwood JH, et al. 2011. Cucumber mosaic virus and its 2b RNA silencing suppressor modify plant-aphid interactions in tobacco. *Sci. Rep.* 1:187
161. Zipfel C. 2014. Plant pattern-recognition receptors. *Trends Immunol*. 35(7):345–51