YopJ Family Effectors Promote Bacterial Infection through a Unique Acetyltransferase Activity

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SUMMARY

Gram-negative bacterial pathogens rely on the type III secretion system to inject virulence proteins into host cells. These type III secreted “effector” proteins directly manipulate cellular processes to cause disease. Although the effector repertoires in different bacterial species are highly variable, the *Yersinia* outer protein J (YopJ) effector family is unique in that its members are produced by diverse animal and plant pathogens as well as a nonpathogenic microsymbiont. All YopJ family effectors share a conserved catalytic triad that is identical to that of the C55 family of cysteine proteases. However, an accumulating body of evidence demonstrates that many YopJ effectors modify their target proteins in hosts by acetylating specific serine, threonine, and/or lysine residues. This unique acetyltransferase activity allows the YopJ family effectors to affect the function and/or stability of their targets, thereby dampening innate immunity. Here, we summarize the current understanding of this prevalent and evolutionarily conserved type III effector family by describing their enzymatic activities and virulence functions in animals and plants. In particular, the molecular mechanisms by which representative YopJ family effectors subvert host immunity through posttranslational modification of their target proteins are discussed.

INTRODUCTION

Microbial pathogens and their hosts are engaged in an endless arms race. The central concept of this battle is the activation of immune responses upon pathogen perception and the subver-

sion of host immunity through the activity of pathogen virulence factors. During bacterial infection, binding of pattern recognition receptors (PRRs) to conserved microbial ligands, called microbe-associated molecular patterns (MAMPs), activates MAMP-triggered immunity (MTI). Well-studied MAMPs include flagellin from flagellated bacteria, lipopolysaccharides (LPS) from the outer membrane of Gram-negative bacteria, and lipoteichoic acid (LTA) from the cell wall of Gram-positive bacteria (1, 2). In animals, recognition of MAMPs in immune cells initiates innate immune responses by activating the mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF- κ B) signaling pathways, which subsequently lead to the production of proinflammatory cytokines (1). The activated inflammatory response results in localized vasodilation, increased blood flow, and recruitment of phagocytes to infection sites for phagocytosis, which is essential to fend off bacterial infection (1). In plants, MAMP recognition also activates the MAPK signaling pathway, leading to the transcriptional reprogramming of defense-related genes, the

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secretion of antimicrobial compounds, and cell wall reinforcement (2).

MTI prevents colonization by the majority of potential pathogens in the surrounding environment. However, successful pathogens produce virulence factors that can effectively defeat MTI. In particular, a multitude of pathogen proteins are delivered into host cells and function as immune suppressors. Collectively, these so-called “effectors” are indispensable for infection because of their ability to directly manipulate specific targets in hosts. Given their essential roles in pathogenesis, substantial efforts have been made to investigate the molecular mechanisms by which effectors promote infection. This research provides key information on the host-pathogen arms race and facilitates the development of disease management strategies.

The best-studied effectors are those injected by Gram-negative bacteria through the type III secretion system (T3SS) into animal or plant cells (3–6). Translocation of pathogen virulence proteins into eukaryotic host cells was first observed during infection of HeLa cells by *Yersinia pseudotuberculosis* (7). Later on, the T3SS from the plant pathogen *Pseudomonas syringae* was revealed to be a needle-like apparatus that allowed the type III secreted effectors (T3SEs) to pass through (8). T3SSs have been identified in most Gram-negative bacteria with a pathogenic lifestyle as well as a few symbiotic bacteria that associate with plants and insects (9, 10). Genes encoding structural proteins of the T3SS machinery are highly conserved among plant and animal pathogens (3, 4). Mutations disrupting T3SS formation often abolish the pathogenicity of bacteria (11, 12), indicating that the T3SS is an evolutionarily conserved virulence determinant. In contrast to the high level of conservation observed in the T3SS structural genes, effector repertoires produced by different bacterial species are variable, likely due to rapid evolution driven by specific host-pathogen interactions (13). Numbers of T3SEs range from as few as 4 in the opportunistic plant and animal pathogen *Pseudomonas aeruginosa* (14) to a remarkable 72 in the plant pathogen *Ralstonia solanacearum* strain GMI1000 (15). Many effectors are species specific (16, 17), and the variability of T3SE repertoires is also evident at the sub-species level (18–21).

Among all the T3SEs identified so far, only three families have members produced by both animal and plant pathogens. The SopE/WxxxE family effectors mimic guanine nucleotide exchange factors (GEFs) and activate G-protein signaling to facilitate pathogen attachment and internalization (22, 23), and the IpaH family effectors function as E3 ubiquitin ligases to promote the proteasome-dependent degradation of host target proteins (24). One of the most widely distributed T3SE families is the *Yersinia* outer protein J (YopJ) family, which has been found in the animal pathogens *Yersinia* spp., *Salmonella enterica*, *Vibrio parahaemolyticus*, and *Aeromonas salmonicida* as well as the plant pathogens *P. syringae*, *Xanthomonas campestris*, *R. solanacearum*, *Erwinia amylovora*, and *Acidovorax citrulli* (25, 26). The YopJ effectors share a conserved cysteine protease-like catalytic triad (27); however, many of them modify their host targets through acetylation (Table 1). Over the years, several members of the YopJ family of effectors have been characterized in depth due to their important virulence functions, unique enzymatic activities, and evolutionary conservation. Here, we review our current knowledge on the enzymatic mechanism and regulation of YopJ effectors, their host targets, and the molecular basis of their virulence functions in

animals and plants. Furthermore, we discuss mechanisms underlying host recognition of YopJ family effectors in plants.

YopJ IS AN EVOLUTIONARILY CONSERVED TYPE III EFFECTOR FAMILY

Phylogenetic analysis clusters known YopJ family effectors into five major groups, with effectors produced by animal pathogens being clearly separated from those produced by plant pathogens (Fig. 1). Group I consists of all the YopJ effectors produced by animal pathogens, including YopJ and YopP from *Yersinia* spp., AvrA from *S. enterica*, AopP from *A. salmonicida*, and VopA from *V. parahaemolyticus*. Group II includes AvrXv4, AvrRxv, AvrBsT, and XopJ from *X. campestris*, PopP1 from *R. solanacearum*, Aave2166 and Aave2708 from *Acidovorax citrulli*, and HopZ2 and HopZ4 from *P. syringae* (28, 29). In addition, NopJ from the micro symbiont *Sinorhizobium fredii* is closely related to XopJ and HopZ4 in this clade. Group III includes HopZ1 alleles and HopZ3 from *P. syringae* as well as ORFB from *E. amylovora* (30). Group IV and V contain members that are found exclusively in *R. solanacearum*, including PopP3 (31), forming group IV, and PopP2, RipAE, and RipJ (32), forming group V.

The full-length protein sequences of YopJ family members are only moderately conserved, with overall identities ranging from 20% (e.g., HopZ1a versus AopP) to 94% (e.g., YopJ versus YopP). In general, YopJ effectors can be divided into three regions: the variable N terminus carrying the secretion and translocation signal required for T3SS-dependent delivery, the central region involved in catalysis, and the C terminus conferring a regulatory function. The central region of YopJ effectors contains a catalytic triad consisting mostly of His/Glu/Cys, with a few exceptions. For example, PopP2 consists of an Asp rather than a Glu residue. This catalytic triad is identical to that of the C55 family of cysteine proteases (27). Furthermore, the predicted secondary structure of the central region of YopJ, which contains the catalytic triad, is reminiscent of those of adenoviral protease (AVP) and ubiquitin-like protease 1 (Ulp1) (27). This structural similarity led to the assumption that the YopJ effectors may function as proteases. Indeed, previous studies reported deubiquitination activities of YopJ and AvrA *in vitro* (27, 33, 34). In addition, weak protease activities (<10% compared to trypsin or proteinase K) were also observed for the *P. syringae* effectors HopZ1a, HopZ2, and HopZ3 (26) as well as the *X. campestris* pv. vesicatoria effector AvrBsT by using generic substrates (35). Furthermore, another *X. campestris* pv. vesicatoria effector, XopJ, causes the degradation of its target protein in the plant host, presumably through a protease activity (36). However, the view that YopJ effectors modify their substrates primarily through proteolytic degradation has changed after the host targets of several YopJ effectors were identified and the consequences of effector-target interactions were characterized. In particular, many YopJ family effectors, including YopJ, AvrA, HopZ1a, HopZ3, and AvrBsT, that were previously shown to possess protease activities modify their host substrates through acetylation. Importantly, the acetylation activities also require the catalytic cysteine residue, suggesting that YopJ effectors may adopt a protease-like catalytic core for a different enzymatic reaction.

YopJ EFFECTORS POSSESS A UNIQUE ACETYLTRANSFERASE ACTIVITY

To date, seven YopJ effectors (YopJ, AvrA, and VopA from the animal pathogens and HopZ1a, HopZ3, PopP2, and AvrBsT from

TABLE 1 YopJ effectors and their virulence targets

YopJ effector	Species	Enzymatic activity(ies)	Target(s) and/or phenotype(s)	Key reference(s)
Produced by animal pathogens				
YopJ/YopP	<i>Y. pestis</i> , <i>Y. pseudotuberculosis</i> , <i>Y. enterocolitica</i>	Acetyltransferase, protease	MKK2, MKK6, IKK β , TAK1; inhibition of MAPK-ERK, -JNK, and -p38 and NF- κ B signaling; reduced cytokine production and inflammatory response; enhanced apoptosis in macrophages	27, 44, 82, 83
AvrA	Several <i>S. enterica</i> serotypes	Acetyltransferase, protease	MKK4, MKK7, p53; inhibition of MAPK-JNK and drosophila defense against Gram-negative bacteria, inhibition of apoptosis, enhanced cell proliferation	85, 92, 93
VopA	<i>V. parahaemolyticus</i>	Acetyltransferase	MKK1, MKK6; inhibition of MAPK-ERK, -JNK, and -p38 signaling	40, 95
AopP	<i>A. salmonicida</i>	Unknown	Inhibition of NF- κ B signaling and drosophila defense against Gram-negative and Gram-positive bacteria, proapoptotic	97, 98
Produced by plant pathogens				
HopZ1a	<i>P. syringae</i> pv. <i>syringae</i> A2	Acetyltransferase, protease	GmHID1, JAZs, tubulin, ZED1; suppression of isoflavone biosynthetic pathway in soybean, activation of JA signaling, inhibition of cell secretion and cell wall-associated defense in <i>Arabidopsis</i> ; activation of defense requires ZED1, which is guarded by ZAR1	41, 43, 119, 123, 124
HopZ3	<i>P. syringae</i> pv. <i>syringae</i> B728a	Acetyltransferase, protease	RIPK, RIN4, AvrB; inhibition of RPM1-mediated defense	47
HopZ4	<i>P. syringae</i> pv. <i>lachrymans</i>	Unknown	RPT6; inhibition of proteasomal activity	28
XopJ	<i>X. campestris</i> pv. <i>vesicatoria</i>	Protease	RPT6; inhibition of proteasomal activity, downregulation of SA-dependent defense	36, 143
AvrBsT	<i>X. campestris</i> pv. <i>vesicatoria</i>	Acetyltransferase, protease	ACIP1, SnRK1; alternation of ACIP1 localization pattern on microtubules, suppression of AvrBs1-dependent HR	35, 42
PopP2	<i>R. solanacearum</i>	Acetyltransferase	WRKY family transcription factors, RRS1-R; inhibition of basal defense, activation of defense upon acetylation on the WRKY domain in RRS1-R	54, 55

the plant pathogens) have been shown to acetylate their corresponding host targets (Table 1). Interestingly, there are no sequence similarities between the YopJ family of acetyltransferases and other known acetyltransferases such as the histone acetyltransferases (HATs) (37) and the N-terminal acetyltransferases (NATs) (38, 39), suggesting that YopJ acetyltransferases evolved independently and utilize a distinctive enzymatic mechanism. Consistently, YopJ family acetyltransferases exhibit unique properties that are not observed for HATs or NATs. For example, YopJ effectors modify multiple substrates on specific Ser/Thr/Lys residues; this is different from HATs, which modify only specific Lys residues on histones. NATs can also acetylate Ser and Thr residues. However, NAT-mediated acetylation occurs strictly on the first residue in the N terminus of a peptide (38, 39). Therefore, YopJ family effectors are the only acetyltransferases that predominantly modify selective Ser and Thr residues in the substrates independent of their position in the polypeptide.

A common assay used to study the acetyltransferase activities of YopJ effectors employs *in vitro* reactions using radiolabeled acetyl coenzyme A (CoA) (AcCoA) as the acetyl group donor. This assay revealed both the “autoacetylation” of the effectors and the “trans-acetylation” of their corresponding substrates (40–47),

leading to the proposal of a two-step catalytic mechanism known as the “ping-pong” model (44) (Fig. 2). In this model, the catalytic reaction starts with the deprotonation of the catalytic cysteine on the thiol group with the neighboring residue, i.e., histidine, in the catalytic triad as the proton group acceptor. The deprotonated cysteine then serves as a nucleophile to attack the carbonyl group of AcCoA, resulting in the formation of an acetyl enzyme intermediate and the release of CoA as a by-product. Finally, the acetyl enzyme intermediate attacks the substrate (i.e., target protein[s] in the host) and transfers the acetyl group to specific Ser/Thr/Lys residues.

Currently, direct evidence supporting the ping-pong model is not available. Even though autoacetylation is observed for all the YopJ family effectors with acetyltransferase activity, it does not necessitate the presence of acetyl enzyme intermediates with the acetyl group attached to the catalytic cysteine. Indeed, acetylation of the catalytic cysteine has not been reported, and residues distant from the catalytic cysteine were proposed to be potential autoacetylation sites. For example, mass spectrometry analyses revealed the acetylation of Lys³⁸³ in PopP2 (48) and Thr³⁴⁶ in HopZ1a (46). However, mutations of Thr³⁴⁶ in HopZ1a did not abolish autoacetylation, and this residue is not conserved among

FIG 1 Phylogeny of YopJ family effectors. The phylogenetic tree was generated with MEGA7 (155), using the neighbor-joining method and the full-length protein sequences of 24 YopJ family effectors. YopJ effectors produced by animal pathogens are all clustered in group I. On the contrary, YopJ family effectors produced by plant pathogens are diversified. AvrXv4, AvrRxv, AvrBsT, and XopJ are found in *Xanthomonas campestris*; all these effectors are clustered in group II. Aave2166 and Aave2708 are found in *Acidovorax citrulli*, and they are found in group II. HopZ1, HopZ2, HopZ3, and HopZ4 are found in *Pseudomonas syringae*; these effectors are distributed in groups II and III. ORFB is produced by *Erwinia amylovora* and is found in group III. *Ralstonia solanacearum* produces PopP1, PopP2, PopP3, RipAE, and RipJ. Apart from PopP1, which is closely related to AvrXv4 in group I, other *R. solanacearum* effectors are found in group IV and group V. NopJ is the only YopJ effector produced by the nonpathogenic symbiotic bacterium *Sinorhizobium fredii* strain NGR234. The GenBank accession numbers of effectors used for this analysis are as follows: [AKN09807](#) for YopJ, [AAN37537](#) for YopP, [AAL21745](#) for AvrA, [AAT08443](#) for VopA, [NP_710166](#) for AopP, [AAG39033](#) for AvrXv4, [AAA27595](#) for AvrRxv, [AAD39255](#) for AvrBsT, [ABM32744](#) for Aave2166, [ABM33278](#) for Aave2708, [WP_011347382](#) for XopJ, [CAF32331](#) for PopP1, [CAD14570](#) for PopP2, [CAF32358](#) for PopP3, [CAD15839](#) for RipJ (also known as Rsc2132), [CAD13849](#) for RipAE (also known as Rsc0321), [AAR02168](#) for HopZ1a (previously known as HopPsyH), [WP_004661226](#) for HopZ1b, [AAL84243](#) for HopZ1c (previously known as HopPmaD), [CAC16700](#) for HopZ2 (previously known as AvrPpiG1), [AAF71492](#) for HopZ3 (previously known as HopPsyV), [EKG29639](#) for HopZ4, [AAF63400](#) for ORFB, and [NP_443964](#) for NopJ (also known as Y4IO).

YopJ effectors (46). Even though Lys³⁸³ in PopP2 is conserved, mutation of this residue in other YopJ effectors, e.g., Lys²⁸² in AvrBsT, affects only *trans*-acetylation and not autoacetylation (42), suggesting that these sites do not participate in catalysis but may be involved in other regulatory functions.

Furthermore, the biological significance of autoacetylation has not yet been clearly demonstrated. If the ping-pong model is correct, autoacetylation should be required for *trans*-acetylation. Even though autoacetylation and *trans*-acetylation are completely abolished in a catalytic mutant, whether these two activities are coupled, as suggested by the ping-pong model, remains unclear. Other acetyltransferase mechanisms different from the ping-pong model cannot be excluded without further evidence. Autoacetylation may play an indirect role in facilitating acetyl group transfer. For example, the *Saccharomyces cerevisiae* HAT Rtt109 is autoacetylated in the regulatory domain, and this autoacetylation is required for acetyltransferase activity by enhancing the binding

affinity of Rtt109 for AcCoA (49). Whether a similar mechanism is also used by YopJ family effectors remains to be tested.

ACTIVATION OF YOPJ EFFECTORS BY THE EUKARYOTIC COFACTOR IP₆

Another significant difference between YopJ family effectors and other acetyltransferases is the requirement for a eukaryote-specific cofactor (45). Recombinant YopJ purified from *Escherichia coli* exhibited only weak acetylation activity, which is inconsistent with its strong virulence function in experiments using human cell cultures (45). This discrepancy suggests the involvement of a eukaryote-specific cofactor for the activation of YopJ. By using a series of size exclusion chromatography and mass spectrometry analyses, inositol hexakisphosphate (IP₆) was identified as the activator (45) (Fig. 2). In addition to YopJ, IP₆ also activates the acetyltransferase activity of AvrA, HopZ1a, HopZ3, and AvrBsT (41, 42, 45–47); this suggests that IP₆-mediated activation is a

FIG 2 Activation and catalysis of YopJ acetyltransferases. YopJ family effectors are presumably in an inactive form in bacterial cells. After being delivered into the eukaryotic host cell by the type III secretion system, IP₆ activates the acetyltransferase activity of YopJ effectors by inducing a conformational change. An exception is the *Ralstonia solanacearum* effector PopP2, which exhibits acetyltransferase activity in the absence of IP₆. The catalysis of YopJ effectors is described by the proposed two-step ping-pong model: the effectors first undergo an autoacetylation process and generate an acetyl enzyme intermediate, and the acetyl group is then passed to the substrates.

general mechanism that evolved in YopJ effectors. IP₆ is abundant in animals and plants but absent in bacteria (50); as such, it is intriguing to predict that the activities of YopJ effectors are greatly enhanced after they enter host cells.

IP₆ is a saturated cyclic compound with six phosphate groups attached to the inositol. Replacement of IP₆ with the lower inositol polyphosphate IP₃ or IS₆, a synthetic compound that has a cyclic structure and overall charge density similar to those of IP₆ but with sulfate replacing the phosphate, failed to activate the acetyltransferase activity of YopJ (45). These results suggest that IP₆ is a specific eukaryote cofactor that activates YopJ activity. Two possible mechanisms could explain IP₆-mediated YopJ activation. First, IP₆ could constitute a structural component of YopJ; however, this is not likely because recombinant YopJ proteins purified from insect cells still require external IP₆ to acetylate their substrates (45). Second, IP₆ could activate YopJ through conformational changes. IP₆-mediated activation has been shown for other virulence factors produced by bacterial pathogens, such as the Repeats-in-toxin (RTX) toxin of *Vibrio cholerae* (51) and toxin A of *Clostridium difficile* (52). In the case of the RTX toxin, IP₆ induces an overall conformational change of the toxin; as a result,

FIG 3 Structure of HopZ1a in complex with the cofactor IP₆ and the acetyl group donor analog CoA. (A) Arrangement of HopZ1a in two distinct domains: the regulatory domain (pink) and the catalytic domain (cyan). (B) Crystal structure of the HopZ1a/IP₆/CoA complex (PDB accession number 5KLQ). The central catalytic domain contains the catalytic triad (His150, Glu170, and Cys216), which is located next to the CoA-binding pocket. The IP₆-harboring regulatory domain is formed by the flanking sequences in both the N and C termini of HopZ1a. The ligands IP₆ and CoA are shown in a ball model.

the catalytic site becomes exposed for autoprocessing, which is required for the activation of the toxin (51). Similar conformational changes have also been observed for the YopJ family effectors YopJ, AvrA, and HopZ1a (45, 46). However, the underlying activation mechanism was not understood until the crystal structure of HopZ1a was finally resolved recently.

A major breakthrough in understanding the biochemical features of YopJ family effectors is attributed to structural analysis of HopZ1a in complex with IP₆ and the acetyl group donor analog CoA (53) (Fig. 3). HopZ1a adopts a two-domain architecture with a central catalytic domain and a regulatory domain formed by flanking sequences from the N- and C-terminal regions. The catalytic domain resembles the fold of the protease ULP1 except that the SUMO-binding region of ULP1 is missing in HopZ1a. Intriguingly, the catalytic domain alone is not sufficient for HopZ1a to function as an acetyltransferase, which requires the regulatory domain that harbors the IP₆-binding site (53). IP₆ binding induces an overall conformational change in HopZ1a that leads to the formation of a stable AcCoA-binding site residing next to the catalytic triad in the catalytic domain (53). As such, the binding affinity of HopZ1a for AcCoA is notably enhanced in the presence of IP₆. Similar conformational changes were also observed for other YopJ effectors (45, 46). In addition, conserved amino acid residues contributing to IP₆ binding are also required for the activation of the *Salmonella* effector AvrA (53), suggesting conserved IP₆-mediated allosteric regulation in YopJ family effectors. It should be noted that the YopJ family effector PopP2 does not seem to require IP₆ as a cofactor since acetyltransferase activity was

FIG 4 Inhibition of MAPK and NF- κ B signaling pathways by YopJ effectors produced by animal pathogens. The *Yersinia* effector YopJ, the *Salmonella enterica* effector AvrA, and the *Vibrio parahaemolyticus* effector VopA directly target specific immune-related kinases in the MAPK and NF- κ B signaling pathways and modify them through acetylation. Common targets of these effectors include MAPKKs and IKK β , which are acetylated on Ser and Thr residues in the activation loop and/or ATP-binding pocket. The MAPKKK TAK1 is also targeted by YopJ. As a result, these effectors inhibit target kinases and suppress the subsequent activation of inflammatory responses. The *Aeromonas salmonicida* effector AopP suppresses the NF- κ B pathway by an unknown mechanism. By suppressing the NF- κ B pathway, YopJ effectors inhibit the activation of the inflammatory response and induce apoptosis in macrophages.

observed when PopP2 and its substrate were coexpressed in *E. coli* (48, 54, 55). PopP2, together with two other effectors produced by *R. solanacearum*, forms a clade that is separated from other YopJ effectors. Further studies are needed to confirm whether the acetyltransferase activity of PopP2 can be further enhanced by IP₆ or whether a bacterium-produced cofactor is utilized by PopP2 for activation instead.

MANIPULATION OF HOST TARGETS BY YopJ EFFECTORS TO FACILITATE INFECTION

Due to their evolutionary conservation and unique enzymatic activity, YopJ effectors have been extensively investigated for their virulence functions in several important pathogens of animals and plants. The majority of YopJ effectors with known host targets modify their substrates through acetylation. As one of the major posttranslational modifications, acetylation influences protein stability, subcellular localization, and enzymatic activity (56). In addition, acetylation has extensive cross talk with other posttranslational modifications, such as methylation, phosphorylation, and ubiquitination, in order to collectively regulate protein function (57). So far, the host targets of seven YopJ acetyltransferases have been characterized (Table 1). These effectors target diverse substrates in their particular hosts, and the acetylation of these targets leads to consequences including the blockage of phosphorylation (44), the promotion of proteasome-dependent protein degradation (43), changes in the subcellular localization pattern (42), and the disruption of interactions with DNA (54, 55). Overall, YopJ effector-mediated acetylation is an important virulence strategy utilized by bacterial pathogens to suppress innate immunity.

YopJ EFFECTORS PRODUCED BY ANIMAL PATHOGENS ACETYLATE KINASES TO SUPPRESS INFLAMMATION

As the founding member of an important effector family, YopJ is one of the best-studied T3SEs. YopJ targets MAPK and NF- κ B signaling to inactivate two pathways involved in the activation of animal innate immunity (58, 59). The MAPK signaling pathway can be divided into three branches, regulated by extracellular signal-regulated protein kinase (ERK), p38, and the c-Jun N-terminal kinase (JNK) (58). The NF- κ B pathway is normally suppressed by I κ B. The phosphorylation of I κ B by I κ B kinase α (IKK α) and IKK β leads to its degradation and the derepression of NF- κ B signaling (59). Activation of the MAPK and NF- κ B pathways leads to the production of proinflammatory cytokines and the recruitment of phagocytes to clear bacterial infection. By targeting the NF- κ B pathway, YopJ also interferes with the apoptotic process to promote cell death of immune cells.

Following pioneering work on YopJ, other family members produced by animal pathogens, including AvrA, AopP, and VopA, have also been characterized for their virulence activity. Current evidence suggests that these effectors all target the MAPK and/or NF- κ B signaling pathways and function as suppressors of inflammation (Fig. 4). However, their target specificities are different: YopJ inhibits all three branches of the MAPK and the NF- κ B pathways, AvrA inhibits only the MAPK-JNK pathway, VopA inhibits all three MAPK pathways but not the NF- κ B pathway, and AopP specifically targets the NF- κ B pathway. Taken together, YopJ effectors produced by animal pathogens serve as inhibitors of immune-associated kinases through acetylation.

***Yersinia* Effector YopJ Blocks Phosphorylation of Defense-Associated Kinases**

YopJ is produced by three pathogenic *Yersinia* species: *Y. pestis* (60), *Y. pseudotuberculosis*, and *Y. enterocolitica* (the YopJ homolog produced by *Y. enterocolitica* is known as YopP, which shares 94% identity with YopJ in amino acid sequence [61]). These *Yersinia* species are extracellular pathogens of humans and rodents. *Y. pestis* is the most virulent species and notoriously known as the etiological agent of bubonic plague, and *Y. pseudotuberculosis* and *Y. enterocolitica* are foodborne pathogens that cause mesenteric lymphadenitis and gastrointestinal diseases, respectively (62). Despite the differences in their infection routes, all three species colonize lymphatic tissue (62).

Upon *Yersinia* infection, the animal host rapidly activates all three branches of the MAPK pathways with the subsequent recruitment of circulating phagocytes to infection sites to phagocytose bacteria as an output of the inflammatory response (63). However, in a YopJ-dependent manner, the activation of MAPKs can be suppressed by *Yersinia* spp. soon (within 45 min) after infection (64). As a result, the number of neutrophils (the major phagocyte) at the initial infection site increases only slightly as the bacteria continue to proliferate (65, 66). The reduced recruitment of neutrophils is correlated with the suppressed release of proinflammatory cytokines, including tumor necrosis factor alpha (TNF- α) (67, 68) and interleukin-8 (IL-8), from macrophages (69). Similar inhibitory effects are recapitulated by YopP (70). By compromising MAPK-dependent immune signaling, YopJ and YopP facilitate colonization by the *Yersinia* pathogen in host tissues in a low-level inflammatory environment.

Consistent with its ability to inhibit MAPK signaling, YopJ directly interacts with kinases associated with these pathways. *In vitro* assays show specific interactions of YopJ with MAPK kinases (MAPKKs), including MKK1/2/3/4/5/6, but not with the upstream MAPKK kinase (MAPKKK) Raf or the downstream MAPKs ERK, JNK, and p38 (71). These results suggest that MAPKKs are direct virulence targets of YopJ. Mass spectrometry analysis further detected YopJ-dependent acetylation on human MAPKKs, confirming that they are substrates of YopJ. In particular, specific residues, i.e., Ser²⁰⁷, Lys²¹⁰, and Thr²¹¹ in MKK6 (44) and Ser²²² and Thr²²⁶ in MKK2 (72), are acetylated by YopJ. Remarkably, the acetylated Ser and Thr residues are situated in the activation loop. In human MAPKKs, the activation loop contains the conserved SxxS/T motif (where x is any amino acid), where the phosphorylation of Ser/Thr is required for kinase activation. As such, acetylation blocks phosphorylation and inactivates the MAPKKs. In addition to immunity-associated processes, YopJ also inhibits MAPK pathways that regulate mating and osmotic stress responses in budding yeast. These findings provide strong support for MAPK kinases as major YopJ targets in eukaryotes (73).

YopJ also acetylates IKK β and inactivates its kinase activity on I κ B. As a result, I κ B is stabilized, and NF- κ B signaling is repressed (71). The NF- κ B pathway plays dual roles in activating the inflammatory response and suppressing apoptosis. By inhibiting the NF- κ B pathway, YopJ can suppress inflammation and induce programmed cell death in infected macrophages so that the phagocytotic machinery is paralyzed in the host (74–78). Interestingly, YopJ inactivates MAPK signaling but has no effect on IKK β -dependent activity within the first

hour of infection by *Y. pseudotuberculosis* (79). Therefore, YopJ appears to target mainly the MAPK pathways during the initial stage of infection as a major means to suppress inflammation. At a later infection stage, YopJ induces apoptosis of immune cells by suppressing the NF- κ B pathway (79). The acetylation site in IKK β has been determined to be Thr¹⁸⁰. Although not a known phosphorylation site, Thr¹⁸⁰ is in the activation loop, adjacent to the two phospho-accepting residues Ser¹⁷⁷ and Ser¹⁸¹. Therefore, YopJ prevents IKK β phosphorylation, likely through its acetylation on Thr¹⁸⁰ (72).

In addition to MKKs and I κ B, YopJ and YopP can also use RIP-like interacting caspase-like apoptosis-regulatory protein kinase (RICK) and the MAPKKK transforming growth factor β -activated kinase 1 (TAK1) as the substrates for acetylation in mice and humans (80–83). The acetylation sites of RICK and TAK1 are Ser and Thr residues within the activation loops, suggesting that YopJ/YopP directly inhibits the phosphorylation of these kinases (83). Impaired activities of RICK and TAK1 by kinase inhibitors result in enhanced intestinal permeability, which is essential for enteropathogens to invade lymphoid tissue known as Peyer's patches (PPs) (83). It is therefore possible that YopJ-mediated inactivation of RICK and TAK1 facilitates the disruption of the integrity of the intestinal barrier and promotes pathogen invasion in the gut (83). Furthermore, TAK1 is a MAPKKK that activates NF- κ B, MAPK-JNK, and MAPK-p38 pathways; as such, the suppression of TAK1 could also contribute to the inhibitory effect of YopJ on inflammation.

***Salmonella enterica* Effector AvrA Inactivates Kinases in the MAPK-JNK Pathway and Suppresses Apoptosis**

AvrA is produced by several serotypes of *Salmonella enterica*, including *S. enterica* serotype Typhimurium, a common causative agent of food poisoning, and *S. enterica* serotype Enteritidis, which is associated with chicken egg contamination (84). Patients infected with *Salmonella* develop symptoms of diarrhea, vomiting, and nausea; infection can be fatal if the bacteria pass from the intestinal barrier into the bloodstream.

In mice, AvrA causes reduced production of serum keratinocyte-derived chemokine (KC) (the homolog of IL-8 in mice) and inhibits the influx of neutrophils into the intestinal mucosa upon infection, indicating that the inflammatory response is dampened (85). In *Drosophila melanogaster*, AvrA suppresses the MAPK-JNK pathway (85). Consistently, AvrA promotes the mortality of flies infected by Gram-negative bacteria only and does not have an effect on flies infected by Gram-positive bacteria (85). This is in agreement with the observation that resistance against Gram-negative bacteria in flies is dependent on the MAPK-JNK pathway (86).

The ability of AvrA to suppress the MAPK-JNK pathway is supported by its direct interactions with MKK7 and MKK4 (85, 87), which are specifically associated with JNK signaling. In particular, AvrA acetylates human MKK4 at the Lys²⁶⁰ and Thr²⁶¹ residues within the activation loop and blocks phosphorylation by a mechanism similar to that of YopJ (85). Interestingly, when the JNK pathway is activated by the constitutively active forms (S326D and T330D) of *drosophila* MKK7, the pathway is no longer inhibited by AvrA. Thr³³⁰ is the corresponding residue of Thr²⁶¹ in human MKK4; it is therefore likely that Thr³³⁰ can also be acetylated by AvrA. As such, the T330D mutation may render *drosophila* MKK7 immune to acetylation and inhibition by AvrA (85).

AvrA is unable to suppress the NF- κ B pathway or induce apoptosis (85). On the contrary, AvrA has been shown to suppress induced apoptosis in mice during infection (88). Unlike *Yersinia* spp., *Salmonella* is an intracellular pathogen (89, 90), and suppression of apoptosis in infected cells is favorable to *Salmonella* survival. AvrA is continuously secreted, up to 8 days postinfection (91), and contributes to the transcriptional reprogramming of a large number of genes related to cell proliferation in the host (92). In addition to MKK4/MKK7, AvrA could also use p53 as a substrate and promote its activity as a transcription factor (92). p53 is a central regulator of the cell cycle. Consistent with its ability to inhibit apoptosis, AvrA triggers cell cycle arrest at the resting stage during *Salmonella* infection. However, since the acetylation site(s) on p53 has not been determined, the underlying mechanism by which AvrA activates p53 through acetylation remains elusive.

Recent studies suggest a link between AvrA-dependent enhancement of cell proliferation and apoptosis suppression with colon tumorigenesis as a consequence of *Salmonella* infection (93). This observation echoes the ability of AvrA to inhibit the ubiquitination-dependent degradation of β -catenin, which leads to the activation of *c-myc*, a gene related to cellular proliferation (34, 93, 94). Together, AvrA suppresses apoptosis and enhances cellular proliferation at infected tissue, which may benefit intracellular colonization by *Salmonella*.

***Vibrio parahaemolyticus* Effector VopA Inhibits Phosphorylation and ATP Binding of MAPK Kinases**

VopA is secreted by *V. parahaemolyticus*, a major causative agent of gastroenteritis that is associated with contaminated seafood. VopA acetylates MKK6 but not the downstream MAPK p38, suggesting that, similarly to YopJ and AvrA, VopA also targets the MAPK pathway at the MAPKK level (40). An intriguing observation was that VopA inhibited MAPK-ERK signaling even in the presence of a phosphomimetic form of MKK1, which is constitutively active (40). This finding indicates that VopA blocks the signal transduction of MKKs through a different mechanism.

VopA acetylates four sites in MKK6. Three acetylated residues, Ser²⁰⁷, Lys²¹⁰, and Thr²¹¹, are located in the activation loop, suggesting that VopA blocks the activation of MKK6 by using a mechanism that is similar to those of YopJ and AvrA. However, the fourth acetylation site, Lys¹⁷², is located in the catalytic loop, which is involved in the binding of ATP with γ -phosphate as the phosphate group donor for phosphorylation (40). Acetylation by VopA inhibits the binding of ATP to the phosphomimetic mutant of MKK1 (40), presumably locking it in a state that cannot phosphorylate downstream MAPKs. Consistent with the fact that VopA acetylates additional residues to simultaneously affect the function of MKKs through two different mechanisms, VopA is a more potent kinase inhibitor than YopJ (95).

***Aeromonas salmonicida* Effector AopP Suppresses the NF- κ B Signaling Pathway by Unknown Mechanisms**

AopP is produced by several isolates of the fish pathogen *A. salmonicida* (96). AopP cannot suppress the MAPK-JNK (97) and MAPK-ERK (98) pathways. It instead suppresses the translocation of the transcription factor p65 from the cytoplasm into the nucleus, which is required for the activation of NF- κ B signaling (98). The inhibitory effect of AopP on the NF- κ B pathway is consistent with a strong proapoptotic effect of AopP on mammalian cells (97). Interestingly, transgenic drosophila flies expressing

AopP are more susceptible to infection by either Gram-negative or Gram-positive bacteria. This is different from AvrA, which affects infection by only Gram-negative bacteria. This finding indicates that the inhibitory effect of AopP goes beyond MAPK-JNK signaling (97). Although AopP has not been examined for acetyltransferase activity, its high sequence similarity to YopJ, AvrA, and VopA and the requirement for the conserved catalytic triad for virulence function make it likely that AopP may also act as a kinase inhibitor to block immune signaling pathways. The identification of direct host targets will determine the virulence mechanism of AopP during *A. salmonicida* infection.

YopJ EFFECTORS PRODUCED BY PLANT PATHOGENS HAVE VARIOUS HOST TARGETS TO PROMOTE PATHOGENESIS OR ELICIT RESISTANCE

Plants lack the mobile immune cells utilized by animals; therefore, they depend on a variety of mechanisms to defend themselves from bacterial infection. Such mechanisms include limiting bacterial entry into the apoplastic space, reinforcing the cell wall by callose deposition, and secreting antimicrobial compounds to defend themselves from bacterial infection (2). So far, 18 YopJ family effectors have been identified in plant pathogens (Fig. 1). They target diverse host proteins and promote infection through various manipulative mechanisms (Fig. 5 and Table 1).

In addition to effector-triggered susceptibility, effector-target interactions in plants are shaped by the complex pathogen-host arms race. In particular, resistance (R) proteins have evolved to recognize the activity of effectors directly or indirectly and activate resistance. Canonical R proteins contain the conserved nucleotide-binding-leucine-rich-repeat (NB-LRR) domain, and their activation leads to effector-triggered immunity (ETI) (99, 100). ETI is often associated with a rapid and localized cell death response called the hypersensitive response (HR), which masks the collective virulence function of the effector repertoires and efficiently limits the proliferation of bacterial pathogens. As the arms race goes on, pathogens change their effector repertoires through sequence diversification and the acquisition of new effectors to suppress and/or evade ETI. Overall, the output of such a coevolutionary arms race is summarized as the zigzag model (101), which explains the expansion of effector repertoires in plant pathogens. In this section, we summarize the virulence mechanisms of YopJ family effectors produced by plant pathogens and discuss how some of these effectors are recognized by their corresponding plant hosts in an acetyltransferase activity-dependent manner.

***Pseudomonas syringae* Effector HopZ1a Acetylates the Hormone Receptor Complex and Modifies Microtubules To Promote Virulence**

HopZ1 alleles (HopZ1a, HopZ1b, and HopZ1c) are produced by isolates of the phytopathogen *P. syringae*, which causes bacterial speck and spot diseases. Phylogenetic and functional analyses suggest that HopZ1 is an ancient effector that has been coevolving with bacterial species, and HopZ1a resembles the ancient allelic form (26, 102, 103). Over the years, the virulence function of HopZ1a has been characterized by using *Arabidopsis thaliana* as a model plant. In *Arabidopsis*, stomata are natural openings for gaseous exchange, and the closure of stomata serves as a preinvasive MTI response to limit bacterial entry into plant tissue (104). HopZ1a inhibits MAMP-triggered stomatal closure and promotes *P. syringae* infection at an early infection stage (46). It also suppresses

FIG 5 YopJ effectors produced by plant pathogens manipulate diverse cellular processes through acetylation. YopJ effectors from plant pathogens target a diverse group of substrates in *Arabidopsis* to promote bacterial infection. The *Pseudomonas syringae* effector HopZ3 suppresses effector-triggered immunity by acetylating multiple components in the RPM1 immune complex, including RIPK. The *Xanthomonas campestris* effector AvrBsT and the *Pseudomonas syringae* effector HopZ1a interfere with the plant microtubule network by acetylating ACIP1 and tubulin, respectively. HopZ1a also acetylates JAZs and induces their proteasome-dependent degradation; the consequent activation of the jasmonate signaling pathway suppresses salicylic acid-dependent plant defenses. The *Ralstonia solanacearum* effector PopP2 acetylates the WRKY transcription factors and impairs their DNA-binding activity; as a result, activation of defense genes is suppressed.

other MTI responses, including the generation of reactive oxygen species (ROS); the activation of MAPK signaling (105); callose deposition, a hallmark of cell wall-associated defense (46); and systemic acquired resistance (SAR) (106), a broad-spectrum and long-lasting induced resistance in systemic tissue (107).

Substantial effort has been invested in identifying the host target(s) of HopZ1a and understanding the molecular mechanisms underlying HopZ1a-mediated virulence. Using yeast two-hybrid

screening, HopZ1a was found to associate with jasmonate (JA) ZIM domain (JAZ) proteins. JAZ proteins are components of the JA receptor complex and key repressors of the JA signaling pathway (108). In plants, JA and salicylic acid (SA) are two major defense hormones (109). SA is responsible for activating defense responses against pathogens that feed on live tissues, i.e., those with a biotrophic lifestyle, whereas JA, together with another hormone, ethylene (ET), plays a major role in defeating pathogens that feed on dead tissues, i.e., those with a necrotrophic lifestyle (109). The SA and JA/ET pathways exhibit antagonism during the plant response to pathogens with a specific lifestyle (110–112). HopZ1a acetylates JAZ and promotes its degradation during bacterial infection (43). As a result, JA signaling is activated, which in turn suppresses SA-mediated defenses and facilitates infection by biotrophic pathogens such as *P. syringae* (43, 106).

JAZ proteins are repetitively identified as targets of pathogen effectors and toxins for degradation (113, 114). Apart from antagonizing the SA pathway, another mechanism for activating JA signaling to promote infection is by facilitating bacterial entry into plant tissues. JA signaling is implicated in regulating stomatal closure triggered by the bacterial MAMP flagellin (115), and HopZ1a suppresses stomatal closure (46), likely through inducing JAZ degradation, so that *P. syringae* can colonize the intercellular space. Furthermore, the virulence activity of HopZ1a is partially redundant with that of the phytotoxin coronatine (43), a potent activator of JA signaling, through structural mimicry. These findings indicate that JAZs are major virulence targets of HopZ1a.

The acetylation site(s) of JAZ has not yet been identified. Nonetheless, interesting hypotheses can be inferred based on the requirement for key residues and motifs for JAZ function. JAZs are regulated through proteasome-dependent degradation, which requires an E3 ligase called COI1. The HopZ1a-JAZ interaction depends on a conserved sequence of the JAZ proteins known as the Jas domain (43), which is also required for JAZ-COI1 interactions, JAZ degradation (116), and JAZ-mediated repression of JA-responsive transcription factors (117). It will be interesting to determine if HopZ1a acetylates any residue(s) in the Jas domain to affect JAZ-COI1 interactions and induce JAZ degradation.

Another substrate of HopZ1a in *Arabidopsis* is tubulin (41). Tubulin is the basic unit that forms the microtubule network, which is essential for the plant secretory pathway. Ectopic expression of HopZ1a in animal cells led to altered cell morphology; infection of *Arabidopsis* by *P. syringae* expressing HopZ1a also resulted in the disruption of the microtubule network. Furthermore, HopZ1a can impair plant secretion in the host and suppress cell wall reinforcement (41), suggesting that it may interfere with the secretion of defense-associated molecules (41). Acetylation of tubulin on a specific lysine residue in animal cells affects microtubule polymerization (118). Since the site(s) of acetylation by HopZ1a on tubulin has not been determined, whether HopZ1a disrupts the microtubule network by acetylating a similar residue(s) of plant tubulin requires further investigation.

In addition to *Arabidopsis*, a target of HopZ1a has also been identified in soybean (*Glycine max*). HopZ1a and another HopZ1 allele, HopZ1b, were found to induce the degradation of hydroxyisoflavanone dehydratase (GmHID1) (119, 120). GmHID1 is associated with the production of daidzein, a major soybean isoflavone (121) and the precursor of antimicrobial phytochemicals (122). Silencing of *GmHID1* enhances soybean susceptibility to *P. syringae*, supporting a virulence contribution of HopZ1a by me-

FIG 6 Activation of HopZ1a- and PopP2-mediated immunity in *Arabidopsis* carrying the cognate R proteins ZAR1 and RRS1-R, respectively. HopZ1a and PopP2 elicit effector-triggered immunity in specific *Arabidopsis* ecotypes carrying the NB-LRR proteins ZAR1 and RRS1-R, respectively. In HopZ1a-triggered immunity, HopZ1a acetylates a pseudokinase, ZED1, which is required for the activation of the CC-NB-LRR protein ZAR1. PopP2 acetylates a chimeric TIR-NB-LRR protein, RRS1-R, which contains a WRKY DNA-binding domain in the C terminus. Acetylation on a conserved lysine residue in the WRKY domain activates RRS1-R.

diating GmHID1 degradation. Since the production of isoflavones is limited to plants in the Fabaceae family, GmHID1 is a specific virulence target of HopZ1a in soybean. However, acetylation of GmHID1 by HopZ1a or HopZ1b has not been reported.

The R Protein ZAR1 Requires the Pseudokinase ZED1 To Elicit HopZ1a-Mediated Resistance

Because HopZ1a resembles the ancient allelic form in *P. syringae*, it is not surprising that many plants have evolved resistance mechanisms and activate ETI in a HopZ1a-dependent manner (26, 106). Through genetic screening of *Arabidopsis* transfer DNA (T-DNA) insertion mutants, *HopZ-activated Resistance 1* (ZAR1), encoding a coiled-coil (CC)-NB-LRR protein, and a pseudokinase-encoding gene, *HopZ-ETI-deficient 1* (ZED1), were identified as genes responsible for the HopZ1a-triggered HR (123, 124).

The current understanding of R-protein-mediated defense against bacterial pathogens is best described by the “guard-and-decoy” model (125), where R proteins (the “guard”) are activated when they recognize the activity of the corresponding effector, i.e., a specific modification on effector targets (the “guardee”). If a guardee has evolved to detect solely effector-mediated modifications without having any function in the absence of the effector, it is called a “decoy” (125). In HopZ1a-triggered immunity, the activation of ZAR1 requires ZED1, which can be acetylated by HopZ1a (124) (Fig. 6). Interestingly, ZED1 is a pseudokinase that shares significant sequence similarity with a class of receptor-like cytoplasmic kinases but has mutations in the aspartate residue of the “HRD” motif (124). The HRD motif is located on the catalytic

loop, which serves as a base to accept protons for catalysis; therefore, it appears that ZED1 is the decoy. Interestingly, ZAR1 is also responsible for immunity triggered by another unrelated T3SE, AvrAC, produced by *Xanthomonas campestris* pv. *campestris*. AvrAC modifies the receptor-like kinase PBS1-like 2 (PBL2) through uridylylation, and uridylylated PBL2 is subsequently recruited to the ZAR1 protein complex, which is associated with another pseudokinase in the same cluster with ZED1 (126). In the case of AvrAC, the pseudokinase is not a direct substrate for uridylylation. It remains to be determined whether the ZAR1-pseudokinase complex is a common strategy of plants to expand the capacity for effector recognition.

Pseudomonas syringae Effector HopZ3 Acetylates Multiple Components of an Immune Receptor Complex To Suppress Immunity

HopZ3 is another YopJ family effector that has been identified in several *P. syringae* isolates. Unlike HopZ1, which is ancestral to *P. syringae*, HopZ3 was likely introduced through horizontal gene transfer from other plant pathogens (26). The virulence function of HopZ3 is best characterized for *P. syringae* pv. *syringae* strain B728a (PsyB728a), which causes diseases on bean and tobacco but can also infect *Arabidopsis* (127). A *hopZ3* deletion mutant of PsyB728a exhibited significantly reduced bacterial growth in *Arabidopsis* (128). This is due at least in part to the ability of HopZ3 to suppress immunity triggered by other effectors produced by PsyB728a, including AvrB3 and AvrRpm1 (47).

AvrB3 and AvrRpm1 can each activate RPM1 (a CC-NB-LRR protein)-mediated resistance in *Arabidopsis*. The activation of RPM1 depends on the phosphorylation of RPM1-interacting protein 4 (RIN4), which also associates with AvrB3 and AvrRpm1. The interactions with these effectors trigger the phosphorylation of RIN4 by RPM1-induced protein kinase (RIPK) (129–131). Through a large-scale pairwise protein-protein interaction survey, HopZ3 was found to interact with multiple components of the RPM1 protein complex, including RIPK, RIN4, AvrRpm1, and AvrB3 (47). Importantly, all these interacting partners can also be acetylated by HopZ3. Mass spectrometry analysis determined that HopZ3 acetylates RIPK on Lys¹²², Thr¹⁶⁴, Ser¹⁷⁴, and Ser²²¹ residues that are close to the ATP-binding site and Ser²⁵¹ and Thr²⁵² residues that are in the activation loop. K122R and S251A/S252A mutants of RIPK have significantly reduced kinase activity; furthermore, phosphorylation of RIN4 by RIPK is reduced in the presence of HopZ3. Taken together, these findings support a potential inhibitory effect of HopZ3 on the kinase activity of RIPK acetylation on functionally important residues.

Similarly to HopZ1a, HopZ3 also seems to be a multitarget enzyme that modifies various substrates with no obvious sequence similarity. In addition to RIPK, HopZ3 also acetylates RIN4 on Ser⁴⁷, Ser⁷⁹, Ser⁸³, and Lys⁸⁶ and the other effector AvrB3 on Thr¹³⁷ and His²²¹ (47). Acetylation of RIN4 is associated with reduced phosphorylation by RIPK, whereas mutations on the acetylated sites also reduced resistance triggered by AvrB3 (47). These findings indicate that HopZ3 may employ multiple strategies to suppress RPM1-dependent immunity. Furthermore, HopZ3 also interacts with other kinases, including PBL1 and BIK1, which have important roles in MTI (132). It will be interesting to examine if HopZ3 could acetylate PBL1 and/or BIK1 to suppress immunity.

***Ralstonia solanacearum* Effector PopP2 Acetylates WRKY Transcription Factors and Inhibits Transcriptional Activation of Defense Genes**

The soilborne vascular pathogen *R. solanacearum* causes bacterial wilt on ~200 plant species, including monocots and dicots. In extreme cases, infection by *R. solanacearum* may result in total yield loss of economic solanaceous plants, e.g., potato and cassava (133). Therefore, *R. solanacearum* is considered one of the most devastating bacterial plant pathogens. Five YopJ family effectors, i.e., PopP1 (134, 135), PopP2, PopP3 (31), RipAE, and RipJ (32), have been identified in *R. solanacearum*, but only PopP2 has been characterized for virulence function.

PopP2 is a unique YopJ family effector in three perspectives. First, the catalytic triad of PopP2 is His/Asp/Cys, instead of His/Glu/Cys, as observed for most YopJ family members. Second, PopP2 specifically modifies Lys residues rather than the predominant acetylation on Ser/Thr residues in other YopJ family members. Third, PopP2 exhibits strong acetylation activity when it is expressed in *E. coli*, i.e., in the absence of IP₆ (48). The noncanonical properties of PopP2 are consistent with its clustering as a phylogenetic group distinct from all other YopJ family members (Fig. 1).

Characterization of PopP2 targets revealed an original strategy utilized by effectors to interfere with the activation of a defense response. PopP2 manipulates a group of transcription factors containing the WRKY domain, which binds to the “W box” in the promoter of stress-related genes, including those contributing to defense (136). In *Arabidopsis*, PopP2 modifies several WRKY transcription factors on the last Lys residue of a conserved heptad sequence, WRKYGQK, in the WRKY domain (54, 55). Nuclear magnetic resonance (NMR) structure analysis shows that the heptad sequence is in direct contact with the major groove of a DNA fragment containing the W box, and the last Lys residue associates with the guanine sugar carbon of DNA through hydrogen bonds and electrostatic interactions (137, 138). Therefore, acetylation of this Lys residue likely neutralizes the electrostatic interactions between the heptad sequence and the DNA. Consistently, coexpression with PopP2 resulted in an inhibition of the DNA-binding activity of WRKY transcription factors and suppressed the induction of WRKY-dependent MTI-responsive genes (55). As such, PopP2 accelerates and enhances wilting symptoms caused by *R. solanacearum* in susceptible *Arabidopsis* plants (55). Interestingly, although the conserved heptad sequence by itself is sufficient as an acetylation substrate of PopP2, it is affected by other sequences/factors. For example, PopP2 interacts with WRKY40 and WRKY60, two proteins carrying the heptad sequence, but does not acetylate them (55). Interestingly, WRKY40 and WRKY60 are negative regulators of plant defense against *P. syringae* (139). Therefore, PopP2 selectively inhibits the function of certain WRKYs whose inactivation is advantageous for bacterial infection.

R Protein RRS1-R Contains a WRKY Domain and Acts as a Trap for PopP2 Acetyltransferase Activity

Prior to the identification of WRKY transcription factors as virulence targets, PopP2 was known to trigger the HR in *Arabidopsis* ecotype Nd-1, and recognition is dependent on the R protein resistance to *Ralstonia solanacearum* 1 (RRS1-R) (140) (Fig. 6). Remarkably, in addition to the Toll and human interleukin-1 recep-

tor (TIR)-NB-LRR domain, RRS1-R also contains a WRKY domain at the C terminus. Furthermore, RRS1-R is located in the nucleus, which is different from the cytoplasmic localization of most R proteins (99, 100). Provided the significant role of WRKY transcription factors in plant immunity, it was an intriguing hypothesis that RRS1-R is a dual-function immune protein that serves as a decoy to detect the acetylation activity of PopP2 on WRKY domains and activates the defense response.

Indeed, PopP2 directly associates with RRS1-R in the nucleus, and the WRKY domain is required for this interaction (140). Similar to the WRKY transcription factors, the WRKY domain of RRS1-R is acetylated by PopP2 on K¹²²¹ (the last Lys residue in the conserved heptad sequence), which subsequently affects its binding to the W box (54). However, the loss of DNA-binding activity *per se* is not sufficient to trigger RRS1-R-dependent immunity. For example, both the acetylation-mimetic mutant RRS1-R^{K1221Q} and the non-acetylation-mimetic mutant RRS1-R^{K1221R} fail to interact with W-box DNA, but only RRS1-R^{K1221Q} causes PopP2-independent stunting and constitutive expression of defense genes (55). Therefore, RRS1-R-activated immunity is likely triggered by PopP2-mediated acetylation. Furthermore, the RRS1-S protein, encoded by the *RRS1-S* allele, is unable to trigger PopP2-dependent immunity (141), even though its DNA-binding activities are also disrupted upon acetylation at the corresponding lysine residue by PopP2 (54, 55). Further investigation of the differences between RRS1-S and RRS1-R will provide mechanistic insight into the activation of RRS1-R-mediated immunity.

***Xanthomonas campestris* Effector AvrBsT Acetylates a Tubulin-Associated Protein**

AvrBsT is a YopJ family effector secreted by *Xanthomonas campestris* pv. *vesicatoria*, the causative agent of bacterial spot disease on pepper and tomato (142). *X. campestris* pv. *vesicatoria* produces three additional YopJ family effectors, XopJ (36, 143), AvrXv4 (144), and AvrRxv (145–147), but only AvrBsT has been demonstrated to possess acetyltransferase activity. AvrBsT interacts with an *Arabidopsis* protein that was previously reported to be copurified with tubulin by affinity chromatography (148). Since this protein is an acetylation substrate of AvrBsT, it was named acetylated interacting protein 1 (ACIP1) (42). In *Arabidopsis*, *P. syringae* infection resulted in changes in ACIP1 localization on microtubules; AvrBsT further induced the aggregation of ACIP1 in puncta-like structures (42). However, how ACIP1 regulates the microtubule network and plant immunity is largely unknown.

Virulence mechanisms of modulating the eukaryotic cytoskeleton by T3SEs and toxins are well established in animal pathogens. These virulence factors either antagonize phagocytosis (149) or induce the internalization of intracellular pathogens by normally nonphagocytic cells (150, 151). However, plants lack phagocytes; both *P. syringae* and *X. campestris* pv. *vesicatoria* are extracellular pathogens. A possible mechanism for HopZ1a and AvrBsT to facilitate infection is through the inhibition of the microtubule-dependent secretion of antimicrobial molecules. Alternatively, disruption of the cytoskeleton may alter defense-related gene expression. For example, harpin (a known MAMP) produced by *E. amylovora* has been shown to reduce microtubule density, which is associated with enhanced expression of defense-related genes. Applications of different cytoskeleton-targeting drugs also result in enhanced expression of defense genes, suggesting that disruption of the cytoskeleton may serve as a signal leading to defense

activation (152). However, alternations to microtubule arrays can either induce or inhibit resistance (153). An understanding of YopJ effector-mediated regulation of the cytoskeleton through acetylation may reveal undiscovered functions of the cytoskeleton network involved in plant immunity.

In addition to ACIP1, AvrBsT also interacts with SNF1-related kinase 1 (snRK1) in tomato and pepper (35). Although neither the degradation nor the acetylation of SnRK1 was detected in the presence of AvrBsT (35), silencing of *SnRK1* abolished the ability of AvrBsT to suppress the hypersensitive response triggered in pepper by another *X. campestris* pv. *vesicatoria* effector, AvrBs1. It remains to be determined how AvrBsT modulates SnRK1 to suppress AvrBs1-mediated defense.

ACETYLTRANSFERASE OR PROTEASE? TWO SIDES OF THE SAME BLADE?

Among the seven YopJ family effectors that can modify their host targets through acetylation, five of them (AvrA, YopJ, HopZ1a, HopZ3, and AvrBsT) were also reported to have protease activities (26, 27, 34, 42). This is not surprising because YopJ family effectors utilize a protease-like catalytic triad, which may allow them to function as acetyltransferases and/or proteases. Indeed, a YopJ family effector, XopJ, produced by *X. campestris* pv. *vesicatoria* was shown to proteolytically degrade the 26S proteasomal subunit regulatory particle ATPase 6 (RPT6) (28, 36, 143). XopJ inhibits proteasome activity in the plant host and manipulates the turnover of nonexpressor of PR1 (NPR1), a central regulator of the SA signaling pathway (154). As a result, XopJ promotes bacterial infection by dampening SA-dependent defenses. Intriguingly, the degradation of RPT6 by XopJ can be inhibited by protease inhibitors (36). Furthermore, *in vitro* acetylation assays did not show autoacetylation of XopJ or *trans*-acetylation of RPT6. These findings support the activity of XopJ as a protease.

As this conclusion was largely based on the inhibition of XopJ-mediated RPT6 degradation by protease inhibitors, these results could have alternative explanations. In particular, since the structures of the catalytic domain of HopZ1a and cysteine proteases are similar (53), protease inhibitors may also inhibit a putative acetyltransferase activity of XopJ. Therefore, the possibility that the degradation of RPT6 is a consequence of potential acetylation by XopJ cannot be excluded without further investigation. Another possibility is that XopJ possesses dual acetyltransferase/protease activities and that acetylation can be observed only when suitable substrates are available.

CONCLUSION

T3SEs are one of the most important groups of virulence factors produced by bacterial pathogens. Although most T3SEs are unique to the pathogenic species or even subspecies that produce them, YopJ family members are widely distributed in both animal and plant pathogens and play essential roles during infection. This rarely observed conservation attracts interest from independent groups to investigate the virulence function of YopJ family effectors. These studies provide a unique opportunity to compare virulence strategies utilized by animal and plant pathogens. When the host targets of YopJ effectors produced by animal and plant pathogens are compared, an obvious difference is that animal pathogens predominantly target kinases, whereas plant pathogens can target a diverse body of proteins with no sequence similarity. While these effectors exhibit promiscuity in substrate specificity, it

would be important to determine whether modifications of these substrates contribute to virulence equally. Furthermore, there is a clear diversification of YopJ effectors in plant pathogens, which is evident by the observation that multiple YopJ family members are produced by the same bacterial species, and they are also clustered in different phylogenetic groups. This reflects the importance of the enzymatic activity of YopJ effectors in suppressing plant immunity.

Accumulating evidence suggests that YopJ family effectors represent a new class of acetylation enzymes without sequence homology to a known acetyltransferase. In particular, YopJ effectors with demonstrated acetyltransferase activities modify specific Ser/Thr/Lys residues in their substrates and require the eukaryotic cofactor IP₆ for activation, which is distinct from other well-characterized acetyltransferases. These unique features are consistent with the adoption of a protease-like catalytic triad in the YopJ effectors, suggesting that they use a distinct catalytic mechanism. A common strategy for effector evolution is to mimic eukaryotic enzymes and hijack cellular processes in hosts (6). It will be interesting to identify Ser/Thr acetyltransferases similar to YopJ effectors in eukaryotes, which would uncover original regulatory mechanisms of protein functions.

Currently, there are two common ways to determine acetylation activities and specific acetylation sites in substrates. First, acetylation can be detected by Western blotting using acetyl-lysine antibodies that are commercially available. However, this approach does not detect acetylation on residues other than lysine, which imposes a major limitation for YopJ family effectors. Alternatively, proteins from *in vitro* acetylation reactions or *in vivo* systems can be analyzed for acetylation by mass spectrometry. This more sensitive approach contributed to the identification of previously uncharacterized acetylation sites, such as Ser/Thr residues in MKKs acetylated by YopJ. With the mass spectrometry technique becoming more affordable, high-throughput acetylome analyses will facilitate the identification of acetylation activities, YopJ effector targets, and mechanisms of target manipulation through acetylation.

The report of the first crystal structure of YopJ family effectors paved the path to understanding the enzymatic activity of this unique acetyltransferase family. A future challenge is to solve the structure of YopJ effector-target complexes, which would provide essential information on the substrate selectivity, target manipulation, and virulence function of this group of evolutionarily conserved effectors.

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