VopA Inhibits ATP Binding by Acetylating the Catalytic Loop of MAPK Kinases*

Jennifer E. Trosky¹, Yan Li³, Sohini Mukherjee¹, Glady Keitany¹, Haydn Ball⁵, and Kim Orth¹²

From the ¹Department of Molecular Biology, ⁵Protein Chemistry Technology Center, and ²Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas 75390 and the ³University of Washington School of Public Health, Seattle, Washington 98195

The bacterial pathogen Vibrio parahaemolyticus manipulates host signaling pathways during infections by injecting type III effectors. One of these effectors, Vibrio outer protein A (VopA), inhibits MAPK signaling via a novel mechanism, distinct from those described for other bacterial toxins, that disrupts this signaling pathway. VopA is an acetyltransferase that potently inhibits MAPK signaling pathways not only by preventing the activation of MAPK kinases (MKKs) but also by inhibiting the activity of activated MKKs. VopA acetylates a conserved lysine found in the catalytic loop of all kinases and blocks the binding of ATP, but not ADP, on the MKKs, resulting in an inactive phosphorylated kinase. Acetylation of this conserved lysine inhibits kinase activity by a new mechanism of regulation that has not been observed previously. Identifying the target of VopA reveals a way that the reversible post-translational modification of lysine acetylation can be used to regulate the activity of an enzyme.

Vibrio parahaemolyticus is a marine bacterium and causative agent of gastroenteritis associated with the consumption of contaminated seafood. It is endemic to Southeast Asia and is the leading cause of gastroenteritis in Japan (1). Sequencing of the V. parahaemolyticus genome revealed the presence of two type III secretion systems each encoded in separate pathogenicity islands (2). Type III secretion systems are used by bacterial pathogens to inject effector proteins into the cytoplasm of the host cells (3). Upon delivery into the host cytosol, the effectors manipulate host signaling to gain an advantage for the infecting pathogen. The bacterial effectors are proposed to usurp or manipulate normal functions in the host cell by either up- or down-regulating signaling pathways (4). However, the bacterial effector proteins must initially be kept in a quiescent state within the bacterium. This is accomplished by a variety of methods, including their association with a chaperone in the bacterial cytosol, their specificity for a substrate, and/or their requirement for an activator or modification that is only provided by a eukaryotic source (4, 5).

VopA (Vibrio outer protein A) is an effector found within one of the V. parahaemolyticus pathogenicity islands, and is a member of a family of type III effectors called YopJ-like proteins that are found in a wide variety of pathogens including other animal pathogens, plant pathogens, and the plant symbiont Rhizobium (5–7). The founding member of the family, YopJ from Yersinia spp., inhibits the mitogen-activated protein kinase (MAPK)³ and NFκB signaling cascades within the host cell, thereby inhibiting the host’s innate immune response (7). All YopJ-like proteins contain a catalytic triad, and it was recently shown that YopJ functions as an acetyltransferase (8–10). It has been speculated that other members of the family of YopJ-like proteins might also possess this activity (8). YopJ was demonstrated to inhibit the MAPK and NFκB signaling pathways by acetylating the critical serine and threonine residues found on the activation loop of MAPK kinases (MKKs), thereby blocking their activation by phosphorylation (8–10). VopA and YopJ share ~55% similarity at the amino acid level although they elicit different functions (11).

VopA inhibits MAPK signaling using a novel mechanism distinct from those described for other bacterial toxins that disrupt this signaling pathway. This effector potently inhibits MAPK signaling but shows no inhibitory effect on the NFκB pathway (11). Herein, we show that VopA acetylates a conserved lysine located in the catalytic loop of all kinases that plays a critical role in the binding of the γ-phosphate of ATP. Modification of this lysine by acetylation inhibits the binding of ATP, but not ADP, to the MAPK kinase, resulting in an inactive kinase. These findings uncover a unique mechanism that targets a critical conserved lysine residue involved in coordinating nucleotide binding that could also be used by eukaryotic enzymes.

EXPERIMENTAL PROCEDURES

Cloning—pSSFV VopA-FLAG and pSFFV VopA-C167A constructs were generated as described previously (11). For bacterial overexpression, VopA and VopA-C167A were ampli-

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2 A Beckman Young Investigator, a Burroughs Wellcome Investigator in Pathogenesis of Infectious Disease, and a W. W. Caruth, Jr. Biomedical Scholar. To whom correspondence should be addressed: University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390. Tel.: 214-648-1685; Fax: 214-648-1488; E-mail: Kim.Orth@utsouthwestern.edu.
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Expression of VopA Constructs—For expression of wild-type VopA, constructs were transformed into pMMB67HE (a kind gift from J. B. Bliska). The resulting construct has a glutathione S-transferase (GST) tag on the N-terminus of VopA with both thrombin and tobacco etch virus cleavage sites in the linker. For expression in *Yersinia pseudotuberculosis*, VopA and VopA-C167A were amplified by PCR using pSFFV VopA or pSFFV VopA-C167A as template with 5′-EcoRI and 3′-stop-XhoI primers and cloned into pGEX-tobacco etch virus (a kind gift from Y. M. Chook). The resulting construct has a glutathione S-transferase (GST) tag on the N-terminus of VopA with both thrombin and tobacco etch virus cleavage sites in the linker. For expression in *Yersinia pseudotuberculosis*, VopA and VopA-C167A were amplified by PCR using pSFFV VopA or pSFFV VopA-C167A as template with 5′-HindIII and 3′-FLAG-stop-BamHI primers and cloned into pMMB67HE (a kind gift from J. B. Bliska).

*Y. pseudotuberculosis Strains—*YP126 (wild type) and YP26 (ΔYopJ) were kind gifts from J. B. Bliska. For expression of pMMB67HE VopA-FLAG and pMMB67HE VopA-C167A-FLAG in YP26, the constructs were transformed into SM10pir. Overnight cultures of SM10 strains were grown at 37 °C, and overnight cultures of YP26 were grown at 26 °C. 200 µl of each strain was mixed and spotted onto an LB agar plate. Plates were incubated at 37 °C right side up for several hours. Bacteria were scraped off plates, replated onto M9 agar plates supplemented with ampicillin (100 µg/ml), and grown at 26 °C. Colonies were picked, and VopA and VopA-C167A expression were verified by Western blot with anti-FLAG antibody (Sigma).

**Macrophage Infections—***Y. pseudotuberculosis* infections were performed as described previously (12). In brief, J774A.1 murine macrophages were seeded into 6-well dishes at a density of 1.0 × 10⁶ cells/ml in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum (Hyclone), penicillin/streptomycin/glutamate (Invitrogen), and sodium pyruvate (Invitrogen). To induce type III secretion, overnight cultures of *Yersinia* strains were diluted back to an OD of 0.1 in LB supplemented with 20 mM sodium oxalate and 20 mM magnesium chloride and grown at 26 °C for 1 h. Cultures were then shifted to 37 °C for 2 h. Bacteria cells (1 × 10⁶ cells) were pelleted and resuspended in Dulbecco's modified Eagle's medium. Bacteria were overlaid on macrophages and plates spun at 500 × g for 5 min to bring bacteria into contact with the cells. Plates were kept at 37 °C for 15 min and 45 min. Plates were put on ice and cells were washed once with cold phosphate-buffered saline. Cells were lysed for 10 min in radioimmunoprecipitation assay buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate, 1 mM Na₃VO₄, 10 mM NaF, 20 mM β-glycerophosphate, 1 µM dithiobis(tetrahydrofurfuryl) disulfide, 0.5% EGTA, and Complete protease inhibitor tablet (Roche Applied Science)). Lysates were cleared by centrifugation at 10,000 rpm, 150 µl of cleared lysate were boiled with 50 µl of 5× Laemmli sample buffer. Samples were separated by SDS-PAGE and analyzed by immunoblot with rabbit anti-phospho-ERK antibody (Cell Signaling Technology, Inc.) and mouse anti-HA antibody (Babco).

**Protein Purification—**GST-VopA wild type and GST-VopA-C167A constructs were expressed in *Escherichia coli* BL21-DE3 cells and purified by GST affinity chromatography. In brief, cells were grown to an A₆₀₀ of 0.6 to 0.8 in 2× yeast extract and tryptophan medium and then induced with 400 µM isopropyl-β-thiogalactopyranoside (Roche Applied Science) for 4 h at 25 °C. The cells were lysed in PBS-T (phosphate-buffered saline (pH 8), 1% (v/v) Triton X-100 (Fisher), 0.1% (v/v) 2-mercaptoethanol (Bio-Rad), and 1 mM phenylmethylsulfonyl fluoride (Sigma)) using a cell disrupter (EmulsiFlex-C5, Avestin, Inc.). The lysates were incubated with glutathione-agarose beads, and bound protein was subjected to GST-tobacco etch virus cleavage overnight in PBS-T supplemented with 1 mM acetyl coenzyme A (acyetyl-CoA) (Sigma) at room temperature. Flow-through was collected and incubated with GST beads to remove any remaining GST-tobacco etch virus. Flow-through was collected and concentrated in a 30-kDa molecular mass cut-off Amicon-Ultra concentrator (Fisher).

rMKK6 construct was expressed in Rosetta Blue cells (Novagen) and purified by nickel affinity chromatography. In brief, cells were grown and lysed as above, except cells were induced with 200 µM isopropyl-β-thiogalactopyranoside and grown at 25 °C for 8 h. Lysates were bound to Ni²⁺-nitrilotriacetic acid beads (Sigma). Bound protein was washed and eluted per the manufacturer’s protocol. His-MKK1 was expressed and purified as His-MKK6 except cells were induced with 400 µM of isopropyl-β-thiogalactopyranoside and grown at 25 °C for 4 h.

**In Vitro Acetylation Assay—**Acetylation assays were performed as described (9). In brief, purified His-MKK6, His-MKK1, or p38 (a kind gift from John Humphreys) was incubated with VopA or VopA-C167A in the presence of 14C-labeled acetyl-CoA for 1 h at 30 °C in acetylation buffer (50 mM Tris, pH 8.0, 10% (v/v) glycerol (Fisher), 100 µM EDTA (Fisher), 1 mM dithiothreitol (Sigma), 1 mM phenylmethylsulfonyl fluoride (Sigma)). The mixtures were resolved by SDS-PAGE, and the gels were incubated with Amplify fluorographic reagent (Amersham Biosciences) and analyzed by autoradiography.

**Molecular Weight Measurement—**The protein molecular weight was measured on a Sciex QSTAR XL mass spectrometer (Applied Biosystems) with an off-line electrospray ionization source. The electrospray tips were purchased from Proxeon Biosystems. Sample solution was desalted using a Millipore C₁₈ ZipTip and eluted with 80% acetonitrile. The eluates were dried, and then resuspended in 1% formic acid, 50% acetonitrile.

**Liquid Chromatography/Tandem Mass Spectrometry—**The tryptic digests of the sample were fractionated using a Dionex LC-Packings HPLC. Peptides were first desalted on a 300-µm × 1-mm PepMap C₁₈ trap column with 0.1% formic acid in HPLC grade water at a flow rate of 20 µl/min. After desalting for 5 min, peptides were flushed onto a LC Packings 75-µm × 15-cm C₁₈ nanocolumn (3 µm, 100 Å) at a flow rate of 200 nl/min. A 45-min gradient was used for the HPLC separation with the acetonitrile concentration increased from 2 to 45%. Elutes were analyzed with a QSTAR XL mass spectrometer (Applied Biosystems). Data were acquired in information...
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**RESULTS**

**VopA Inhibits Mammalian MAPK Activation**—We have shown previously that VopA is able to inhibit the ERK-MAPK signaling pathway in mammals and the high osmolarity growth pathway in yeast (11). To confirm that VopA inhibits other mammalian MAPK pathways, we analyzed the activation of the p38-MAPK and JNK-MAPK pathways by assessing the phosphorylation of p38 and JNK in the presence and absence of VopA or the catalytically inactive form of VopA (VopA-C167A) where the catalytic cysteine is mutated to an alanine (Fig. 1). For this analysis, VopA or VopA-C167A was cloned into the bacterial expression vector pMMB67HE and conjugated into a

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**Figure 1. VopA inhibits JNK and p38 activation.** A, inhibition of JNK signaling. J744A.1 murine macrophages infected at a multiplicity of infection of 50 with *Y. pseudotuberculosis* strains overexpressing VopA and VopA-C167A and assessed for phosphorylation of JNK by immunoblot (upper panel). Immunoblot for total JNK in the lower panel shows equal loading. B, inhibition of p38 signaling. Same as in A except assessed for phosphorylation of p38 (upper panel). Total p38 in the lower panel shows equal loading.

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**Y. pseudotuberculosis* strain that is deleted for YopJ (12). These strains were used to infect J744A.1 murine macrophages to test the ability of VopA to rescue the deleted YopJ strain by restoring the inhibition of the p38 or JNK pathways. Infection with the wild type *Yersinia* strain that contains YopJ efficiently inhibits both the p38 and JNK-MAPK pathways (Fig. 1A, lanes 2 and 3, and B, lanes 2 and 3, respectively), whereas the *Yersinia* strain deleted for YopJ does not inhibit these signaling pathways (Fig. 1A, lanes 4 and 5, and B, lanes 4 and 5, respectively) (12). Inhibition of the p38 and JNK phosphorylation was restored in the YopJ deletion strain by induced expression of VopA (Fig. 1A, lanes 8 and 9, and B, lanes 8 and 9, respectively) but not the catalytically inactive VopA-C167A (Fig. 1A, lanes 12 and 13, and B, lanes 12 and 13, respectively).

**VopA Inhibits Downstream of MKK Activation**—To determine the point at which VopA blocks the MAPK pathway, we performed a series of epistasis experiments. Consistent with previous findings, when the MAPK pathway is stimulated with an external stimulus (such as epidermal growth factor) VopA, but not VopA-C167A, is able to inhibit the ERK-MAPK pathway as demonstrated by the lack of phosphorylated HA-ERK in treated cells (Fig. 2A) (11). Phosphorylation of endogenous ERK is observed because of the background from nontransfected, stimulated cells (Fig. 2A) (11). Constitutive activation of this pathway by transfected RasV12 or B-Raf BxB is also inhibited by wild type VopA, but not mutant VopA-C167A, as demonstrated by analysis of ERK phosphorylation (Fig. 2, B and C, respectively). Surprisingly, when an activated form of MKK1, MKK1 ED, is used to activate the pathway just upstream of ERK, VopA is still able to inhibit phosphorylation of HA-ERK (Fig. 2D). This observation is in contrast to the inhibitory profile of YopJ, which is unable to inhibit signaling in the presence of an activated MKK (7). Based on these findings, we concluded that VopA inhibits MAPKs downstream of MKK1 activation and upstream of ERK activation.

**VopA Acetylation of MKK6 Inhibits Activation**—Given that VopA only affects the MAPK signaling pathways and that it also inhibits the activation of MAPKs, we predicted the molecular target of VopA would be MAPKs (11). In addition, because of the fact that VopA is a member of the family of YopJ-like proteins and that YopJ is an acetyltransferase, we hypothesized that VopA functions as an acetyltransferase that acetylates and inhibits MAPK activation (8). To test this hypothesis, we used purified p38 in an *in vitro* acetylation assay with purified wild type and mutant VopA. However, we observed that neither VopA nor the catalytically inactive VopA-C167A acetylates p38 (Fig. 3A). However, as a control we included rMKK6 as a substrate and discovered that VopA, but not mutant VopA-C167A, is able to acetylate rMKK6.

Previously, YopJ was observed to acetylate rMKK6 on its activation loop, thereby preventing phosphorylation by upstream kinases (9, 10). To determine whether VopA modifies rMKK6 in a similar manner, we tested whether rMKK6 is phosphorylated by upstream kinases in the presence or absence of VopA using a previously established *in vitro* signaling assay (9). rMKK6 was preincubated with GST-VopA or GST-VopA-C167A in the presence or absence of acetyl-CoA followed by incubation with membrane-free lysates derived from serum-
stimulated cells. In control samples without VopA, rMKK6 was phosphorylated and activated by upstream kinases as indicated by immunoblot analysis with an anti-phospho-MKK6 antibody (Fig. 3B, lanes 1 and 2). However, when rMKK6 is preincubated with wild type VopA in the presence of acetyl-CoA, rMKK6 is no longer activated by phosphorylation (Fig. 3B, lane 4). By contrast, incubation of the kinase with mutant VopA-C167A has no inhibitory effect on the phosphorylation of rMKK6 (Fig. 3B, lanes 5 and 6). Although these results demonstrate that VopA can inhibit activation of MKKs by acetylation, they do not explain how VopA is able to inhibit activated MKKs.

VopA Acetylates Four Conserved Residues in MKK6—To determine how VopA acetylates MKK6, we first assessed the total mass of rMKK6 isolated from bacterial cells expressed either alone (rMKK6) or in the presence of GST-VopA (rMKK6/V11001Y) for total mass spectrometric analysis. The mass of rMKK6 is observed as one strong peak at 39,655 kDa, but the mass of rMKK6/V11001Y is divided among five peaks, with mass differences in increments of 42 Da, equal to the mass of one acetyl group (Fig. 4A). These results indicate that VopA acetylates four residues on rMKK6.

To determine which four residues of rMKK6 are modified by GST-VopA, the samples were subjected to liquid chromatography/tandem mass spectrometry analyses. No acetylation was observed for rMKK6, whereas rMKK6/V11001Y was acetylated on three residues in the activation loop (Ser-207, Lys-210, and Thr-211) and on one residue in the catalytic loop (Lys-172) (Fig. 4B) (13). The three residues in the activation loop are the same three residues that are modified by YopJ, and as shown in Fig. 3A, the acetylation by VopA on the activation loop of the kinase directly competes with phosphorylation on the serine and threonine residues. The fourth residue is a conserved lysine that is involved in the coordination of the γ-phosphate of ATP upon nucleotide binding.

To confirm that acetylation of the lysine residue on the catalytic loop can occur on an active kinase, we used the purified active phosphomimic, HisMKK1(S218E,S222D), as a substrate in an acetylation assay (7). We were able to detect acetylation of HisMKK1(S218E,S222D) by VopA but not VopA-C167A (Fig. 4C). We were also able to detect autoacetylation of VopA, which is not seen in the assay with rMKK6. This observed difference is likely because of the extended exposure required to see HisMKK1(S218E,S222D) acetylation (1.5 weeks). The longer exposure time is required because only one acetylation site on HisMKK1(S218E,S222D) is available for modification, due to the mutations of both serines on the acti-
mation of MANT-ADP bound to His6-MKK1(S218E,S222D). We both wild type VopA and VopA-C167A does increase the emission in the presence of MANT-ADP (Fig. 5)

FIGURE 4. VopA acetylates the catalytic loop of MKK6. A, the total mass spectrometric analysis of His-MKK6 expressed in bacteria alone or in the presence of VopA is shown. B, a ribbon diagram showing the representative kinase structure, MKK1, with modified residues starred. Residues starred in blue are modified by both VopA and YopJ, and the residue starred in pink is modified only by VopA. C, VopA acetylates rMKK1. Same as in Fig. 3A except His-MKK1(S218E,S222D) is used as a substrate. His-MKK1 ED is His-MKK1(S218E,S222D).

vitation loop and the lack of a lysine residue on the activation loop in MKK1.

Acetylation of Lys-172 by VopA Disrupts ATP Binding to MKK—Based on the location of the conserved lysine in the kinase (Lys-172 in MKK6) and its interaction with the γ-phosphate of ATP, we hypothesized that acetylation of this lysine would negatively affect the binding of ATP, but not ADP, to MKKs. Therefore, we analyzed the binding of the fluorescent nucleotide analogs MANT-ATP and MANT-ADP to constitutively activated His-MKK1(S218E,S222D). When excited at 290 nm the MANT-labeled nucleotides, ATP and ADP, emit strong fluorescent peaks (Fig. 5, A and B, respectively) (14). When MANT-ATP or MANT-ADP is incubated with either His-MKK1(S218E,S222D) or His-MKK1(S218E,S222D) that is preincubated with VopA-C167A, an increase in the emission maximum at ~450 nm is observed (Fig. 5, A and B, respectively). This is similar to the emission profile observed for binding of these nucleotide analogs to other kinases (14). However, this increase in fluorescence in the presence of MANT-ATP is abrogated when His-MKK1(S218E,S222D) is preincubated with wild type VopA (Fig. 5A). This inhibitory effect is not seen in the presence of MANT-ADP (Fig. 5B), although binding of both wild type VopA and VopA-C167A does increase the emission of MANT-ADP bound to His-MKK1(S218E,S222D). We propose this difference be attributed to the disruption of the coordination of binding for the γ-phosphate of ATP by the acetylated lysine residue (Lys-192) in MKK1.

DISCUSSION

VopA, a Multi-tasking Acetyltransferase—

VopA Functions as an Acetyltransferase—

VopA, an Ideal Molecular Inhibitor—For decades, scientists have been, and still are, trying to design specific inhibitors that target the nucleotide binding site of kinases. Attempts to make selective inhibitors, such as imatinib, have been focused on targeting the inactive state of a kinase because of its unique fold (15). In their active states many kinases conform to a canonical structure, which makes it difficult to target a specific kinase or even a family of kinases. Natural selection has once again proved to be the champion chemist by creating VopA. This bacterial effector disrupts not only the inactive form of the kinase by phosphorylation, similar to what has been observed before with Yersinia YopJ (9, 10). The lysine in the activation loop of the MKK6 is not conserved and, therefore, not thought to be important for the inhibitory activity of the effector (9). Based on the architecture of their catalytic site, VopA and other YopJ-like proteins are predicted to transfer the acetyl group from acetyl-CoA to a protein substrate using a ping-pong mechanism (8). For this mechanism, a covalent acetyl-enzyme intermediate is formed that is attacked by the R group of an amino acid. Supported by the presented experiments, VopA must efficiently coordinate two distinct nucleophiles for attack on the acetyl-enzyme intermediate: an amine from a lysine residue or a hydroxyl from a serine or threonine. Although the structure/function studies required to understand the biochemical mechanism used by these enzymes are beyond the scope of this study, from a molecular point of view the mechanism used by this group of enzymes is intriguing and will be interesting to decipher.

VopA, an Ideal Molecular Inhibitor—For decades, scientists have been, and still are, trying to design specific inhibitors that target the nucleotide binding site of kinases. Attempts to make selective inhibitors, such as imatinib, have been focused on targeting the inactive state of a kinase because of its unique fold (15). In their active states many kinases conform to a canonical structure, which makes it difficult to target a specific kinase or even a family of kinases. Natural selection has once again proven to be the champion chemist by creating VopA. This bacterial effector disrupts not only the inactive form of the kinase but the active form as well.

VopA efficiently inhibits the MKKs two ways by targeting different sites on the kinase. First, VopA targets the pharmacological “sweet spot” (i.e. the nucleotide binding site) of a kinase by masking the charge on a lysine residue with an acetyl moiety. This change in the charge and shape of the nucleotide binding pocket of a kinase drastically changes the activity of the enzyme.
The modified kinase is no longer able to bind ATP but is still able to bind ADP. Therefore, acetylated MKKs are covalently locked into an inactive state and are no longer able to modify their substrates. VopA also targets the activation of the MKKs by acetylation of the activation loop on the serine and threonine residues, directly competing with phosphorylation, resulting in a kinase that cannot be activated. Perhaps it is understandable that VopA appears to be a more potent inhibitor of MAPK signaling than YopJ because VopA is able to inactivate MKKs by two mechanisms, whereas YopJ only uses one (11).

Recently another effector, the Yersinia kinase YpkA, has been shown to block the binding of a GTP to G-protein Gαq. In contrast to VopA that modifies the nucleotide binding site with a neutral acetyl group, YpkA phosphorylates a serine residue found in the proximity of the GTP binding site (16). Phosphorylation of Ser-47 on Gαq is predicted to reduce its affinity for GTP, thereby inactivating the G-protein. The molecular mechanisms are quite distinct for these two effectors, but the global outcome is the same: they disrupt the binding of the nucleotide to a host enzyme.

**Diversity of Acetyltransferase Effector Family**—Bacterial effectors, much like viral oncoproteins, target and manipulate host signaling by usurping or mimicking a host activity (4, 5). The bacterial effectors contain a variety of enzymatic activities that includes hydrolases, phosphatases, GTPase-activating proteins, guanine nucleotide exchange factors, and kinases (4, 5, 17). Recently, another enzyme, a serine/threonine acetyltransferase, was observed, and now we find lysine acetylation is used to manipulate host signaling 8–10, 18. Some of these effectors, like VopA, belong to a large family of effectors that are expressed by a variety of pathogens (11, 19). These families are arranged by the homology found in their primary amino acid sequence. As with other families of enzymes, the molecular activity of a bacterial effector can be predicted based on the biochemical activity of one or more of the family members. Proving an effector has the predicted activity requires direct biochemical experiments.

Even though the substrates of many eukaryotic enzymes can be predicted because of the conservative nature of signaling pathways throughout evolution, the identification of substrate for bacterial effectors is challenging to say the least. These enzymes have been mutated and molded over time to contain an activity that is custom designed by the pathogen that expresses it. For example, Yersinia YopJ cripples the innate immune response by inhibiting all MAPK pathways and the NFκB pathway, whereas Vibrio VopA targets only MAPK pathways, albeit in a more potent manner (7, 11). Homologues of these acetyltransferases are expressed by Rhizobium, a plant symbiont that may use this activity to attenuate host-signaling pathways to facilitate a commensal relationship between the bacteria and host (5, 7). Plant pathogens seem to be the most aggressive with regard to this family of effectors because some of the bacteria that target plants contain over a half-dozen genes encoding YopJ-like effectors (20).

Modifying a protein with lysine acetylation is a reasonable way to regulate the nucleotide binding to a kinase because lysine acetylation, like phosphorylation, is reversible. We propose that VopA exemplifies an effector that usurped the eukaryotic activity of lysine acetylation. Consistent with this hypothesis is the observation that expression of VopA in yeast suspends cell growth, but the growth suppression can be reversed when expression of VopA is stopped. In addition, these observations support by inference that O-acetylation (serine and threonine acetylation), like N-acetylation (lysine acetylation), will be reversible. Therefore, serine, threonine, and lysine acetylation might be reversible post-translational
modifications that are used to modulate the enzymatic activity of eukaryotic signaling machinery.

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