Plant Signaling & Behavior
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kpsb20

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Published online: 07 Jan 2014.

Plant Signaling & Behavior 9, e27563; January; © 2014 Landes Bioscience

The rise of the undead

Pseudokinases as mediators of effector-triggered immunity

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Keywords: *Pseudomonas syringae*, HopZ1a, ZAR1, ZED1, ZRK, kinase, immunity, effector, Resistance gene, Yersinia, Xanthomonas

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Submitted: 12/06/2013

Accepted: 12/17/2013

Published Online: 01/07/2014

Citation: Lewis JD, Lo T, Bastedo P, Guttman DS, Desveaux D. The rise of the undead: Pseudokinases as mediators of effector-triggered immunity. Plant Signaling & Behavior 2014; 9:e27563; PMID: 24398910; http://dx.doi.org/10.4161/psb.27563

Addendum to: Lewis JD, Lee AH-Y, Hassan JA, Wan J, Hurley B, Jhingree JR, Wang PW, Lo T, Youn J-Y, Guttman DS, et al. The Arabidopsis ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the Pseudomonas syringae type III effector HopZ1a. Proc Natl Acad Sci U S A 2013; 110:18722-7; http://dx.doi.org/10.1073/pnas.1315520110; PMID:24170858

Pathogens use effector proteins to suppress host immunity and promote infection. However, plants can recognize specific effectors and mount an effector-triggered immune response that suppresses pathogen growth. The YopJ/HopZ family of type III secreted effector proteins is broadly distributed in bacterial pathogens of both animals and plants. These effectors can either suppress host immunity or elicit defense responses depending on the host genotype. In a recent report, we identified an *Arabidopsis thaliana* pseudokinase ZED1 that is required for the recognition of the *Pseudomonas syringae* HopZ1a effector. Here we discuss the role of ZED1 in HopZ1a recognition, and present models of effector recognition in plants. We draw parallels between HopZ1a and YopJ effector proteins, and between ZED1 and other immunity-related kinases that can be targeted by pathogen effectors.

Pathogens and their hosts are engaged in a dynamic molecular arms race where the growth and reproductive success of one usually comes at a cost to the other. *Pseudomonas syringae* is a Gram-negative bacterial pathogen that infects a wide range of plant species. It employs a type III secretion system to secrete and translocate type III secreted effector (T3SE) proteins into its hosts. T3SEs primarily function to suppress plant immunity.[1,2,3] Plants protect themselves from pathogens using 2 layers of immunity. The first layer of immunity relies on recognition of microbe-associated molecular patterns (MAMPs; e.g., flagellin), and leads to pattern recognition receptor (PRR)-triggered immunity (PTI).[4] The second layer of immunity results from recognition of specific T3SEs by nucleotide-binding leucine-rich repeat receptors (NLRs), and leads to effector-triggered immunity (ETI).[5] ETI typically includes a rapid form of programmed cell death called the hypersensitive response (HR) that restricts bacterial proliferation.[5,6]

Recognition of T3SEs can occur through direct interaction between a T3SE and an NLR, or indirectly where the T3SE and NLR both interact with an intermediate protein. The "guard" model was first proposed to account for indirect recognition of a T3SE, and postulates that the modification of a host virulence target ("the guardee") by a T3SE is recognized by an NLR.[5,7] An extension of the guard model is the "decoy" model, in which an effector target undergoes duplication and neo – or non-functionalization to evolve a protein that has no inherent function except to serve as a sentinel for effector activity.[8,9] Importantly, the decoy model predicts that modification of the decoy by a T3SE can activate an NLR-mediated ETI response, but does not promote pathogen growth in the absence of recognition.

The YopJ/HopZ family of bacterial T3SEs is evolutionarily diverse and found in both mammalian and plant pathogens.[10] The *P. syringae* HopZ1a T3SE is recognized by the *Arabidopsis*

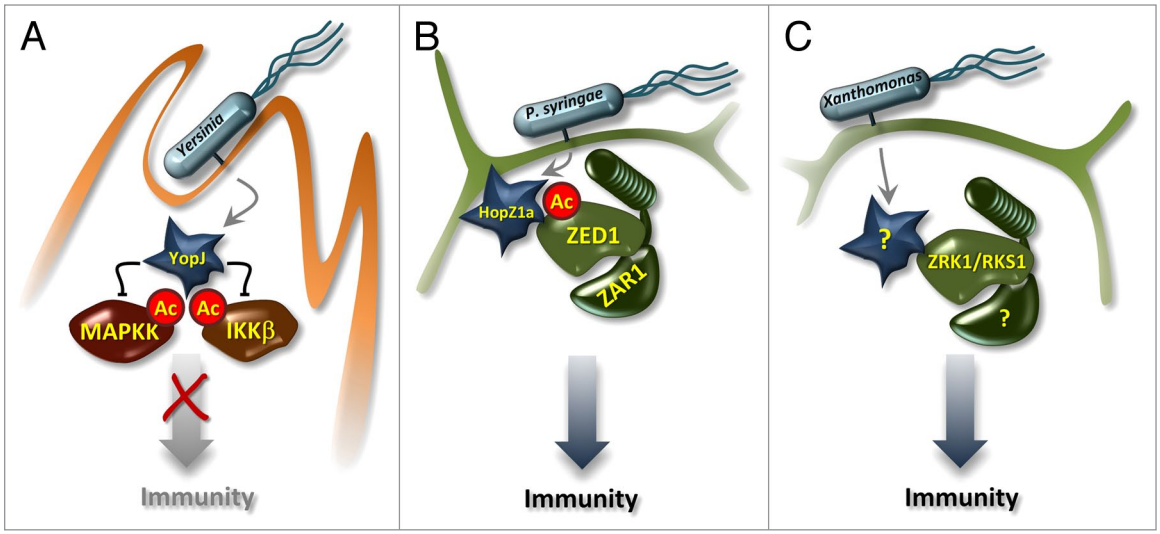

Figure 1. Recognition of YopJ, HopZ1a, and an unknown *Xanthomonas* T3SE. (**A**) *Yersinia spp.* injects YopJ into mammalian host cells. YopJ interferes with mitogen-activated protein kinase kinase (MAPKK) cascades via acetylation of MAPKK and IKKβ in the kinase binding site, thereby blocking downstream signaling and suppressing immune signaling. (**B**) *Pseudomonas syringae* injects HopZ1a into plant cells, where it is myristoylated and membrane-associated. HopZ1a acetylates ZED1, which triggers ZAR1-mediated immunity. (**C**) *Xanthomonas spp.* injects an unknown T3SE into plant cells that is recognized by ZRK1/RKS1 (a homolog of ZED1). The T3SE may modify ZRK1 to trigger immunity by an unknown NLR protein.

NLR protein ZAR1.[11] We hypothesized a model of indirect recognition of HopZ1a by ZAR1 since the acetyltransferase activity of HopZ1a is required to activate ZAR1.[11,12,13] To identify other host components necessary for HopZ1a recognition, we performed a forward genetic screen and identified multiple mutants in one locus that were deficient for HopZ1a ETI. We named this locus *ZED1* to reflect the *hopZ ETI-deficient* mutant phenotype.[14] Sequence analysis of ZED1 indicates that it is a pseudokinase that lacks the catalytic aspartic acid residue of the conserved HRD kinase motif.[15] We showed that ZED1 interacts directly with both HopZ1a and ZAR1, and that HopZ1a acetylates ZED1 at threonine 125 and threonine 177. Importantly, while ZED1 is required for HopZ1a-associated ETI, loss of ZED1 does not alter HopZ1a-associated virulence or PTI. Consequently, we propose that ZED1 acts as a decoy for HopZ1a, allowing the immune system to trap HopZ1a into the ZAR1 recognition complex. We hypothesize that once in this complex, ZED1 acetylation by HopZ1a contributes to ZAR1-mediated resistance (**Fig. 1B**).

ZED1 is a member of a clade of closely related kinases we named ZED1-related kinases (ZRKs). Seven ZRKs and ZED1 are co-localized in a 14kbp region of *Arabidopsis* chromosome 3. At least some of the ZRK family members are predicted to be functional kinases, including ZRK10, which we validated experimentally. As discussed above, recognition of HopZ1a requires both ZED1 and ZAR1,[11,14] but the absence of ZED1 does not eliminate HopZ1a's ability to promote bacterial growth. This strongly suggests that ZED1 is not the virulence target of HopZ1a, but is instead a decoy.[11,14] Consequently, we speculate that one or several other kinases, possibly in the ZED1/ZRK genomic cluster, are the true virulence targets of HopZ1a. We predict that HopZ1a acetylates these kinases, and that this acetylation promotes bacterial growth. This would be similar to the function of YopJ from *Yersinia pestis*, which acetylates and inactivates kinases to suppress their immunity-related functions (**Fig. 1A**).[14,15,16]

One of the ZRK family has recently been implicated in immunity against *Xanthomonas campestris*. Huard-Chauveau and colleagues independently identified resistance related kinase 1 (RKS1/ZRK1) as contributing to quantitative resistance against several strains of *Xanthomonas campestris*.[16] Like ZED1, ZRK1 appears to be a pseudokinase, although while ZED1 lacks a key catalytic residue, ZRK1 lacks residues involved in ATP binding.[14,16] It is possible that ZRK1 triggers ETI when modified by an unidentified *X. campestris* T3SE (**Fig. 1C**).

While pseudokinases such as ZED1 and ZRK1 are catalytically dead, they are not necessarily non-functional. Some pseudokinases lack catalytic sites but retain binding faces for protein-protein interaction.[17] Pseudokinases can also act as scaffolding proteins,[15] or bind ATP, and may also act as allosteric switches to regulate functional kinases.[18,19] With respect to ZED1, it is unlikely that it only acts as a scaffold to help recruit ZAR1 or other kinases to a resistance signaling complex since loss-of-function point mutations occur in the predicted ATP binding pocket of ZED1, suggesting that an ATP-dependent ZED1 function may be involved in HopZ1a recognition.

Pseudokinases are emerging as crucial components of numerous biological processes, demonstrating that these catalytically inactive proteins are anything but non-functional.[15] The studies discussed here have extended the roles of pseudokinases to plant immunity. Given the inherent structural conservation of kinase active sites,[20] the deployment of pseudokinase decoys may prove to be irresistible traps for pathogen effectors

that promiscuously target kinases to promote pathogen virulence. Further studies will show if plants commonly use this elegant mechanism to effectively turn the activity of a foreign molecule against itself.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Work on HopZ function and recognition is supported by Natural Sciences and Engineering Research Council of Canada awards to Guttman DS and Desveaux D; a Canada Research Chair in Plant-Microbe Systems Biology (Desveaux D) or Comparative Genomics (Guttman DS); the Centre for the Analysis of Genome Evolution and Function (Desveaux D and Guttman DS); United States Department of Agriculture Agricultural Research Service 5335–21000–040–00D (Lewis JD).

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