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# Pathogens manipulate host autophagy through injected effector proteins

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**ABSTRACT**

Macroautophagy/autophagy plays a dual role in many physiological processes of multicellular eukaryotes. In plants, autophagy can be used by both host and pathogen for a beneficiary infection outcome. Plants employ a two-tier innate immune system to defend against invading pathogens. Cell surface localized pattern recognition receptors recognize conserved pathogen-associated molecular patterns (PAMPs) and launch pattern-triggered immunity (PTI) to provide broad-spectrum resistance. Pathogens inject a battery of effector proteins into their hosts to counter PTI and compromise the primary immune response. Hosts induce a second layer of defense called effector-triggered immunity (ETI) to counter the effects of these effectors. In addition to ETI and PTI, autophagy is emerging as a central cellular process modulated by both host and pathogens toward their respective advantage. Pathogens lacking the ability to inject effectors are compromised in virulence. However, molecular targets and biochemical characterization of most of these effector proteins remain elusive. In a recent paper we presented a systematic analysis of interaction between autophagy proteins of *Arabidopsis thaliana* with effectors from bacterial, fungal, oomycete and nematode pathogens.

**Abbreviations**: ATG, autophagy related; BiFC, bimolecular fluorescence complementation; ETI, effector-triggered immunity; PAMPs, pathogen-associated molecular patterns; PTI, pattern-triggered immunity

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ATG4; ATG8; autophagy; host-microbe interaction; pathogenesis; *Pseudomonas syringe*

The homeostatic cellular role of autophagy is well studied in both unicellular and multicellular eukaryotes. However, the physiological role of autophagy beyond its housekeeping role in multicellular eukaryotes is perplexing. Autophagy plays a dual role in many physiological and disease states. In various cancers, autophagy can have a pro- or anti-tumor growth role depending on the context. Cell death mediated by autophagy has an anti-tumor effect while at the same time autophagy can help tumor growth by suppressing metabolic stress encountered by cancerous cells. Similarly, in host pathogen interaction, autophagy plays both pro- and anti-defense roles. Both host and pathogen can induce or inhibit autophagy to their own advantage during infection. However, the mechanistic basis of how hosts and pathogens regulate autophagy during the course of infection remains largely unknown. In a recent paper we showed that pathogens dedicate a significant proportion of their resources to modulate host autophagy [1].

Pathogens translocate effector proteins into host cells to manipulate cellular processes that benefit pathogen growth and disrupt immunity. Although 100's of effectors have been identified in different pathogens that infect plants, the molecular targets and the mechanistic basis of how they manipulate cellular processes that avoid host immune response to favor disease development is poorly understood. Because pathogens inject a limited number of effectors inside their host target, we hypothesized that if autophagy plays an important role in the overall outcome of infection, a good proportion of injected effectors must target autophagy. We designed a directed yeast two-hybrid screen to test interaction between different autophagy-related (ATG) proteins from *Arabidopsis thaliana* with effectors from various pathogens. We used 184 effector proteins from bacterial, fungal, nematode and oomycete pathogens, and 25 *Arabidopsis* core ATG proteins. We found a total of 88 interactions, confirming our hypothesis that pathogens use a significant proportion of their effector arsenal to target autophagy during infection. We further confirmed a subset of these interactions *in vitro* using purified proteins and *in planta* using a bimolecular fluorescence complementation (BiFC) assay.

To better understand the mechanism of these interactions we characterized the molecular basis of 3 effectors from the bacterial pathogen *Pseudomonas* in more detail. The first effector we characterized, HrpZ1, belongs to the harpin superfamily and has homologs in prominent human pathogens such as *Salmonella, Shigella* and *Yersinia*. In the yeast two-hybrid screen HrpZ1 showed strong interaction with ATG8. In contrast to a single *ATG8* gene in yeast, multiple *ATG8* orthologs have been identified in the plant kingdom. *Arabidopsis thaliana* has nine ATG8 isoforms, and it has been thought that most of these ATG8s have

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redundant functions. We tested the specificity of HrpZ1 and ATG8 interaction using purified proteins and *in planta* BiFC experiments. HrpZ1 specifically interacted with all *Arabidopsis* ATG8s except ATG8c and ATG8e. We further confirmed the interaction using purified proteins to show the specificity of interactions. Conditional expression of HrpZ1 leads to enhanced accumulation of cleaved GFP in GFP-ATG8 *Arabidopsis* lines. Consequently, HrpZ1 expression makes plants more susceptible to bacterial infection. We further showed that HrpZ1 forms a calcium-dependent oligomeric form to expedite ATG4b cleavage of ATG8a *in vitro*. We proposed a model in which oligomeric HrpZ1 holds ATG8a in a stretched form to expedite cleavage by ATG4b. Because HrpZ1 has homologs in multiple human pathogens it will be interesting to see if they also modulate autophagy through similar mechanisms in the mammalian system.

We next characterized the *Pseudomonas* effector HopF3, that forms one of the most diverse family of *P. syringae* effectors with more than 100 different forms. Similar to HrpZ1-ATG8 interaction, we found only a subset of ATG8 orthologs can interact with HopF3. Interestingly, whereas HrpZ1 could interact with all ATG8s except ATG8c and e, HopF3 could interact with all ATG8s except ATG8c and ATG8g. Further bolstering the idea that different ATG8s, despite having redundant functions, do show some level of specificity. Conditional expression of HopF3 in GFP-ATG8 lines showed that HopF3 selectively inhibits autophagy. To our surprise we found that although HopF3 inhibits autophagy it still makes plants more susceptible to infection. This was in stark contrast to HrpZ1 that enhances autophagy to increase virulence. To rule out the possibility that HopF3 has an autophagy-independent role in enhancing virulence, we generated HopF3 conditional expression transgenic lines in the *atg5* mutant background. We showed that the enhanced virulence phenotype of HopF3 requires a functional autophagy process.

Finally, we characterized an AvrPtoB-ATG1 interaction. AvrPtoB inhibits the kinase activity of multiple kinases involved in immune responses in *Arabidopsis*. To our surprise, when we tested if AvrPtoB can directly interact with the ATG1 kinase domain, we found that instead of the kinase domain, AvrPtoB interacts with the C-terminal MIT domain of ATG1. Using yeast two-hybrid, *in planta* BiFC and purified proteins we showed that a previously uncharacterized N-terminal domain of AvrPtoB interacts with the MIT domain of ATG1 and inhibits ATG1 auto-phosphorylation. Similar to HopF3, AvrPtoB suppresses autophagy to enhance virulence.

In summary, we provided evidence for multiple ways through which pathogens precisely manipulate autophagy for a favorable infection outcome. Single pathogens inject multiple effectors that target autophagy, and one effector enhances autophagy whereas another decreases it. The temporal and contextual differences of how these effectors are deployed during infection to manipulate autophagy will be of interest for future exploration.

## Disclosure statement

No potential conflict of interest was reported by the authors

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