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Research Highlight

Unraveling the mysteries of (L)WY-domain oomycete effectors

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Oomycetes (“egg fungi”), also known as Water Molds, form a large group of filamentous microorganisms, including many plant pathogens that pose serious threats to global food security. The oomycete *Phytophthora* species have been listed among the most destructive plant pathogens due to their capacity to cause huge damages to global crop production. The pandemic of potato late bight disease caused by *Phytophthora infestans* (*P. infestans*) was responsible for the infamous Irish potato famine from 1845 to 1852 [1]. Among a total of over 140 species in the genus of *Phytophthora*, *P. infestans* alone currently causes an annual economic loss of more than US$ 6 billion. To prevent crop losses by oomycete pathogens, it is imperative to have a better understanding on how they infect plants so that scientists can develop effective strategies for controlling their epidemics.

Plant pathogenic oomycetes rely on hundreds of secreted effectors to cause diseases. A major characteristics of oomycete effectors is that many of them contain a single or more than one (L)WY domains that consist of several leucine (L), one tryptophan (W), and one tyrosine (Y) [2]. Win et al. [3] found that 44 % of annotated RXLR effectors, which are defined by their conserved N terminal Arg-Xaa-Leu-Arg (RXLR) motif, in *P. infestans*, *Phytophthora ramorum*, and *Phytophthora sojae* (*P. sojae*) contain one or more (L)WY domain. In addition, (L)WY domain is also found in oomycete effectors without the canonical RXLR motif [4]. These (L)WY-domain effectors were also found in the grapevine downy mildew pathogen *Plasmopara viticola*, the lettuce downy mildew pathogen *Bremia lactucae*, the downy mildew of hop pathogen *Pseudoperonospora humuli*, and the *Arabidopsis* downy mildew pathogen *Hyaloperonospora arabidopsidis*, but not in *Pythium ultimum*, *Albugo laibachii*, and *Saprolegnia parasitica*, suggesting that they are restricted in *Peronosporales* [3–5]. Even though effectors with (L)WY domain are only found in oomycetes, structural studies have already provided evidence that two naturally occurring variants of avirulence protein M (AvrM), AvrM-A and avrM form an L-shaped fold consisting of a tandem duplicated four-helix motif, which is similar to the (L)WY domain core in oomycete effectors [6]. AvrM is a secreted effector protein from the flax rust fungal pathogen *Melampsora lini*. As it is well known, the functions of proteins are determined by their tertiary structures. Therefore, a deeper understanding of the (L)WY domain-containing oomycete effectors could provide insights on these fungal effectors and contribute to the control of plant fungal diseases.

Early studies have revealed the molecular functions of some of these (L)WY-domain effectors. The RXLR-(L)WY effector avirulence protein 3a (AVR3a) from *P. infestans*, for example, has been found to prevent cell death during the biotrophic phase of infection by stabilizing the E3 ligase CYS, MET, PRO, and GLY 1 (CMPG1) [7]. Meanwhile, AVR3a associates with the dynamin-related protein 2 (DRP2), a plant GTPase required for the endocytosis of the pathogen-associated molecular pattern (PAMP) flagellin receptor FLAGELLIN-SENSING (FLS2), to suppress PAMP-triggered immunity (PTI). Additionally, another (L)WY-domain RXLR effector suppressor of early flg22-induced immune response (SFI3) from *P. infestans* targets U-box-kinase (UBK) protein to dampen early immune transcriptional responses, thereby attenuating PAMP-triggered immunity [8]. Furthermore, PexRD31 from *P. infestans* has been shown to target vesicle trafficking [9], while the RXLR effector PsAvh240 from *P. sojae* inhibits the secretion of soybean aspartic protease 1 (GmAP1) to promote infection [10]. However, despite these studies, our understanding of the functions of the majority of these (L)WY domain-containing effectors in promoting disease development and the evolution of these effectors remains very limited. Recently, a fascinating *Cell* paper uncovered unique functions of a group of oomycete effectors [2].

Phytophthora suppressor of RNA silencing 2 (PSR2) is an effector from *Phytophthora sojae*, which is the causal agent of stem and root rot in soybean. Earlier structural studies revealed that PSR2 consists of α-helical folds, forming a stick-like structure [11]. To identify PSR2-interacting proteins in *Arabidopsis thaliana* plants, Li et al. [2] performed immunoprecipitation assays followed by mass spectrometry analysis and found serine/threonine protein phosphatase 2A (PP2A) in the PSR2-associated protein complex.

PP2A is a highly conserved heterotrimeric core phosphatase in eukaryotes, with its active form consisting of a structural A subunit, a regulatory B subunit that confers substrate specificity, and a catalytic C subunit [2]. In mammals, PP2A regulates diverse cellular functions, such as cell proliferation, signal transduction, and apoptosis, by catalyzing over 50% Ser/Thr dephosphorylation in most cell types [2]. *Arabidopsis* genome encodes three A subunits, seventeen B subunits, and five C subunits. Li et al. [2] revealed that

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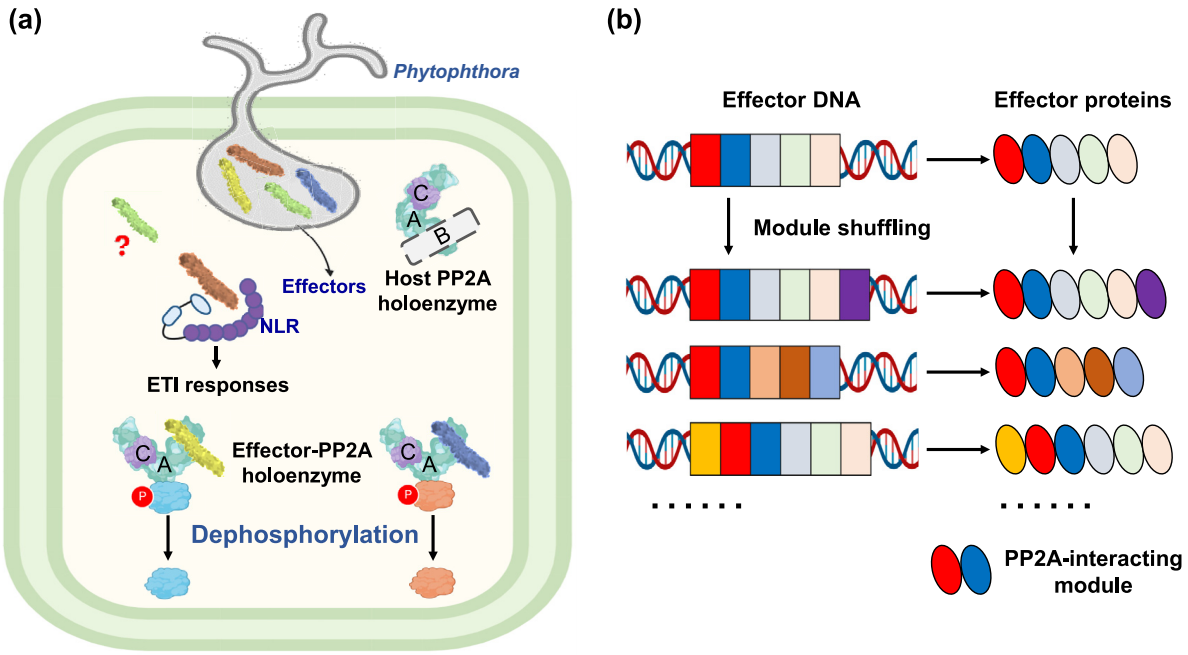

Fig. 1. A proposed model illustrating how *Phytophthora* effectors hijack the plant core phosphatase PP2A and the role of domain shuffling in driving functional diversity of *Phytophthora* effectors with tandem repeats of (L)WY units. (a) Two *Phytophthora* effectors with tandem repeats of (L)WY units, PSR2 and PITG_1512, mimic and outcompete the B subunit of plant core phosphatase PP2A. They interact with PP2A and hijack PP2A to form effector-PP2A holoenzymes. The effector-PP2A holoenzymes alter the phosphorylation status of host proteins by dephosphorylating different substrates to favor pathogenesis. It has been shown that a large group of *Phytophthora* (L)WY effectors act as avirulence proteins, and they are presumably recognized by nucleotide binding leucine-rich repeat (NLR) resistance proteins to activate effector-triggered immunity (ETI). The biochemical functions and molecular targets of many *Phytophthora* (L)WY effectors remain unknown. (b) Shuffling of functional modules promotes diversity of the *Phytophthora* effectors with tandem repeats of (L)WY units. The relatively conserved PP2A-interacting module is in the N terminus of these effectors. The functional diversity of these effectors is promoted by the rearrangement of their C terminal regions through domain shuffling. The figure was created with the software BioRender (BioRender.com).

PSR2 forms a protein complex with the A subunit of PP2A through its LWY2 and LWY3 units (Fig. 1a). Notably, the binding affinity of PSR2 to PP2A was much higher than an *Arabidopsis* PP2A B subunit. Thus, PSR2 mimics and outcompetes the B subunits of PP2A, thereby hijacking the PP2A to form a new effector-PP2A holoenzyme (Fig. 1a) [2].

Detailed structural analysis showed that ten residues in LWY3 and two residues in LWY2 "WY" portion of PSR2 were involved in the interaction between PSR2 and PP2A [2]. Therefore, the authors speculated that any effectors with the (L)WY2-LWY3 domain would target the PP2A core phosphatase. After thorough analyses, Li et al. [2] selected 15 effectors and found 12 out of these 15 effectors associate with the PP2A core enzyme. All these 12 effectors and PSR2 have a minimum of 5 WY/LWY tandem repeats and the predicted PP2A-interacting module is exclusively located on the N terminus. These 13 effectors show diverse localization patterns, suggesting that they have different functions.

To investigate the impact of effector-PP2A holoenzyme on host proteins, Li et al. [2] made *Arabidopsis* transgenic plants expressing PSR2 or another effector with tandem repeats of (L)WY units, PITG_1512. They found that both PSR2 and PITG_1512 promote pathogen infection by regulating the phosphorylation of proteins in the host plants (Fig. 1a). However, PSR2 and PITG_1512 affect the phosphorylation of almost totally different sets of proteins. Consistent with these observations, Li et al. [2] used immuno-precipitation in conjunction with mass spectrometry assays to identify PSR2 and PITG_1512 interacting plant proteins and they found that the only common protein that associates with both PSR2 and PITG_1512 is PP2A, further confirming functional divergence between PSR2 and PITG_1512.

Why do *Phytophthora* pathogens carry so many effectors with (L)WY tandem repeats? How were these effectors generated in *Phytophthora* species? Li et al. [2] proposed that functional diversification of *Phytophthora* effectors with (L)WY tandem repeats could be the result of domain shuffling (Fig. 1b). The PP2A-interacting (L)WY2-LWY3 module is in the N terminal of these effectors, while the diversity of these effectors is largely derived from the rearrangement of C terminal LWY units.

Mimicking eukaryotic molecules is a common strategy that is deployed by many pathogens, including viruses, bacteria, oomycetes, and fungi, to cause diseases [12]. Many plants pathogens secret mimics of plant peptides, plant proteins, or produce mimics of plant hormones and small RNAs to cause diseases [12]. In this study, Li et al. [2] have shown that at least thirteen *Phytophthora* effectors target PP2A, which is also a common virulence target of oncogenic viruses and *Pseudomonas syringae* type III effector AvrE and its homolog DspA/E in *Erwinia amylovora* [2]. As *PP2A* functions as an important host susceptibility gene, genome editing of *PP2A* using CRISPR/Cas9 to prevent the targeting of the PP2A protein by these pathogens is likely to create hosts that are resistant to these pathogens. This is especially important for *Phytophthora* species since at least thirteen *Phytophthora* effectors all target PP2A. In this case, genome editing of effector-interacting region in PP2A to prevent the interaction between *Phytophthora* effectors and PP2A holds promise for creating *Phytophthora*-resistant plants.

The swift evolution of these effectors may be attributed to the presence of (L)WY tandem repeats. It is well known that tandem repeats are extremely unstable since their mutation rates are usually 10–100,00 times higher than the rest of the genome [13]. The

authors proposed that domain shuffling drives the functional diversification of *Phytophthora* effectors with tandem repeats of (L)WY units (Fig. 1b) [2]. What is the molecular mechanism of this domain shuffling? The available literature indicates that the genes encoding *Phytophthora* effectors tend to reside in gene-sparse genomic regions with a lot of transposons, which could generate high levels of diversity through recombination [14,15].

Li et al. [2] discovered in their study that PSR2-PP2A and PITG_1512-PP2A holoenzymes are capable of altering the phosphorylation of hundreds of host proteins either directly or indirectly, potentially affecting the functions of downstream unidentified host targets. Effector-PP2A holoenzyme might promote the functions of plant susceptibility genes and suppress the roles of plant resistance genes. Identifying the targets of effector-PP2A holoenzyme should benefit the identification of positive and negative regulators of plant immunity. It is worthwhile to emphasize that the biochemical functions and molecular targets of many *Phytophthora* (L)WY effectors remain obscure (Fig. 1a).

In addition, it has been shown a large group of *Phytophthora* (L)WY effectors function as avirulence (Avr) proteins to induce effector-triggered immunity (ETI) in plants (Fig. 1a). Moreover, the nucleotide binding leucine-rich repeat (NLR) proteins recognizing these effectors have also been identified. For instance, *Arabidopsis* NLR protein RPP1 has been found to recognize the effector ATR1 with tandem repeats of (L)WY domain from *H. arabidopsidis* through direct interaction. The interaction between ATR1 and the C-terminal jelly roll/Ig-like domain (C-JID) as well as the LRR of RPP1 leads to the RPP1 tetrameric assembly that is essential for the nicotinamide adenine dinucleotide hydrolase (NADase) activity [16]. Furthermore, a unique NLR protein Rpi-amr3 has been shown to confer resistance to multiple *Phytophthora* pathogens including the tobacco black shank disease pathogen *P. parasitica* and cacao black pod disease pathogen *P. palmivora*, in addition to *P. infestans*, through its association with the conserved RLXR-(L)WY effector AVRamr3 [17].

More in-depth structural studies could help us better understand how (L)WY-domain oomycete effectors, such as PSR2 and PITG_1512, alter PP2A's functions to favor pathogenicity. Future research on the identification of molecular targets of more oomycete (L)WY-domain effectors and characterizations of more NLR resistance proteins recognizing this type of effectors should contribute to the development of novel and effective strategies for controlling devastating plant diseases caused by oomycetes.

Conflict of interest

The authors declare that they have no conflict of interest.

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