Effector–Decoy Pairs: Another Countermeasure Emerging during Host–Microbe Co-evolutionary Arms Races?

Plant pathogenic microbes pose a significant threat to food production, collectively affecting all cultivated crops. Given the impact of these pathogens on food security, there continues to be an urgent need to understand and exploit the biology of pathogenesis, plant susceptibility, and immunity in crop systems. Consequently, intense research efforts have helped define the molecular and evolutionary events that underpin plant-microbe interactions.

To thrive in their biotic environments, plants have acquired and evolved a robust multi-tier immune system, able to keep most would-be pathogens at bay. Microbes, able to overcome or compromise the structural and/or chemical barriers of the plant, are perceived by pattern recognition receptors (PRRs) that populate the host cell membrane. These PRRs recognize conserved microbe-associated molecular patterns (MAMPs) or damage-associated molecular patterns (DAMPs), and signal a microbial threat. PRR activation initiates a cellular defense response (pattern-triggered immunity [PTI]) that in most cases limits microbial ingress and prevents disease (Jones and Dangl, 2006).

Per definition, plant pathogens are adapted microbes able to infect, colonize, and reproduce on their hosts despite PTI. This suggests the presence of specialized pathogen strategies employed to inflict structural damage, promote nutrient loss, or suppress plant immunity. Genome sequencing, functional genomics, and detailed biochemical studies have firmly implicated secreted proteins (effectors) in these processes, leading to the widely held view that pathogen effectors promote host susceptibility (effector-triggered susceptibility, ETS) and enable pathogen-virulence (effector-triggered immunity, ETI) in its host soybean (Glycine max) and solanaceous species (Ma et al., 2015). These results, combined with known prevalence of secreted glucanase inhibitors in plants, prompted an immunoprecipitation-based approach to identify candidate PsXEG1 inhibitors in the host. Analyses of host proteins in complex with PsXEG1-EGFP resulted in the identification of soybean Glucanase Inhibitor Protein 1 (GmGIP1), a protein with 37% amino acid identity to tomato-XEG1.

Subsequent co-immunoprecipitation (CoIP), in vitro pull-down assays, and biochemical assays enabled the authors to establish that binding of GmGIP1 to PsXEG1 is strictly correlated with inhibition of xyloglucanase activity, providing strong evidence that inhibition is a direct consequence of GmGIP1 binding. Given that deletion or inactivation of PsXEG1 in vivo (by CRISPR/Cas9-mediated gene knockout and gene replacement, respectively) as well as GmGIP1 overexpression impairs P. sojae infection, GmGIP1 appears to be an important player in apoplastic immunity against P. sojae. It also raises the critical question as to why native levels of GmGIP1 are not sufficient to limit P. sojae infection or indeed PsXEG1 activity in vivo (Ma et al., 2017).

PsXLP1, AN INACTIVE PARALOG OF PsXEG1 IN P. SOJAE, CONTRIBUTES TO VIRULENCE

The inability of native levels of GmGIP1 to limit P. sojae infection suggests the presence of pathogen-encoded co-factors that...
inhibit GmGIP1 and thereby protect PsXEG1. Since PsXEG1 is a member of a protein family in *P. sojae*, the ability of PsXEG1 paralogs to bind GmGIP1 was tested. From these analyses one paralog (*P. sojae* XEG1-Like Protein 1 or PsXLPI) was found to bind GmGIP1. PsXLPI has 67% amino acid identity with PsXEG1 and, importantly, has a 52-residue truncation at the C terminus that results in a loss of hydrolase activity when tested in *Nicotiana benthamiana*. Despite a loss in catalytic activity, gene expression analyses of PsXLPI in time-course infection assays revealed tight correlation with PsXEG1 gene expression patterns, suggestive of a function beyond xyloglucan degradation. Indeed, mutants in which PsXLPI was disrupted or replaced were found to be severely restricted in their ability to infect host plants, whereas PsXLPI overexpression increased pathogen virulence. This indicated that despite a lack of catalytic activity toward xyloglucan, PsXLPI contributes to virulence (Ma et al., 2017).

**COUNTER-COUNTERMEASURES REVEALED: *P. SOJAЕ DEPLOYS PSXLPI AS A PSXEG1 DECOY***

To understand the mechanism by which PsXLPI contributes to virulence, two PsXLPI mutants were generated. In PsXLPI<sup>E136A</sup>, a mutation in the remaining theoretical active site was introduced (E to A), whereas in PsXLPI<sup>X1,2,3</sup> three theorized GmGIP1 contact sites were mutated (regions X1, X2, and X3). CoIP of both mutants showed that PsXLPI<sup>E136A</sup> binds very weakly to GmGIP1 whereas PsXLPI<sup>X1,2,3</sup> binds as strongly as wild-type PsXLPI. Overexpression of the mutants demonstrated correlation between PsXLPI’s ability to bind GmGIP1 and enhance virulence. This suggests that PsXLPI exerts its virulence function by binding to the inhibitor of PsXEG1. These results invoke a model in which PsXLPI binding to GmGIP1 protects catalytically active PsXEG1 from being inactivated.

To test this model, competition assays were performed in which PsXEG1 binding to GmGIP1 was assessed in the presence or absence of PsXLPI. CoIP of PsXEG1 showed that PsXLPI displaces PsXEG1. Critically, subsequent measurements of dissociation constants (K<sub>d</sub>) revealed that PsXLPI bound more tightly to GmGIP1 than to PsXEG1. These results suggest that PsXLPI is required for PsXEG1 activity, by acting on the host inhibitor GmGIP1. Importantly, transgenic soybean plants overexpressing PsXLPI did not boost virulence of PsXEG1-defective mutants, showing that PsXLPI binding to GmGIP1 is solely intended to protect PsXEG1 activity and does not contribute to virulence independent of PsXEG1. Indeed, in planta experiments showed that both PsXEG1 and PsXLPI are required to increase apoplastic sugar levels (indicative of PsXEG1 activity) during infection. These results thus invoke a virulence strategy that relies on the simultaneous secretion of an effector–decoy pair during infection (Ma et al., 2017).
IMPLICATIONS AND PERSPECTIVES

It is widely accepted that both plants and their pathogens can be engaged in a co-evolutionary arms race in which molecular innovations are made. Perhaps, therefore, it is not surprising that pathogens have adopted counter-defense strategies that resemble those found in plants. It is well known that plants deploy decoys in their bid to counter pathogen infection (Figure 1). A good example is the Avr2, RCR3, PIP1 interaction (Shabab et al., 2008). AVR2 is a protease inhibitor secreted by the plant pathogen *Cladosporium fulvum* to inhibit the action of the protease PIP1. During infection, AVR2 is secreted into the apoplast where it binds PIP1 and RCR3. Although RCR3 does not act to capture or inactivate AVR2, it acts as a decoy by triggering Cf2-dependent cell death and ETI. Importantly and in contrast to the *PsXEG1* and *PsXLP1* model, RCR3 and PIP1 do not appear to be co-dependent. Although gene duplication and subsequent divergence can lead to decoy strategies in plant and pathogen that are largely analogous (Van Der Hoorn and Kamoun, 2008), important differences remain on both a mechanistic and functional level. Nonetheless, gene duplication and diversification are emerging as key events that could lead to the evolution of effector–decoy pairs. The discovery of truncated transcription activator like effectors (TALEs) in the plant pathogen *Xanthomonas oryzae*, able to suppress TALE-induced ETI (Ji et al., 2016; Read et al., 2016), is a pertinent example of this phenomenon in plant pathogens. Thus, deployment of seemingly inactive pseudogenes to protect key effector activities and undermine the host immune system represents a new virulence strategy to achieve ETS in host plants. The presence of large and complex effector gene families undoubtedly points to analogous and as yet undefined molecular innovations that are likely to emerge in the future.

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