Bodyguards: pathogen-derived decoys that protect virulence factors

Judith K. Paulus, Jiorgos Kourelis, Renier A. L. van der Hoorn

The Plant Chemetics Laboratory, Department of Plant Sciences, University of Oxford, OX1 3RB Oxford, UK

Recent studies on plant-pathogen interactions have exposed a new strategy used by plant pathogens: decoy effectors that protect virulence factors. Examples of these "bodyguards" include the recently discovered PsXLP1 from *Phytophthora sojae* and truncated TALEs from *Xanthomonas oryzae*. These examples suggest important roles for seemingly non-functional effector proteins in distracting the host.

The Xyloglucan-specific Endo Glucanase (*Ps*XEG1) acts as an important virulence factor of the soil-borne oomycete pathogen *Phytophthora sojae* [1]. During infection, *Ps*XEG1 is secreted into the apoplast of its host soybean (*Glycine max*) to macerate the host cell wall. However, *Ps*XEG1 is recognized by the plant's recognition machinery as a Pathogen-associated Molecular Pattern (PAMP), independent of its enzymatic activity [1]. *P. sojae* is able to suppress PAMP-triggered immune responses caused by *Ps*XEG1, presumably by injecting multiple intracellular RxLR effectors [1]. But in addition to being recognized as a PAMP, *Ps*XEG1 is targeted by a secreted endoglucanase inhibitor (*Gm*GIP1) of soybean. *Gm*GIP1 binds to *Ps*XEG1 and strongly inhibits XEG1-catalyzed depolymerisation of xyloglucan, reducing the virulence role of *Ps*XEG1 for *P. sojae* [2].

The pathogen, however, protects *Ps*XEG1 from host inhibition by secreting a more tightly binding, truncated paralog of *Ps*XEG1, called *Ps*XLP1. *Ps*XLP1 has lost one of the residues critical for enzyme activity and has no known function in pathogenicity, other than to intercept *Gm*GIP1 [2]. This leads to a 'bodyguard' model in which *Ps*XLP1 has evolved as a paralogous decoy to neutralize *Gm*GIP1 and prevent inhibition of *Ps*XEG1 (**Figure 1A**). The conservation of *Ps*XLP1 paralogs across *Phytophthora* species suggests that these oomycete pathogens use these paralogous decoys frequently to bodyguard *Ps*XEG1-like effectors [2].

The publication of Ma et al., 2017 [2] strengthens this exciting new area in the field of host-pathogen interactions. The described pathogen decoy is distinct from the decoy function of host-derived factors as described in the Decoy Model, where decoys act as co-receptors or sensors with immune receptors [3]. Both host- and pathogen-derived decoys are specialized, often paralogous forms of a functional effector target or effector, respectively. But in contrast to host-derived decoys that trap effectors to mount effector-triggered immunity (ETI), pathogen decoys protect virulence factors as bodyguards.

Two recent publications describe a distinct but similar bodyguard strategy used by the gram-negative bacterium *Xanthomonas oryzae* [4,5]. *X. oryzae* pathovars *oryzae* (*Xoo*) and *oryzicola* (*Xoc*) cause devastating bacterial blight (BB) and bacterial leaf streak (BLS) in rice (*Oryza sativa*), respectively. *Xanthomonas* strains express a variety of Type-III effectors, including many Transcription activator-like effectors (TALEs) [6,7]. TALEs are major virulence determinants that act by *trans*-activating host genes in the plant cell nucleus by binding to promoter elements (Effector-binding Elements, EBEs) in a sequence-specific manner.

TALEs contain several nuclear localization signals (NLSs) that target these effectors to the host cell nucleus. TALEs also carry a C-terminal acidic activation domain (AD) that activates host gene expression, and a central repeat domain that directly binds to the EBE in the DNA. Resistance to *Xoo* has mostly been attributed to the action of TALEs, either by polymorphisms in EBEs that prevent the induction of susceptibility (*S*) genes, or by the induction of executor genes that carry EBEs embedded in their promoter and induce immune responses as dominant *R* genes [8].

However, some TALEs are recognised by classical *R* genes that encode NLRs (Nod-like Receptors). The TALE AvrBs4 of *X. euvesicatoria* is recognized by the *Bs4*-encoded NLR protein in tomato [9], whilst all tested, full length TALEs of *Xoo* are recognized by the *Xa1*-encoded NLR in rice [4]. Interestingly, however, both the Yang (Iowa State University, USA) and Bogdanove (Cornell University, USA) laboratories found a new class of truncated TALEs that could suppress TALE recognition by these NLRs.

The Yang group demonstrated that TALE perception by the NLR Xa1 is suppressed by two groups of truncated TALE variants that interfere with Xa1, which they coined “iTALEs”. The frequent occurrence of iTALEs could explain the narrow resistance spectrum of Xa1, even though it appears to perceive most, if not all TALEs [4]. Similarly, the Bogdanove group found that *Xo1* resistance in a different rice variety is mediated by the perception of TALEs, and that this perception can be blocked by truncated TALEs – “truncTALEs” - such as Tas2h from *Xoc* strain BLS256. In contrast to *Xa1*, *Xo1* has not been cloned yet, but the *Xo1* locus is allelic to *Xa1*, confers resistance to both *Xoc* and *Xoo*, and contains six candidate *R* genes encoding NLRs, indicating that *Xo1* encodes an NLR conferring TALE perception [4,10,11].

The truncated Tas2h TALE lacks N- and C-terminal regions that are important for DNA binding and this implies that Tas2h does not act by binding host DNA [5]. Both iTALEs and truncTALEs were previously annotated as pseudogenes due to their N- and C-terminal deletions. Both studies conclude that the truncated TALE proteins bind to TALE-recognizing NLR proteins to block the TALE binding site without activating the NLR receptor, to prevent the recognition of full length TALEs that act as virulence factors (**Figure 1B**). Even though there is strong genetic evidence, this hypothesis still requires experimental support to demonstrate a direct interaction of TALEs with the Xa1/Xo1 NLRs

and that truncated TALEs interfere with this interaction by having a higher affinity for NLRs. These are notoriously challenging experiments as NLR proteins are difficult to produce and purify.

Both XLP1 and truncated TALEs illustrate that paralogous decoys can evolve to protect important virulence factors to prevent their inactivation or recognition. Theoretically, these bodyguards have no other function in disease development, meaning that they have no role in the absence of the virulence factor they are mimicking, or in the absence of host proteins that inactivate or recognize this virulence factor. These bodyguard effectors probably have a higher affinity to the host protein and/or higher abundance when compared to the corresponding virulence factor. Both mechanisms allow the virulence factor to remain unaffected or undetected.

The evolution of a bodyguard that protects a single virulence factor may seem very costly. From an evolutionary standpoint, it might seem more efficient for a pathogen to develop effectors that interfere broadly with immune signalling. However, this bodyguard strategy might be worthwhile for critical effectors. In addition, the example of truncated TALEs illustrates how one bodyguard can simultaneously protect a larger class of virulence factors.

The production of decoy effector proteins by two unrelated pathogens highlights a common strategy of pathogens to protect virulence factors. These observations encourage new searches in pathogen genomes to investigate truncated or seemingly inactive effector proteins that were previously considered pseudogenes. Increased knowledge on these mechanisms will enable us to engineer the host immune system to circumvent manipulation by bodyguard effectors.

Figure 1 Two bodyguards at work: effector decoy XLP1 prevents host protein GIP1 from inhibiting glucanase XEG1 (A); and truncated TALEs prevent TALE effectors from being recognized by immune receptors Xa1/Xo1 (B).

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