Decoy engineering: the next step in resistance breeding

Jiorgos Kourelis, Renier A. L. van der Hoorn, Daniela J. Sueldo

The Plant Chemetics Laboratory, Department of Plant Sciences, University of Oxford, Oxford, UK

Correspondence: renier.vanderhoorn@plants.ox.ac.uk (R.A.L.van der Hoorn)

Keywords: PBS1 // AvrPphB // RPS5 // Disease resistance // Decoy model // Protein engineering

The recent finding that decoy engineering can expand the recognition specificity of a plant immune receptor opens a wealth of opportunities for resistance breeding. In this Spotlight we discuss which factors should be considered to successfully translate decoy engineering into crop species.

Until recently it was not possible to engineer novel recognition specificities of classical plant immune receptors to completely unrelated effectors. In a recent publication in Kim *et al.* engineered a plant effector target to increase novel recognition specificities by trapping unrelated pathogen-derived proteases in their act [1].

RESISTANCE TO PSEUDOMONAS SYRINGAE 5 (RPS5) is a plant immune receptor of the Nucleotide-binding, leucine-rich repeat (NLR) type which perceives the *Pseudomonas syringae* Type-III effector AvrPphB, a papain-like cysteine protease belonging to the Peptidase C58 family [2]. The perception of AvrPphB by RPS5 requires one additional host-derived factor known as AVRPPHB SUSCEPTIBLE 1 (PBS1), which belongs to Subfamily VII of Receptor-like Cytoplasmic Kinases (RLCK VII). Upon bacterial infection, PBS1, which binds to RPS5 in its pre-activation state, is cleaved by AvrPphB. PBS1 cleavage exposes a five amino acid loop in PBS1 that is believed to activate RPS5, triggering an immune response characterized by the hypersensitive response (HR), a form of programmed cell death [3]. Interestingly, RPS5-mediated immune signaling requires both PBS1 fragments, and the conformational change induced by cleavage can be mimicked by insertion of five amino acids in the AvrPphB cleavage site [4]. Therefore, perception of AvrPphB follows a mouse-trap mechanism where cleavage of PBS1 (bait) sets off the trap and activates RPS5, triggering immune responses.

RPS5 detects PBS1 cleavage through its concomitant conformational change, irrespective of the presence of AvrPphB. Based on this, Kim *et al*. inferred that an RPS5-mediated immune response against other pathogens could be engineered by exchanging the AvrPphB-cleavage sequence with the cleavage sequence of other pathogen-secreted proteases (Figure 1). To test this, three PBS1 mutant alleles were generated, containing

cleavage sites of three different pathogen-derived proteases: the Type-III effector AvrRpt2 of *P. syringae* (PBS1^{RCS2}); the NIa protease of Tobacco Etch Virus (TEV) (PBS1^{TCS}); and the NIa protease of Turnip Mosaic Virus (TuMV) (PBS1^{TuMV}). As predicted, these PBS1 mutant proteins were specifically cleaved only upon co-expression with the corresponding pathogen-derived protease, triggering RPS5-dependent HR. Importantly, stable expression of PBS1^{RCS2} under its own promotor in Arabidopsis provided resistance against *P. syringae* expressing AvrRpt2 in the absence of RPS2 and RIN4, two proteins required for AvrRpt2-triggered immunity. In addition, these lines recognize AvrPphB, as they still carry the wild-type *PBS1* allele, indicating that expression of *PBS1* mutant alleles does not interfere with the RPS5-dependent perception of AvrPphB by wild-type PBS1. Likewise, Arabidopsis lines stably expressing PBS1^{TuMV} mounted an immune response upon infection with TuMV, which expresses the TuMV-Nla protease. The ease by which novel specificities are generated by PBS1 modifications makes one wonder if PBS1 homologs might have evolved to confer different resistance specificities.

But there are important considerations when modifying a decoy to engineer novel pathogen recognition specificities. First, for an immune response to be effective and timely, all components involved in perception should localize to the same subcellular compartment. Indeed, whereas the RPS5/PBS1 complex localizes to the plasma membrane (PM), the TuMV-Nla protease mainly resides in the nucleus [5]. Hence, though cleavage of PBS1^{TuMV} by TuMV-NIa activates RPS5, the induction of the immune response is delayed to a late stage of infection, when enough TuMV-Nla protein has accumulated in the cytoplasm and the virus has spread. As a result of this, Arabidopsis lines overexpressing PBS1^{TuMV} suffered from trailing necrosis upon infection with TuMV, a phenomenon wherein HR trails the spread of the virus. Kim *et al.* suggest targeting RPS5/PBS1 to the same subcellular location as TuMV-NIa protease to circumvent this issue. However, several studies have shown that subcellular localization is important for the proper activation of multiple NLR proteins, including RPS5 [5, 6]. Targeting RPS5 to a different subcellular compartment could impair its

downstream signaling. Second, there must be constraints as to how much PBS1 can be altered without triggering auto-immune responses. For example, engineering PBS1 to recognize a pathogen-derived protease with a similar substrate specificity as a host protease would inevitably trigger auto-immunity. In addition, a slight alteration of the PBS1 sequence may be enough to trigger HR. For instance, inserting five amino acids in the AvrPphB cleavage site triggers auto-immune responses [4]. Finally, in case an effector target has an important physiological function, modifications of such an effector target should not impair its original function or interfere with the wild-type effector target when both alleles are present.

PBS1-based protease traps may be easily extended to perceive proteases from other pathogens. Pathogen-derived proteases are important effectors, and are produced by many unrelated pathogens. Given the fact that these effector proteases alter the host cell, they must have a specificity or spatial/temporal regulation that makes their activity distinct from endogenous host proteases. This specificity can be exploited while building other PBS1-like protease traps. Interestingly, plants have evolved additional mechanisms to recognize pathogen-derived proteases through their activity. RIN4, for instance, is an effector target whose cleavage by AvrRpt2 triggers immunity mediated by the NLR RPS2 [6]. It would be interesting to explore whether replacing the AvrRpt2 cleavage site in RIN4 for that of AvrPphB activates RPS2-mediated HR. However, we predict that engineering RIN4 is more challenging than PBS1, as the AvrRpt2 cleavage site overlaps with a region that is crucial for other immune-related functions [7]. Several pathogen-derived apoplastic proteases, such as *Xanthomonas campestris* argC and *Clavibacter michiganensis* Chp-7 and ChpG are perceived in an activity-dependent manner in *Brassicaceae* and *Nicotiana* subspecies, respectively [8, 9]. Identification of the molecular components underlying perception of these extracellular proteases could facilitate engineering the perception of apoplastic pathogen-derived proteases, mediating recognition in subcellular compartments other than the cytoplasm.

In contrast to effectors with other enzymatic activities, the identification of preferential cleavage motifs of effector proteases can be relatively straightforward, for example by simple proteomic techniques such as PICS (Proteomic Identification of protease Cleavage Sites) [10]. Furthermore, only a few amino acids in the host target need to be substituted, as protease specificity is usually determined by short peptide sequences, thereby reducing the chance of unintended side effects. Engineered decoy proteins can be quickly screened by transient expression for specific cleavage by effector proteases and triggering HR to facilitate pre-selection before generating stable lines.

Also other decoys can be engineered to change their specificity. Apart from effector target cleavage, also inhibition, phosphorylation and other post-translational modifications of guarded effector targets have been reported. For example, tomato Cf-2 is a transmembrane immune receptor that perceives the interaction of the apoplastic cysteine protease Rcr3 with Avr2, a protease inhibitor produced by the fungus *Cladosporium fulvum* [11]. It would be interesting to investigate if the concept of decoy engineering can be extended to this perception mechanism. Likewise, the promotors of the so-called executor genes have been successfully engineered to trap unrelated TAL (Transcription-activator like) effectors produced by *Xanthomonas spp* [12[].

In conclusion, the work of Kim *et al.* provides a proof-of-principle for new crop protection strategies, by showing that pathogen-derived proteases can be tricked into cleaving PBS1, thus turning this host kinase into a versatile protease trap.

ACKNOWLEDGEMENTS

The authors are financially supported by ERC Consolidator grant 616449 ‘GreenProteases’, the University of Oxford and The Clarendon Fund.

REFERENCES

1. Kim, S. H., *et al.* (2016) Using decoys to expand the recognition specificity of a plant disease resistance protein. *Science.* 351, 684–687.
2. Shao, F. *et al.* (2003) Cleavage of Arabidopsis PBS1 by a bacterial type III effector. *Science.* 301, 1230–1233.
3. Qi, D. *et al.* (2014) Recognition of the protein kinase AVRPPHB SUSCEPTIBLE1 by the disease resistance protein RESISTANCE TO PSEUDOMONAS SYRINGAE5 Is dependent on S-acylation and an exposed loop in AVRPPHB SUSCEPTIBLE. *Plant Physiol.* 164, 340–351.
4. Deyoung, B. J. *et al.* (2012) Activation of a plant nucleotide binding-leucine rich repeat disease resistance protein by a modified self protein. *Cell. Microbiol.* 14, 1071–1084.
5. Qi, D. *et al.* (2012) Structure-function analysis of the coiled-coil and leucine-rich repeat domains of the RPS5 disease resistance protein. *Plant Physiol.* 158, 1819-1832.
6. Gao, Z *et al*. (2011) Plant intracellular innate immune receptor Resistance to *Pseudomonas syringae* pv. *maculicola* (RPM1) is activated at and functions on the plasma membrane. *Proc. Natl. Acad. Sci. USA.* 18, 7619-7624.
7. 9. Li, M. *et al*. (2014). Proline isomerization of the immune receptor interacting protein RIN4 by a cyclophilin inhibits effector-triggered immunity in Arabidopsis. *Cell Host Microbe*. 16, 473-483
8. 10. Cheng, Z. *et al.* (2015) Pathogen-secreted proteases activate a novel plant immune pathway. *Nature.* 521, 213–216.
9. Lu, Y., *et al.* (2015) Putative serine protease effectors of *Clavibacter michiganensis* induce a hypersensitive response in the apoplast of *Nicotiana* species. *Mol. Plant-Microbe Interact.* 28, 1216–1226.
10. Schilling, O. and Overall, C. M. (2008) Proteome-derived, database-searchable peptide libraries for identifying protease cleavage sites. *Nat. Biotechnol.* 26, 685–694.

11. Dixon, M. S *et al*. (2000) Genetic complexity of pathogen perception by plants: The example of Rcr3, a tomato gene required specifically by Cf-2. *Proc. Natl. Acad. Sci. USA.* 97, 8807-8814

12. Zheng, X. *et al.* (2015). Genetic engineering of the Xa10 promoter for broad-spectrum and durable resistance to *Xanthomonas oryzae* pv. *oryzae. Plant Biotechnology Journal*. 13, 993-1001.

.

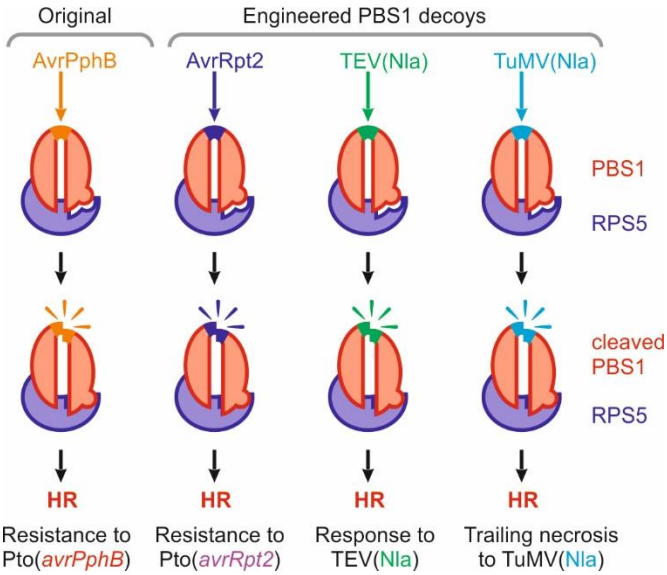

Figure 1. Engineering of the PBS1 decoy by introducing cleavage sites for unrelated proteases results in novel recognition specificities of the RPS5 immune receptor. The native PBS1 (left) was engineered by replacing the AvrPphB cleavage site (orange) by cleavage sites targeted by unrelated pathogen-derived proteases: AvrRpt2 (blue) from *Pseudomonas syringae* (Pto); Nla protease (green) of Tobacco Etch Virus (TEV) and the Nla protease (cyan) of Turnip Mosaic Virus (TuMV).