

PREreview of bioRxiv article “Convergent evolution of effector protease recognition by Arabidopsis and barley”

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Abstract

This is a review of Carter, Helm et al. bioRxiv 374264; doi: <https://doi.org/10.1101/374264> posted on July 23, 2018. In this paper, the authors showed that diverse barley cultivars are able to respond to the *Pseudomonas syringae* effector AvrPphB and they characterized both the effector target (PBS1) and the receptor (PBR1) responsible for this recognition. Furthermore, their phylogenetic analyses revealed that the immune receptor involved in this response is not orthologous to a previously characterized receptor (RPS5) from *Arabidopsis thaliana* that has a functionally analogous AvrPphB recognition mechanism. This leads the authors to conclude that recognition of the AvrPphB protease has evolved independently in *Arabidopsis* and barley.

Summary

This study builds on previous publications (Ade et al., 2007)(Zhang et al., 2010) that describe the activity of the *Pseudomonas syringae* Type 3 host-translocated effector AvrPphB during infection of *Arabidopsis thaliana*. AvrPphB is a protease that cleaves the *Arabidopsis* receptor like cytoplasmic kinase PBS1, which in turn is guarded by the nucleotide-binding leucine-rich repeat (NLR) intracellular receptor RPS5. Upon sensing kinase cleavage by the effector, RPS5 is activated and triggers the hypersensitive immune response. Previous studies by Roger Innes group (Kim et al., 2016), discussed by (Giannakopoulou et al., 2016) have provided a proof of concept for the use of this system as a platform to develop disease resistance to diverse plant pathogens. RPS5 recognition spectrum can be broadened by introducing cleavage sites recognized by different effector proteases into PBS1. In this paper, the authors explore natural responses to AvrPphB in plants other than *Arabidopsis*, notably barley (see Figure below). They uncovered a recognition system of AvrPphB in barley that is mechanistically analogous to *Arabidopsis* RPS5/PBS1 and potentially exploitable. Two barley orthologs of PBS1, HvPBS1-1 and HvPBS1-2, are cleaved by AvrPphB. By employing genome-wide association studies (GWAS) they singled out PBR1, a functional homolog of RPS5, as the NLR that mediates AvrPphB recognition. They further characterized this PBR1 and identified a PBR1 homolog in wheat implying that the system could be functional in this important cereal crop.

We found the paper to be well written and we particularly enjoyed the original genetic mapping strategies employed to identify the candidate NLR responsible for AvrPphB recognition. One important implication of the findings reported by Carter, Helm et al. is that PBS1-derived decoys can be developed in barley and other cereals such as wheat, to detect host-translocated protease effectors.













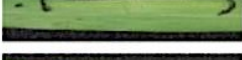




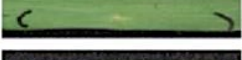

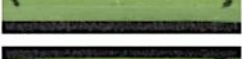




| Line | Response | AvrPphB | Catalytically inactive |
|-------------|----------|---|--|
| Rasmusson* | HR |  |  |
| Haruna Nijo | HR |  |  |
| Gorak | HC |  |  |
| PI061533 | HC |  |  |
| PI584977 | C |  |  |
| PI163409 | C |  |  |
| Clho15600* | LC |  |  |
| CI 16151 | LC |  |  |
| PI386650 | N |  |  |
| PI362207* | N |  |  |
| Morex | N |  |  |
| PI329000* | N |  |  |

Figure 1: “AvrPphB, and not a catalytically inactive derivative, triggers defense responses in barley.” Carter, Helm et al. bioRxiv, 2018.

Comments

Phylogenetic analyses. The authors report that RPS5 and PBR1 are not orthologous based on phylogenetic analysis (Figure 4), and as a consequence conclude that the capacity to recognize AvrPphB activity is an example of convergent evolution in Arabidopsis and barley. However, the phylogenetic trees shown in Fig. 4a and 4b lack the broader context necessary to determine the relatedness of the depicted NLRs. Phylogenetic analyses that include a wider diversity of Arabidopsis and barley NLRs are necessary to conclusively define the evolutionary relationship between AtRPS5 and PBR1.

Cell-death assays. To study PBR1 role in AvrPphB recognition, the authors carried out cell-death assays (Figure 6) by transiently expressing both the NLR and the effector in *N. benthamiana*. However, the presence of an endogenous PBS1 homolog in these assays limit the level of mechanistic insight that can be gained from these experiments. We suggest using a PBS1-silenced or loss-of-function background to obtain unambiguous results.

Figure 2A. In the experiments depicted in Figure 2A, what was the rationale behind employing a Bayesian phylogenetic analysis as opposed to other statistical methods? In general, it would be good to justify or to

mention whether other phylogenetic methods have yielded similar findings.

Figure 3A. In the experiments depicted in Figure 3A, what was the rationale behind including the barley line with the Low Chlorosis (LC) response phenotype to AvrPphB in the GWAS as opposed to just using non-AvrPphB responsive lines?

Reviewers

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