DISPATCH

Plant immunity: crosstalk between plant immune receptors

Marta Bjornson[1] and Cyril Zipfel[1,2,*]

[1]Institute of Plant and Microbial Biology and Zürich-Basel Plant Science Center, University of Zürich, Zürich, Switzerland
[2]The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, UK
***Correspondence: Cyril.zipfel@botinst.uzh.ch**

Plant immunity has long been divided into two 'tiers', involving cell-surface versus intracellular immune receptors. Although both systems can induce similar diagnostic responses, they have been considered independent pathways. Recent work challenges this view, showing a striking requirement for both recognition layers to achieve maximum immune output.

Plants recognize the presence and effects of pathogens. This can occur at the surface of plant cells, where pattern recognition receptors (PRRs) recognize conserved microbial signatures ('patterns') and induce pattern-triggered immunity (PTI). To inhibit PTI, pathogens secrete effector proteins into host cells, leading to effector-triggered susceptibility (ETS). Plants have evolved to recognize these effectors, directly or indirectly, *via* intracellular nucleotide-binding leucine-rich repeat receptors (NLRs) that activate a generally stronger effector-triggered immunity (ETI) response[1]. Although PTI and ETI share many components, these pathways are induced on different time scales and at different quantitative levels, and PTI and ETI are frequently thought of as being qualitatively different[2]. Complicating this picture, many molecules recognized by plants blur the line between ETI and PTI, and proposals to reduce conceptual boundaries between these pathways have been made[3]. Recent studies by Ngou *et al*.[4] and Yuan *et al*.[5] use multiple elegant approaches to recharacterize plant immunity, finding that PTI is required for full induction of ETI and that ETI in turn induces and stabilizes key PTI signaling components.

Although PTI signaling can easily be studied in isolation by applying purified or synthetic recognized patterns, ETI requires intracellular recognition of pathogen effectors. ETI has generally been studied through infection with virulent pathogens or microbes engineered to deliver such effectors. Since these microbes are themselves recognized at the cell surface by native PRRs, most ETI studies to date have thus shown the effects of ETI in combination with PTI.

To circumvent this issue, some studies have expressed effector proteins in transgenic *Arabidopsis thaliana* plants under the control of an inducible promoter[6,7]. Ngou *et al.*[4] also used this approach, expressing the bacterial effector AvrRps4 under the control of an estradiol-inducible promoter. Surprisingly, estradiol treatment alone was not sufficient to trigger either rapid or long-term production of reactive oxygen species (ROS) or full deposition of cell-wall-fortifying callose, hallmark outputs of both PTI and ETI. These defense responses could be restored by applying estradiol with a pathogen pattern to co-induce PTI and ETI. Yuan *et al.*[5] started with a different approach, using *Arabidopsis* backgrounds mutated in either two major PRRs and a coreceptor, or three major coreceptors of PRRs. They found that, in these PTI-compromised mutant plants, ETI-mediated bacterial resistance and the hypersensitive response (HR), a form of programmed cell death typical of ETI, were both severely impaired. Yuan *et al.*[5] then also used inducible effector expression, in this case dexamethasone-mediated induction of the bacterial effector AvrRpt2 – and found similar results for ROS production as Ngou *et al*.[4].

Yuan *et al*.[5] went on to explore the ROS phenotypes of ETI alone versus ETI and PTI. In PTI, it is known that ROS is primarily produced by the NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD), which is regulated by several mechanisms, most prominently *via* direct phosphorylation by the cytoplasmic receptor-like kinase BOTRYTIS-INDUCED KINASE 1 (BIK1)[8,9]. Yuan *et al*.[5] found that *rbohd* and *bik1* mutants, like major coreceptor mutants, were compromised in ETI-mediated bacterial resistance. In keeping with a role for RBOHD in ETI, dexamethasone-induced ETI caused low levels of phosphorylation of RBOHD at known BIK1 phosphosites, and this activation was greatly enhanced when PTI was triggered along with ETI. These results are in good agreement with previous studies of RBOHD regulation during ETI[7], and together this suggests that a major part of the dependence of ETI on PTI is in fact attributable to dependence on BIK1-regulated RBOHD-produced ROS. BIK1 has many other targets in PTI, and accordingly Yuan *et al.*[5] identified several defense genes for which ETI-triggered induction was also *BIK1*-dependent.

Expanding upon the observation that PTI-associated genes are induced when ETI is triggered alone, Yuan *et al*. performed RNA-seq of either wild-type plants or PTI coreceptor mutants, after infection with either a bacterial strain engineered to produce no effectors or the same strain with a single effector re-introduced ***(AU:OK?)***. Through this analysis, they identified a subset of genes that were induced by PTI, and strongly induced by ETI regardless of PTI induction. These genes were associated with immune function and specifically PTI signaling ***(AU:OK?)***. This agreed well with the results of Ngou *et al.*[4] who conducted a similar experiment, investigating transcriptional effects of ETI alone through their estradiol-inducible AvrRps4 expression.

Ngou *et al*.[4] extended this result to a time-course study of transcript and protein levels of several PTI-associated genes after induction of ETI alone. They found multiple characteristic profiles for each, including stable induction of both transcript and protein levels, unaffected transcript and protein levels, and, interestingly, a transient increase in transcript levels associated with stable increase in protein levels. These findings implicate both transcriptional and post-translational mechanisms in increasing PTI signaling competency during ETI activation, with ETI potentiating PTI. Yuan *et al.*[5] similarly found that ETI upregulated both transcript and protein levels of key PTI signaling components. This mechanism is thus proposed to alleviate ETS imposed during infection by virulent pathogens (Figure 1).

These two studies raise several questions. For example, although the studies of Ngou *et al*.[4] and Yuan *et al*.[5] reported largely similar results, Ngou *et al.* found that, with ETI induced by AvrRps4, no ROS were produced at all, whereas both studies reported that, with ETI induced by AvrRpt2, some ROS were produced and some HR could be observed in the absence of PTI signaling. AvrRps4 and AvrRpt2 have broadly different strengths of response in *Arabidopsis* and are also recognized by different classes of NLRs that involve different modes of activation. Dependence on PTI may thus be a new axis on which to dissect and understand mechanisms of ETI activation.

Another question raised by these studies is the mechanism by which PTI enables 'ETI'-like responses, and particularly HR. While PTI does not necessarily lead to HR, several patterns induce HR in *Arabidopsis* and other plant species, and this feature is most prominent with patterns recognized by receptor-like proteins as the PRRs. Important roles of NLRs downstream of these particular PRRs as well as biochemical associations between cell-surface receptors and NLRs were previously reported[10–15]. Furthermore, recent studies reported that key ETI signaling components associate with PRR complexes to activate PTI[16,17]. Together, these studies provide potential mechanistic links between PTI signaling and the activation of ETI — a point not explicitly addressed in the Ngou *et al.*[4] and Yuan *et al.*[5] studies.

Finally, the molecular mechanisms that link NLR activation to PTI potentiation, as well as to HR, are also still unclear. Indeed, several recent studies have proposed that 'sensor' or 'helper' NLRs can form pores or channels — potentially within the plasma membrane — leading to calcium influx within the cytosol as a main driver of HR[18,19]. As such, it is tempting to speculate that the increase in intracellular calcium concentration during ETI mediates the observed effect on PTI components, consistent at least with the previously reported calcium-dependent transcriptional regulation of immune genes during ETI[20]. In this context, it will be important to clarify the role played by RBOHD-dependent ROS in HR[4,5]— for example, by regulating NLR-mediated calcium channel activity or other as yet uncharacterized processes (Figure 1).

Beyond improving our understanding of plant immune signaling, the model proposed by these studies further suggests that ectopic overexpression, perhaps of only a few key PTI components, could lead to ETI-like immune activation after pattern perception alone, an intriguing prospect for crop biotechnology. In a similar scenario, if ETI triggered by different effectors quantitatively enhances PTI signaling, then simultaneous transformation of recognition proteins for multiple effectors into crops ***(AU: OK?)*** may not only make the evolution of pathogen resistance more difficult, but also quantitatively increase plant defense responses when more than one effector is recognized.

Together, these two papers elegantly redefine the nature of two intertwined pathways in plant immunity, with implications and opportunities from the study of signaling mechanisms to crop protection.

References

1. Lu, Y., and Tsuda, K. (2021). Intimate association of PRR- and NLR-mediated signaling in plant immunity. MPMI *34*, 3–14.

2. Li, P., Lu, Y.-J., Chen, H., and Day, B. (2020). The lifecycle of the plant immune system. CRC. Crit. Rev. Plant Sci. *39*, 72–100.

3. Thomma, B.P.H.J., Nürnberger, T., and Joosten, M.H.A.J. (2011). Of PAMPs and effectors: the blurred PTI-ETI dichotomy. Plant Cell *23*, 4–15.

4. Ngou, B.P.M., Ahn, H.-K., Ding, P., and Jones, J.D.G. (2021). Mutual potentiation of plant immunity by cell-surface and intracellular receptors. Nature *592*, 110–115.

5. Yuan, M. Jiang, M., Bi, G., Nomura, K., Liu, M., Wang, Y., Cai, B., Zhou, J.M., He, S.Y., and Xin, X.-F. (2021). Pattern-recognition receptors are required for NLR-mediated plant immunity. Nature *592*, 105–109.

6. Ngou, B.P.M., Ahn, H.K., Ding, P., Redkar, A., Brown, H., Ma, Y., Youles, M., Tomlinson, L., and Jones, J.D.G. (2020) Estradiol-inducible AvrRps4 expression reveals distinct properties of TIR-NLR-mediated effector-triggered immunity. J. Exp. Bot. *71*, 2186–2197.

7. Kadota, Y. Liebrand, T.W.H., Goto, Y., Sklenar, J., Derbyshire, P., Menke, F.L.H., Torres, M.A., Molina, A., Zipfel, C., Coaker, G., and Shirasu, K. (2019). Quantitative phosphoproteomic analysis reveals common regulatory mechanisms between effector- and PAMP-triggered immunity in plants. New Phytol. *221*, 2160–2175.

8. Kadota, Y., Sklenar, J., Derbyshire, P., Stransfeld, L., Asai, S., Ntoukakis, V., Jones, J.D.G., Shirasu, K., Menke, F., Jones, A., and Zipfel, C. (2014). Direct regulation of the NADPH

oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. Mol. Cell *54*, 43–55.

9. Li, L. Li, M., Yu, L., Zhou, Z., Liang, X., Liu, Z., Cai, G., Gao, L., Zhang, X., Wang, Y., *et al.* (2014). The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. Cell Host Microbe *15*, 329–338.

10. Qi, Y., Tsuda, K., Glazebrook, J., and Katagiri, F. (2011). Physical association of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) immune receptors in Arabidopsis. Mol. Plant Pathol*. 12*, 702–708.

11. Leibman-Markus, M., Pizarro, L., Schuster, S., Lin, Z.J.D., Gershony, O., Bar, M., Coaker, G., and Avni, A. (2018). The intracellular nucleotide-binding leucine-rich repeat receptor (SlNRC4a) enhances immune signalling elicited by extracellular perception. Plant Cell Environ. *41*, 2313–2327.

12. Huang, Y., Yin, C., Liu, J., Feng, B., Ge, D., Kong, L., Ortiz-Morea, F.A., Richter, J., Hauser, M.T., Wang, W.M. *et al.* (2020). A trimeric CrRLK1L-LLG1 complex genetically modulates SUMM2-mediated autoimmunity. Nat. Commun. *11*, 4859.

13. Liu, J., Huang, Y., Kong, L., Yu, X., Feng, B., Liu, D., Zhao, B., Mendes, G.C., Yuan, P., Ge, D., *et al.* (2020). The malectin-like receptor-like kinase LETUM1 modulates NLR protein SUMM2 activation via MEKK2 scaffolding. Nat. Plants *6*, 1106–1115.

14. Gabriëls, S.H.E.J., Vossen, J.H., Ekengren, S.K., van Oijen, G., Abd-El-Haliem, A.M., van den Berg, G.C.M., Rainey, D.Y., Martin, G.B., Takken, F.L.W., de Wit, P.J.G.M., and Joosten, M.H.A.J. (2007). An NB-LRR protein required for HR signalling mediated by both extra- and intracellular resistance proteins. Plant J. *50*, 14–28.

15. Wu, CH., Belhaj, K., Bozkurt, T.O., Birk, M.S., and Kamoun, S. (2016). Helper NLR proteins NRC2a/b and NRC3 but not NRC1 are required for Pto-mediated cell death and resistance in Nicotiana benthamiana. New Phytol. *209*, 1344–1352.

16. Pruitt, R. N., Zhang, L., Saile, S.C., Karelina, D., Frohlich, K., Wan, W.L., Rao, S., Gust, A.A., Locci, F., Joosten, M.H.A.J., *et al.* (2020). Arabidopsis cell surface LRR immune receptor signaling through the EDS1-PAD4-ADR1 node. bioRxiv, https://doi.org/10.1101/2020.11.23.391516.

17. Tian, H., Chen, S., Wu, Z., Ao, K., Yaghmaiean, H., Sun, T., Huang, W., Xu, F., Zhang, Y., Wang, S., *et al.* (2020). Activation of TIR signaling is required for pattern-triggered immunity. bioRxiv, https://doi.org/10.1101/2020.12.27.424494.

18. Jacob, P., Kim, N.K., Wu, F., El-Kasmi, F., Walton, W.G., Furzer, O.J., Lietzan, A.D., Sunil, S., Kempthorn, K., Redinbo, M.R., *et al.* (2021). The plant immune receptors NRG1.1 and ADR1 are calcium influx channels. bioRxiv, https://doi.org/10.1101/2021.02.25.431980.

19. Wang, J., Hu, M., Wang, J., Qi, J., Han, Z., Wang, G., Qi, Y., Wang, H.W., Zhou, J.M., and Chai, J.(2019). Reconstitution and structure of a plant NLR resistosome conferring immunity. Science *364*, eaav5870.

20. Gao, X., Chen, X., Lin, W., Chen, S., Lu, D., Niu, Y., Li, L., Cheng, C., McCormack, M., Sheen, J., *et al.* (2013). Bifurcation of Arabidopsis NLR immune signaling via Ca^{2+}-dependent protein kinases. PLoS Pathog. *9*, e1003127.

Figure 1. Molecular logic of an integrated plant immune system.
Depending on the host and microbe considered, at least three potential interactions exist. (A) For a non-adapted microbe, plant recognition of conserved microbial molecules at the cell surface induces pattern-triggered immunity (PTI), leading to hallmark immune outputs including production of reactive oxygen species (ROS) and activation of MAP kinase (MPK) signaling cascades. (B) Compatible pathogens secrete effectors, suppressing PTI and leading to effector-triggered susceptibility (ETS). (C) In an incompatible host–pathogen interaction, plant recognition of effectors induces effector-triggered immunity (ETI), strongly enhancing PTI via increasing transcription, translation and stability of PTI signaling components and immunity including the hypersensitive response (HR) potentially via ROS. This bolstering of PTI may be partially achieved via feedback signaling mediated by Ca^{2+}. ***(AU: this description of panel C is somewhat brief for what is shown – could you expand a little please to include a mention of all of the items present?)***