

MicroCommentary

New effects of type III effectors

Roger Innes

Department of Biology, Indiana University, Bloomington, IN 47405-7107, USA.

Summary

The enzymatic activities and/or targets of four type III effector proteins from plant pathogens have been reported in a flurry of new papers. In this issue, XopD is shown to remove SUMO groups from host cell proteins, while in previous issues of *Molecular Microbiology*, HopPtoD2 was shown to function as a tyrosine phosphatase and AvrRpt2 as probably a cysteine protease that targets the host RIN4 protein. Finally, AvrPphB is revealed in a recent *Science* paper to function as a cysteine protease that targets the host PBS1 kinase. This work is providing some of the first insights into how plant pathogens subvert host cell signalling machinery to cause disease.

For most Gram-negative bacterial pathogens, a type III secretion system (TTSS) is essential for causing disease (Galan and Collmer, 1999). The TTSS is used to inject a cocktail of proteins, referred to as effectors, into cells of the host, be it plant or animal. Once inside the host cell, these effectors co-opt signalling pathways to promote responses beneficial to the pathogen (Galan and Collmer, 1999). Understanding the specific functions of these effectors has thus become a top priority in both the animal and plant pathology communities. Such knowledge will probably lead to new methods of disease control and will provide new insights into host cell biology.

With the advent of whole genome sequencing, the catalogue of putative type III effectors is growing rapidly. For example, *Pseudomonas syringae* strain DC3000 possesses a minimum of 36 TTSS effector proteins (Collmer *et al.*, 2002). Despite this extensive catalogue, we have very limited knowledge as to what these effector proteins actually do. Studies are most advanced in the enteric pathogens, where enzymatic activities and host cell substrates have been assigned to several effectors (Cornelis,

2002; Zaharik *et al.*, 2002). By comparison, identification of the activities and targets of plant pathogen TTSS effectors has been slow in coming. Thankfully, a recent burst of publications is now changing this picture. In this issue, Hotson *et al.* (2003) show that the XopD effector from *Xanthomonas campestris* functions to remove SUMO groups (a ubiquitin-like post-translational modification) from plant proteins via an isopeptidase activity. Similarly, the AvrPphB effector from *Pseudomonas syringae* has recently been shown to function as a cysteine protease that specifically cleaves the PBS1 kinase of Arabidopsis (Shao *et al.*, 2002; 2003) and indirect evidence suggests that the *P. syringae* AvrRpt2 protein also functions as a cysteine protease (Axtell *et al.*, 2003). Lastly, the *P. syringae* HopPtoD2 was recently shown to possess tyrosine phosphatase activity (Bretz *et al.*, 2003; Espinosa *et al.*, 2003).

Hotson *et al.* (2003) were led to their discovery by BLAST searches that revealed sequence similarity between the C-terminal third of XopD and a group of eukaryotic cysteine proteases, including the yeast Ulp1 protease. This homology suggested that XopD might possess SUMO protease activity and/or SUMO isopeptidase activity (removal of SUMO from SUMOylated proteins). Both XopD and Ulp1 belong to the 'C48' peptidase family of the CE clan of cysteine proteases, as defined by the MEROPS Protease Database (<http://merops.sanger.ac.uk/>). Significantly, this family is made up almost exclusively of eukaryotic proteins. The only exceptions come from pathogens and the plant symbiont *Mesorhizobium loti*, which suggests that these proteases function only in eukaryotic cells. In a series of elegant experiments, Hotson *et al.* (2003) showed that XopD specifically cleaves plant SUMO isoforms and not animal SUMOs, and more significantly, that XopD can remove SUMO from SUMOylated plant proteins. This last observation is particularly noteworthy because YopJ-like type III effectors, which belong to a different protease family, but the same clan as XopD, are also proposed to function as SUMO isopeptidases (Orth *et al.*, 2000). However, similar experiments with YopJ and mammalian cell extracts failed to show SUMO isopeptidase activity; thus Hotson *et al.* (2003) provide the first unambiguous data demonstrating that deSUMOylation of host proteins represents an important virulence strategy.

If XopD can remove SUMO from the majority of SUMOylated plant proteins in an extract, does it also target these proteins when delivered by *X. campestris* during a normal infection? Probably not. Using XopD-EYFP protein fusions, Hotson *et al.* (2003) showed that XopD is targeted to the nucleus of plant cells, where it concentrates in subnuclear foci. This targeting is mediated by the N-terminal non-protease domain of XopD. Likely the only proteins that are deSUMOylated by XopD during a normal infection are located in these subnuclear foci. The major questions remaining from this work are the identity of these proteins and how their deSUMOylation contributes to pathogenesis.

Continuing with the protease theme, the *P. syringae* AvrPphB and *Yersinia* YopT effectors were shown last year to belong to the C58 family of cysteine proteases (Shao *et al.*, 2002). This family belongs to the CA clan, which is structurally unrelated to the CE clan described above. YopT targets small GTPases belonging to the Rho-family, resulting in the depolymerization of the actin cytoskeleton and prevention of phagocytosis by macrophages (Shao *et al.*, 2002). As *P. syringae* does not need to worry about macrophages in a plant, the targets of AvrPphB were expected to be different. Prior genetic analyses in Arabidopsis had suggested that the PBS1 kinase might be a substrate, as PBS1 is required for the recognition of AvrPphB by the RPS5 disease resistance protein (Swiderski and Innes, 2001). This hypothesis has now been confirmed in the new work of Shao *et al.* (2003), where PBS1 was shown to be a direct substrate of AvrPphB both in whole plants and *in vitro*. The latter work used purified recombinant proteins isolated from *Escherichia coli*, demonstrating that no additional protein factors are required for the proteolytic cleavage of PBS1. Significantly, mutations in PBS1 that block cleavage also block recognition of AvrPphB, indicating that the PBS1 cleavage event is a prerequisite for activation of RPS5-mediated resistance. Important questions not addressed by Shao *et al.* (2003) are how the cleavage of PBS1 contributes to the virulence of *P. syringae* and whether there are other targets of AvrPphB in plants. Unpublished work from my laboratory has shown that AvrPphB can cleave PBS1 orthologues found in monocots, but does not cleave the most closely related paralogue in Arabidopsis, indicating that AvrPphB is highly specific, and that its target is highly conserved among plants.

Also possibly belonging to the CA clan of cysteine proteases is the AvrRpt2 protein of *P. syringae* (Axtell *et al.*, 2003). At least one of the virulence functions of AvrRpt2 appears to be elimination of the host cell protein RIN4 (Axtell and Staskawicz, 2003; Mackey *et al.*, 2003). It was not clear from this work, however, how AvrRpt2 causes the elimination of RIN4. In the new work from Axtell *et al.* (2003) they report a predicted structural similarity

between AvrRpt2 and staphopain, a well-characterized CA clan member. Importantly, mutation of the predicted catalytic cysteine and histidine residues abolishes the ability of AvrRpt2 to trigger elimination of RIN4, consistent with RIN4 being an AvrRpt2 substrate. However, recombinant AvrRpt2 produced from *E. coli* cannot cleave RIN4. The authors speculate that an additional eukaryotic protein is required to activate AvrRpt2 protease activity.

Two recent papers in *Molecular Microbiology* independently demonstrated that HopPtoD2 contains a functional protein tyrosine phosphatase (PTP) domain, along with a second domain homologous to AvrPphD (Bretz *et al.*, 2003; Espinosa *et al.*, 2003). Mutation of HopPtoD2 in *P. syringae* pv. *tomato* strain DC3000 reduces its virulence on susceptible tomato and Arabidopsis, while overexpression partially suppresses resistance responses induced on non-host plants. Both activities are eliminated by mutation of the PTP catalytic site, indicating that phosphatase activity is required for these effects. Although the host cell targets of HopPtoD2 are not yet known, Espinosa *et al.* (2003) found that HopPtoD2 can suppress programmed cell death induced by an activated form of MEKK, a MAPK kinase, raising the intriguing possibility that MAPK proteins could be targets of HopPtoD2.

This collection of papers represents a quantum leap in our understanding of how TTSS effectors contribute to plant disease. A year ago no direct 'effects' of plant pathogen TTSS effectors were known. This work not only enhances our understanding of pathogenesis, but is providing exciting new insights into basic plant cell biology.

References

- Axtell, M.J., and Staskawicz, B.J. (2003) Initiation of RPS2-specified disease resistance in Arabidopsis is coupled to the AvrRpt2-directed elimination of RIN4. *Cell* **112**: 369–377.
- Axtell, M.J., Chisholm, S.T., Dahlbeck, D., and Staskawicz, B.J. (2003) Genetic and molecular evidence that the *Pseudomonas syringae* type III effector protein AvrRpt2 is a cysteine protease. *Mol Microbiol* **49**: 1537–1546.
- Bretz, J.R., Mock, N.M., Charity, J.C., Zeyad, S., Baker, C.J., and Hutcheson, S.W. (2003) A translocated protein tyrosine phosphatase of *Pseudomonas syringae* pv. *tomato* DC3000 modulates plant defence response to infection. *Mol Microbiol* **49**: 389–400.
- Collmer, A., Lindeberg, M., Petnicki-Ocwieja, T., Schneider, D.J., and Alfano, J.R. (2002) Genomic mining type III secretion system effectors in *Pseudomonas syringae* yields new picks for all TTSS prospectors. *Trends Microbiol* **10**: 462–469.
- Cornelis, G.R. (2002) The *Yersinia* Ysc-Yop 'type III' weaponry. *Nat Rev Mol Cell Biol* **3**: 742–752.
- Espinosa, A., Guo, M., Tam, V.C., Fu, Z.Q., and Alfano, J.R. (2003) The *Pseudomonas syringae* type III-secreted protein HopPtoD2 possesses protein tyrosine phosphatase

- activity and suppresses programmed cell death in plants. *Mol Microbiol* **49**: 377–387.
- Galan, J.E., and Collmer, A. (1999) Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* **284**: 1322–1328.
- Hotson, A., Chosed, R., Shu, H., Orth, K., and Mudgett, M.B. (2003) *Xanthomonas* type III effector XopD targets SUMO-conjugated proteins *in planta*. *Mol Microbiol* doi:10.1046/j.1365-2958.2003.03730.x
- Mackey, D., Belkhadir, Y., Alonso, J.M., Ecker, J.R., and Dangl, J.L. (2003) Arabidopsis RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. *Cell* **112**: 379–389.
- Orth, K., Xu, Z., Mudgett, M.B., Bao, Z.Q., Palmer, L.E., Bliska, J.B., *et al.* (2000) Disruption of signaling by *Yersinia* effector YopJ, a ubiquitin-like protein protease. *Science* **290**: 1594–1597.
- Shao, F., Merritt, P.M., Bao, Z., Innes, R.W., and Dixon, J.E. (2002) A *Yersinia* effector and a *Pseudomonas* avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis. *Cell* **109**: 575–588.
- Shao, F., Golstein, C., Ade, J., Stoutemyer, M., Dixon, J.E., and Innes, R.W. (2003) Cleavage of Arabidopsis PBS1 by a bacterial type III effector. *Science* **301**: 1230–1233.
- Swiderski, M.R., and Innes, R.W. (2001) The *Arabidopsis* *PBS1* resistance gene encodes a member of a novel protein kinase subfamily. *Plant J* **26**: 101–112.
- Zaharik, M.L., Gruenheid, S., Perrin, A.J., and Finlay, B.B. (2002) Delivery of dangerous goods: type III secretion in enteric pathogens. *Int J Med Microbiol* **291**: 593–603.