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Type III effector proteins: doppelgangers of bacterial virulence

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Bacterial pathogens have co-evolved with their hosts in their ongoing quest for advantage in the resulting interaction. These intimate associations have resulted in remarkable adaptations of prokaryotic virulence proteins and their eukaryotic molecular targets. An important strategy used by microbial pathogens of animals to manipulate host cellular functions is structural mimicry of eukaryotic proteins. Recent evidence demonstrates that plant pathogens also use structural mimicry of host factors as a virulence strategy. Nearly all virulence proteins from phytopathogenic bacteria have eluded functional annotation on the basis of primary amino-acid sequence. Recent efforts to determine their three-dimensional structures are, however, revealing important clues about the mechanisms of bacterial virulence in plants.

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Introduction

The ability of a microbe to cause disease is an exception in nature and is the result of co-evolution between pathogens and hosts. Successful pathogens must cope with myriad host defense responses in order to survive and multiply. As a result, pathogens possess sophisticated virulence factors that can actively thwart defense responses and highjack host cell machinery for the pathogen's benefit. Many Gram-negative bacterial pathogens use a molecular syringe known as the type III secretion system to inject virulence factors, termed type III effectors, directly into host cells, where they suppress host responses and promote the growth and dissemination of the pathogen [1,2]. Unfortunately for the pathogen, these proteins are ideal recognition molecules for the host surveillance system. Plant resistance proteins can 'recognize' the presence of specific type III effector proteins, triggering the plant immune system and, often, the localized cell death known as the hypersensitive response (HR) [3].

Type III effectors were originally identified by their ability to trigger the HR in a host genotype-specific manner, and were thus termed avirulence (Avr) proteins. Since the cloning of the first avirulence gene in 1984 [4], hundreds of type III effectors have been identified from various plant pathogens. For a few, function has been ascribed from primary sequence similarity to known eukaryotic proteins [5•,6]. However the biochemical functions of the majority remain elusive. Recent structural studies of type III effectors from both mammalian and plant pathogens have revealed important functional information. We highlight recent structural efforts aimed at understanding the roles of bacterial virulence proteins in plant pathogenesis, focusing on recently published three-dimensional structures of *Pseudomonas syringae* type III effectors. We address the potential clues revealed by these studies about the strategies employed by plant pathogens to promote virulence.

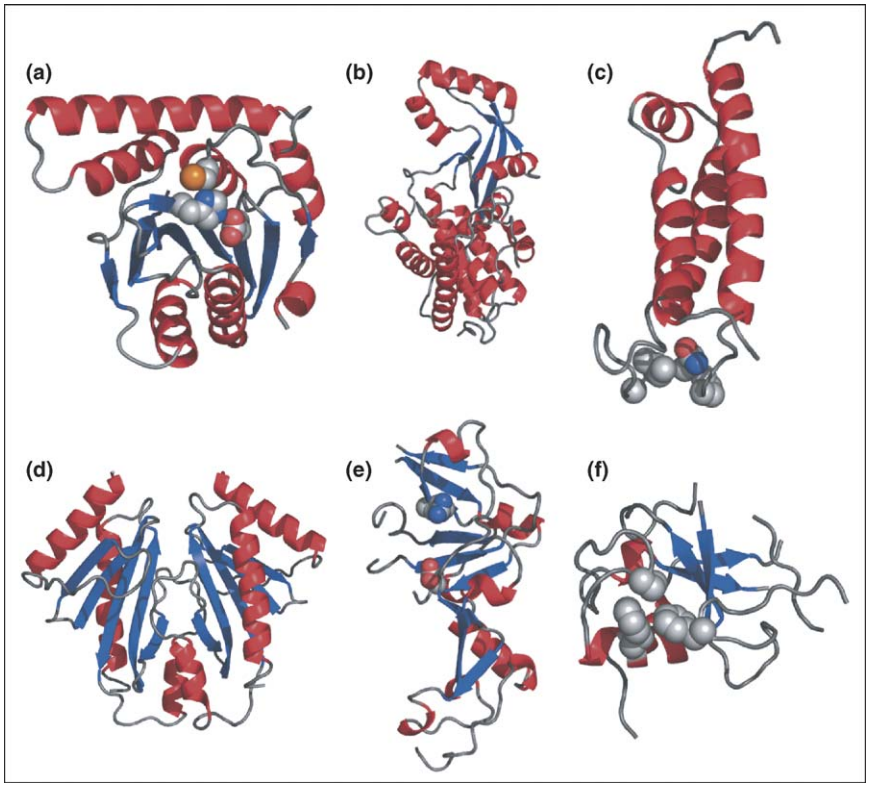
Function from the fold: still the wake of the flood

Genome-wide screens have identified several hundred putative and confirmed type III effectors distributed across the many pathovars of *P. syringae* [7]. *In silico* predictions of possible biochemical functions that are based on persistent scrutiny of primary amino-acid sequence allows prediction of biochemical functions for only a handful of effector domains [5•,6]. These include sumo- and cysteine-proteases, tyrosine phosphatases and ADP-ribosyltransferases [5•,6,8]. These predictions relied on the conservation of either catalytic amino acids or predicted secondary structures between type III effectors and known enzyme families.

AvrPphB: function confirmed

AvrPphB is recognized indirectly by the *Arabidopsis* RPS5 (RESISTANT TO *P. SYRINGAE* 5) protein and was the first *P. syringae* type III effector with a published crystal structure [9,10•]. AvrPphB is a member of a novel class of papain-like proteases that also includes the *Yersinia* type III effector YopT [11]. AvrPphB undergoes autoproteolytic processing between Lys-62 and Gly-63, and cleaves its host target, the *Arabidopsis* serine/threonine kinase PBS1, at a similar sequence [11,12]. This cleavage is required to initiate RPS5-mediated disease resistance [12]. The crystal structure of AvrPphB (Figure 1) resembles that of the papain-like cysteine proteases, with the

Figure 1



Ribbon diagrams of the *P. syringae* type III structures solved to date with α-helices in red and β-strands in blue, and side chains and Cα atoms of critical residues are shown for clarity. **(a)** AvrPphB [10•], with the residues of the catalytic triad, namely Cys-98, His-212 and Asp-227, highlighted. **(b)** AvrB [17•], showing the bilobal fold (large lobe all-helical and small lobe containing a five-stranded β-sheet plus three helices). **(c)** AvrPto [22•] with residues Gly-95, Ile-96, Asn-97 and Pro-98 (GINP), which are involved in Pto recognition, highlighted. **(d)** SchF [29•], a type III chaperone, **(e)** HopF1 [29•] with critical residues Arg-72 and Asp-174 highlighted. **(f)** AvrPtoB [32••] with the three critical residues involved in E2 recognition (Phe-479, Phe-525 and Pro-533).

core of the structure containing the active-site catalytic triad Cys-98, His-212 and Asp-227 ([10•]; Table 1). This fold is predicted to be adopted by all members of the widespread YopT family, despite their low primary sequence similarity. The similarity of the AvrPphB active site to that of papain-like proteases suggests a similar molecular mechanism for proteolysis. A number of PBS1-like kinases from *Arabidopsis* that have nearly identical sequences around the AvrPphB cleavage site are not cleaved by AvrPphB, indicating that additional tertiary structural determinants in PBS1 are required for specific cleavage [12].

AvrB: a novel fold

The type III effector AvrB is recognized by the products of the *Arabidopsis RPM1* and soybean *RPG1* resistance genes, resulting in a disease resistance response [13,14], and can add virulence to a soybean pathogen on susceptible cultivars [15]. AvrB interacts with the RPM1-interacting protein RIN4, leading to RIN4 phosphorylation [16]. The simplest explanation for this observation is that AvrB is a kinase. However, AvrB shares no obvious sequence similarity to known kinases. The structure of AvrB revealed a novel bilobal fold, with a large lobe that contains a deep cleft and a distinct small lobe attached to the back and side of the large lobe ([17•]; Figure 1, Table 1). Many solvent-exposed residues in the major cleft of the large lobe are conserved among AvrB homologs, leading to speculation that this region might represent a conserved enzyme catalytic site that is required for substrate or cofactor binding. The small lobe contains weaker sequence homology among alleles. Chimeric proteins of AvrB and the homolog AvrPphC, which does not induce *RPM1*-mediated resistance responses, demonstrated that sequences in the small lobe of AvrB are required to confer recognition by RPM1. The biochemical function of AvrB remains elusive and awaits the determination of its crystal structure in complex with RIN4 or other host targets.

AvrPto: knowing when to fold

AvrPto was originally characterized by its ability to elicit gene-for-gene resistance on tomato plants expressing the *Pto* disease resistance gene [18]. It also confers virulence

Table 1

***Pseudomonas syringae* type III structures.**

Type III effector	Structure type (PDB identity code/resolution Å)	Fold description	Function
AvrPphB [10•] (full length)	Crystal structure (1UKF/1.35 Å)	Papain-like fold: two-lobe structure with a central anti-parallel β-sheet packed on both sides by α-helices (Figure 1a).	Cysteine protease
AvrB [17•] (full length)	Crystal structure (1NH1/2.2 Å)	Novel bilobal-fold: small lobe of three α-helices and five β-strands, and an all helical larger lobe containing a deep cleft of over 900 $Å^3$ (Figure 1b).	Unknown
AvrPto [22•] (truncated: 29-133)	NMR structure (1R5E/rmsd 0.97 ± 0.2 Å for backbone and 1.32 ± 0.2 Å for all heavy atoms)	Compact three-helix bundle: similar to folds in proteins involved in protein recognition, such as DnaK (Figure 1c).	Unknown
SchF [29•] (full length)	Crystal structure (1S28/3.0 Å)	Dimer similar to other type III chaperone structures; each protomer adopts α-β-β-α-β-β-α topology with α2 contributing major intermolecular interactions (Figure 1d).	Type III chaperone
HopF1 [29•] (full length)	Crystal structure (1S21/2.0 Å)	Novel mushroom-like structure with 'head' and 'stalk' subdomains: head subdomain shares limited structural similarity to ADPRT toxins (Figure 1e).	Unknown (ADP-RT?)
AvrPtoB [32••] (protease-resistant fragment: 436-553)	Crystal structure (2FD4/1.8 Å)	Overall globular fold with four-stranded β-sheet that packs against two helices on one face and three very extended loops. The structure includes core-fold of RING-finger and U-box E3 ligases, which involve a three-stranded β-sheet with a single helix and two extended loops (Figure 1f).	E3 ligase

PDB, The Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (http://www.rcsb.org/pdb/Welcome.do).

on tomato plants lacking *Pto* [19]. AvrPto interaction with the Pto serine/threonine kinase is required for activation of disease resistance [20,21]. The solution structure of a truncated AvrPto that is capable of eliciting Pto-mediated defense responses has been solved by NMR ([22•]; Table 1). The AvrPto fragment adopts a conformation that shows similarity to structures in other proteins that are involved in protein recognition, such as the molecular chaperone DnaK. The AvrPto sequence 95-GINP-98 is required for recognition by Pto and is found on a large loop that is enriched in hydrophobic amino acids on one end of the helical bundle ([23,24]; Figure 1). Docking models of AvrPto with Pto provide a likely scenario of interaction, but this interaction awaits confirmation by crystallization of a AvrPto–Pto complex. Two sets of NMR resonances for AvrPto indicated the presence of both folded and unfolded states in solution, and demonstrated that these interconverted approximately every 0.3 seconds. These results indicate a small energy difference between the folded and unfolded state that might allow partially unfolded type III effector proteins to pass through the type III pilus, which has an estimated diameter of only ~2 nm [25].

AvrPphF: bean there, done that

AvrPphF was originally identified from the bean pathogen *P. syringae* pv. *phaseolicola* strain 1449B. It consists of two open reading frames (ORFs) that encode the type III chaperone *SchF* and the effector *HopF1*, which are both required for avrPphF function [26,27]. Secondary structure predictions indicate that SchF is a probable type III chaperone, and studies of the AvrPphF allele from *P. syringae* pv. *tomato* strain DC3000 (HopPtoF2) demonstrated that SchF interacts with and stabilizes the HopF2 protein in the bacterial cell [28]. On the other hand, the HopF1 primary sequence provided no clues about its biochemical function. HopF1 is a virulence factor for pathogens on many bean and soybean cultivars [27]. However, the *R1* disease resistance gene in some bean cultivars confers recognition of AvrPphF (HopF1). The crystal structures of the products of both *avrPphF* ORFs, the ShcF and HopF1 proteins from *P. syringae* pv. *phaseolicola* strain 1449B, have been solved to 3.0 Å and 2.0 Å resolution, respectively ([29•]; Figure 1). The structure of ShcF revealed a high degree of structural similarity to type III chaperones from mammalian pathogens. The overall structure of HopF1 did not display significant similarity to known protein structures. It adopts a novel mushroom-like three-dimensional structure that can be subdivided into 'head' and 'stalk' subdomains (Table 1). Closer analysis of these subdomains revealed limited structural similarity of the head subdomain to the catalytic domain of the ADP-ribosyltransferase (ADPRT) diphtheria toxin. However, HopF1 displayed neither ADPRT-associated activities *in vitro* nor NAD-binding activity. Nevertheless, we used the structural similarity to diphtheria toxin, as well as sequence similarity between HopF1 alleles, to predict amino acids that are important for function. Arg-72 and Asp-174 of HopF1 are absolutely conserved among alleles and are both spatially and charge

conserved with the catalytic amino acids His-21 and Glu-148 of diphtheria toxin. Mutation of either Arg-72 or Asp-174 compromised both the virulence of AvrPphF and the *R1*-dependent avirulence activities of bean plants. Thus, Arg-72 and Asp-174 might represent catalytic residues that are important for enzymatic activity or, alternatively, might be required for interaction with specific host proteins or cofactors.

AvrPtoB: Bingo! An E3 ligase

AvrPtoB inhibits programmed cell death (PCD) that is associated with plant immunity and also acts as a general suppressor of eukaryotic cell death that is capable of inhibiting PCD in yeast [30]. AvrPtoB, like AvrPto, is recognized by the resistance protein Pto, leading to activation of the HR and disease resistance [31]. The primary amino-acid sequence of AvrPtoB provided no clues about its biochemical function (a familiar refrain, by now!). The amino-terminal (N-terminal) region (1-387) mediates recognition by Pto, whereas the carboxy-terminal (C-terminal) region (308-553) is responsible for inhibition of PCD [30]. The structure of the functional protease-resistant sub-fragment of this C-terminal domain of AvrPtoB was recently determined ([32••]; Figure 1, Table 1). Remarkably, this domain displayed structural homology to RING-finger and U-box families of proteins that are components of eukaryotic E3 ubiquitin ligases. This similarity included the 'core-fold' of this protein family and further extended to a potential binding site for the E2 'ubiquitin-conjugating' enzymes. In particular, a surface patch of three residues found in E3 ligases forms a conserved potential E2-binding patch in AvrPtoB. *In vitro*, full-length AvrPtoB and the crystallized C-terminal fragment possess auto-ubiquitination activity that depends on an E1 'ubiquitin-activating enzyme' and an E2 enzyme, a common characteristic of eukaryotic E3 ligases. Mutation of the conserved E2-binding patch abrogated this auto-ubiquitination activity. *In vivo*, mutation of the conserved E2-binding patch resulted in a loss of the cell-death-inhibiting activity of AvrPtoB, and a loss of bacterial virulence activity on *pto* mutant tomato plants. However, these mutations did not effect recognition by Pto [32••,33]. Therefore, AvrPtoB functions as an E3 ubiquitin ligase by structurally mimicking RING-finger or U-box E3 ubiquitin ligases. This important finding confirms how a structural approach can successfully reveal the biochemical functions of *P. syringae* type III effectors that share no sequence similarity to known proteins. E3 ligases are well-known inhibitors of PCD [34]. An intriguing hypothesis is that AvrPtoB ubiquitinates a host protein(s), possibly a positive regulator of PCD that is recruited to the degradation machinery via the AvrPtoB N-terminus. Alternatively, AvrPtoB might prove to be a dominant negative E3 ligase that blocks a normal host E3 ligase activity that is required to initiate defense responses by removing a negative regulator.

Future directions for structural approaches

A major challenge for understanding the functions of type III effector proteins remains the identification of their host protein targets. The virulence proteins of mammalian pathogens adopt two major structural strategies to mimic the activities of host proteins [35]. One group of virulence proteins shares sequence and structural similarity to known eukaryotic enzymes. Examples of this class are the tyrosine phosphatases YopH from *Yersinia* spp., the C-terminal half of SptP from *Salmonella* spp., and now AvrPphB from *P. syringae*.

The second group of virulence proteins does not show sequence similarity to known proteins, and their mechanism of action is not apparent from their primary sequence. However, the structures of these proteins in complex with their host targets have revealed that their interaction surfaces are excellent mimics of eukaryotic host proteins, even though they possess different overall 'folds' from these host proteins. A classic example is the N-terminal domain of SptP, which acts as a GTPase-activating protein (GAP) for Rac1 and Cdc42 by strategically contacting residues that are similar to those contacted by different protein structures within host GAPs [36,37]. A number of *P. syringae* type III effectors, such as AvrPtoB, might belong to this latter class of structural mimics, and their structures when complexed with identified targets will reveal important functional domains.

In this vein, the recently described crystal structure of the *P. aeruginosa* ADPRT exotoxin A (ETA), bound to its eukaryotic target translation elongation factor 2 (eEF2), demonstrates that structural mimicry is not limited to protein sequences, but can also involve small molecules bound to the virulence protein [38•]. ETA catalyzes the transfer of ADP-ribose to the diphthamide residue, a post-translationally modified histidine residue found only in eEF2 and essential for its function. Ribosylation of diphthamide inactivates eEF2 and thus inhibits translation. In an elegant example of molecular mimicry, the NAD^+ substrate molecule bound to ETA (or its non-hydrolyzable analog βTAD in the crystal structure) ensures its recognition of eEF2 by mimicking two backbone phosphates of the eEF2 ribosome binding site, which are universally conserved in 16S/18S rRNA and essential for tRNA recognition at the A site. Another important observation from this study is that the ADPRT reaction of ETA is substrate-assisted by amino-acid residues of eEF2. The structure of the ETA–eEF2 complex reveals two important concepts for virulence protein function: the use of small molecules for mimicry and the requirement of residues from both the virulence protein and the host target for enzymatic function.

Similar considerations might be operative in plant pathogens. For example, at least three unrelated *P. syringae*

type III effectors target the negative regulator of basal defenses RIN4 [16,39–41]. In fact, two of these, AvrB and AvrRpt2, both target a motif of RIN4 that is conserved in a family of at least 11 *Arabidopsis* proteins [42]. *P. syringae* therefore uses multiple virulence proteins to modify this conserved motif differentially. It will be important to know if RIN4 and the 11 other *Arabidopsis* proteins that have this motif are also virulence targets for these, or additional, type III effectors.

As a start, we co-crystallized AvrB in complex with a RIN4 fragment containing this interaction domain. We further demonstrated that AvrB binds nucleotides, that nucleotide-binding residues identified from the co-crystal structure are required for AvrB activity, and that AvrB is phosphorylated in the presence of plant extract (D Desveaux, AU Singer, AJ Wu, B McNulty, Z Nimchuk, L Musselwhite, J Sondek, JL Dangl, unpublished). It might be the case that AvrB is a kinase mimic that does not have this activity in isolation.

Careful curation can pay dividends: can computational approaches as well?

Type III effectors often fall into families that contain multiple alleles which have evolved by descent from a common predecessor or have horizontally transferred within or across species [7,43]. A careful curation approach (see Box 1) might suggest functions for particular type III effector protein families that are diverged enough to reveal commonalities characteristic of known functional domains. However, most *P. syringae* type III effector families possess large tracts of conservation that thwart functional predictions. Nevertheless, some successes have been reported. For the type III effector AvrRpt2, the presence of a catalytic triad that is characteristic of cysteine proteases and a predicted secondary structure similar to that of staphopain foreshadowed the determination of its activity as a cysteine protease [44].

Box 1 A curation case study

Identification of important functional domains of virulence proteins from thoughtful manual analysis of primary sequences has been elegantly demonstrated by the recent identification of a 24-member protein family of Gram-negative pathogens. These possess an invariant WxxxE motif [45]. The enterohaemorrhagic *Escherichia coli* type III effector map was used as an index protein for a BLAST search. This identified a small number of protein homologs primarily from the attaching and effacing (A/E) pathogen group. These shared the common Trp-x-x-x-Glu (WxxxE) motif. PSI-BLAST iterations were then conducted by including WxxxE-containing proteins manually identified from the original search that fell below the significance threshold. This revealed a total of 24 distinct proteins that were subdivided into six classes and included IpgB2 and IpgB1 from *Shigella*. The WxxxE motif was demonstrated to be required for IpgB2-, IpgB1- and Map-dependent phenotypes in human embryonic kidney cell lines. However, the precise molecular function of the WxxxE motif remains unknown and will await further structural and biochemical characterization of this family of virulence proteins.

Box 2 Tortuous technicalities

Structural approaches will have to contend with the insolubility of many *P. syringae* type III effectors when expressed in *E. coli*. The production of delimited functional domains for expression might partially overcome this problem, as was the case for AvrPtoB [32••]. In addition, various affinity tags can be used to enhance solubility [46]. Structure determination can then be attempted with affinity tags still attached to proteins of interest to avoid the possibility of protein insolubility when tags are removed [47]. The horizontal transfer of type III effectors between strains of *P. syringae* and even distantly related plant pathogens provides an important resource to obtain soluble protein; the incorporation of various family members into structural proteomics projects has been demonstrated to increase the probability of obtaining soluble/crystallizable proteins [48]. Co-expression of type III effectors with interacting proteins can also change their solubility characteristics. In these cases, delineating the minimal interaction domains of host targets by limited proteolysis or deletion analysis can increase the probability of obtaining structural data by reducing the size of unstructured regions in these protein complexes.

And, as discussed above for the *P. syringae* effector AvrPphF (HopF1), a combination of structural and primary sequence alignments of protein family members can highlight important functional domains.

Myriad technical issues must also be solved before many type III effector structures can be obtained (see Box 2). Of the five effectors whose structures have been solved, four (AvrB, AvrPto, AvrPphB and AvrPphF) are small proteins (i.e. <40 kDa) containing a single folded domain. However, of the type III effectors described by Chang *et al.* [5•] from the fully sequenced genomes of *P. syringae* pv. *tomato* (*Pto*) and *P. syringae* pv. *phaseolicola* (*Pph*), almost half (8 of 19 in *P. syringae* pv. *phaseolicola* strain 1448A, and 13 of 28 in *P. syringae* pv. *tomato* strain DC3000) are large (>400 amino acids) and likely to be multi-domain proteins. In such cases, crystallization of full-length proteins could be attempted but, in addition, efforts might be expended in subdividing the protein into individual predicted domains, as for AvrPtoB [32••].

Conclusions

Determination of the structure of type III effectors from *P. syringae* has yielded one novel fold (from AvrB), and another fold (from AvrPphF) that is sufficiently different from that of its closest relatives (ADPRT enzymes) to be no longer functionally identical. As more effector structures are solved, we will probably discover more unusual protein structures, complicating discovery of their functions. Although some might display similarity to known enzymes, such as AvrPphB and AvrPtoB, a number could function through interaction with, and mimicry of, small eukaryotic protein domains. Therefore, a combination of primary sequence analyses, biochemical assays, *in vivo* functional studies and identification of host targets will complement structural studies in providing an

understanding of the complexity of events that result from type III effector activity in host cells.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Nomura K, Melotto M, He SY: **Suppression of host defense in compatible plant–*Pseudomonas syringae* interactions**. *Curr Opin Plant Biol* 2005, **8**:361-368.

2. Chisholm ST, Coaker G, Day B, Staskawicz BJ: **Host–microbe interactions: shaping the evolution of the plant immune response**. *Cell* 2006, **124**:803-814.

3. Dangl JL, Jones JD: **Plant pathogens and integrated defence responses to infection**. *Nature* 2001, **411**:826-833.

4. Staskawicz BJ, Dahlbeck D, Keen N: **Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr**. *Proc Natl Acad Sci USA* 1984, **81**:6024-6028.

5. • Chang JH, Goel AK, Grant SR, Dangl JL: **Wake of the flood: ascribing functions to the wave of type III effector proteins of pytopathogenic bacteria**. *Curr Opin Microbiol* 2004, **7**:11-18.
This thorough review of type III effector functions of the plant pathogen *P. syringae* covers pre-structural data and is a complement to this review. Of interest is the review of homology-based approaches for functional annotation of proteins.

6. Mudgett MB: **New insights to the function of phytopathogenic bacterial type III effectors of plants**. *Annu Rev Plant Biol* 2005, **56**:509-531.

7. Lindeberg M, Stavrinides J, Chang JH, Alfano JR, Collmer A, Dangl JL, Greenberg JT, Mansfield JW, Guttman DS: **Proposed guidelines for a unified nomenclature and phylogenetic analysis of type III Hop effector proteins in the plant pathogen *Pseudomonas syringae***. *Mol Plant Microbe Interact* 2005, **18**:275-282.

8. Petnicki-Ocwieja T, Schneider DJ, Tam VC, Chancey ST, Shan L, Jamir Y, Schechter LM, Janes MD, Buell CR, Tang X *et al.*: **Genomewide identification of proteins secreted by the Hrp type III protein secretion system of *Pseudomonas syringae* pv. DC3000**. *Proc Natl Acad Sci USA* 2002, **99**:7652-7657.

9. Simonich MT, Innes RW: **A disease resistance gene in *Arabidopsis* with specificity for the *avrPph3* gene of *Pseudomonas syringae* pv. *phaseolicola***. *Mol Plant Microbe Interact* 1995, **8**:637-640.

10. • Zhu M, Shao F, Innes RW, Dixon JE, Xu Z: **The crystal structure of *Pseudomonas* avirulence protein AvrPphB: a papain-like fold with a distinct substrate-binding site**. *Proc Natl Acad Sci USA* 2004, **101**:302-307.
The first report of the crystal structure of a type III effector from a plant pathogen demonstrates the structural similarity of AvrPphB to the papain-like cysteine proteases and suggests a common catalytic mechanism.

11. Shao F, Merritt PM, Bao Z, Innes RW, Dixon JE: **A *Yersinia* effector and a *Pseudomonas* avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis**. *Cell* 2002, **109**:575-588.

12. Shao F, Goldstein C, Ade J, Stoutemyer M, Dixon JE, Innes RW: **Cleavage of *Arabidopsis* PBS1 by a bacterial type III effector**. *Science* 2003, **301**:1230-1233.

13. Grant MR, Godiard L, Straube E, Ashfield T, Lewald J, Sattler A, Innes RW, Dangl JL: **Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance**. *Science* 1995, **269**:843-846.

14. Ashfield T, Ong LE, Nobuta K, Schneider CM, Innes RW: **Convergent evolution of disease resistance gene specificity in two flowering plant families**. *Plant Cell* 2004, **16**:309-318.

15. Ashfield T, Keen NT, Buzzell RI, Innes RW: **Soybean resistance genes specific for different *Pseudomonas syringae* avirulence genes are allelic, or closely, at the *RPG1* locus**. *Genetics* 1995, **141**:1597-1604.

16. Mackey D, Holt BF III, Wiig A, Dangl JL: **RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in *Arabidopsis***. *Cell* 2002, **108**:743-754.

17. • Lee CC, Wood MD, Ng K, Andersen CB, Liu Y, Luginbühl P, Spraggon G, Katagiri F: **Crystal structure of the type III effector AvrB from *Pseudomonas syringae***. *Structure* 2004, **12**:487-494.
The crystal structure of AvrB revealed a novel fold that did not display significant similarity to known proteins. This highlights one of the current challenges of type III effector functional annotation. The structure revealed a structural domain that is responsible for recognition by *RPM1*.

18. Ronald P, Salmeron J, Carland F, Staskawicz BJ: **The cloned avirulence gene *avrPto* induces disease resistance in tomato cultivars containing the *Pto* resistance gene**. *J Bacteriol* 1992, **174**:1604-1611.

19. Chang JH, Rathjen JP, Bernal AJ, Staskawicz BJ, Michelmore RW: **avrPto enhances growth and necrosis caused by *Pseudomonas syringae* pv. *tomato* in tomato lines lacking either Pto or Prf**. *Mol Plant Microbe Interact* 2000, **13**:568-571.

20. Scofield SR, Tobias CM, Rathjen JP, Chang JH, Lavelle DT, Michelmore RW, Staskawicz BJ: **Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato**. *Science* 1996, **274**:2063-2065.

21. Tang X, Frederick RD, Zhou J, Halterman DA, Jia Y, Martin GB: **Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase**. *Science* 1996, **274**:2060-2063.

22. • Wulf J, Pascuzzi PE, Fahmy A, Martin GB, Nicholson LK: **The solution structure of type III effector protein AvrPto reveals conformational dynamic features important for plant pathogenesis**. *Structure* 2004, **12**:1257-1268.
The first solution structure of a type III effector reveals the dynamics of effector populations. AvrPto exists in populations of disordered states and a defined three-helix bundle, suggesting a low stability that might have implications for type III secretion mechanisms.

23. Kim YJ, Lin NC, Martin GB: **Two distinct *Pseudomonas* effector proteins interact with the Pto kinase and activate plant immunity**. *Cell* 2002, **109**:589-598.

24. Shan L, He P, Zhou JM, Tang X: **A cluster of mutations disrupt the avirulence but not the virulence function of AvrPto**. *Mol Plant Microbe Interact* 2000, **13**:592-598.

25. Cordes FS, Komoriya K, Larquet E, Yang S, Egelman EH, Blocker A, Lea SM: **Helical structure of the needle of the type III secretion system of *Shigella flexneri***. *J Biol Chem* 2003, **278**:17103-17107.

26. Jackson RW, Athanassopoulos E, Tsiamis G, Mansfield JW, Sesma A, Arnold DL, Gibbon MJ, Murillo J, Taylor JD, Vivian A: **Crystal structures of the type III effector protein AvrPphF and its chaperone reveal residues required for plant pathogenesis**. *Structure* 1999, **12**:1669-1681.

27. Tsiamis G, Mansfield JW, Hockenhull R, Jackson RW, Sesma A, Athanassopoulos E, Bennett MA, Stevens C, Vivian A, Taylor JD, Murillo J: **Cultivar-specific avirulence and virulence functions assigned to AvrPphF in *Pseudomonas syringae* pv. *phaseolicola*, the cause of halo-blight disease**. *EMBO J* 2000, **19**:3204-3214.

28. Shan L, Oh HS, Chen J, Guo M, Zhou J, Alfano JR, Collmer A, Jia X, Tang X: **The *HopPtoF* locus of *Pseudomonas syringae* pv. *tomato* DC3000 encodes a type III chaperone and a cognate effector**. *Mol Plant Microbe Interact* 2004, **17**:447-455.

29. • Singer AU, Desveaux D, Betts L, Chang JH, Nimchuk Z, Grant SR, Dangl JL, Sondek J: **Crystal structures of the type III effector**

protein AvrPphF and its chaperone reveal residues required for plant pathogenesis. *Structure* 2004, **12**:1669-1681.
The structure of HopF1 reveals a novel mushroom-like fold that is divided into 'head' and 'stalk' subdomains. This study demonstrates that a combination of structural information and homology-based approaches can predict important functional domains of type III effectors. The type III chaperone SchF shares remarkable structural similarity to type III chaperones of mammalian pathogens despite sharing limited sequence similarity.

30. Abramovitch RB, Kim YJ, Chen S, Dickman MB, Martin GB: ***Pseudomonas* type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death**. *EMBO J* 2003, **22**:60-69.

31. Pedley KF, Martin GB: **Molecular basis of Pto-mediated resistance to bacterial speck disease in tomato**. *Annu Rev Phytopathol* 2003, **41**:215-243.

32. •• Janjusevic R, Abramovitch RB, Martin GB, Stebbins CE: **A bacterial inhibitor of host programmed cell death defenses is an E3 ubiquitin ligase**. *Science* 2006, **311**:222-225.
The structure of the type III effector AvrPtoB reveals its enzymatic function as an E3-ligase. This demonstrates the role of structural mimicry in bacterial pathogens of plants and emphasizes the importance of structural studies for the functional annotation of phytopathogenic type III effectors. This study also adds a new enzymatic activity to the list of those adopted by bacterial virulence proteins.

33. Abramovitch RB, Janjusevic R, Stebbins CE, Martin GB: **Type III effector AvrPtoB requires intrinsic E3 ubiquitin ligase activity to suppress plant cell death and immunity**. *Proc Natl Acad Sci USA* 2006, **103**:2851-2856.

34. Estelle M: **Proteases and cellular regulation in plants**. *Curr Opin Plant Biol* 2001, **4**:254-260.

35. Stebbins CE, Galan JE: **Structural mimicry in bacterial virulence**. *Nature* 2001, **412**:701-705.

36. Fu Y, Galan JE: **A *Salmonella* protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion**. *Nature* 1999, **401**:293-297.

37. Stebbins CE, Galan JE: **Modulation of host cell signalling by a bacterial mimic. Structure of the *Salmonella* effector SptP bound to Rac1**. *Mol Cell* 2000, **6**:1449-1460.

38. • Jørgensen R, Merrill AR, Yates SP, Marquez VE, Schwan AL, Boesen T, Andersen GR: **Exotoxin A–eEF2 complex structure indicates ADP ribosylation by ribosome mimicry**. *Nature* 2005, **436**:979-984.
Four crystal structures of the *P. aeruginosa* toxin exotoxin A with its eukaryotic target eEF2 reveal important insights into the catalytic mechanism of this ADP-ribosyltransferase. Importantly, the catalysis appears to be substrate assisted, meaning that the bacterial virulence protein requires residues from its eukaryotic target eEF2 in order to function. Also, the complex of exotoxin A with eEF2 and the non-hydrolyzable NAD analog βTAD reveals that a small molecule can structurally mimic the ribosome.

39. Mackey D, Belkhadir Y, Alonso JM, Ecker JR, Dangl JL: ***Arabidopsis* RIN4 is a target of the type III effector AvrRpt2 and modulates *RPS2*-mediated resistance**. *Cell* 2003, **112**:379-389.

40. Axtell MJ, Staskawicz BJ: **Initiation of RPS2-specified disease resistance in *Arabidopsis* is coupled to the AvrRpt2-directed elimination of RIN4**. *Cell* 2003, **112**:369-377.

41. Kim MG, da Cunha L, McFall AJ, Belkhadir Y, DebRoy S, Dangl JL, Mackey D: **Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in *Arabidopsis***. *Cell* 2005, **121**:749-759.

42. Kim HS, Desveaux D, Singer AU, Patel P, Sondek J, Dangl JL: **The *Pseudomonas syringae* effector AvrRpt2 cleaves its C-terminally acylated target, RIN4, from *Arabidopsis* membranes to block RPM1 activation**. *Proc Natl Acad Sci USA* 2005, **102**:6496-6501.

43. Rohmer L, Guttman DS, Dangl JL: **Diverse evolutionary mechanisms shape the type III effector virulence factor repertoire in the plant pathogen *Pseudomonas syringae***. *Genetics* 2004, **167**:1341-1360.

44. Axtell MJ, Chisholm ST, Dahlbeck D, Staskawicz BJ: **Genetic and molecular evidence that the *Pseudomonas syringae* type III effector protein AvrRpt2 is a cysteine protease**. *Mol Microbiol* 2003, **49**:1537-1546.

45. Alto NM, Shao F, Lazar CS, Brost RL, Chua G, Mattoo S, McMahon SA, Ghosh P, Hughes TR, Boone C, Dixon JE: **Identification of a bacterial type III effector family with G protein mimicry functions**. *Cell* 2006, **124**:133-145.

46. Korf U, Kohl T, van der Zandt H, Zahn R, Schleeger S, Ueberle B, Wanschneider S, Bechtel S, Schnolzer M, Ottleben H *et al.*: **Large-scale protein expression for proteome research**. *Proteomics* 2005, **5**:3571-3580.

47. Smyth DR, Mrozkiewicz MK, McGrath WJ, Listwan P, Kobe B: **Crystal structures of fusion proteins with large-affinity tags**. *Protein Sci* 2003, **12**:1313-1322.

48. Savchenko A, Yee A, Khachatryan A, Skarina T, Evdokimova E, Pavlova M, Semesi A, Northey J, Beasley S, Lan N *et al.*: **Strategies for structural proteomics of prokaryotes: quantifying the advantages of studying orthologous proteins and using both NMR and X-ray crystallography approaches**. *Proteins* 2003, **50**:392-399.