

The long and winding road: virulence effector proteins of plant pathogenic bacteria

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Abstract Plant pathogenic bacteria inject about 30 virulence effector proteins into the host cell using a specialized secretion apparatus. Bacteria which are unable to do this elicit host immunity and cannot grow inside living plant tissue. Thus, the primary function of the effectors is to suppress host immunity. The identity of individual effectors within each complement varies even between closely related bacterial strains, and effectors themselves act redundantly and are apparently interchangeable. Many effectors are known to target components of plant defense pathways, but it is difficult to study their role in molecular terms. For some of them, there is controversy about their mode of action. We propose that effectors act promiscuously by targeting host molecules with low specificity and affinity.

Keywords Type III secretion system · Effector proteins · MAMPs · Innate immunity · Virulence factors

Introduction

Plant pathogenic bacteria, in contrast to many obligate eukaryotic pathogens, can choose whether or not to deploy their arsenals of virulence weaponry. Normally

resident in soil as saprophytes, or on leaves as epiphytes, bacteria move opportunistically into the leaf in response to unknown cues [1]. The attraction of this niche is the access to host nutrients which can be exploited to drive bacterial replication to an extent that is impossible in the other niches. The drawback is the enforced proximity to the host immune system, which responds to the bacterial presence through both extracellular and intracellular perception mechanisms [2]. Given the right conditions, bacteria replicate exponentially to high levels and cause the typical spots, wilts, and canker symptoms of infection. The ability to opt out of the pathogenic lifestyle, and the generalist nature of bacterial infectivity, means that hosts can be relieved of selective pressure from pathogens. Similarly, selection pressures on the virulence components of bacteria can be modified by infection of alternate hosts, or released from selection pressure entirely during non-pathogenic phases. This is a remarkably different selection framework when compared to eukaryotic pathogens which are obligate and frequently restricted to a single host species, and hence the forces which shape evolution of their respective virulence machineries may vary significantly.

The most important strategy for virulence of plant pathogenic bacteria is deployment of proteinaceous ‘effectors’ within the host cell. On entry to the leaf, bacteria form tight associations with the host cell wall [3]. This sets into play a chain of host perception events that must be avoided or suppressed by the pathogen, in a relationship that is often characterized as a molecular arms race [2]. Thus, bacteria are betrayed by the presence of characteristic elicitors, which are detected at the cell surface by specific receptor proteins that activate immunity. Immunity triggered by elicitor recognition is suppressed by bacterial effector proteins, but these can

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themselves act as elicitors after recognition by cytoplasmic receptors of the nucleotide-binding-leucine rich repeats (NB-LRR) class. Predictably, some effectors suppress NB-LRR signaling; more surprisingly, it was shown recently that a host defense protein can modify a pathogen effector to ensure its cytoplasmic detection [4]. Although the molecular activities and host targets of most effectors have not been defined, there is an emerging theme of degradative activities and weak specificities, congruent with the idea of bacteria as generalist, non-obligate pathogens. This complicates the identification of effector targets, because it is difficult to discriminate between false and true positives. However, everything that is worthwhile in life is also difficult, as the Beatles remind us in “The Long and Winding Road”; this review takes their works as themes to explore current topics in effector biology.

Anti-bacterial immunity: “A beginning”

In the initial steps of an infection, plants perceive microbes through detection of conserved elicitors known as PAMPs or MAMPs (for pathogen- or microbe-associated molecular patterns) [5]. Recognition of bacterial MAMPs occurs through specific pattern recognition receptors (PRRs) [6]. The PRRs that respond to bacterial MAMPs so far as is known are receptor kinase proteins, many of which form a complex with a second transmembrane kinase called BAK1 (for BRI1-associated receptor kinase 1) immediately after elicitation [7–9]. Upon formation of the recognition complex, a cascade of responses is mounted which includes an increase in cytosolic calcium from external stores, the generation of a burst of reactive oxygen species, activation of a mitogen-activated protein kinase (MAPK) cascade, induction of defense-associated genes, and deposition of callose into cell walls at the site of infection [10, 11]. There are some dependency relationships amongst these responses, but it is not yet clear whether they follow a linear pathway or comprise a signaling network. The outcome of PRR activation is PAMP-triggered immunity (PTI), although the specific mechanisms that curtail bacterial growth are unknown [5, 12]. To counteract PTI, pathogens of all kinds deposit so-called effector molecules into the plant cell, which suppress immunity through various strategies, most of which are unknown. As noted previously, effectors can also act as elicitors due to direct or indirect recognition by NB-LRR proteins. This review concentrates on the virulence activities of bacterial effectors with a focus on the model pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000), which is pathogenic on tomato and the model plant *Arabidopsis thaliana*.

Effector secretion by pathogenic bacteria: “All things must pass”

Phytopathogenic bacteria remain extracellular throughout the life cycle. They enter the intercellular spaces called the apoplast through stomatal openings or wounds. Once inside the leaf, they adhere to the plant cell wall and form dense colonies [13]. Early insights with non-pathogenic *hrp* mutants (hypersensitive response and pathogenicity) outlined the fundamental characteristics of bacterial pathogenicity [14]. The hypersensitive response (HR) is a rapid localized cell death response at the infection site that is associated with immunity. Bacteria mutated in *hrp* genes do not induce the HR on resistant hosts, and behave as non-pathogens (i.e., do not replicate or cause symptoms) on susceptible hosts. Thus, there is a link between bacterial pathogenicity and recognition by the host immune system leading to the HR [15]. A highly conserved subset of *hrp* genes is present in all major gram-negative bacterial phytopathogens [e.g., *Pseudomonas syringae*, *Xanthomonas* spp., *Ralstonia solanacearum*, and *Pectobacterium* (formerly *Erwinia*) spp.]. Those conserved with animal pathogens are called *hrc* genes (hypersensitive response and conserved). *Hrc* genes encode structural components of the Type-III secretion system (TTSS), a supramolecular, filamentous needle-like structure that is the conduit for effector delivery [16, 17]. They are regulated collectively by the transcription factors *hrpR/S* and *hrpL* which bind to the *hrp* box motif in the respective promoter regions [18–20]. The Type-III pilus is composed of *hrpA* building blocks and grows progressively from its tip [16, 17]. It extends across both bacterial cell membranes and the plant cell wall, and apparently penetrates the host plasma membrane. The pilus injects around 30 virulence effector proteins directly into the host cytoplasm, the expression of which are also regulated by *hrpL* [21–23]. Injected effector proteins localize to various cellular compartments where they manipulate the host in favor of pathogen growth.

To date, 62 Type-III effectors of *P. syringae* have been identified, which fall into 29 families, 10 of which contain multiple members (significant variants), and 16 of which are singletons (<http://www.pseudomonas-syringae.org/>; [24]). Genome sequencing of three *P. syringae* strains [pvs *tomato* DC3000, *syringae* B728a (*Psy* B728a), and *phaseolicola* 1448a (*Pph* 1448a)] and the draft genome sequence of a fourth [pv. *tabaci* 11528 (*Pta* 11528)] has revealed important information on the diversity of effectors encoded by these strains [24–27]. Each strain encodes about 20–30 effectors. Despite very high levels of sequence identity over the genome as a whole, remarkably few effector genes are shared between genomes. For example, *Pph* 1448a and *Pta* 11528 share 97% nucleotide identity over the alignable portions of their genomes, but share only

9 effector genes [24]. This scenario is generally true, and despite the concept of “core” and “variable” effector compliments in the literature [28], the four genomes share only four effectors, and it seems likely that this will be diminished by sampling further genome sequences. The inherent variability of effector complements between strains may reflect selective pressure from the host’s immune system, particularly via intracellular recognition. In addition, the lack of overlap between effector complements of strains that share a common host, such as *Pto* DC3000 and *Psy* B728a, suggests that effectors are interchangeable.

Effector redundancy: “You won’t see me”

The first bacterial effector proteins were identified based on the ability to cause the HR—a conspicuous immune reaction—on certain host plants [2]. Why should pathogens secrete proteins which curtail their infectivity so spectacularly? Because of the extreme selection pressure active against recognized effectors, it was clear that they must play important roles in the absence of recognition; that is, on susceptible plant hosts. Suppression of immunity by virulent bacteria is easy to demonstrate: bacteria as extracellular pathogens elicit PTI responses, which are suppressed by intact pathogens but not by those lacking a functional TTSS [29]. Demonstration of virulence roles for individual effectors has proven to be more difficult, with only quantitative contributions to bacterial growth conferred by even the strongest effectors. This is likely due to effector redundancy [31, 32]. For example, both AvrPto and AvrPtoB target PRR receptor complexes to abrogate signal transduction [30–32]. Likewise, individual mutations of the conserved effector locus (CEL) comprising the effector genes AvrE and HopM1 did not affect pathogen growth [33]. However, a complete knockout of the CEL locus compromised bacterial growth strongly [34], suggesting that AvrE and HopM1 act redundantly. Overall, individual knockouts tend not to be informative, whereas multiple knockouts can begin to identify redundancy relationships between various effectors [35].

Detecting effector activity on plants: “Within you without you”

It has been difficult to study the physiological and molecular activities of Type III effectors. This is due in part to the quantitative contribution of individual effectors to bacterial virulence [2, 36]. Therefore, methods to study individual effectors in isolation are necessary, although a completely satisfactory method for achieving this is not

currently available. Two common approaches are to express effector genes transgenically, either from stably transformed plants expressing the effector gene from an inducible promoter, e.g., in *Arabidopsis*, or by *Agrobacterium*-mediated transient transformation of *Nicotiana benthamiana* leaves [11, 37, 38]. Another popular approach is to transform plant protoplasts with constructs for effector gene expression [39, 40]. These approaches are very powerful, as they allow elicitation of transformed tissue with MAMPs coupled with assays for suppression of defined immune responses by effectors. Transient gene expression methods have the additional benefit of being relatively quick, allowing higher throughput. However, in all cases, the effector is present before the MAMP stimulus—different from how the natural infection is supposed to progress—and super-accumulates to unrealistic levels. *Agrobacterium*-based transient expression assays have the additional drawback that *Agrobacterium* has endogenous MAMPs of its own, which might obscure the response to exogenous MAMPs. The use of protoplasts is also controversial, because they are under stress and lack the cell wall which is a hallmark of the plant–bacterial interaction. Nevertheless, all three systems have delivered important data, and results obtained with these systems can be tested independently using genetically altered bacterial strains on intact plants. An alternative strategy for effector delivery is to use *P. fluorescens* complemented by a TTSS from *P. syringae* [41], allowing individual delivery of each effector at presumed biological levels. However, this system pairs poorly with most assays of MAMP-triggered immunity. Finally, effectors are not normally delivered individually, and such reductionist experiments might miss informative interactions between effectors.

Virulence targets and search for function: “Here, there, and everywhere”

The molecular functions or host virulence targets of most Type-III effectors are poorly defined. We summarize the effectors with known functions and/or targets in Table 1. Probably the best understood effector is AvrPtoB of *Pto* DC3000 (Fig. 1). This is a multidomain protein with an N-terminal region that is a general kinase-binding domain and a C-terminal ubiquitin ligase domain [42–44]. AvrPtoB suppresses perception of bacterial flagellin and fungal chitin through targeting of their respective receptor kinases FLS2 and CERK1 [30, 45, 46]. Its E3 ligase activity is required for ubiquitination of CERK1 leading to its lysosomal degradation [46]. For suppression of FLS2, the role of the E3 ligase domain is less clear. Several workers showed that the AvrPtoB E3 domain is dispensable for suppression of flagellin responses [11, 47], yet Gohre et al.

Table 1 Molecular activities of bacterial Type-III effector proteins

Bacterial strain(s)	Effector	Function	Action on plant cell	Virulence target(s)	Function of target(s)	Ref
<i>Pto</i> DC3000	AvrPto	Unknown	Stops initiation of MAMP signaling	BAK1, FLS2, EFR and possibly other PRRs	MAMP perception and signaling	[30, 32]
<i>Pto</i> DC3000	AvrPtoB	E3 protein ligase	Stops initiation of MAMP signaling	BAK1, FLS2, EFR, CERK1 and possibly other PRRs	MAMP perception and signaling	[30, 45, 46]
<i>Pto</i> DC3000	HopAII	Phosphothreonine lyase	Suppresses MAPK signaling	MAPKs	Kinase activity/MAMP signaling	[49]
<i>X. campestris</i> pv. <i>vesicatoria</i>	XopJ	Unknown	Suppresses exocytosis	Unknown	Probably involved in exocytosis	[52]
<i>Pto</i> DC3000	HopM1	Unknown but initiates target degradation	Suppression of callose deposition	AtMIN7 and others	ARF-GEF involved in exocytosis	[37]
<i>Pto</i> DC3000	HopU1	ADP-ribosyl transferase (ADP-RT)	Suppression of flagellin-induced callose deposition and cell death	GRP7	RNA binding protein	[54]
<i>Xanthomonas</i> spp.	TAL effectors	Transcription factors	Interfere with host gene transcription	Diverse host genes	Development and immunity	[55–57]
<i>X. campestris</i> pv. <i>vesicatoria</i>	XopN	Unknown	Suppression of defense genes	TARK1	Atypical receptor kinase of unknown function	[66]
<i>Pto</i> DC3000	HopAA1-1	Potential GTPase-activating protein (GAP) domain	Involved in speck lesion formation	Unknown	Unknown	[67]
<i>P. syringae</i> spp.	HopI1	Putative J domain	Thylakoid remodelling and suppression of SA	Unknown	Unknown	[73]
<i>Pto</i> DC3000	HopAMI	Unknown	Suppression of ABA and aids adaptation to drought	Unknown	Unknown	[74]

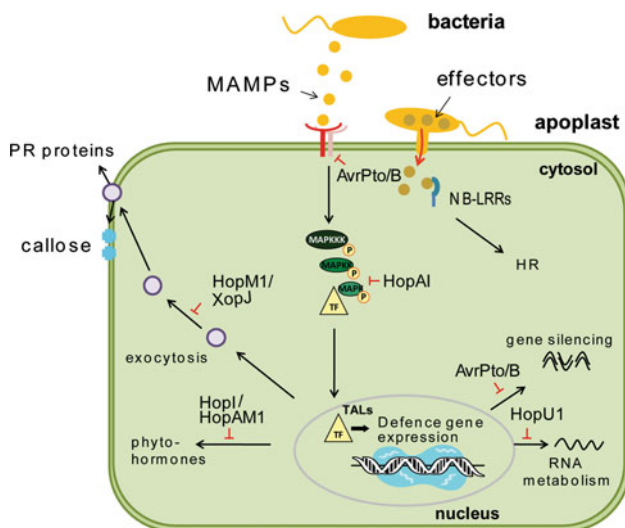


Fig. 1 Suppression of defense signaling through effector proteins at different stages of signaling

[45] reported that AvrPtoB ubiquitinates FLS2 for subsequent proteasomal degradation. FLS2 levels were reduced only slightly in the presence of AvrPtoB, whereas CERK1 was completely degraded [45, 46]. In an alternative model, Shan and colleagues reported that AvrPtoB suppresses flagellin signaling by targeting the FLS2 co-factor BAK1, thus preventing complex formation [30]. This model is attractive because it provides an explanation of how AvrPtoB might suppress multiple MAMP-responsive pathways. However, as the FLS2 complex assembles subsequent to MAMP perception but before effector delivery, it is unclear how this model might work in vivo, and additionally, BAK1 levels were unaffected by AvrPtoB expression [45].

The *Pto* DC3000 effector AvrPto, which is structurally unrelated to AvrPtoB, also suppresses flagellin-dependent responses (Fig. 1). Competing models to explain this suppression exist. Constitutive overexpression of AvrPto in stable transgenic *Arabidopsis* lines causes dwarfing that phenocopies mutants deficient in perception of the growth hormone brassinosteroid (BR). As BAK1 participates in both pathways as a co-factor of FLS2 or the brassinosteroid receptor BRI1, inhibition of BAK1 by AvrPto was a likely hypothesis [30]. Indeed, Shan et al. [30] showed that AvrPto targets BAK1 leading to disruption of the BAK1-FLS2 complex. However, another study showed that AvrPto interacts directly with FLS2, inhibiting its kinase activity and subsequent signaling [32]. It is not possible at this stage to reconcile these models, and in fact they are not exclusive. Shan et al. [30] also found that AvrPto interacted with FLS2, albeit at a lower affinity compared with BAK1 interaction. BAK1 is a plausible target because all known BAK1-dependent MAMP perception systems are suppressed by AvrPto, and AvrPto also suppressed signaling

downstream of BR perception [30]. However, the growth defect conferred by constitutive expression of AvrPto in *Arabidopsis* plants was far greater than the mild *bak1* mutant phenotype [8]. Other hormonal pathways involved in plant growth such as auxin were not affected as shown by analysis of gene expression [30]. Despite the discrepancies between the proposed mechanisms it is clear that both AvrPto and AvrPtoB target receptor complexes [11, 30, 38, 46]. In general, it is likely that both effectors target multiple protein kinases, because they also interact with members of the cytosolic Pto kinase family [48]. This observation is of potentially considerable importance because it introduces the idea that effectors may lack specificity and have many host targets.

Disrupting the initial steps of pathogen detection is one strategy to ensure successful infection. However, bacteria rely not only on disruption of MAMP perception but also interfere with downstream components of the signaling cascade. For example, the *Pto* DC3000 effector HopAI1 was reported to suppress MAPK activation via its phosphothreonine lyase activity (Fig. 1) [49]. However, He et al. [38] observed neither MAPK suppression nor reductions in defense gene expression in the presence of HopAI1. Further studies are necessary to resolve the activities of this effector.

Vesicle transport to the plasma membrane is associated with plant defense against bacteria [50, 51]. Two effectors, namely the *Pto* DC3000 effector HopM1 and the *Xanthomonas campestris* pv. *vesicatoria* effector XopJ interfere with this pathway (Fig. 1) [37, 52]. While the molecular target(s) of XopJ remain(s) to be identified, HopM1 interacts with an adenosine diphosphate (ADP) ribosylation factor–guanine nucleotide exchange factor (ARF–GEF) protein named AtMIN7 (*Arabidopsis thaliana* HopM interactor 7). ARF–GEFs are well-known components of vesicle trafficking [37]. The importance of AtMIN7 was underlined by studies on knockout mutants which no longer exhibited callose deposition in response to flagellin and allowed increased growth of the TTSS-deficient strain *Pto* DC3000 *hrcC*. In addition, AtMIN7 participates in brefeldin A-dependent vesicle formation and localization of the auxin efflux carrier PIN1 [53]. It is as yet unknown which defense compounds require AtMIN7 for trafficking, and it is conceivable that it plays multiple roles in plant immunity.

Another effector which targets a known host protein is HopU1, an ADP-ribosyl transferase (ADP-RT) which interacts with and modifies the RNA-binding protein GRP7 (Fig. 1) [54]. *Pto* DC3000 requires HopU1 for cell death suppression on *N. tabacum*, and this is dependent on the ADP-RT catalytic residues of HopU1. Similarly, suppression of flagellin-dependent callose deposition by HopU1 required enzymatic activity. Consistent with these

observations, *grp7* mutant *Arabidopsis* lines were more susceptible to both *Pto* DC3000 and a non-pathogenic *hrcC* mutant, and deposited less callose in response to flagellin [54]. GRP7 has characteristics of an RNA binding protein, but what is its role in plant immunity? The question remains open; possible roles include acting as an important post-transcriptional regulator of gene expression through the trafficking, stabilization or processing of specific mRNAs. A second important question is the role of ADP-ribosylation in modulating GRP7 function. A final question is whether HopU1 has other targets in host immunity.

A recent important discovery made on the transcription activator-like (TAL) effectors of *Xanthomonas* spp. should also be mentioned here [55–57]. These effectors activate transcription of host genes directly through a central tandem repeat domain which mediates DNA binding (Fig. 1). Some of the genes activated by TAL effectors appear to be involved in plant development and lead to changes in cell division, cell enlargement, and hypertrophy, thus contributing to disease symptoms [57–60]. However, many more target genes of TAL effectors remain to be identified. The striking discovery is that two hypervariable amino acid residues in each repeat domain specify interaction with a characteristic nucleotide within the effector recognition site. Thus, the amino acid sequence of the tandem repeats predicts the nucleotide sequence of the target DNA binding site with complete accuracy. This enables precise modification of gene expression in vivo using biotechnological methods, including turning this system against *Xanthomonas* spp. by engineering TAL recognition sites upstream of active resistance genes [57].

The examples above are based on classical studies of plant immunity with a conceptual bias toward linear signal perception, transduction and response pathways. Plants also respond to pathogens through an endogenous mechanism called gene silencing [61, 62]. Gene silencing is mediated by short single-stranded RNA molecules which target complementary sequences within the genome to down-regulate gene expression [63]. One prominent subclass of small RNAs are the so-called microRNAs (miRNAs), which are transcribed and subsequently processed from non-coding genes, and can target one or more coding genes in order to suppress their expression [63, 64]. For example, the endogenous miRNA miR393 is MAMP-induced and contributes to bacterial resistance by interference with auxin signaling [61]. Furthermore, *Arabidopsis* plants mutated for components of the miRNA silencing machinery were found to be hypersusceptible to adapted and unadapted bacterial pathogens [65]. Several effectors from *Pto* DC3000, including AvrPto, suppressed expression of MAMP induced and even endogenous miRNA at various steps (Fig. 1). In light of what we know about AvrPto, one might suspect that this effect is due solely to suppression of

the MAMP signal at the level of perception [65]. However, Navarro et al. also found that the miRNA precursor molecules were not affected, and that miRNAs involved in endogenous pathways not regulated by MAMPs were likewise downregulated. Therefore, the authors argued that AvrPto might target a component of the silencing machinery directly, or that components of PRR complexes such as BAK1 might somehow cross talk with endogenous silencing pathways [65]. The role of RNA silencing in defense against phytopathogenic bacteria is in its infancy and needs further work to be firmly established.

Most effectors have no detected or defined roles in pathogen virulence. One confounding factor could be the timepoint during infection at which some effectors are active. Bacterial growth is typically measured within the first few days of inoculation between lag phase and stationary phase, and what happens after this is largely disregarded. One interesting example is the effector XopN of *X. campestris* pv. *vesicatoria*, which conferred growth benefits that could be measured only after 6 days post-inoculation [66]. XopN targets the *Solanum lycopersicum* atypical receptor kinase (TARK1) at the plasma membrane. Plants silenced for *TARK1* were more susceptible to *X. campestris* pv. *vesicatoria* infection. The role of TARK1 in immunity is unknown. As MAMP responses are important in the very early stages of infection, it seems likely that the XopN-TARK1 interaction must play some other role in bacterial pathogenesis.

Other scenarios for absent effector phenotypes are possible. For example, the respective effector might be dispensable for infection of the plant species under investigation, or require additional pathogen-derived molecules for function. Others may play roles in disease symptom formation, such as the *Pto* DC3000 effector HopAA1-1, which promotes the formation of bacterial speck lesions and carries a potential GTPase-activating protein (GAP) domain [67]. In addition, HopAA1-1 suppresses flagellin-induced NHO1 expression and is localized to mitochondria in yeast [34, 68]. Overall, there are many open hypotheses and this field is only beginning to be explored.

Roles for effectors outside of defense suppression: “Magical mystery tour”

A long-standing hypothesis is that effectors may facilitate nutrient release to aid pathogen growth. However, experimental evidence for such a role is still absent. However, some evidence for alterations in metabolic activities comes from transcriptome studies comparing plant responses to virulent and non-pathogenic (*hrcC*) bacteria indicating that some genes involved in sugar metabolism and transport were induced by the *Pto* DC3000 effector repertoire

[69–71]. However, this is a descriptive approach, and the role of these genes in host susceptibility has not been validated. Furthermore, these experiments compared intact virulent bacteria with mutant strains lacking the TTSS, which prevents insights into the activities of individual effectors. It would be interesting to perform similar microarray analyses on transgenic plants expressing individual effectors with previously unidentified function, with and without prior MAMP treatment. Interestingly, in a proteomic study, it was found that virulent *Pto* DC3000 specifically induced the secretion of some enzymes of primary metabolism in *Arabidopsis* cell cultures [72]. These enzymes were not secreted in response to either the non-virulent *Pto* DC3000 *hrcC* mutant or *Pto* DC3000 carrying *AvrRpm1* which induces ETI in this system. Therefore, it is tempting to speculate that bacterial Type III effectors manipulate the host's secretion machinery to release these enzymes into the intercellular space.

Another potential role of effectors might be to aid environmental adaption of plants. Data supporting this have been presented for two effectors, HopI1 and HopAM1 (Fig. 1) [73, 74]. HopI1 is a ubiquitous *P. syringae* effector which localizes to the chloroplast where it promotes thylakoid remodeling and suppression of salicylic acid production [73]. The C-terminus of HopI1 has J domain activity which is commonly found in heat shock protein 70 (Hsp70) co-chaperones, and was required for alterations in thylakoid structure. Consistently, HopI1-expressing transgenic plants show increased heat tolerance indicating that HopI1 can engage the plant stress-response machinery. Likewise, HopAM1 appears to aid the adaptation of infected plants to drought and salinity stress by manipulation of ABA signaling [74]. In general, effectors are studied under standard laboratory conditions which might obscure some activities associated with specific environments.

Effector delivery and host cell death:

“All together now”

A surprising number of effectors suppress the HR associated with effector recognition [75–77]. However, cell death is not always associated with immunity but can be a consequence of disease. For hemibiotrophic pathogens such as *P. syringae*, it may be important not only to suppress the HR but also to induce disease-associated cell death late in the infection cycle. It therefore seems logical that such pathogens invest significant resources to keep such cell death-inducing effectors in check. For example, the cell death induced by the *Pto* DC3000 effector HopAI on *N. tabacum* was suppressed by at least 26 of the 35 *Pto* DC3000 effectors [76]. To fully understand the interplay of

these effectors it will be important to investigate the order and speed they are secreted into the host. What is the timing of effector delivery and does it vary for different effectors?

Perspective: “All I’ve got to do”

Although investigation of the effectors of plant pathogenic bacteria is at a relatively early stage, enough progress has been made to sketch their strategy in a tentative way. The key characteristics are that effectors are redundant and interchangeable. Individual effectors do not impact pathogenicity significantly except in a negative sense, if they are recognized by the host immune system, but collectively are indispensable for pathogenicity. Pairs of unrelated effectors such as *AvrPto* and *AvrPtoB*, or *HopM1* and *AvrE*, work redundantly [77]. There is no evidence that individual effectors provide specificity on a particular host, in fact, the opposite may be true. Thus, *Pto* DC3000, which is not an efficient pathogen on *N. benthamiana*, can be converted into one by deletion of the recognized effector *HopQ1*, and hence, the remaining effectors which are not evolved to suppress *N. benthamiana* defense responses, can do so efficiently [78]. A second emerging theme is the probable lack of specificity of individual effectors, exemplified by *AvrPtoB*. This effector currently has five recognized cellular protein kinase targets, and likely more because of the high relatedness of the plant protein kinase family. This makes obvious sense for bacteria as generalist or occasional pathogens—to gain the most advantage from each effector, firstly that they have multiple targets, and moreover, that they have enzyme activity so each effector can process multiple targets. The overall strategy behind effector deployment may be a brute force one—to disable the host proteome, particularly the plasma membrane proteome where the PRRs reside, as comprehensively as possible, with little regard to the identity of individual targets. Of course, evolution will select those effector–target interactions that are most beneficial, but bacteria, unlike obligate pathogens, can release such selective pressures by infecting alternate hosts or eschewing the pathogenic lifestyle. We therefore propose that promiscuous, low affinity interactions will be characteristic of binding events between effectors of plant pathogenic bacteria and their host targets.

What remains to be done? We know so little about effector targets, but these will be hard to detect and validate, especially if the model above holds. But this has much to teach us about immune pathways, of which we know equally little. We need to know more about the pathogenic niche, and how the pathogen manipulates cell death and other environmental variables to its advantage.

We know nothing about nutrient acquisition and whether effectors act to manipulate this. Lastly, effector detection systems embodied by NB-LRR proteins and their co-factors, which appear to react to the activity of specific effectors, are truly interesting. For these studies and others which we are yet to foresee, we need to invent new approaches and tool kits. To borrow a final line from the Fab Four, “We can work it out”!

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