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Review

The targeting of plant cellular systems by injected type III effector proteins

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ABSTRACT

The battle between phytopathogenic bacteria and their plant hosts has revealed a diverse suite of strategies and mechanisms employed by the pathogen or the host to gain the higher ground. Pathogens continually evolve tactics to acquire host resources and dampen host defences. Hosts must evolve surveillance and defence systems that are sensitive enough to rapidly respond to a diverse range of pathogens, while reducing costly and damaging inappropriate misexpression. The primary virulence mechanism employed by many bacteria is the type III secretion system, which secretes and translocates effector proteins directly into the cells of their plant hosts. Effectors have diverse enzymatic functions and can target specific components of plant systems. While these effectors should favour bacterial fitness, the host may be able to thwart infection by recognizing the activity or presence of these foreign molecules and initiating retaliatory immune measures. We review the diverse host cellular systems exploited by bacterial effectors, with particular focus on plant proteins directly targeted by effectors. Effector–host interactions reveal different stages of the battle between pathogen and host, as well as the diverse molecular strategies employed by bacterial pathogens to hijack eukaryotic cellular systems.

Contents

Abbreviations: ABA, abscisic acid; CC, coiled-coil; ETI, effector-triggered immunity; ETS, effector-triggered susceptibility; FRET, Förster resonance energy transfer; GEF, guanine exchange factor; HR, hypersensitive response; LRR, leucine-rich-repeat; MAMP, microbe-associated molecular pattern; MAP kinase, mitogen-activated protein kinase; NBS, nucleotide-binding-site; PAMP, pathogen-associated molecular pattern; *Pgy*, *Pseudomonas syringae* pv. glycinea; *Pma*, *Pseudomonas syringa*e pv. maculicola; *Pph*, *Pseudomonas syringae* pv. phaseolicola; PTI, PAMP-triggered immunity; *Pto*DC3000, *Pseudomonas syringae* pv. tomato DC3000; R, resistance; RIN4, RPM1-interacting protein 4; RCS, AvrRpt2 cleavage site; TAL, transcription-activator-like; T3SE, type III secreted effector protein; TIR, Toll and interleukin-1 receptor.

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1. Introduction

Many Gram-negative bacterial pathogens use type III secretion systems to inject virulence proteins directly in the cells of their hosts [1]. Phytopathogenic bacteria can inject as many as thirty distinct type III secreted effector (T3SE) proteins into host cells, which can manipulate host cellular processes to promote infection [2–5]. These effectors target various plant cellular systems, including plant innate immunity, transcription, cell death, proteasome and ubiquitination systems, RNA metabolism, hormone pathways, and chloroplast function [6–9]. As our understanding of the breadth of T3SE targets increases, a number of general patterns are emerging. First, single T3SEs may target multiple host factors. Second, T3SEs target critical steps in key host processes. The system most commonly targeted is, perhaps not surprisingly, the immune system. Third, distinct T3SEs can converge on specific host targets, perhaps providing redundancy and robustness. Finally, important host targets of T3SEs can directly interact with nucleotide-binding-site leucine-rich-repeat (NBS-LRR) containing resistance (R) proteins.

While the initial identification of putative T3SE targets via *in vivo* or *in vitro* assays is often technically challenging, this effort is frequently dwarfed by the subsequent characterization and validation of biological function and relevance. In this review we focus mainly on the type III-mediated interactions between the model plant *Arabidopsis thaliana* and the widely studied plant pathogenic bacterium *Pseudomonas syringae*. We explore the data in this rapidly developing field and attempt to distill general biological principles out of the complex molecular, biochemical, and genomic data.

2. Multiple ways to target plant immunity

Plant immune systems are emerging as dominant targets of T3SEs, and it is very likely that the selective pressures imposed by pathogens in general are responsible for shaping and driving the evolution of these systems [10–12]. Initially, pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) trigger an innate immunity response in the host (PAMP-triggered immunity or PTI) [11]. PAMPs are conserved epitopes presented by pathogen molecules, which are recognized by host pattern recognition receptors (PRRs). Well characterized phytopathogenic PAMPs include the flagellin subunit from the bacterial flagella and the elongation factor Ef-Tu [13]. PTI (a.k.a. basal defence) is usually effective at preventing infection, and includes responses such as pathogen-induced gene expression, the production of reaction oxygen species, and the reinforcement of the plant cell wall.

The suppression of bacterial growth by the innate immune system invariably imposes selective pressures on the invading microbe to overcome these defences [14]. One very successful strategy employed by bacterial pathogens relies on PTI-suppressing T3SEs. This tactic has been documented for multiple T3SEs, and is referred to as effector-triggered susceptibility (ETS) [10,12,15].

The second branch of plant immunity relies on the recognition of T3SEs by host R proteins in a process termed effector-triggered immunity (ETI) [16]. ETI typically initiates a rapid and localized programmed cell death response termed the hypersensitive response (HR). R proteins rarely recognize T3SEs directly, and instead, most R proteins physically interact with and monitor the host targets of bacterial effectors. ETI is triggered by T3SE-mediated modification of a host target monitored by an R protein [16,17]. This 'guarding' activity adds a level of robustness to the immune system since it removes the need for R proteins to chase an evolving target, and instead focuses on the integrity of its own systems. While rare, direct interaction between an R protein and a T3SE has been demonstrated for the *Ralstonia solanacearum* T3SE PopP2 and the R protein RRS1-R [18].

Pathogens can respond to ETI by evolving away from recognition. This can occur by evolving or acquiring new allelic variants either through pathoadaptation (the mutational process) or horizontal gene transfer (recombination). Alternatively, strains may acquire or evolve additional T3SEs that specifically block this ETI, resulting in a second level of effector-triggered susceptibility (ETS) [12,14,19,20].

In this first section, we discuss effectors that have been demonstrated to target plant immunity. We initially discuss the direct targeting of PTI and/or ETI by T3SEs, and subsequently, how plants have evolved to recognize the presence of pathogens via their T3SEs. We then present examples of other host systems targeted by T3SEs that indirectly alter host immunity. Although the line between direct and indirect targeting of plant immunity can be difficult to define, we base our discussion on whether the T3SE directly alters a component of PTI and/or ETI signalling, or usurps another plant system to indirectly affect plant immunity (Fig. 1).

2.1. Directly targeting plant immunity

2.1.1. Targeting PTI

2.1.1.1. HopAI1. The recognition of the flagellin flg22 epitope by the FLS2 receptor induces PTI in *Arabidopsis* via the activation of the MAP kinases MPK3 and MPK6 [21]. These MAP kinases are targeted by the *P. syringae* pv. tomato DC3000 (*Pto*DC3000) T3SE HopAI1$_{PtoDC3000}$ (hereafter HopAI1), which is a member of a family of proteins that display a novel phosphothreonine lyase function [22]. This activity, which involves the irreversible removal of phosphate groups from phosphothreonines, was first demonstrated in the *Shigella* T3SE OspF on phosphothreonine residues in the activation loop of MAP kinases [22]. HopAI1 is an OspF-related T3SE that physically interacts with MPK3 and MPK6 during *in vitro* and *in vivo* co-precipitation assays [23]. HopAI1-mediated phosphothreonine lyase activity inactivates MPK3 and MPK6, and consequently suppresses downstream events associated with PTI [23].

2.1.2. Targeting PTI and ETI

2.1.2.1. AvrPto and AvrPtoB. AvrPto1 and AvrPtoB (a.k.a. HopAB2) are unrelated effectors carried by *Pto*DC3000, a strain virulent on *Arabidopsis* and tomato [24]. AvrPto1$_{PtoDC3000}$ and AvrPtoB$_{PtoDC3000}$ (hereafter, AvrPto and AvrPtoB, respectively) can suppress very early immune responses mediated by MAPK cascades, suggesting that suppression occurs immediately after signal perception or before MAPKKK signalling [25]. Recent publications have elegantly demonstrated that AvrPto and AvrPtoB target *r*eceptor-*l*ike *k*inases (RLKs) and/or PAMP receptors to interfere with their downstream signalling during infection. These receptors include the brassinolide-associated RLK BAK1 (*B*RI1 *a*ssociated *k*inase 1), the flagellin receptor FLS2, the Ef-Tu receptor EFR, and the chitin receptor CERK1 [26–29].

AvrPto and AvrPtoB interact with BAK1 in the split-ubiquitin yeast two-hybrid assay, by co-immunoprecipitation from protoplasts, and in *in vitro* pull-down assays [26–28]. BAK1 and the brassinolide receptor BRI1 (*b*rassinosteroid *in*sensitive *1*) form a complex that is necessary for brassinolide-mediated plant growth and development [30]. BAK1 contributes to innate immunity through its association with the flagellin receptor FLS2 *in vivo* [31–34]. Perception of flagellin or the flg22 peptide is necessary for flagellin-induced signalling and defence, and for the elicitation of defence signalling to other PAMP inducers like Ef-Tu, HrpZ, peptidoglycan and lipopolysaccharide [27,31,33,35]. Suppression of PAMP signalling is correlated with the direct binding of AvrPto or AvrPtoB to BAK1. AvrPto mutants (i.e. S46P and Y89D) or AvrPtoB truncations do not suppress PAMP signalling and display reduced

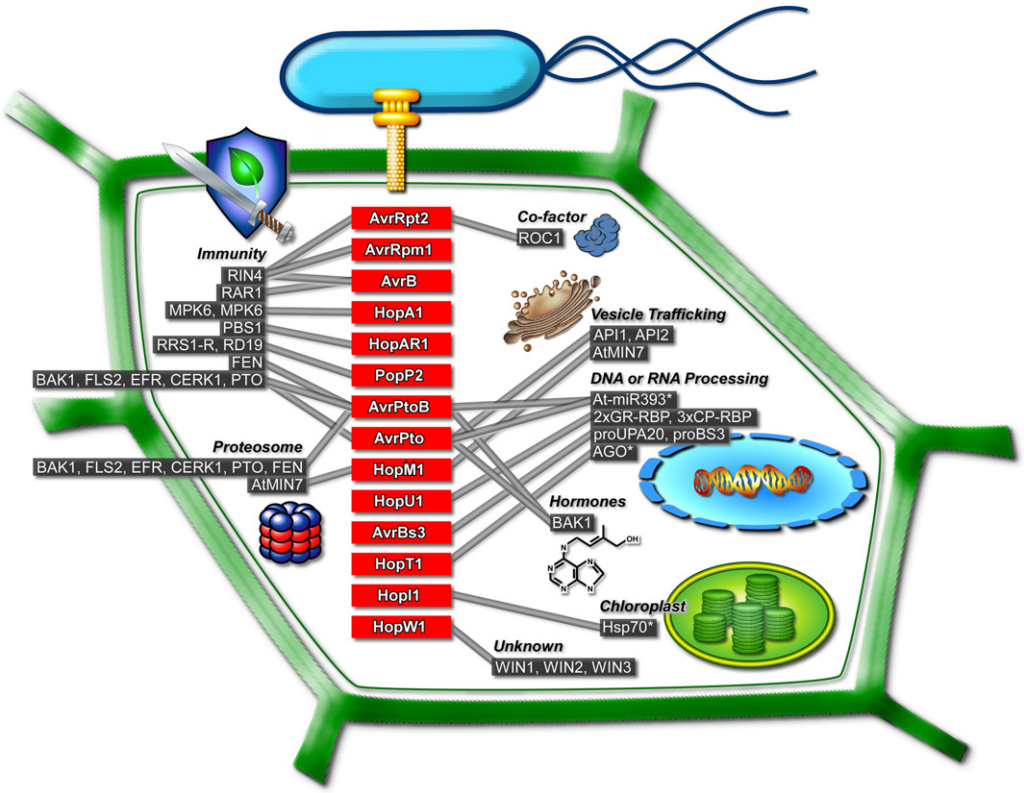

Fig. 1. Plant systems targeted by phytopathogenic type III effector proteins. The direct targets of phytopathogenic T3SEs are grouped according to the plant systems to which they belong as outlined in the review. Lines represent interactions between T3SEs and host target proteins or genes. The HopW interactors, WIN1-3, have yet to be assigned a biological function in *Arabidopsis*. The *Arabidopsis* protein ROC1 is required for the activation of AvrRpt2 but does not appear to be a virulence target of AvrRpt2 and has been designated as a co-factor. Effectors are shown with a red background, while host interactors are shown with a grey background. Asterisks indicate proteins or genes predicted to be targets of the corresponding effector but for which a direct interaction has not yet been demonstrated.

binding to BAK1 [27,36]. Both AvrPto and AvrPtoB are believed to block PTI by interfering with the interaction between BAK1 and FLS2 [26,27]. In support of this, *P. syringae* knockouts of AvrPto and AvrPtoB induce a slightly increased interaction between BAK1 and FLS2, and consequently increased PTI [26,27].

AvrPto and AvrPtoB also inhibit PTI signalling through their direct interaction with the PAMP receptors FLS2, EFR and CERK1 [26–29]. AvrPtoB is an E3 ligase, a function identified by its structural similarity to the eukaryotic E3 ligases [37,38]. AvrPtoB ubiquitinates FLS2, EFR and CERK1 *in vitro*, ubiquitinates FLS2 *in planta*, and degrades CERK1 *in planta*, the latter presumably by ubiquitination [26,29]. The immunity-suppression function of AvrPtoB is dependent on its E3 ligase activity, as a *Pto*DC3000Δ*avrPtoB* strain complemented with non-catalytically active AvrPtoB shows reduced bacterial virulence compared to *Pto*DC3000 [26,29].

In addition to their PTI-suppression abilities, AvrPto and AvrPtoB also induce ETI through the R protein PRF, via interactions with their host targets PTO and/or FEN [39–43]. The co-crystal structure of the AvrPto–PTO complex revealed two key interfaces that mediate their interaction: the P+1 loop of PTO with the GINP motif of AvrPto, and a second PTO loop with an AvrPto helical bundle [44]. Interestingly, mutations which disrupt either of the AvrPto-interacting loops in PTO result in constitutive activation of PRF-dependent defences [44]. Thus, it appears that the two PTO loops negatively regulate PRF-mediated defences in tomato in the absence of AvrPto [44–46]. Binding of AvrPto to PTO is proposed to change the conformation of PTO, causing PTO to release PRF from its inactive state [44].

The N-terminal domain of AvrPtoB interacts with FEN and PTO kinases to initiate PRF-mediated defences [36,43,47]. Like PTO, FEN interacts directly with PRF [48]. The C-terminal E3 ubiquitin ligase domain of AvrPtoB specifically targets FEN kinase for degradation by the proteasome, thereby inhibiting ETI [37,38,43]. As such, FEN/PRF-mediated defences are termed Rsb for *R*esistance suppressed by AvrPto*B* C terminus [47].

2.1.2.2. AvrRpt2, AvrRpm1 and AvrB. *P. syringae* T3SEs $AvrRpt2_{PtoJL1065}$ and $AvrRpm1_{PmaM2}$ (hereafter AvrRpt2 and AvrRpm1, respectively) also suppress PAMP-triggered innate immunity, enhancing the growth of otherwise virulence-attenuated strains [49,50]. Immune suppression by AvrRpt2 and AvrRpm1 likely occurs in several ways, one of which is mediated through their common host target, RIN4 (RPM1-interacting protein 4), which is monitored by at least two distinct R proteins (see below). RIN4 is a negative regulator of basal defences. The *in planta* overexpression of RIN4 inhibits PTI, while PTI is enhanced in *rin4* plants [49,50]. RIN4 has no apparent functional or enzymatic motifs; therefore, it has been suggested that RIN4 acts as an adaptor protein that negatively modulates the signal transduction from several PAMP receptors [50].

AvrRpt2 is a cysteine protease which is self-cleaved *in planta* to form a stable active 21 kDa protein [51,52]. AvrRpt2 must be activated *in planta* via its interaction with the cyclophilin ROC1, a peptidyl-prolyl cis–trans isomerase involved in protein folding [53]. ETI is induced by the R protein RPS2 upon AvrRpt2-mediated cleavage of RIN4 [54,55]. This cleavage occurs at two AvrRpt2 cleavage site (RCS1 and RCS2), which are similar in sequence to the AvrRpt2-self processing site [53,56–58]. RIN4 negatively regulates RPS2, as *rin4* plants exhibit constitutive activation of RPS2 and are seedling lethal [54,57].

The *P. syringae* pv. glycinea effector $AvrB_{Pgyrace0}$ also interacts with RIN4 but appears to suppress innate immunity via its interaction with another protein RAR1 [59]. RAR1, along with SGT1 and

HSP90, have previously been shown to regulate the stability of R proteins [60]. RAR1 also negatively regulates PTI as *rar1* mutants display enhanced callose deposition when treated with flg22 [59]. AvrB transgenic *Arabidopsis* treated with flg22 show an 80% reduction in callose deposition which is lost in *rar1* mutant lines [59].

AvrRpm1 co-immunoprecipitates with RIN4, while AvrB interacts with RIN4 in yeast two-hybrid and co-immunoprecipitation assays [49,61,62]. Both effectors induce the phosphorylation of RIN4 and induce RPM1-mediated ETI [49,63]. Protein crystallography shows that AvrB residues interacting with RIN4 are required for RPM1 activation, and that AvrB has an ADP-nucleotide-binding domain necessary for RPM1-mediated defences and for phosphorylation by an unknown protein in *Arabidopsis* [62,64]. In soybean, AvrB is recognized by the RPG1 R protein [65]. AvrB mutants in the *Arabidopsis* RIN4 or ADP binding domains are no longer recognized by the soybean R protein, suggesting that AvrB is recognized in a conserved manner in these divergent hosts [61,62].

The complex interplay of multiple T3SEs with RIN4 clarified the observation that AvrRpt2 can interfere with RPM1-mediated ETI [66,67]. AvrRpt2 cleaves RIN4 from the membrane, leaving only a fragment of membrane-embedded RIN4, which presumably cannot be phosphorylated by AvrB or AvrRpm1 [49,58,68]. Thus AvrRpt2 triggers RPS2-mediated ETI, while suppressing RPM1-mediated ETI.

RIN4 is not the only virulence target of AvrRpm1, AvrB and AvrRpt2, as virulence is not lost in *rin4* plants [56,68–70]. RIN4 homologues are found in most plant species, and eleven RIN4 paralogs are present even in the small genome of *Arabidopsis* [58]. Most of these have the RCS sequence and some have been demonstrated to be cleaved by AvrRpt2 [56,58]. Like RIN4, they are predicted to be membrane-associated by palmitoylation or prenylation and are predicted to bind AvrB [57,58]. It has been suggested that RIN4 acts as a decoy to trigger *R* gene-mediated defences, while the true virulence target may be one of the RIN4-like proteins [71].

2.1.3. Targeting ETI

2.1.3.1. HopAR1. The *P. syringae* pv. phaseolicola effector HopAR1$_{Pph\text{race}3}$ (formerly AvrPphB, hereafter HopAR1), part of the *Yersinia pestis* YopT superfamily, is a papain-like cysteine protease produced as a 35 kDa protein, which self-cleaves to a mature 28 kDa form, revealing a functional myristoylation site for membrane targeting [69,72–75]. HopAR1 cleaves the serine/threonine protein kinase PBS1 at a site similar to its autoprocessing sequence, an activity recognized by the R protein RPS5 which guards PBS1 [76–80].

In the absence of HopAR1, PBS1 and RPS5 interact *in planta* through the coiled-coil (CC) domain of RPS5 [78,80]. PBS1 protein kinase autophosphorylation is necessary for the interaction of PBS1 with RPS5 [78,80]. Constitutive defence signalling through RPS5 is prevented by the LRR domain of RPS5, as RPS5 truncations lacking this domain exhibit a constitutive HR [80]. Based on mutant studies, Ade and colleagues [80] proposed that RPS5, in a complex with phosphorylated PBS1, remains in an inactive state by interaction of the LRR domain with the NBS domain. HopAR1 cleavage of PBS1 likely changes the conformation of RPS5 making the NBS domain accessible for nucleotide exchange and allowing downstream signalling events and defence induction to occur.

2.1.3.2. PopP2. The *Ralstonia solanacearum* PopP2 effector is part of the *Yersinia pestis* YopJ superfamily. PopP2 is an example of a bacterial T3SE that interacts directly with its cognate R protein, RRS1-R [18]. RRS1-R is an unusual R protein with a WRKY motif characteristic of plant WRKY transcription factors and a TIR-NBS-LRR structure (the TIR domain has homology to the *Drosophila T*oll and mammalian *i*nterleukin-1 *r*eceptors) [81]. RRS1-R is re-localized to the nucleus following PopP2 interaction [18].

PopP2 also interacts with RD19, an *Arabidopsis* cysteine protease whose expression is induced during *R. solanacearum* infection [82], as shown by Förster resonance energy transfer (FRET). RD19 is normally found in motile vacuole-associated vesicles, but is re-localized to the nucleus upon co-expression with PopP2 [82]. While RD19 does not interact with RRS1-R, these two host proteins seem to have an additive affect on PopP2-mediated resistance [82].

2.1.3.3. Other effectors. A number of T3SEs suppress cell death responses, including cell death induced by the T3SE HopPsyA or the pro-apoptotic protein Bax [83], nonhost-associated cell death [47,84–87], and cell death induced by specific plant *R* genes [47,66,88,89]. It is clear that many more cell death-associated targets of phytopathogenic T3SEs remain to be identified.

2.2. Indirectly targeting plant immunity

2.2.1. Ubiquitination/proteasome

Eukaryotic ubiquitination/proteasome systems have been recognized as important targets of microbial effectors [90,91]. One of the clearest examples of hijacking the plant ubiquitin pathway is the structural mimicry of eukaryotic E3 ligases by AvrPtoB [38]. As discussed above, AvrPtoB possesses E3 ligase activity against a number of proteins involved in the immune response *in vitro*, and requires this activity to suppress plant cell death and immunity *in vivo* [26,29,37,38,43,92] (see Section 2.1.2.1).

Another example of hijacking the plant proteasome to interfere with immunity comes from the *P. syringae* T3SE HopM1$_{Pto\text{DC}3000}$ (hereafter HopM1), which induces the degradation of the ARF guanine exchange factor (GEF) family protein AtMIN7 [93]. AtMIN7 is required for a robust immune response against *P. syringae*. Finally, seven T3SEs from *R. solanacearum* termed the GALA effectors possess an F-box domain and can interact with different *Arabidopsis* Skp1-like proteins that are components of SCF-type E3 ubiquitin ligase complexes [94]. These effectors are required for optimal virulence on *Arabidopsis* and tomato; however, the specific targets of these proteins remain to be identified.

2.2.2. RNA processing

The *P. syringae* T3SE HopU1$_{Pto\text{DC}3000}$ (hereafter HopU1) is a mono-ADP-ribosyltransferase (ADP-RT) that ribosylates at least three chloroplast RNA-binding proteins (CP-RBP) and two glycine-rich RNA-binding proteins (GR-RBPs) [95]. One of the GR-RBPs, GRP7, is required for optimal resistance to *P. syringae*, indicating a role in plant immunity, and suggests that *P. syringae* may alter RNA metabolism in order to promote pathogen virulence.

The miRNA pathway is a component of RNA metabolism that is important for plant immunity [96]. The effector AvrPtoB, which suppresses both PTI and ETI, also suppresses the transcription of the PAMP-inducible miRNAs *At-miR393a* and *At-miR393b* in *Arabidopsis*. This transcriptional suppression is independent of the E3 ligase activity of AvrPtoB [96]. In addition, AvrPto interferes with the accumulation of mature miR393, however unlike AvrPtoB, AvrPto interference appears to be posttranscriptional [96]. The T3SE HopT1$_{Pto\text{DC}3000}$ (hereafter HopT1) also suppresses miRNA activity. HopT1 may interfere with the splicing activity of ARGONAUTE1 (AGO1) towards its targets and with miRNA-directed translational inhibition [96]. The direct targets of AvrPtoB, AvrPto and HopT1 related to the miRNA pathway remain to be determined.

2.2.3. Plant hormones

A number of T3SEs manipulate plant hormone signalling pathways; however, direct targets remain to be identified. Upregulation of auxin signalling contributes to disease susceptibility, and numerous pathovars of *P. syringae* produce auxin presumably to promote infection [97,98]. Additionally, the T3SE AvrRpt2 upregulates auxin

levels in *Arabidopsis* and contributes to increased disease susceptibility [99]. The hormone abscisic acid (ABA), responsible for drought tolerance and growth suppression, also contributes to disease susceptibility and is upregulated by AvrPtoB [100]. The T3SE HopAM1$_{PtoDC3000}$ (formerly AvrPpiB, hereafter HopAM1) induces hypersensitivity to ABA in *Arabidopsis* plants and promotes *P. syringae* virulence in drought stressed plants indicative of manipulation of ABA signalling [101].

As previously discussed, the *P. syringae* T3SEs AvrPtoB and AvrPto interact with the *Arabidopsis* RLK BAK1, which associates with the BRI1 brassinosteroid receptor. This hormone plays an important role in defence against a broad range of pathogens in tobacco and rice [27,102]. AvrPto and AvrPtoB also affect the ethylene pathway via their induction of tomato ACC oxidases involved in ethylene biosynthesis [103].

The extensive crosstalk that exists between hormone signalling pathways in plants makes it challenging to distinguish between direct and indirect effects. Clearly, identifying the direct targets of these effectors will be essential for teasing apart these complex interactions [104].

2.2.4. Other target systems of interest

The *P. syringae* T3SE HopI1$_{PmaES4326}$ (hereafter HopI1) is targeted to the plant chloroplast and can induce structural remodeling of the thylakoids and suppress salicylic acid accumulation [105]. HopI1 may target chloroplastic Hsp70 since it possesses a J domain which is typically involved in mediating interactions with Hsp70 proteins [105]. The J domain of HopI1 can functionally substitute for the J domain of yeast Hsp40 (Ydj1) which stimulates the ATPase activity of Hsp70; however, a direct interaction between HopI1 and Hsp70 has yet to be demonstrated [105].

Numerous other T3SEs possess N-terminal sequences that predicted chloroplast localization, suggesting that this organelle may be a critical target for many T3SEs [106]. Conversely, the T3SE HopAA1 is currently the only effector known to localize to the mitochondria, where it inhibits respiration in yeast [107].

The cytoskeleton is a common host target of mammalian pathogens [108]. In plants, the cytoskeleton plays an important role in plant defence responses, cellular trafficking and plasma membrane organization [109–111]; however, to date no phytopathogenic T3SE has been demonstrated to target the plant cytoskeleton. However, T3SEs do target the secretory system of plants. AtMIN7 (targeted by HopM1) is an ARF GEF [93]. Since ARF GEFs are involved in intracellular vesicle trafficking in eukaryotes, it is possible that HopM1 may alter intracellular trafficking to promote pathogen virulence. In support of this, *atmin7* plants show decreased plant immunity, and the virulence of *P. syringae* lacking HopM1 could be restored by the treatment of *Arabidopsis* plants with Brefeldin A, an inhibitor of vesicle trafficking [93]. Additionally a yeast two-hybrid analysis of AvrPto against a tomato cDNA library identified two Ras-related putative GTP-binding proteins (API1 and API2), similar to those involved in vesicular trafficking [112].

Various studies have identified other host proteins that interact with effectors, though the plant systems specifically targeted are not always clear. Bogdanove and Martin [112] identified a stress-related protein (API3) and a putative N-myristoyltransferase (API4) as putative targets of AvrPto. The T3SE HopW1-1$_{PmaES4326}$ (formerly HopPmaA, hereafter HopW1-1) interacts with three *Arabidopsis* proteins; WIN1 a putative acetylornithine transaminase, WIN2 a predicted protein phosphatase 2C, and WIN3 a member of the firefly luciferase superfamily [113]. Although these proteins have yet to be assigned to a specific host cellular system, genetically modifying their expression levels results in altered host susceptibility [113]. Finally, the DspA/E effector from the fire blight pathogen *Erwinia amylovora* interacts with several apple RLKs in yeast two-hybrid screens and in *in vitro* pull-down assays [114]. As these RLKs are found in susceptible and resistant cultivars of apple [114], it is still unclear how they may be manipulated by the pathogen.

3. Targeting transcription: nuclear-based recognition of transcription-activator-like (TAL) effectors

The *Xanthomonas* AvrBs3 family of TAL effectors are characterized by a central repeat domain, a nuclear localization signal, and an acidic transcriptional activation domain, and are localized to the nucleus via their interaction with importin α [115–119]. Unlike the effectors discussed thus far, AvrBs3 targets plant gene promoters. In infected pepper plants, the central repeat domain of AvrBs3 binds to a conserved element in the *upa20* promoter and the AvrBs3 activation domain induces *upa20* expression. Upa20 is a bHLH transcription factor and master regulation of cell expansion. Consequently, AvrBs3-mediated induction results in hypertrophy of the mesophyll tissue [120,121].

Recognition of AvrBs3 is mediated by the pepper BS3 resistance protein in a unique manner [122,123]. In addition to binding to the *upa20* promoter, the repeat domain of AvrBs3 also binds to the *BS3* resistance gene promoter and induces its expression, initiating ETI [124]. Therefore, by mimicking the *upa20* promoter, *BS3* subverts AvrBs3's virulence function and instead initiates plant defences.

Several other *Xanthomonas* effectors also have characteristics of transcription factors suggesting that they act by modulating gene transcription [119]. The XopD SUMO protease has effects on host transcription and additionally can target SUMO-conjugated proteins *in planta* [125,126]. As well, *avrBs3*-like genes including *avrXa27*, *pthXo1*, *pthXo6* and *pthXo7* induce the expression of their cognate resistance genes or other putative targets although they have not yet been shown to bind any plant promoters [127–129]. These *Xanthomonas* effectors illustrate that the nuclear targeting of host genes and/or proteins is an effective strategy that can be used to modify host metabolism [4].

4. Emerging and established themes of phytopathogenic T3SE targets

Over 200 T3SEs and 60 effector families and subfamilies have been identified in *P. syringae* alone [3,130]. One of the major challenges facing molecular plant pathologists is to identify the host targets of these effectors, and assess their role in disease and defence. From the examples outlined above, there are established and emerging themes regarding T3SE targets that may help to guide future studies (Table 1).

First, a single T3SE may have multiple targets in the plant host [7]. This is best exemplified by the multiple targets of AvrPtoB and AvrPto described above. Additionally, HopM1 has 21 strong interacting partners identified by yeast two-hybrid assays, many of which may represent true targets [93]. Additionally, the five RNA-binding proteins ribosylated by HopU1 may all represent virulence targets [95]. AvrRpm1 and AvrRpt2 have as yet unidentified targets besides RIN4, and AvrB can target at least RIN4 and RAR1 [59,68]. The multiple *upa* genes upregulated by the TAL effector AvrBs3 are all potential host targets.

Second, T3SEs target multiple critical nodes of essential host systems. For example, T3SEs target multiple steps of PTI, including PAMP receptors (FLS2 and CERK1), receptor-associated proteins (BAK1), and downstream signalling components (MAPKs and RAR1). Importantly, each of the PTI components targeted by T3SEs is required for optimal plant immunity, highlighting the use of effectors as probes to identify important components of plant systems [6]. Pathogenic bacteria can use either single effectors (e.g. AvrPtoB) or multiple effectors (e.g. AvrPtoB and HopAI1) to disrupt PTI [15]. This strategy has resulted in very effective and robust suppression of

Table 1
Host targets of phytopathogenic type III effector proteins.

Effector	Strain	Target	Potential interaction indicated by	Ref	Direct interaction demonstrated by	Ref
AvrB	*Pgy* race0[a]	RIN4			Co-immunoprecipitation	[49]
		RAR1	Genetics (EMS)	[59]	Co-immunoprecipitation	[59]
AvrBs3	*Xcv* 85-10[b]	Bs3	Genetics (natural diversity)	[133]	Electrophoretic mobility shift assay, chromatin immunoprecipitation	[124]
		Upa20	Transcriptional upregulation	[121]	Electrophoretic mobility shift assay, chromatin immunoprecipitation	[121]
AvrPto	*Pto* DC3000[c]	BAK1			Split-ubiquitin yeast two-hybrid, co-immunoprecipitation	[27]
		FLS2			*In vitro* pull-down, co-immunoprecipitation, split-YFP	[28]
		EFR			*In vitro* pull-down, co-immunoprecipitation, split-YFP	[28]
		CERK1		[27]	Co-immunoprecipitation	[27]
		Pto	Genetics (natural diversity)	[40]	Gal4 yeast two-hybrid; LexA	[134,135]
		Api1, Api2, Api3, Api4			LexA yeast two-hybrid	[112]
AvrPtoB	*Pto* DC3000[c]	BAK1			Co-immunoprecipitation; *in vitro* pull-down	[27,26]
		FLS2	Ecotypic diversity	[136]	*In vitro* pull-down	[26]
		EFR			*In vitro* ubiquitination	[26]
		CERK1			Gal4 yeast two-hybrid	[29]
		Pto			LexA yeast two-hybrid	[42]
		Fen	Candidate gene approach	[43]	LexA yeast two-hybrid	[43]
AvrRpm1	*Pma* M2[d]	RIN4			Co-immunoprecipitation	[49]
AvrRpt2	*Pto* JL1065[c]	RIN4			Co-immunoprecipitation	[54,55]
		ROC1			Substrate of enzyme	[53]
DspA/E	*Ea* CFBP1430[e]				LexA yeast two-hybrid, *in vitro* pull-down	[114]
HopAI1	*Pto* DC3000[c]	MPK3/6			*In vitro* pull-down, co-immunoprecipitation	[23]
HopAR1	*Pph* race3[f]	PBS1	Genetics (EMS)	[77]	Co-immunoprecipitation	[79]
HopI1	*Pma* ES4326[d]	HSP70	Yeast complementation	[105]		
HopM1	*Pto* DC3000[c]	AtMIN7			LexA yeast two-hybrid, *in vivo* pull-down	[93]
HopU1	*Pto* DC3000[c]	GRP7			Substrate of enzyme	[95]
HopW1-1	*Pma* ES4326[d]	WIN1, WIN2, WIN3			SRS yeast two-hybrid, *in vitro* pull-down	[113]
PopP2	*Rs* GMI1000[g]	RRS1	Genetics (natural diversity)	[81]	Split-ubiquitin yeast two-hybrid, colocalization	[18]
		RD19			Gal4 yeast two-hybrid, FLIM	[82]

[a] Pgy: *Pseudomonas syringae* pv. glycinea.
[b] Xcv: *Xanthomonas campestris* pv. vesicatoria.
[c] Pto: *Pseudomonas syringae* pv. tomato.
[d] Pma: *Pseudomonas syringae* pv. maculicola.
[e] Ea: *Erwinia amylovora*.
[f] Pph: *Pseudomonas syringae* pv. phaseolicola.
[g] Rs: *Ralstonia solanacearum*.

PTI by virulent pathogens, with multiple effectors apparently suppressing the immune response in a redundant (at least to our crude level of resolution) manner [131]. It remains to be determined if a similar strategy is employed to target other plant systems besides PTI.

Third, multiple T3SEs converge on important host targets. This is exemplified by the convergent targeting of RIN4 by the evolutionarily unrelated effectors AvrB, AvrRpm1 and AvrRpt2. Another example of convergence onto important host targets is the targeting of PTO, BAK1, FLS2, EFR and CERK1 by the T3SEs AvrPto and AvrPtoB.

Fourth, host targets of T3SEs can directly interact with NBS-LRR containing R proteins (originally proposed as the "guard hypothesis") [16,17]. This is exemplified by RIN4 and the R proteins RPM1 and RPS2, PTO and FEN with PRF, and PBS1 with RPS5. It remains to be established whether effector targets associated with resistance proteins are decoys for the T3SEs or whether they are true virulence targets [71]. For TAL effectors the resistance gene promoter can mimic an important promoter element of a virulence target as exemplified by the *BS3* promoter [121,124].

5. Conclusions

Disease and immunity processes of plants share many commonalities (derived both from homology as well as simply due to convergence) with those seen in animal systems. The host systems exploited by phytopathogens described above are also common targets of animal pathogens, including the targeting of host immunity and ubiquitin/proteasome systems. Furthermore, the themes outlined for phytopathogenic T3SE targets also hold true for animal T3SEs, with the exception of the guarding of T3SE targets by host resistance proteins.

Up to now, the host cytoskeleton is a significant exception to the above systems. While it is a critical component of both animal and plant cells, and is commonly targeted by T3SEs from animal pathogens, it has not yet been found to be targeted by plant pathogens despite its important role in cellular integrity and immunity. Recent work in our group, however, has shown that the *P. syringae* T3SE HopZ1a (a YopJ family member) is an acetyltransferase that is activated by tubulin and can destroy host microtubule networks (A.H. Lee, D.S. Guttman, D. Desveaux, in preparation), adding one more important commonality between targets of plant and animal pathogens.

Evolutionarily conserved and convergent host targets attacked by bacterial pathogens of plants and animals emphasize the common, fundamental strategies that exist to exploit eukaryotic hosts despite significant differences in host cell physiology and architecture. As such, comparative analysis of T3SE function in diverse hosts, and effector-associated phenotypes in heterologous model systems such as yeast will provide powerful means to study effec-

tor function, identify important host targets, and gain insight into conserved and fundamental infection strategies [132]. These studies are essential for understanding the fundamental principles of host–pathogen interactions, as well as for the development of effective anti-virulence treatments.

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