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Microbiology

Review

The conserved AvrE family of bacterial effectors: functions and targets during pathogenesis

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The AvrE family of type III secreted effectors are highly conserved among many agriculturally important phytopathogenic bacteria. Despite their critical roles in the pathogenesis of phytopathogenic bacteria, the molecular functions and virulence mechanisms of these effectors have been largely unknown. However, recent studies have identified host-interacting proteins and demonstrated that AvrE family effectors can form water-permeable channels in the plant plasma membrane (PM) to create a hydrated and nutrient-rich extracellular space (apoplast) required for disease establishment. Here, we summarize these recent discoveries and highlight open questions related to AvrE-targeted host proteins.

Highlights

The type III secreted AvrE effector family is highly conserved among many agriculturally important phytopathogenic bacteria and contributes significantly to their virulence.

AvrE effectors induce water-soaking, immune suppression, and cell death.

AvrE effectors induce water-soaking by manipulating abscisic acid (ABA) signaling and interacting with plant proteins.

AvrE effectors form water- and solute-permeable channels, directly contributing to water perturbations across membranes.

AvrE effectors interact with conserved plant receptor kinases and protein phosphatases potentially to optimize AvrE-induced outputs.

Blocking AvrE-channel function or manipulation of AvrE-targeted plant proteins increases resistance against AvrE-translocating bacterial pathogens.

Introduction

Most Gram-negative bacterial pathogens require the type III secretion system (T3SS) to cause disease. This needle-like structure translocates effectors into the cytoplasm of host cells. Often, these type III-secreted effectors (T3SEs) modulate the host immune system and physiological processes to promote virulence [1,2]. Pathogens encode diverse effector repertoires together contributing to overall bacterial virulence; among them, a few 'core' effectors and their contribution to virulence are more highly conserved than others [3,4]. Of particular interest is the AvrE effector family that is highly conserved among many phytopathogenic bacteria [5]. Thus, understanding the virulence function of the AvrE effectors and identification of their plant targets is essential to combat AvrE-secreting pathogens.

AvrE family as a conserved bacterial T3SE family

The AvrE family is highly conserved among many agriculturally important phytopathogenic bacteria, with AvrE from *Pseudomonas syringae* pathovar *tomato* (*Pto*) being its founding member [6,7]. Searched through sequence similarity, AvrE homologs were found in other species of *Pseudomonas* as well as in diverse genera such as *Pantoea*, *Erwinia*, *Dickeya*, and *Pectobacterium* [5,8–14]. AvrE family effectors are genetically linked to T3SS clusters in these pathogens, suggesting an early evolutionary association with infection initiation [5,15,16]. Distant AvrE homologs that are not located next to T3SS gene clusters have been identified in *Pseudomonas* [17], other bacterial genera (*Xanthomonas*, *Ralstonia*) and even in the oomycete *Hyaloperonospora arabidopsidis* [17–20].

Despite low sequence similarity, AvrE effectors are functionally conserved among different bacterial species. For instance, AvrE from *P. syringae* and the AvrE family member DspA/E from *Erwinia amylovora* share only 28% amino acid sequence identity [9]. Nevertheless, AvrE can partially complement the virulence of *E. amylovora DspA/E*$^{-}$, AvrE-like WtsE from *Pantoea stewartii* subsp. *stewartii* (*Pnss*) complements that of *Pto* DC3000 ΔCEL (conserved effector locus that

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contains AvrE in *Pseudomonas*), and DspA/E complements that of *Pnss WtsE*$^{-}$, indicating their functional conservation [9,21,22].

To unravel the functional conservation of AvrE effectors, several studies attempted to identify conserved amino acid motifs; however, only few motifs were identified in subsets of AvrE effectors, possibly due to low sequence similarity between orthologs [23,24]. Among them, two motifs – the endoplasmic reticulum membrane retention signals (ERMRS) and a WxxxE – were present in most AvrE family members [23]. Mutation of WxxxE motifs and deletion of the ERMRS in WtsE partially compromised the ability to suppress defense responses and to promote disease caused by *Pnss* in maize, indicating that these motifs are important for the virulence function of the AvrE family [23]. The WxxxE motif locates at the N terminus of AvrE effectors, and some AvrE homologs have two WxxxE motifs [23]. Interestingly, this motif was also shown to be required for AvrE-induced effector-triggered immunity (ETI) activation in *Arabidopsis* [25]. T3SEs with a WxxxE motif from human bacterial pathogens were suggested to modify the actin skeleton. This hypothesis was based on the observation that the WxxxE motif provides a structural fold in proteins that have GTPase activity [26]; and indeed, DspA/E affected cell trafficking in yeast [27]. Interestingly, despite the predicted C-terminal ERMRS motif and the lack of known PM-targeting signals, AvrE was shown to localize to the plant PM [28], indicating that AvrE effectors might employ other mechanisms to locate to the PM or have uncharacterized PM-targeting signals.

Initial structural prediction methods predicted a conserved β-propeller domain in the N-terminal portion of AvrE, DspA/E, WtsE, and other analyzed AvrE family effector sequences [24,29]. The structural similarity to eukaryotic, membrane-binding PROPPINS suggested phosphatidylinositol phosphate-binding ability, and AvrE was shown to bind specific phosphatidylinositol phosphates [29].

Recent progress in structure-prediction tools, such as AlphaFold2 [30], enabled the more detailed prediction of the tertiary structure of DspA/E, DspE, AvrE, and WtsE. Surprisingly, these proteins were predicted to resemble a pore with an N-terminal putative protein–protein interaction interface [30]. These predicted similar structures, supported by cryogenic electron microscopy, explain their functional conservation despite relatively low sequence similarity and enabled new avenues for investigating this effector family [5,8,9,30].

Virulence and avirulence functions of AvrE effectors

AvrE effectors are central – sometimes essential – virulence factors for AvrE-encoding pathogens. Mutations in *DspA/E* of *E. amylovora*, *WtsE* of *Pnss*, *DspA/E* of *Pantoea agglomerans*, and *DspE* of *Pectobacterium carotovorum* alone significantly compromise the virulence of these pathogens on their host plants, suggesting a core function of AvrE effectors in promoting pathogenesis [8–10,14]. In *Pto* DC3000, AvrE is functionally redundant to the sequence-unrelated effector HopM1 [16,31,32]. The parallel deletion of *AvrE* and *HopM1* significantly impacts the pathogenicity on *Arabidopsis thaliana* (hereafter Arabidopsis), and complementation with either AvrE or HopM1 can reconstitute virulence [31–33]. The presence of functionally redundant HopM1 in addition to AvrE suggests the importance of AvrE virulence function, and putative presence of other functionally redundant effectors in different pathogens masking the core virulence effect of AvrE effectors [31,32,34].

The phenotypic consequences and physiological effects associated with AvrE effector virulence have been examined in various plant species. Multiple AvrE effectors can suppress host defense responses (Figure 1). For example, both DspA/E and WtsE delay *PATHOGENESIS-RELATED 1* (*PR1*)-gene expression and callose deposition induced upon bacterial infection [15,35,36]. In

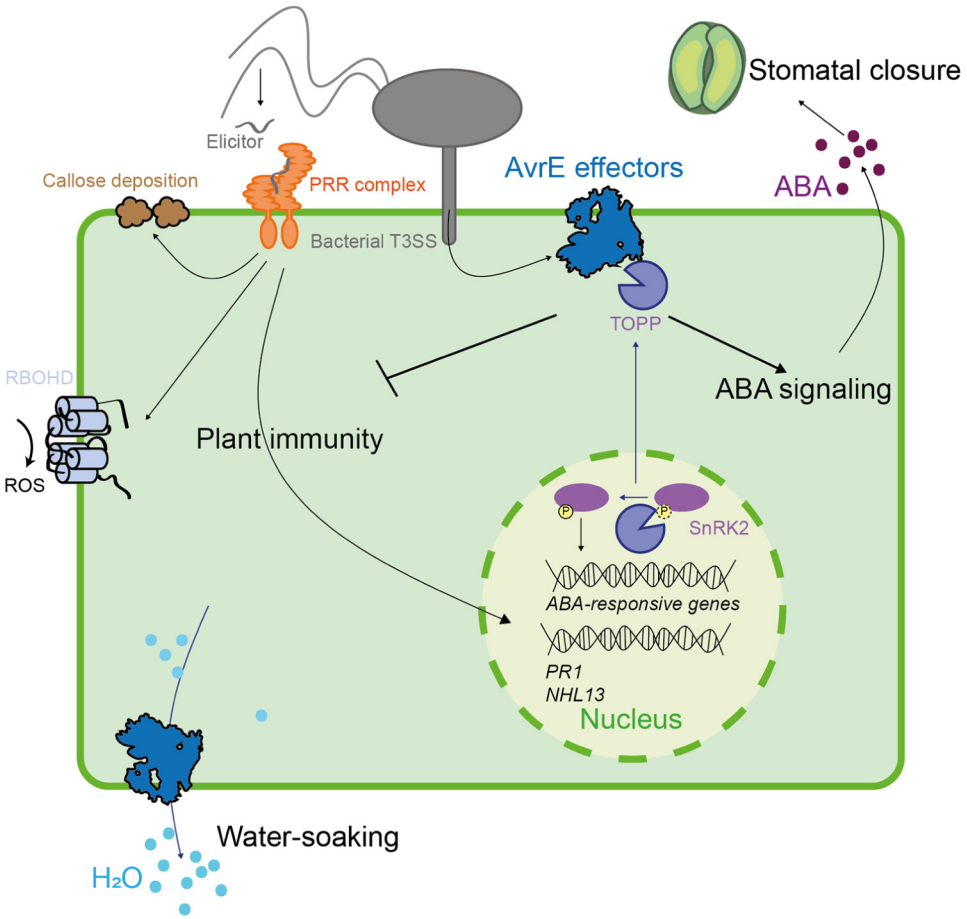

Figure 1. Graphical summary of reported AvrE functions during pathogenesis. Upon perception of bacterial pathogens, plant immune responses are initiated by plasma membrane localized pattern-recognition receptor (PRR) complexes perceiving elicitors from pathogens, leading to the production of reactive oxygen species (ROS) via RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD), callose deposition, and defense-related gene induction such as *PR1* and *NONRACE-SPECIFIC DISEASE RESISTANCE1/HARPIN-INDUCED1-LIKE13 (NHL13)*. AvrE effectors are delivered into the plant cell via bacterial type III secretion system (T3SS) and contribute to the suppression of plant defense responses including the suppression of callose deposition, ROS production, and defense gene expression. Additionally, AvrE effectors modulate abscisic acid (ABA) signaling by targeting type one protein phosphatases (TOPPs) to regulate stomata closure which is important for apoplastic water-soaking. TOPPs are localized in the nucleus and repress the induction of ABA-responsive genes by dephosphorylating the ABA master regulator SUCROSE NON-FERMENTING (SNF1)-RELATED PROTEIN KINASE (SnRK2). Upon interaction with AvrE, TOPPs are retained in the cytoplasm, which prohibits the dephosphorylation of SnRK2, resulting in the expression of ABA-responsive genes. AvrE effectors also function as water- and solute-permeable channels, which directly contributes to the generation of a water-soaked and nutrition-rich apoplastic environment. Altogether, AvrE effectors function as critical virulence factors regulating multiple facets of plant immunity and physiology.

Pto DC3000, AvrE suppresses salicylic acid (SA)-dependent immunity, callose deposition, production of reactive oxygen species (ROS), and immune-related gene expression [28,32,34,37]. WtsE disturbs aromatic compound metabolism and induces cell wall reinforcements [36,38], while DspA/E affects actin dynamics and vesicle trafficking in yeast [27], suggesting that AvrE effectors modulate various plant pathways to the benefit of the pathogen. In addition, AvrE effectors are involved in creating water-soaking symptoms, which are associated with disease development in Arabidopsis [39].

Interestingly, many AvrE effectors trigger cell death when overexpressed in both host and non-host plants [21,23,36,37,40]. In addition, AvrE-effector expression causes yeast growth arrest, suggesting that the cell toxicity or cell-death-triggering function is conserved in both plant and yeast and potentially suggesting the existence of conserved effector targets or activities in eukaryotes [22,41]. Whether such cell death phenotype in plants is due to cell toxicity related to the virulence function or due to recognition of the effector by plant immune receptors (leading to avirulence) is still unclear. AvrE from *Pto* DC3000 was initially named after its contribution to avirulence when transferred to *P. syringae* pv. *glycinea* in soybean [6,7]. In addition, expression of DspA/E in the non-host plant Arabidopsis induces expression of SA-dependent defense genes [40]. However, AvrE-induced cell death symptoms occur slowly and are not strictly correlated with ETI induction, indicating a complex regulation of AvrE-induced cell death [5,34,42]. Interestingly, some AvrE effector variants from *P. syringae* strains, including the AvrE allele from *Pto* DC3000, are recognized in Arabidopsis by the nucleotide-binding and leucine-rich repeat receptor protein (NLR) CAR1 (CEL-ACTIVATED RESISTANCE 1) triggering ETI [25]. When expressed alone, *Pto* DC3000 AvrE can restrict growth of effectorless *Pto* DC3000 D36E mutant in *Nicotiana benthamiana*, and such AvrE-induced ETI and cell death is suppressed by the effector HopI1 [34], Therefore, it is plausible that AvrE-effector-triggered cell death is associated with ETI, which could however be affected by the presence of other effectors.

Role of AvrE effectors in creating aqueous and nutrient-rich apoplast

Despite their importance to pathogen virulence, the molecular mechanisms of how AvrE effectors contribute to the virulence of these bacteria have been unknown for almost 30 years. However, recent research began to elucidate the biological and molecular functions of AvrE during pathogenesis.

One of the common features shared by AvrE effectors is their ability to induce apoplastic hydration leading to macroscopic water-soaking [30,39,43,44] (Figure 1). This macroscopic apoplastic hydration is one of the earliest and most common symptoms of bacterial infections in plant leaves and is associated with sites of early bacterial proliferation that later result in disease lesions [39,45]. In addition, water-soaking is an actively induced, likely highly regulated process. In the Arabidopsis-*Pto* DC3000 pathosystem, for instance, water-soaked lesions occur transiently during the early infection phase and disappear before the development of late disease symptoms [39,45]. DspA/E, WtsE, and AvrE have all been shown to be required and sufficient to induce water-soaking symptoms *in planta*, potentially contributing to a feedforward connection between water and solute synergetic accumulation in the apoplast [21,23,30,39,43,46].

Pto DC3000 AvrE is the best studied example of an AvrE effector that can induce an aqueous apoplast. AvrE was recently shown to induce water-soaking by manipulating the signaling pathway of the plant phytohormone abscisic acid (ABA), which is involved in stomata closure (Figure 1). During initial pathogenesis, stomata rapidly close upon perception of pathogens, then the jasmonic acid (JA)-mimicking toxin coronatine, produced by *Pto* DC3000, re-opens the stomata to aid the entry of bacteria [47]. Both AvrE and its functionally redundant effector HopM1 induce a secondary stomata closure after this re-opening stage by inducing ABA accumulation and ABA-induced transcriptional responses in guard cells, thereby creating a hydrated apoplast required for bacterial proliferation [48]. This AvrE-dependent stomatal closure is required and sufficient to induce water-soaking [48,49]. To activate ABA signaling, AvrE de-represses the activity of the ABA signaling master regulator SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2 (SnRK2) by interacting with inhibitory type-one protein phosphatases (TOPPs) [49]. The interaction of AvrE with TOPPs reduces their accumulation in the nucleus by retaining them in the cytoplasm. Therefore, SnRK2 remains phosphorylated in the nucleus and induces ABA-responsive genes [49].

In addition to ABA regulation, a recent study suggested a direct role for AvrE effectors in inducing apoplastic water-soaking. The structural model of AvrE effectors by AlphaFold2 [50] and cryogenic electron microscopy (cryo-EM) analysis of DspA/E revealed that they form a mushroom-like structure with a C-terminal β-barrel stem having a predicted pore size of 15–20 Å, suggesting a channel function [30]. Indeed, DspA/E and AvrE form channels that are water- and ion-permeable in *Xenopus* oocytes, and DspA/E-formed channels allow the passive passage of molecules larger than water or ions but smaller than the predicted pore size (15–20 Å), similarly to aquaporins [30]. During *Pnss* infection, WtsE is required for the accumulation and release of metabolites to the apoplast, potentially suggesting that the AvrE effector family could not only form water- and solute-permeable channels but also nutrient-permeable pores [46]. Inhibition of AvrE-effector channel function by either chemical inhibitor treatment or by introducing mutations affecting the potential pore function compromised their ability to induce water soaking *in planta* [30]. These data solved a long-standing puzzle of the biochemical function of AvrE effectors as water- and solute-permeable channels, possibly explaining their direct contribution to the creation of osmotic and water potential perturbations at the PM.

AvrE effector-interacting receptor kinases and protein phosphatases

The recently reported biochemical function of AvrE effectors in inducing apoplast hydration enables us to discuss the reported AvrE interactors in the context of this novel effector function. In addition to AvrE effector's channel-forming function, which directly regulates apoplastic hydration via manipulating water and solute permeability, AvrE effectors seem also to be able to manipulate ABA signaling to regulate indirectly the apoplastic hydration. For instance, independently of AvrE channel function, ABA treatment alone was also shown to induce water-soaking lesions [48]. This suggests that AvrE might have functions in addition to its channel activity to regulate water-soaking by inducing ABA production and/or signaling, potentially via the interaction with host proteins. Interestingly, water infiltration into the apoplast is sufficient to suppress callose deposition induced by the bacterial flagellin-derived epitope flg22 [30], indicating that several AvrE-induced virulence phenotypes might be directly linked to AvrE effector channel activity or apoplast hydration. AvrE effectors are large proteins (>200 kDa) with potentially many protein-interacting interfaces in their N-terminal domain [15,22,29,30]. Indeed, they were reported to interact with different host proteins, which are discussed later (Figure 1). Based on the genetic and functional conservation of AvrE effectors, it was hypothesized that the AvrE effector family shares conserved virulence targets across plant species.

Leucine-rich repeat receptor kinases

The first identified interactors of the AvrE effector family were leucine-rich repeat receptor kinases (LRR-RKs). A yeast-two hybrid (Y2H) screen with the N-terminal part of DspA/E against an apple cDNA library identified four LRR-RKs, named DIPM1–4 (DspA/E-interacting proteins of *Malus* x *domestica* 1–4) [51]. Silencing of these genes or mutation of *DIPM4* by CRISPR/Cas9 in apple led to increased disease resistance against *E. amylovora*, suggesting that these RKs might serve as virulence targets [52,53]. Interestingly, a Y2H screen for WtsE interactors using a maize cDNA library identified three LRR-RKs, WIP3–5 (WtsE-interacting proteins 3–5), as interacting with the N terminus of WtsE [22]. All LRR-RKs identified belong to the subfamily III of LRR-RKs (LRR-RK III). LRR-RK III members are characterized by a short extracellular domain and are predicted to be putative pseudokinases due to their altered amino acid sequence compared with the canonical kinases at the catalytic motif (HRD to HGN) [54]. Although predicted to be pseudokinases, a few LRR-RK III proteins were nevertheless reported to have kinase activity *in vitro* [55,56]. Therefore, it remains to be determined if AvrE-interacting LRR-RKs are active kinases, if their kinase activities are required for their functions, and if AvrE is targeting them to modulate the kinase activity as a virulence strategy.

While the functions of the majority of LRR-RK III family members are poorly described, some members in Arabidopsis are known to regulate water and metabolite transport, ABA, and immune signaling, which would be in line with AvrE's *in planta* effects. For example, Arabidopsis SIRK1 (SUCROSE-INDUCED RECEPTOR KINASE 1) and the WIP5 homolog QSK1 (QIANSHOU KINASE 1) positively regulate aquaporin activities upon external sucrose stimulation via reciprocal phosphorylation [55]. In addition, other members of the Arabidopsis LRR-RK III family, RKL1 (RECEPTOR-LIKE KINASE 1) and RLK902, interact with aquaporins, and coexpression of RKL1 with the aquaporin PIP2 (PLASMA MEMBRANE INSTINSIC PROTEIN 2) enhanced its water transport activity [57]. In addition to aquaporins, QSK1 phosphorylates the ABC transporter ABCG36 and represses export of the auxin precursor indole-3-butyric acid allowing export of the antimicrobial compound camalexin [58]. Altogether, these results indicate potential conserved roles of LRR-RK III proteins in the regulation of water or metabolite transport via the interaction with different transporters. Additionally, LRR-RK III members have reported roles in ABA signaling. GHR1 (GUARD CELL HYDROGEN PEROXIDE-RESISTANT 1) is required for ABA- and H_2O_2-induced stomatal closure during drought tolerance [59,60]. RDK1 (RECEPTOR DEAD KINASE 1) acts as a positive regulator of ABA signaling via the recruitment of the protein phosphatase type-2C (PP2C) ABI1 (ABSCISIC ACID INSENSITIVE 1) [61]. Interestingly, the interaction of QSK1 with the K^+ channel TPK1 (TWO PORE K^+ CHANNEL 1) mediates K^+ efflux from the vacuoles, thus positively regulating ABA-induced stomatal closure [62].

Protein phosphatases

Another group of AvrE host interactors are protein phosphatases (PPs). In maize, protein phosphatase type-2A (PP2A) B′ subunits (WIP1/2) interacted with the N terminus of WtsE. This interaction was also confirmed for Arabidopsis PP2As with the N-terminal fragment of AvrE [22]. PP2As are types of PPs composed of three units of which the subunit B′ determines substrate specificity, indicating that AvrE may modulate the interaction between PP2A with specific substrate targets [63]. *In planta*, PP2As of both Arabidopsis and maize were shown to be required for the virulence function of AvrE and HopM1 as well as for WtsE, respectively [22]. This indicates that PP2As are essential for the ability of AvrE effectors to manipulate the host metabolism and cause disease symptoms. Interestingly, DspA/E expression in yeast activated the PP2A Cdc55 and mutation of this phosphatase suppressed DspA/E-induced growth arrest [41]. Similarly, WtsE-induced growth arrest of yeast was suppressed by the mutation of four PP2A-related genes, suggesting that PP2A is a conserved target in eukaryotes [22].

In addition to PP2As, AvrE also interacts with TOPPs resulting in the derepression of the ABA signaling master regulator SnRK2 [49]. Interestingly, regulation of TOPPs by AvrE to induce ABA signaling seems to be partially independent of AvrE's channel function, as the N-terminal part of AvrE was sufficient to activate SnRK2 derepression preventing TOPPs to go to the nucleus [49]. Both TOPPs and PP2As are proposed as negative regulators of ABA signaling [64–66]. PP2As in plants are also associated with the negative regulation of plant immune responses as well as the dephosphorylation of aquaporins indicating their putative roles in water movement and AvrE functions in disease suppression [67–69].

Other potential interactors

While both LRR-RKs and PPs have been identified as interactors of the AvrE effector family in different plant species, other potential interactors have been identified using different screening methods. One ankyrin repeat family protein (NM_001175852) and one ankyrin domain-containing protein (NM_001154512) were identified as potential WtsE interactors in maize in a Y2H screen [22], which requires further investigation. Co-immunoprecipitation of transiently expressed AvrE *in planta* followed by mass spectrometry analysis identified aquaporins (PIP1B,

PIP2A, PIP3A), AHA1 (H^+-ATPase 1), and CPK32 (CALCIUM-DEPENDENT PROTEIN KINASE 32), in addition to TOPPs, as being part of the AvrE complex(es) [29]. This indicates diverse *in planta* AvrE targets, which might be linked to AvrE's effects on water/solute movement, ABA signaling, and/or immune responses, although this remains to be tested.

Concluding remarks and future directions

Collectively, the recent discovery of the AvrE channel structure and function, and the AvrE virulence mechanisms, have significantly advanced our knowledge of how AvrE effectors serve as functionally conserved 'core' virulence factors. However, some important questions are still outstanding: to what extent is AvrE channel function connected to AvrE-induced ABA accumulation, inhibition of immune signaling, and elicitation of cell death? Are these phenotypes downstream of one another, and what are the consequences or independent responses? Do AvrE effectors additionally use plant target proteins to modulate AvrE's channel function or to modulate the responses independently of channel activity (Figure 2) (see Outstanding questions)?

An investigation of the functional roles of AvrE's plant target proteins would allow us to understand AvrE-induced virulence mechanisms and to apply such knowledge in a broader context. Here, we highlight the putative roles of AvrE-interacting plant RKs and PPs (Figure 2). We find it intriguing that multiple AvrE-family effectors target LRR-RK III proteins in different host plants, suggesting the potential functional overlap between targeted LRR-RK III members. Such conserved targets also may imply the roles of targeted LRR-RK III members in AvrE effector core function: creating hydrated apoplast and manipulating solute transport to the apoplast. Notably, several LRR-RK III homologous to DIPMs and WIPs are known to interact with plant aquaporins [55,57]. This may suggest that AvrE effectors may target RKs to further increase water/nutrient transport uptake by manipulating the interaction between RKs and aquaporins. AvrE could also potentially target RKs and PPs to regulate positively ABA signaling as shown in the case of TOPPs. Alternatively, the AvrE target RKs, for instance, could be involved in extracellular ABA sensing. It should also be investigated whether the identified interacting RKs and PPs regulate AvrE's channel function by dynamic phosphorylation.

Conversely, some AvrE-induced phenotypes seem independent of AvrE's channel function. For example, the N-terminal part of AvrE, which does not form the β-barrel part – and is thus not expected to form a channel by itself – partially modulates ABA signaling [49]. Therefore, at least partial functions of AvrE might require the action of host AvrE-interacting proteins.

Interestingly, several, but not all, AvrE effectors are recognized by the NLR CAR1 [25]. It would be interesting to investigate whether AvrE can escape recognition by this NLR through the effect of interacting RKs and/or PPs. Other remaining questions are how AvrE localizes to the plant PM and becomes incorporated into the PM as a water channel. The N-terminal part of AvrE is required for PM localization, driving the C-terminal part to go there as well [28]. AvrE-targeted proteins may also allow AvrE's PM localization, as previously reported [28].

As the AvrE-effector family is highly conserved among many important phytopathogenic bacteria, an understanding of how AvrE effectors work, and how they manipulate plant proteins, can have wide applications for crop protection against bacterial disease. Notably, the dendrimer PAMAM G1 was identified as a potent inhibitor of the AvrE-family channel activity in plants. PAMAM G1 suppressed AvrE-effector-induced water-soaking and fire blight disease symptoms on pears caused by *E. amylovora* while not affecting bacterial growth [30]. These results suggest that plant diseases induced by AvrE-encoding pathogens could be controlled using synthetic channel blocker identified based on AvrE's molecular function. Conversely, mutation of the DspA/E-plant

Outstanding questions

Is the recently reported AvrE's channel function connected to AvrE-induced ABA signaling manipulation, inhibition of plant immune responses, and elicitation of cell death?

How do AvrE effectors localize to the PM? And further, how are they incorporated in the PM as channels upon translocation?

Is AvrE's channel activity regulated? Does this involve AvrE-targeted host proteins?

Do AvrE channels also modulate nutrient (sugars, amino acid) transport?

What are the roles of AvrE interactors *in planta*? Which pathways are they involved in? How does AvrE manipulate these targets to increase virulence?

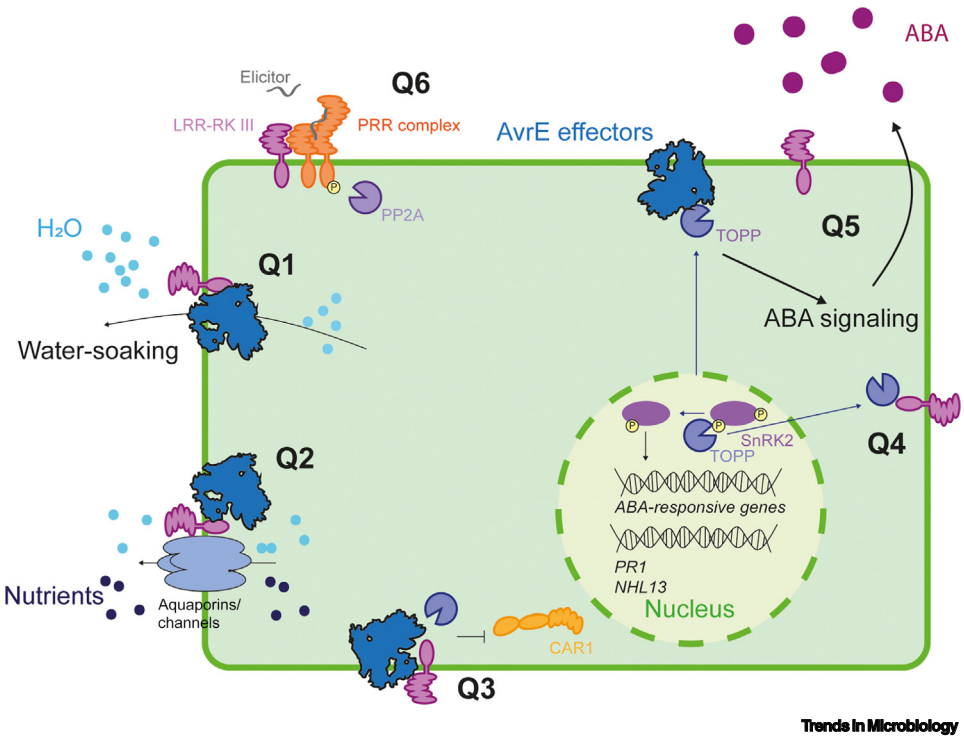

Figure 2. Open questions about potential roles of AvrE-interacting proteins. In addition to their function as water- and solute-permeable channels, AvrE effectors were shown to interact with plant leucine-rich repeat receptor kinases from the subfamily III (LRR-RK III) and protein phosphatases (PPs), including members of the family of type-one protein phosphatases (TOPPs) and of the protein phosphatase 2A (PP2A). AvrE interacts with TOPPs and relocalizes those from the nucleus to the cytoplasm, resulting in the induction of abscisic acid (ABA)-responsive genes. However, there are many open questions as to whether and how other AvrE-interacting proteins contribute to AvrE virulence functions or to plant immunity: Q1. Could LRR-RK III members directly manipulate AvrE function? Are LRR-RK III involved in the creation of a hydrated apoplast and the manipulation of solute transport to the apoplast by modulating AvrE channel function? Q2. Could LRR-RK III members interact with aquaporins/channel proteins and manipulate water and nutrient uptake? Q3. Some AvrE effectors are recognized by the nucleotide-binding and leucine-rich repeat (NLR) receptor CEL-ACTIVATED RESISTANCE 1 (CAR1). Could AvrE-interacting proteins contribute to suppression of CAR1-mediated AvrE recognition or to modulation of CAR1 activation? Q4. Both TOPPs and PP2As were shown to modulate ABA signaling. AvrE interaction with LRR-RK III and PPs could therefore result in the positive regulation of ABA signaling. Could AvrE interaction with LRR-RK IIIs also modulate ABA-signaling by interacting with and relocalizing PPs? Q5. Could AvrE interactors be involved in sensing extracellular ABA? Q6. Could interaction between AvrE and PPs or LRR-RK IIIs modulate plant immune responses by directly or indirectly regulating phosphorylation dynamics of PRR complexes? Abbreviation: PRR, pattern-recognition receptor.

interactor RKs (DIMPs) in apple increased disease resistance against *E. amylovora* [53] and SNPs in DIPM-encoding genes have been further identified as robust markers for both increased and decreased susceptibility towards *E. amylovora.* The importance of DIPMs in resistance against *E. amylovora* highlights the potential for genetic modulation of these RKs to control fire blight [70,71]. In contrast to DIPMs, the transfer of the NLR-encoding *FB_MR5* gene from the wild apple genotype *Malus x robusta 5* into the susceptible 'Gala' variety resulted in stronger reduction of fire blight symptoms. However, a single mutation in the effector gene AvrRpt2$_{EA}$ of *E. amylovora* was shown to overcome this resistance, again resulting in susceptibility [72,73]. Therefore, editing of DIPM-encoding genes might provide a long-term solution against *E. amylovora*. Taken together, understanding the function of the AvrE-plant interactors could provide innovative solutions to fight AvrE-encoding pathogens in various plant species.

Acknowledgments

We thank Giovanni Broggini and all the members of the Zipfel group for fruitful discussions during the project. L.H. was funded by a Zürich-Basel Plant Science Center-Syngenta Fellowship. C.Z. was supported by the University of Zürich and the Swiss National Science Foundation grants no. 31003A_182625 and 310030_212382. S.Y.H. was supported by the United States National Institutes of Health grant 1R01AI155441. S.Y.H. is an investigator at the Howard Hughes Medical Institute.

Declaration of interests

No interests are declared.

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