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Microreview

The AvrE superfamily: ancestral type III effectors involved in suppression of pathogen-associated molecular pattern-triggered immunity

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SUMMARY

The AvrE superfamily of type III effectors (T3Es) is widespread among type III-dependent phytobacteria and plays a crucial role during bacterial pathogenesis. Members of the AvrE superfamily are vertically inherited core effectors, indicating an ancestral acquisition of these effectors in bacterial plant pathogens. AvrE-T3Es contribute significantly to virulence by suppressing pathogen-associated molecular pattern (PAMP)-triggered immunity. They inhibit salicylic acid-mediated plant defences, interfere with vesicular trafficking and promote bacterial growth *in planta*. AvrE-T3Es elicit cell death in both host and non-host plants independent of any known plant resistance protein, suggesting an original interaction with the plant immune system. Recent studies in yeast have indicated that they activate protein phosphatase 2A and inhibit serine palmitoyl transferase, the first enzyme of the sphingolipid biosynthesis pathway. In this review, we describe the current picture that has emerged from studies of the different members of this fascinating large family.

Keywords: AvrE, PAMP-triggered immunity, protein phosphatase 2A, receptor-like kinase, sphingolipid.

INTRODUCTION

To induce disease in their respective plants, many Gram-negative plant bacterial pathogens depend on a functional type III secretion system (T3SS), which allows the injection of bacterial proteins, named type III effectors (T3Es), inside plant host cells.

Collectively, the T3E repertoire modulates cellular processes and suppresses host defences for the benefit of the pathogen (Block and Alfano, 2011). Plants, however, have developed effective immune defences to resist pathogen attack. The plant immune system recognizes pathogen-associated molecular patterns (PAMPs) of the invading pathogen, and triggers PAMP-triggered immunity (PTI). Bacterial pathogens inject T3Es to counteract and suppress PTI, but plant resistance (R) proteins can sometimes recognize some of the injected T3Es and this triggers a second line of defence, called effector-triggered immunity (ETI). To counteract ETI, pathogens either lose the recognized T3Es or acquire new T3Es to suppress ETI (Chisholm *et al*., 2006; Jones and Dangl, 2006; Lindeberg *et al*., 2012). As a result of this endless arms race, the repertoire of T3Es has been shaped by evolution, is incredibly diverse and varies greatly among bacteria. The AvrE family of T3Es is the only family of TE3s present in all type III-dependent, agriculturally important phytobacteria, including gammaproteobacteria (enterobacteria, xanthomonads and pseudomonads) and betaproteobacteria (*Ralstonia* spp.). In this review, we describe the current picture that has emerged from studies of the different members of this large family.

HISTORICAL DISCOVERY OF THE AvrE FAMILY OF T3Es

AvrE was originally cloned from *Pseudomonas syringae* pv. *tomato* (*Pto*) PT23 on the basis of its ability to confer avirulence to *P. syringae* pv. *glycinea* race 4 in soybean (Kobayashi *et al*., 1989). A BLAST-P search revealed that AvrE shared 28% identity with the DspA/E protein required for *Erwinia amylovora* pathogenicity, and cross-complementation analysis confirmed conservation of function (Bogdanove *et al*., 1998a; Gaudriault *et al*., 1997). Both encoding genes are located next to the T3SS cluster, and orthologues, also linked to the T3SS cluster, were later described in other plant-pathogenic bacteria: *Pantoea stewartii* ssp. *stewartii* (WtsE) (Frederick *et al*., 2001), *Pantoea agglomerans* pv. *gypsophilae* (DspE/A) (Mor *et al*., 2001), *Dickeya dadantii* (DspE) (Glasner *et al*., 2011) and *Pectobacterium atrosepticum* and

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carotovorum (DspE) (Holeva *et al*., 2004; Kim *et al*., 2011). Combinatorial deletion analysis of *Pto* DC3000 T3Es identified HopR1 as a distant AvrE orthologue sharing 14% identity with AvrE. BLAST-P search with HopR1 from *Pto* DC3000 generates hits in *Xanthomonas* spp. (XopAM) and *Ralstonia solanacearum* (PopS, also named RipR) (Kvitko *et al*., 2009; Peeters *et al*., 2013). The genes encoding HopR1, XopAM and PopS are not located next to the T3SS cluster.

Phylogenetic analysis indicates that AvrE (pseudomonads)/ DspA/E, DspE or WtsE (enterobacteria)/PopS (*Ralstonia* spp.)/HopR (pseudomonads)/XopAM (xanthomonads) sequences can be grouped into five different clades (Jacobs *et al*., 2013). The phylogenetic trees obtained with PopS or AvrE mirror the phylogenies of *Ralstonia* and *Pseudomonas* species, respectively, indicating that the genes were vertically inherited (Jacobs *et al*., 2013; Rohmer *et al*., 2004). This highlights the ancestral and crucial role played by this T3E family in plant bacterial pathogenesis.

CONTRIBUTION TO VIRULENCE

Members of the AvrE superfamily contribute significantly to virulence on host plants. DspA/E, WtsE, DspE/A and DspE are essential for the pathogenicity of *E. amylovora*, *Pa. stewartii* ssp. *stewartii*, *Pa. agglomerans* pv. *gypsophilae* and *Pe. carotovorum*, respectively (Frederick *et al*., 2001; Gaudriault *et al*., 1997; Kim *et al*., 2011; Mor *et al*., 2001). The crucial role played by these effectors could probably be explained by the small repertoire of T3Es found in the genome of these pathogens. However, this result is surprising for the gall-forming pathogen *Pa. agglomerans* pv. *gypsophilae* and the soft rot pathogen *Pe. carotovorum*, and suggests that disease depends on both WtsE and DspE injection inside the host cell and on the production of phytohormones and plant cell wall-degrading enzymes (Barash and Manulis-Sasson, 2007; Kim *et al*., 2011). Such a dependence was, however, not observed with DspE of *Pe. atrosepticum*, as DspE makes only a minor contribution to the virulence of this soft rot pathogen on potato stems and tubers (Holeva *et al*., 2004). Disruption of *R. solanacearum* PopS, *Xanthomonas* spp. XopAM or *P. syringae* AvrE leads to slightly reduced virulence of the mutant strains (Badel *et al*., 2006; Jacobs *et al*., 2013; Jiang *et al*., 2009; Kvitko *et al*., 2009; Qian *et al*., 2005). The subtle phenotype observed could be explained by functional overlap among effectors as a result of the large T3E repertoire of the latter bacterial strains. For example, HopR1 and AvrE are part of the same redundant effector group (REG) and both partially complement the ΔIV-ΔCEL *Pto* DC3000 mutant, multi-deleted of several T3Es, including HopR1 and AvrE, for growth on *Nicotiana benthamiana* (Cunnac *et al*., 2011; Kvitko *et al*., 2009). Interestingly, HopR1 does not complement the ΔIV-ΔCEL *Pto* DC3000 mutant for growth on tomato, suggesting that the importance of HopR1 may differ in different plants (Kvitko *et al*., 2009).

SECRETION, TRANSLOCATION AND CHAPERONE REQUIREMENT

The secretion of effectors of the AvrE family can be observed *in vitro* when bacteria are cultivated in minimal medium (Bogdanove *et al*., 1998b; Gaudriault *et al*., 1997; Ham *et al*., 2006). As observed for most T3Es studied to date, N-terminal uncleaved secretion and translocation signals are described in DspA/E and AvrE (Badel *et al*., 2006; Bocsanczy *et al*., 2008; Ham *et al*., 2009; Oh *et al*., 2010; Triplett *et al*., 2009). Genes encoding DspA/E, DspE, AvrE and WtsE are associated with a type III secretion chaperone (T3SC) gene. These T3SCs are required for full accumulation of *Pa. stewartii* WtsE or *E. amylovora* DspA/E inside the bacterial cytoplasm. Mutation of the T3SC gene almost abolishes the secretion of *E. amylovora* DspA/E, but has only a slight impact on *Pa. stewartii* WtsE secretion, indicating that additional T3SCs are probably involved in WtsE secretion (Gaudriault *et al*., 2002; Ham *et al*., 2006). The T3SC of *P. syringae* AvrE has not been studied in detail; however, mutation in the transcriptional unit that encodes this T3SC abolishes the ability of AvrE to induce a hypersensitive reaction (HR) on soybean cultivar, suggesting that this T3SC is important for AvrE secretion (Lorang and Keen, 1995). *Erwinia amylovora* DspA/E interaction with its T3SC has been studied in detail. Two independent groups identified a chaperone-binding domain (CBD) within amino acids 51–200 of DspA/E (Oh *et al*., 2010; Triplett *et al*., 2009). Neither disruption of this N-terminal DspA/E CBD nor deletion of the T3SC gene has a significant impact on translocation levels of N-terminal DspA/E–CyaA fusions (Triplett *et al*., 2009), but the presence of T3SC strongly enhances the translocation of a DspA/E–AvrRpt2 fusion protein into plant cells (Oh *et al*., 2010). These contradictory results indicate that the requirement for the chaperone is influenced by the C-terminal part of the protein. In accordance with this, it was found that the T3SC also interacts with the C-terminal half of DspA/E (Triplett *et al*., 2010). Furthermore, another T3SC has also been found to interact with the C-terminal domain of DspA/E (Oh *et al*., 2010). The interaction with several T3SCs and the presence of several CBDs may be required because effectors of the AvrE family are very large proteins.

T3SC genes are not found next to genes encoding HopR1, PopS or XopAM. Whether these effectors do not need chaperone assistance or whether T3SCs encoded elsewhere in the genome assist the secretion/injection of these effectors remains to be determined.

REGULATION OF EXPRESSION

The different genes of the AvrE family are co-regulated with the T3SS (Gaudriault *et al*., 1997; Hogan *et al*., 2013; Jacobs *et al*., 2013; Lorang and Keen, 1995; McNally *et al*., 2012; Mor *et al*., 2001; Yang *et al*., 2004, 2010). *In vitro*, their expression is

repressed in rich medium and induced in minimal medium (Gaudriault *et al*., 1997; Peng *et al*., 2006; Wei *et al*., 1992). *In planta*, their expression is detected as early as 2 h following inoculation and is still observed 2–3 days post-infection, indicating that AvrE-like effectors are probably required at both the beginning and throughout the infection process (Hogan *et al*., 2013; Kim *et al*., 2011; Peng *et al*., 2006; Pester *et al*., 2012; Yang *et al*., 2004). Interestingly, Peng *et al*. (2006), by analysing the expression of green fluorescent protein (GFP) fused to the *dsp*E promoter of *D. dadantii* with a fluorescence-activated cell sorter, observed that a proportion of *D. dadantii* cells never expressed *dsp*E in various media or host plants. This raises the possibility that individual bacterial cells may play different roles and work together as a community effort to ensure disease development. Wang *et al*. (2010) also noticed that the expression level of *E. amylovora dsp*A/E correlates with the aggressiveness of the strain, suggesting that this effector induces a dose-dependent effect.

PHYSIOLOGICAL EFFECTS OF AvrE-LIKE EFFECTORS: SUPPRESSION OF DEFENCE ASSOCIATED WITH PERTURBATION OF CELLULAR TRAFFIC AND SLOW DEATH INDUCTION

Several members of the AvrE family are able to suppress salicylic acid-dependent defence responses, such as callose deposition or *PR1* expression (Boureau *et al*., 2006, 2011; DebRoy *et al*., 2004; Ham *et al*., 2008; Jacobs *et al*., 2013). In contradiction with this general scheme, DspE of *Pe. carotovorum* is unable to suppress callose deposition induced by *Pe. carotovorum* along the leaf vein of *N. benthamiana* (Kim *et al*., 2011). Whether this reflects intrinsic differences among AvrE effectors, or whether the callose deposition induced by *Pe. carotovorum* is different from the callose deposition induced by *P. syringae*, remains to be determined. In support of the second hypothesis, it is known that callose deposition in *Arabidopsis thaliana* involves more than one pathway (Luna *et al*., 2011). Furthermore, DspA/E of *E. amylovora* suppresses *P. syringae*-induced, but not *E. amylovora*-induced, callose deposition on *A. thaliana*, further indicating that callose deposition induced by different pathogens is somehow different (Degrave *et al*., 2013).

When ectopically expressed, most effectors of the AvrE family elicit cell death on host and non-host plants (Badel *et al*., 2006; Boureau *et al*., 2006; Ham *et al*., 2006, 2008; Hogan *et al*., 2013; Oh *et al*., 2007). Whether the cell death observed following expression on non-host plants is inherent to the toxicity of this effector family, or whether effectors of the AvrE family trigger an HR on non-host plants as a result of recognition by an R protein, has been a matter of debate. First, one should keep in mind that the answer could vary between non-host plants, but nobody has yet identified R proteins involved in such recognition. For example, although AvrE was discovered on the basis of its ability to confer avirulence to *P. syringae* pv. *glycinea* race 4 in soybean (Kobayashi *et al*., 1989), it was later found to confer such avirulence to all soybean cultivars tested, raising the possibility that the phenotype observed is simply a result of AvrE toxicity on soybean (Lorang and Keen, 1995). Against recognition by an R protein and ETI triggering, it should be noted that the induced cell death develops slowly (approximately 36 h) on both host and non-host plants (Badel *et al*., 2006; Boureau *et al*., 2006). This 'slow death' phenotype is also observed with an *A. thaliana* transgenic line expressing *E. amylovora* DspA/E under an oestradiol-inducible promoter (Degrave *et al*., 2013). Oestradiol induction allows an investigation of the dose-dependent effect. When the expression of DspA/E is induced by low oestradiol concentrations, transgenic plants suffer growth reduction without associated cell death. With high oestradiol concentrations, which lead to stronger DspA/E expression, leaves start to wilt at 24 h post-induction and are completely necrotic at 5 days post-induction (Degrave *et al*., 2013). Most surprisingly, transient expression of an *R. solanacearum* 35S promoter–PopS–haemagglutinin tag (HA) construct does not induce cell death, suggesting that this particular member of the AvrE family could have evolved to avoid cell death induction (Jacobs *et al*., 2013). This is consistent with the fact that *R. solanacearum* causes a non-necrotic wilt. However, one may also question whether the addition of an HA tag at the end of the protein is or is not deleterious for PopS function. Indeed, deletion of the four penultimate amino acids of *Pa. stewartii* WtsE or *E. amylovora* DspA/E inactivates the cell death-inducing ability of these effectors (Ham *et al*., 2008; A. Degrave *et al*., unpublished observations).

The expression of DspA/E in transgenic *A. thaliana* lines is also associated with the inhibition of protein synthesis (Degrave *et al*., 2013). This is consistent with the fact that *Pa. stewartii* WtsE-induced symptoms in corn seedlings are not inhibited by cycloheximide, which blocks *de novo* protein synthesis in eukaryotic cells (Ham *et al*., 2006), and the observation that cycloheximide partially rescues the growth of an *E. amylovora dsp*A/E mutant on *A. thaliana* (Degrave *et al*., 2013).

AvrE TYPE III FAMILY EFFECTORS, LARGE ENIGMATIC PROTEINS

Effectors of the AvrE family are very large proteins of approximately 2000 amino acids, without sequence similarity with proteins of known function. Although their large size would be consistent with a multifunctional activity, their biochemical function remains unknown. However, the importance of this family of T3Es for pathogenicity on host plants and cell death elicitation on non-host plants allowed us to test the functionality of mutated effector versions. Mutational analysis of *Pa. stewartii* WtsE

showed that the full-length protein is required for function (Ham *et al*., 2006, 2008). Consistent with this, we could not identify a subdomain of *E. amylovora* DspA/E sufficient to elicit cell death when transiently expressed (A. Degrave *et al*., unpublished observation). One exception is the first N-terminal 200 amino acids dedicated to secretion and translocation, as described above, which could be removed from *Pa. stewartii* WtsE without affecting the cell death induction capacity of the transiently expressed construct (Ham *et al*., 2008). Following this N-terminal secretion/translocation domain, protein-threading software predicts a conserved double β-propeller domain in all AvrE orthologues (Siamer *et al*., 2013). It is possible that this predicted domain acts as a platform-binding domain for interactors (Chen and Chan, 2011).

Motif searches identified nuclear localization signals (NLSs), endoplasmic reticulum membrane retention signals (ERMRSs), leucine zipper motifs (LZs), peroxisome targeting signals (PTSs) and WXXXE motifs, but none of these motifs are present in all the AvrE effectors analysed. The ERMRS signal, when present, is always positioned in the penultimate four amino acids and its deletion impairs DspA/E or WtsE protein function (Ham *et al*., 2006, 2009; A. Degrave *et al*., unpublished observations). The position of other motifs is generally not conserved between orthologues (Ham *et al*., 2006; Siamer *et al*., 2013). WXXXE motifs are of particular interest, because they were also described in T3Es of animal-pathogenic bacteria, which functionally mimic guanine nucleotide exchange factors (GEFs), the endogenous activators of Rho family GTPases (Orchard and Alto, 2012). Simultaneous mutagenesis of the two WXXXE motifs of *Pa. stewartii* WtsE alters the function of the protein (Ham *et al*., 2009). When the WXXXE motifs are not detected in orthologous proteins, as in *Pe. carotovorum* DspE or *R. solanacearum* PopS, tryptophan residues are nevertheless conserved at the same position (Hogan *et al*., 2013; Jacobs *et al*., 2013), and site-directed mutagenesis of these two tryptophan residues is deleterious for the function of *Pe. carotovorum* DspE (Hogan *et al*., 2013). Therefore, it appears that at least these tryptophan residues are important to maintain the function of the protein. Whether these residues are important for correct folding of the protein or are involved in a putative catalytic GEF function remains to be determined.

LOCALIZATION INSIDE THE EUKARYOTIC CELL

Attempts to localize AvrE family effectors inside plant cells following *Agrobacterium*-mediated transient expression were unsuccessful (Boureau *et al*., 2006; Degrave *et al*., 2013; Ham *et al*., 2009). This is probably because of the toxic mode of action of AvrE-T3Es, which leads to a rapid repression of *de novo* protein synthesis (Degrave *et al*., 2013). A transient GFP signal has nevertheless been detected inside the plant nucleus in a transgenic *A. thaliana* line expressing a GFP–DspA/E fusion (Degrave *et al*., 2013). This is consistent with predictions made with the PSORTII program, which predict that all AvrE family effectors, except PopS from *R. solanacearum*, may localize to the plant cell nucleus (Ham *et al*., 2006). However, not all AvrE-T3Es harbour an NLS signal, and mutagenesis of the *Pa. stewartii* WtsE and *E. amylovora* DspA/E NLS indicates that this motif is not required for protein function (Ham *et al*., 2006; A. Degrave *et al*., unpublished observations). Therefore, if the protein is transported inside the plant nucleus, this could be achieved without a functional NLS. It is also possible that the function of AvrE family effectors is not dependent on nucleus localization and may be linked to localization in another cellular compartment. AvrE-like effectors may also localize to the endoplasmic reticulum because most effectors of the family harbour an ERMRS motif at their C-terminus.

YEAST AS A TOOL TO UNRAVEL THE FUNCTION OF AvrE FAMILY EFFECTORS

Induction of the expression of *E. amylovora* DspA/E or *Pa. stewartii* WtsE in *Saccharomyces cerevisiae* leads to growth arrest (Ham *et al*., 2008; Meng *et al*., 2006; Oh *et al*., 2007; Siamer *et al*., 2011) and strongly affects cellular traffic (Siamer *et al*., 2011). This phenotype is reminiscent of the growth inhibition observed on *A. thaliana* transgenic lines expressing a low level of DspA/E (Degrave *et al*., 2013), and suggests that AvrE-like effectors probably target a general pathway conserved in eukaryotes.

Yeast toxicity precludes yeast two-hybrid experiments with full-length effectors of the AvrE family. However, yeast two-hybrid assays performed with protein fragments of *Pa. stewartii* WtsE and *E. amylovora* DspA/E identified leucine-rich repeat receptor-like serine/threonine kinases (RLKs) as putative interactors (Ham *et al*., 2006; Meng *et al*., 2006). Whether these interactions occur *in vivo* remains to be determined. Interactions of WtsE with two maize regulatory subunits of protein phosphatase 2A (PP2A) have also been reported in a recent review (Roper, 2011). Although this awaits publication of the data, it is tempting to speculate that these latter interactions occur *in vivo*, as it has been shown that *E. amylovora* DspA/E expression in yeast activates PP2A (Siamer *et al*., 2014). Activated PP2A dephosphorylates and activates Orm proteins, which are the negative regulators of serine palmitoyltransferase (SPT), the first enzyme of the sphingolipid pathway (Breslow *et al*., 2010; Sun *et al*., 2012). This, in turn, leads to the inhibition of SPT activity and decreases the level of long-chain bases (LCBs), the products of SPT activity. Feeding yeast cells expressing DspA/E with LCB, or expressing DspA/E in yeast mutants that accumulate LCB, rescues yeast cell growth and cellular traffic, indicating that DspA/E toxicity is linked to LCB depletion (Siamer *et al*., 2014). Inhibition of SPT could explain all the toxic phenotypes observed. Indeed, SPT inhibition leads to the perturbation of cellular traffic (Zanolari *et al*., 2000), inhibition of translation initiation (Meier *et al*., 2006), growth arrest and, ultimately, cell death (Dickson *et al*., 2006; Pinto *et al*., 1992).

Given the functional complementation observed between effectors of the AvrE family (Bogdanove *et al*., 1998a; Ham *et al*., 2006; Kvitko *et al*., 2009), it is likely that the expression of other members of the AvrE family could also activate PP2A and decrease the LCB level, but this remains to be tested.

FUTURE PROSPECTS

Yeast studies allow the proposal of several non-exclusive hypotheses to explain how AvrE-T3Es could inhibit PTI (Fig 1). First, yeast two-hybrid studies indicate that effectors of the AvrE family could interact with RLKs (Ham *et al*., 2006; Meng *et al*., 2006). As the predicted structure of these RLKs is similar to a class of surface-localized immune receptors (Afzal *et al*., 2008; Dangl and Jones, 2001), a possible consequence of direct interactions with these RLKs of host plants during infection could be to block signal transduction leading to defence responses. Effectors of the AvrE family might also interact with and activate PP2A (Roper, 2011; Siamer *et al*., 2014). Activation of PP2A by effectors of the AvrE family would reinforce PTI inhibition as PP2A is involved in the negative control of PTI *in planta* (Segonzac *et al*., 2014; Trotta *et al*., 2011). The involvement of several putative substrates of PP2A could now be tested *in planta* following bacterial infection. Yeast studies indicate that ORM proteins, which negatively regulate SPT activity and sphingolipid biosynthesis, could be important PP2A substrates *in planta* (Siamer *et al*., 2014). This would make sense as the sphingolipid biosynthetic pathway is induced during the hypersensitive response that blocks pathogen attack at the site of infection (Berkey *et al*., 2012; Peer *et al*., 2010; Shi *et al*., 2007). Therefore, inhibition of this pathway could be seen as a mechanism that delays hypersensitive response cell death and allows bacterial development *in planta*. Perturbation of the sphingolipid pathway *in planta* could also explain why effectors of the AvrE family induce a slow plant cell death, because sphingolipids are structural lipids of plant membranes and prolonged inhibition of the sphingolipid pathway ultimately leads to plant cell death (Chen *et al*., 2006). Another substrate involved in PTI that could be targeted following AvrE-mediated activation of PP2A *in planta* is BAK1, whose steady-state phosphorylation is important to trigger a defence reaction and whose dephosphorylation requires PP2A (Segonzac *et al*., 2014). Overall, yeast studies suggest that effectors of the AvrE family could reinforce PTI inhibition at different levels. This remains to be tested *in planta* with different members of the family. In particular, as most of the studies were performed with AvrE (pseudomonads) and DspA/E, DspE or WtsE (enterobacteria), it would be very interesting to check whether PopS (*Ralstonia* spp.), HopR1 (pseudomonads) and XopAM (xanthomonads), which are more distantly related, function similarly.

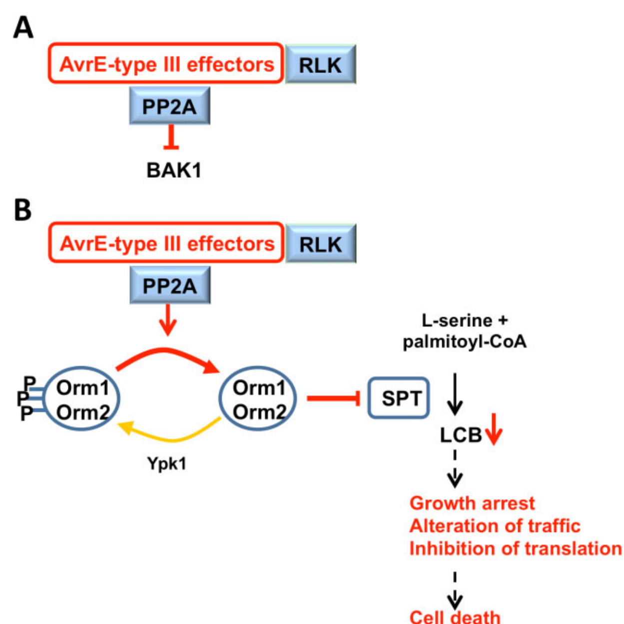

Fig. 1 Proposed model for AvrE-like effector-mediated toxicity and inhibition of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI deduced from yeast studies. Yeast two-hybrid studies indicate that AvrE-type III effectors (T3Es) possibly interact with receptor-like serine/threonine kinases (RLKs) and protein phosphatase 2A (PP2A) (Ham *et al*., 2006; Meng *et al*., 2006; Roper, 2011). Direct binding to RLKs could potentially block signal transduction, leading to plant defence (Meng *et al*., 2006). (A) Binding and activation of PP2A could also reinforce PTI inhibition indirectly through dephosphorylation and inactivation of BAKI (Segonzac *et al*., 2014; Siamer *et al*., 2014). As RLKs and BAK1 are localized to the plasma membrane (PM), PM is a potential site of action for AvrE-T3Es. (B) Once activated, PP2A could also dephosphorylate and activate Orm proteins. As ORM proteins are associated with serine palmitoyltransferase (SPT) at the endoplasmic reticulum (ER), the ER is another potential site of action of AvrE-T3Es. Dephosphorylated Orm proteins could, in turn, inhibit SPT, leading to long-chain base (LCB) depletion. As the sphingolipid pathway is induced during the hypersensitive reaction (HR), inhibition of this pathway could delay HR cell death and participate in PTI inhibition (Berkey *et al*., 2012). Prolonged inhibition of the sphingolipid pathway will ultimately lead to plant cell death (Chen *et al*., 2006), explaining the 'slow death' phenotype observed on host and non-host plants.

Beyond the role of the AvrE effector family during plant bacterial pathogenesis, it is interesting to note that several bacteria which associate with plants, but which are not reported to be pathogens, also harbour an effector of the AvrE family. For example, *Marinomonas mediterannea*, a bacterium reported to associate with sea grass, harbours a gene showing 26% identity with DspA/E of *E. amylovora* (Siamer *et al*., 2013). The same is true of *P. fluorescens* SBW25, which harbours DspE, although it is non-pathogenic and does not elicit an HR in any host plant tested (Preston *et al*., 2001). As the low expression of effectors of the AvrE family is not associated with plant cell death (Degrave *et al*., 2013), it would be interesting to determine whether a low expression level of effectors of the AvrE family could be beneficial for the association of these non-pathogenic bacteria with plants.

Finally, an intriguing question is the origin of the AvrE family of T3Es. Until now, no homology to any known protein has been reported. Interestingly, T3Es of the AvrE family and several viruses inhibit the whole sphingolipid pathway of their hosts (Rosenwasser *et al*., 2014). This could be a case of convergent evolution; however, it would be interesting to determine whether T3Es of the AvrE family share similarity with a viral protein.

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