Microreview

The HopF family of *Pseudomonas syringae* type III secreted effectors

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SUMMARY

Pseudomonas syringae is a bacterial phytopathogen that utilizes the type III secretion system to inject effector proteins into plant host cells. *Pseudomonas syringae* can infect a wide range of plant hosts, including agronomically important crops such as tomatoes and beans. The ability of *P. syringae* to infect such numerous hosts is caused, in part, by the diversity of effectors employed by this phytopathogen. Over 60 different effector families exist in *P. syringae*; one such family is HopF, which contains over 100 distinct alleles. Despite this diversity, research has focused on only two members of this family: HopF1 from *P. syringae* pathovar *phaseolicola* 1449B and HopF2 from *P. syringae* pathovar *tomato* DC3000. In this study, we review the research on HopF family members, including their host targets and molecular mechanisms of immunity suppression, and their enzymatic function. We also provide a phylogenetic analysis of this expanding effector family which provides a basis for a proposed nomenclature to guide future research. The extensive genetic diversity that exists within the HopF family presents a great opportunity to study how functional diversification on an effector family contributes to host specialization.

Keywords: HopF, *Pseudomonas syringae*, type III secreted effectors.

INTRODUCTION

The Gram-negative bacterial phytopathogen *Pseudomonas syringae* causes disease on a broad range of host plants, including grasses, trees, crop plants, as well as the model plant *Arabidopsis thaliana* (Mansfield *et al.*, 2012; O'Brien *et al.*, 2011). The pathogenic success of *P. syringae* relies, in part, on the type III secretion

system, which is used to inject a complement of effector proteins into host cells to suppress immunity and to promote pathogenesis. The pangenome of *P. syringae* encodes approximately 60 families of type III secreted effectors (T3SEs) which are distributed in various combinations within each *P. syringae* strain (O'Brien *et al.*, 2011).

In response to the detrimental action of pathogenic effectors, plants have evolved nucleotide-binding leucine-rich repeat resistance proteins (NLRs) that recognize either specific T3SEs, or the cellular perturbations that are caused by the T3SEs, to induce effector-triggered immunity (ETI) (Dangl and Jones, 2001; Deslandes *et al.*, 2003; Dodds and Rathjen, 2010). ETI often culminates in a localized programmed cell death response, termed the hypersensitive response (HR), which is thought to limit pathogen growth to the site of infection (Heath, 2000). Selective pressure imposed by host ETI responses is believed to have driven the diversification of T3SE families to avoid host recognition, whilst maintaining their virulence functions. As a result, nearly every T3SE family includes genetically diverse alleles that have been shaped by evolutionary pressures imposed by the plant immune systems (McCann and Guttman, 2008).

T3SE diversification is exemplified by the HopZ family of T3SEs that contains four evolutionarily and functionally distinct allelic subfamilies: HopZ1, HopZ2, HopZ3 and HopZ4 (Ma *et al.*, 2006; Üstün *et al.*, 2014). In *Arabidopsis*, HopZ1 targets the ZAR1 resistance complex, which includes the ZED1 pseudokinase (Lewis *et al.*, 2010, 2013), transcriptional repressors (Jiang *et al.*, 2013) and the microtubule network (Lee *et al.*, 2012), HopZ2 targets the immune protein MLO2 (Lewis *et al.*, 2010), HopZ3 targets the RPM1 immune complex (Lee *et al.*, 2015) and HopZ4 targets the proteasome (Üstün *et al.*, 2014). This diversity of host targets emphasizes the functional diversification that can occur within a T3SE family. To date, the functional diversification of the HopZ/YopJ family has been exceptionally well documented relative to most other T3SE families, and has emphasized that a single T3SE family member cannot functionally represent an entire family (Lewis *et al.*, 2011).

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Like HopZ, the HopF T3SE family is broadly distributed among pathovars of *P. syringae* and exhibits extensive genetic diversity; however, the functional diversification of this

family has yet to be surveyed (Fig. 2, see 'HopF diversification' section). In this review, we provide a characteristic overview of HopF functions and present a phylogenetic analysis

Fig. 1 Ribbon diagrams of the SchF_{Pph1449B} type III secreted effector (T3SE) chaperone (A) and HopF1_{Pph1449B} (B) with α -helices depicted in red, β -sheets in blue and loop regions in yellow. Spherical depictions of the ADP-ribosyltransferase (ADP-RT) catalytic residues, R72 in blue and D174 in yellow, are shown for HopF1_{Pph1449B}. (C) HopF homologue alignment. An amino acid alignment was performed using MUSCLE with representative *Pseudomonas syringae* HopF subgroup members. The chimeric subgroups, AvrRpm2 and HopBB1, were trimmed to the region of HopF homology retaining 104 amino acids from the N-terminus. HopF homologue amino acid sequences were then aligned to the *P. syringae* HopF alignment. The *Ralstonia* and *Acidovorax* homologues were trimmed to regions of HopF homology, removing 89 and 34 amino acids from the N-terminus, respectively. The glycine at position 2 is essential for myristoylation and is conserved in all sequences aligned, except HopF2c. This residue is not shown for the *Ralstonia* and *Acidovorax* sequences because of trimming of their N-terminal sequences. The ADP-RT catalytic residues, R72 and D174, are boxed in red (for sequences, see Supporting Information 3).

of the HopF family based on recent *P. syringae* genome sequencing efforts.

HopF MOLECULAR FUNCTIONS

HopF1

HopF1 (formerly AvrPphF), from the halo blight pathogen *P. syringae* pv. *phaseolicola* 1449B (Pph1449B), was the first characterized member of the HopF family (Jackson *et al.*, 1999). HopF1_{Pph1449B} plays a dual role in beans; it can promote bacterial growth in susceptible cultivars by suppressing immunity (Hou *et al.*, 2011; Singer *et al.*, 2004), whereas cultivars harbouring the *R1* resistance gene elicit HopF1_{Pph1449B}-dependent ETI (Tsiamis *et al.*, 2000). Cloning of *hopF1*_{Pph1449B} identified two open reading frames (ORFs) which encode the type III chaperone *schF1*_{Pph1449B} (ORF1) and the effector *hopF1*_{Pph1449B} (ORF2) (Arnold *et al.*, 2011; Shan *et al.*, 2004; Tsiamis *et al.*, 2000).

The crystal structures of SchF1_{Pph1449B} and HopF1_{Pph1449B} have been solved (Singer *et al.*, 2004). SchF1_{Pph1449B} adopts a fold similar to that of other type III chaperones, suggestive of a common mode of action (Fig. 1A). The structure of HopF1_{Pph1449B} resembles a mushroom with a head and stalk subdomain (Fig. 1B). Although the stalk subdomain does not resemble any structures of known function, the head subdomain possesses structural similarity to diphtheria toxin, an ADP-ribosyltransferase (ADP-RT) (Singer *et al.*, 2004). This structural similarity was used to predict two putative catalytic residues: Arg72 and Asp174. Mutation of either of these two sites was shown to abolish both the virulence and avirulence activities of HopF1_{Pph1449B} in various cultivars of bean (Singer *et al.*, 2004). Although ADP-RT activity has yet to be demonstrated for HopF1_{Pph1449B}, it has been shown for HopF2 from the bacterial speck pathogen *P. syringae* pv. *tomato* DC3000 (PtoDC3000) (Wang *et al.*, 2010).

HopF2

HopF2_{PtoDC3000} contains an alternative ATA start codon, which limits protein production in *P. syringae* (Shan *et al.*, 2004). Mutation of the ATA start codon to ATG results in increased protein production, which enhances the virulence of *P. syringae* in tomato and *Arabidopsis* (Shan *et al.*, 2004; Wilton *et al.*, 2010). In addition, HopF2_{PtoDC3000} contains an N-terminal myristoylation signal

that localizes the T3SE to host membranes (Robert-Seilaniantz *et al.*, 2006). The myristoylation site, as well as the ADP-RT catalytic residues, are both required for HopF2_{PtoDC3000} virulence activity in tomato (Robert-Seilaniantz *et al.*, 2006) and *Arabidopsis* (Wilton *et al.*, 2010), avirulence activity in tobacco (Wang *et al.*, 2010) and the ability of HopF2_{PtoDC3000} to suppress host immune responses (Guo *et al.*, 2009; Wu *et al.*, 2011), suggesting that this T3SE ADP-ribosylates membrane-localized targets. These include the immunity regulators RIN4 and MKK5, which are targeted by HopF2_{PtoDC3000} (Wang *et al.*, 2010; Wilton *et al.*, 2010). BAK1 may also be a target, but ribosylation by HopF2_{PtoDC3000} has yet to be shown (Zhou *et al.*, 2014). In addition, HopF2_{PtoDC3000} has also been shown to enhance sense-post-transcriptional gene silencing to interfere with host RNA silencing, but the host targets of this activity have yet to be identified (Sarris *et al.*, 2011).

MKK5 is part of a mitogen-activated protein (MAP) kinase signalling cascade that is required for immunity in plants (Asai *et al.*, 2002). HopF2_{PtoDC3000} has been shown to interact with and ADP-ribosylate MKK5 to inhibit its kinase activity, thereby suppressing downstream immune responses, such as callose deposition (Wang *et al.*, 2010). HopF2_{PtoDC3000} also targets BAK1, which acts upstream of MKK5. BAK1 is a receptor-like kinase (RLK) which can heterodimerize and act as a co-receptor that is required for the perception of many microbe-derived molecules, including flagellin (Chinchilla *et al.*, 2007; Heese *et al.*, 2007; Postel *et al.*, 2010; Roux *et al.*, 2011). HopF2_{PtoDC3000} interacts directly with BAK1 to suppress multiple downstream immunity pathways, including the MKK5 pathway (Zhou *et al.*, 2014).

HopF2_{PtoDC3000} is also able to interfere with ETI elicited by the unrelated T3SE AvrRpt2 (Wilton *et al.*, 2010). AvrRpt2 is a cysteine protease that cleaves the negative regulator of immunity RIN4, leading to activation of ETI by the NLR RPS2 (Axtell and Staskiewicz, 2003; Chisholm *et al.*, 2005; Day *et al.*, 2005; Mackey *et al.*, 2003). HopF2_{PtoDC3000} interacts with RIN4 and prevents its degradation by AvrRpt2, leading to the suppression of AvrRpt2-induced ETI (Wilton *et al.*, 2010). In addition to its ETI-suppressing capability, HopF2_{PtoDC3000} also promotes virulence in *Arabidopsis* in a RIN4-dependent manner that is independent of ETI suppression. Both of these functions are dependent on the ADP-RT activity of HopF2_{PtoDC3000} (Wilton *et al.*, 2010).

HopF2_{PtoDC3000} may also possess functions that are independent of ADP-RT activity. *PtoDC3000* utilizes the phytotoxin coronatine to open stomata and gain entry into the apoplastic space. As

Fig. 2 Phylogeny of the *Pseudomonas syringae* HopF type III secreted effector (T3SE) family. One hundred unique HopF alleles were obtained from a collection of 386 *P. syringae* strains and a codon alignment was performed using MUSCLE. For the chimeric subgroups, AvrRpm2 and HopBB1, the sequences were trimmed to the region of HopF homology retaining 312 bp from the 5' end of the alignment. All sequences were then realigned using the trimmed sequences. The maximum-likelihood tree was then built using the GTR model and gamma distributed with 1000 bootstrap replications. Although 268 HopF genes were found, identical alleles were not included in this tree for simplicity (see Table 1). The numbers in parentheses beside HopF specific members indicate the numbers of identical alleles including the member represented in the tree. Some plant hosts of HopF containing *P. syringae* pathovars are shown (see Supporting Information 1 and 2 for FASTA sequences).

such, a *PtoDC3000* strain lacking coronatine (*PtoDC3118*) is severely compromised in virulence when sprayed onto leaves. However, transgenic expression of HopF2_{PtoDC3000} in *Arabidopsis* is able to restore the virulence of *PtoDC3118* to levels equivalent to those of wild-type *PtoDC3000* (Hurley *et al.*, 2014). Interestingly, *PtoDC3118* growth promotion is not dependent on ADP-RT catalytic residues (Hurley *et al.*, 2014), suggesting that HopF2_{PtoDC3000} may represent a multifunctional T3SE that possesses activities that are independent of its ADP-RT function.

HOPF DIVERSIFICATION

Based on approximately 400 sequenced *P. syringae* strains (D. S. Guttman, unpublished data), the HopF family currently includes 100 unique alleles from pathovars that infect a wide variety of plant species, including herbaceous and woody plants (Fig. 2; Table 1). The HopF family can be broken down into six distinct subfamilies: HopF1, HopF2, HopF3, HopF4, HopBB1 and AvrRpm2. HopBB1 and AvrRpm2 are chimeric T3SEs with N-terminal regions homologous to those of the HopF4 family (including myristoylation sites), and C-terminal regions similar to proteins of unknown function in other phytopathogens and an unrelated T3SE family AvrRpm1, respectively. All members of the HopF family, including the chimeric subgroups, contain *schF* upstream of their promoters. HopF2_{PtoDC3000} requires the chaperone SchF for stability in bacteria and efficient type III secretion (Shan *et al.*, 2004). ADP-RT catalytic residues are conserved throughout the HopF family, with the exception of the chimeric effector families. As such, it is predicted that all non-chimeric HopF subfamilies function as ADP-RTs.

HopF1 and HopF3 are the two subgroups with the least genetic diversity within the HopF family. HopF1_{Pph1449B} has been shown to play a role in common bean and many *hopF1* alleles originate from *P. syringae* strains isolated from bean, pea and soybean (Singer *et al.*, 2004). Little is known about the functions of the remaining HopF1 members and any of the HopF3 subgroup. HopF3_{Pph1448A} was tested in an *Agrobacterium*-mediated transient infection assay in over 50 plant genotypes, including multiple

Table 1 List of currently known HopF alleles.

Subfamily	Member	Pathovar	Plant host of isolation
HopF1	HopF1_PhoICMP7840 (2)	<i>photiniae</i>	<i>Photinia glabra</i> (Japanese photinia)
	HopF1_PhoICMP7847	<i>photiniae</i>	<i>Photinia glabra</i> (Japanese photinia)
	HopF1_Pta11528_NCB1 (5)	<i>tabaci</i>	<i>Nicotiana tabacum</i> (tobacco)
	HopF1_PtaICMP2835	<i>tabaci</i>	<i>Nicotiana tabacum</i> (tobacco)
	HopF1_PtaICMP4525	<i>tabaci</i>	<i>Nicotiana tabacum</i> (tobacco)
	HopF1_Pta11528	<i>tabaci</i>	<i>Nicotiana tabacum</i> (tobacco)
	HopF1_Pta6606	<i>tabaci</i>	<i>Nicotiana tabacum</i> (tobacco)
	HopF1_PdeICMP9150	<i>dendropanacis</i>	<i>Dendropanax trifidus</i> (tree ivy)
	HopF1_PcsiCMP9420 (2)	<i>castaneae</i>	<i>Castanea crenata</i> (Japanese chestnut)
	HopF1_PcsiCMP9419	<i>castaneae</i>	<i>Castanea crenata</i> (Japanese chestnut)
	HopF1_PgylCMP807	<i>glycinea</i>	<i>Glycine max</i> (soybean)
	HopF1_PmeICMP5711 (3)	<i>mellea</i>	<i>Nicotiana tabacum</i> (tobacco)
	HopF1_PmeN6801	<i>mellea</i>	<i>Nicotiana tabacum</i> (tobacco)
	HopF1_PamICMP3918	<i>amygdali</i>	<i>Prunus dulcis</i> (almond)
	HopF1_PrelCMP16945 (3)	<i>retacarpa</i>	<i>Retama sphaerocarpa</i> (broom-like shrub)
	HopF1_PrelCMP16946	<i>retacarpa</i>	<i>Retama sphaerocarpa</i> (broom-like shrub)
	HopF1_PrelCMP16947	<i>retacarpa</i>	<i>Retama sphaerocarpa</i> (broom-like shrub)
	HopF1_PthICMP3923 (3)	<i>theae</i>	<i>Camellia sinensis</i> (tea)
	HopF1_PthNCPB2598	<i>theae</i>	<i>Camellia sinensis</i> (tea)
	HopF1_PthICMP3934	<i>theae</i>	<i>Camellia sinensis</i> (tea)
	HopF1_PgylCMP9923 (2)	<i>glycinea</i>	<i>Glycine javanica</i> (perennial soybean)
	HopF1_PgylCMP4324	<i>glycinea</i>	<i>Glycine wightii</i> (perennial soybean)
	HopF1_PphISPaVe596	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF1_PphY5_2 (6)	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF1_PphICMP637	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF1_PphK4	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF1_PphKN86	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF1_PgylCMP4332	<i>glycinea</i>	<i>Glycine javanica</i> (perennial soybean)
	HopF1_PgylCMP4333	<i>glycinea</i>	<i>Glycine max</i> (soybean)
	HopF1_PphICMP2740	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF1_PanICMP18883 (11)	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_PanICMP19094	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_PanICMP19095	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_PanICMP19099	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_PanICMP18807	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_PanICMP18803	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_PanICMP18804	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_PanICMP19096	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_PanICMP19098	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_PanICMP19100	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_PsycCMP18806	<i>syringae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF1_Pph1449B (6)	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF1_PpilCMP2788	<i>pisi</i>	<i>Pisum sativum</i> (pea)
	HopF1_PpilCMP4433	<i>pisi</i>	<i>Pisum sativum</i> (pea)
	HopF1_Ppi895A	<i>pisi</i>	<i>Pisum sativum</i> (pea)
	HopF1_PpiH5E3	<i>pisi</i>	<i>Pisum sativum</i> (pea)
	HopF1_PttlCMP11935	<i>aptata</i>	<i>Beta vulgaris</i> (beet)
	HopF1_PsolCMP16926 (3)	<i>solidagae</i>	<i>Solidago altissima</i> (tall golden rod)
	HopF1_PsolCMP16925	<i>solidagae</i>	<i>Solidago altissima</i> (tall golden rod)
	HopF1_PsolCMP16927	<i>solidagae</i>	<i>Solidago altissima</i> (tall golden rod)
	HopF1_PcalCMP2855 (3)	<i>caricapapayae</i>	<i>Carica papaya</i> (papaya)
	HopF1_PcalCMP4086	<i>caricapapayae</i>	<i>Carica papaya</i> (papaya)
	HopF1_PcalCMP2854	<i>caricapapayae</i>	<i>Carica papaya</i> (papaya)
	HopF1_PcalCMP7496	<i>caricapapayae</i>	<i>Carica papaya</i> (papaya)
	HopF1_PheICMP3549	<i>helianthi</i>	<i>Helianthus annuus</i> (sunflower)
	HopF1_PtgcCMP6369 (3)	<i>tagetis</i>	<i>Tagetes erecta</i> (African marigold)
	HopF1_PtgcCMP11161	<i>tagetis</i>	<i>Tagetes erecta</i> (African marigold)
	HopF1_PtgcCMP4091	<i>tagetis</i>	<i>Tagetes erecta</i> (African marigold)

Table 1 Continued

Subfamily	Member	Pathovar	Plant host of isolation
HopF2	HopF1_PheICMP3263	<i>helianthi</i>	<i>Helianthus annuus</i> (sunflower)
	HopF1_PcdICMP12341 (4)	<i>coriandricola</i>	<i>Coriandrum sativum</i> (coriander)
	HopF1_PcdICMP12471	<i>coriandricola</i>	<i>Coriandrum sativum</i> (coriander)
	HopF1_Pci0788_9	<i>cilantro</i>	<i>Coriandrum sativum</i> (coriander)
	HopF1_PmaM6	<i>maculicola</i>	Brassicaceae
	HopF2a_Pac302273 (3)	<i>aceris</i>	<i>Acer</i> sp. (maple)
	HopF2a_PacA10853	<i>aceris</i>	<i>Acer</i> sp. (maple)
	HopF2a_PaciCMP2802	<i>aceris</i>	<i>Acer</i> sp. (maple)
	HopF2a_PpoiCMP8960	<i>porri</i>	<i>Allium porrum</i> (leek)
	HopF2a_PsyCC1583	<i>syringae</i>	Environmental, epilithon
	HopF2b_PmaM6 (8)	<i>maculicola</i>	Brassicaceae
	HopF2b_Pma4981	<i>maculicola</i>	Brassicaceae
	HopF2b_PmaH7608	<i>maculicola</i>	Brassicaceae
	HopF2b_PmalCMP11281	<i>maculicola</i>	<i>Brassica rapa</i>
	HopF2b_PmalCMP2744	<i>maculicola</i>	<i>Brassica nigra</i> (mustard)
	HopF2b_PmalCMP3935	<i>maculicola</i>	<i>Brassica oleracea</i> (cauliflower)
	HopF2b_Pla302278PT	<i>lachrymans</i>	<i>Cucumis sativus</i> (cucumber)
	HopF2b_Pla3988	<i>lachrymans</i>	<i>Cucumis sativus</i> (cucumber)
	HopF2b_PtoDC3000 (4)	<i>tomato</i>	<i>Lycopersicon esculentum</i> (tomato)
	HopF2b_PtoiCMP2844	<i>tomato</i>	<i>Lycopersicon esculentum</i> (tomato)
	HopF2b_Pma88_10	<i>maculicola</i>	Brassicaceae
	HopF2b_Pma90_32	<i>maculicola</i>	Brassicaceae
	HopF2b_Pap1089_5_2 (2)	<i>apii</i>	<i>Apium graveolens</i> (celery)
	HopF2b_PapiCMP1657_2	<i>apii</i>	<i>Apium graveolens</i> (celery)
	HopF2b_PtgiCMP6369	<i>tagetis</i>	<i>Tagetes erecta</i> (African marigold)
	HopF2c_PsyUSA007	<i>syringae</i>	Environmental, stream water
	HopF2c_PdplCMP529 (2)	<i>delphinii</i>	<i>Delphinium</i> sp. (delphinium)
	HopF2c_PdplCMP4330	<i>delphinii</i>	<i>Delphinium</i> sp. (delphinium)
	HopF2c_PdplCMP13052 (2)	<i>delphinii</i>	<i>Delphinium</i> sp. (delphinium)
	HopF2c_PdplCMP5014	<i>delphinii</i>	<i>Delphinium elatum</i> (candle larkspur)
	HopF2c_PaniCMP18807	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF2c_PsyCC1559	<i>syringae</i>	Environmental, snow
	HopF2c_PpeiCMP5786	<i>persicae</i>	<i>Prunus persica</i> (nectarine)
	HopF2c_PbbiCMP8899 (5)	<i>berberidis</i>	<i>Berberis gagnepainii</i> (barberry)
	HopF2c_PbbiCMP4116	<i>berberidis</i>	<i>Berberis</i> sp. (barberry)
	HopF2c_PbbiCMP4065	<i>berberidis</i>	<i>Berberis</i> sp. (barberry)
	HopF2c_PbbiCMP13291	<i>berberidis</i>	<i>Berberis</i> sp. (barberry)
	HopF2c_PpdiCMP8903	<i>philadelphii</i>	<i>Philadelphus coronarius</i> (mock orange)
	HopF2c_PapliCMP2814	<i>apii</i>	<i>Apium graveolens</i> (celery)
	HopF2c_PpriCMP2732 (3)	<i>primulae</i>	<i>Primula</i> × <i>polyantha</i> (polyanthus)
	HopF2c_PpriCMP8670	<i>primulae</i>	<i>Primula</i> × <i>polyantha</i> (polyanthus)
	HopF2c_PpriCMP18417	<i>primulae</i>	<i>Primula</i> × <i>polyantha</i> (polyanthus)
	HopF2c_PapliCMP1659 (3)	<i>apii</i>	<i>Apium graveolens</i> (celery)
	HopF2c_Pap1089_5_1	<i>apii</i>	<i>Apium graveolens</i> (celery)
	HopF2c_PapliCMP1657_1	<i>apii</i>	<i>Apium graveolens</i> (celery)
	HopF2c_Pmp19322	<i>morsprunorum</i>	<i>Prunus avium</i> (sweet cherry)
	HopF2c_Pmp302280PT	<i>morsprunorum</i>	<i>Prunus avium</i> (sweet cherry)
	HopF2c_PsyCC1544	<i>syringae</i>	Environmental, lake water
	HopF2c_PtoiCMP7230	<i>tomato</i>	<i>Lycopersicon esculentum</i> (tomato)
	HopF2c_PsplCMP16928 (2)	Unknown	Unknown
	HopF2c_PsplCMP16929	Unknown	Unknown
	HopF2c_PatliCMP2813 (6)	<i>antirrhini</i>	<i>Antirrhinum majus</i> (snapdragon)
	HopF2c_PatliCMP4303	<i>antirrhini</i>	<i>Antirrhinum majus</i> (snapdragon)
	HopF2c_PatliCMP4453	<i>antirrhini</i>	<i>Antirrhinum majus</i> (snapdragon)
	HopF2c_PatliCMP4959	<i>antirrhini</i>	<i>Antirrhinum majus</i> (snapdragon)
	HopF2c_PtgiCMP4092	<i>tagetis</i>	<i>Tagetes erecta</i> (African marigold)
	HopF2c_PtoiCMP2841	<i>tomato</i>	<i>Lycopersicon esculentum</i> (tomato)
	HopF2c_PmaM4a	<i>maculicola</i>	Brassicaceae
	HopF2c_PtoT1 (5)	<i>tomato</i>	<i>Lycopersicon esculentum</i> (tomato)

Table 1 Continued

Subfamily	Member	Pathovar	Plant host of isolation
HopF3	HopF2c_Pto1108	<i>tomato</i>	<i>Lycopersicon esculentum</i> (tomato)
	HopF2c_PtoICMP4263	<i>tomato</i>	<i>Lycopersicon esculentum</i> (tomato)
	HopF2c_PtoK40	<i>tomato</i>	<i>Lycopersicon esculentum</i> (tomato)
	HopF2c_PtoMax13	<i>tomato</i>	<i>Lycopersicon esculentum</i> (tomato)
	HopF3_PgylCMP867 (3)	<i>glycinea</i>	<i>Glycine max</i> (soybean)
	HopF3_PgylCMP4966	<i>glycinea</i>	<i>Glycine max</i> (soybean)
	HopF3_PgylCMP10449	<i>glycinea</i>	<i>Glycine max</i> (soybean)
	HopF3_PgylCMP807 (2)	<i>glycinea</i>	<i>Glycine max</i> (soybean)
	HopF3_PgylCMP9589	<i>glycinea</i>	<i>Glycine max</i> (soybean)
	HopF3_Pav631	<i>avellanae</i>	<i>Corylus avellana</i> (common hazel)
	HopF3_PphICMP5059 (3)	<i>phaseolicola</i>	<i>Vigna radiata</i> (mung bean)
	HopF3_PmaKN91	<i>maculicola</i>	Brassicaceae
	HopF3_PgylCMP4324	<i>glycinea</i>	<i>Glycine javanica</i> (perennial soybean)
	HopF3_PmplCMP4983	<i>morsprunorum</i>	<i>Prunus avium</i> (sweet cherry)
	HopF3_PmplCMP3897	<i>morsprunorum</i>	<i>Prunus domestica</i> (European plum)
	HopF3_PmylCMP7120	<i>myricae</i>	<i>Myrica rubra</i> (Chinese bayberry)
	HopF3_PdelCMP9150	<i>dendropanacis</i>	<i>Dendropanax trifidus</i> (tree ivy)
	HopF3_PdalCMP9757	<i>daphniphylli</i>	<i>Daphniphyllum teijsmannii</i> (evergreen shrub)
	HopF3_PrhlCMP9756	<i>raphiolepidis</i>	<i>Raphiolepis umbellata</i> (Japanese hawthorn)
	HopF3_PmelCMP6289	<i>meliae</i>	<i>Melia azedarach</i> (chinaberry)
	HopF3_PaelCMP8947	<i>aesculi</i>	<i>Aesculus indica</i> (horse chestnut)
	HopF3_PgylCMP2185	<i>glycinea</i>	<i>Glycine max</i> (soybean)
	HopF3_Pph1448A (11)	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF3_PphICMP2740	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF3_PphICMP637	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF3_PphK4	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF3_PphKN86	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF3_PphHB10Y	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF3_PphNPS3121	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF3_Pph1302A	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF3_PphY5_2	<i>phaseolicola</i>	<i>Phaseolus vulgaris</i> (green bean)
	HopF3_PgylCMP4332	<i>glycinea</i>	<i>Glycine javanica</i> (perennial soybean)
	HopF3_PmeN6801	<i>meliae</i>	<i>Nicotiana tabacum</i> (tobacco)
	HopF3_PcelCMP17524	<i>cerasicola</i>	<i>Prunus x yedoensis</i> (Yoshino cherry)
	HopF3_PcelCMP13928	<i>cerasicola</i>	<i>Prunus x yedoensis</i> (Yoshino cherry)
	HopF3_PmplCMP568	<i>morsprunorum</i>	<i>Prunus avium</i> (sweet cherry)
	HopF3_PcsiCMP9421	<i>castaneae</i>	<i>Castanea crenata</i> (Japanese chestnut)
	HopF3_PfrcCMP7711 (5)	<i>fraxini</i>	<i>Fraxinus excelsior</i> (European ash)
	HopF3_PfrcCMP7712	<i>fraxini</i>	<i>Fraxinus excelsior</i> (European ash)
	HopF3_PfrcCMP9129	<i>fraxini</i>	<i>Fraxinus excelsior</i> (European ash)
	HopF3_PfrcCMP9132	<i>fraxini</i>	<i>Fraxinus excelsior</i> (European ash)
	HopF3_PsvICMP4352	<i>savastanoi</i>	<i>Olea europaea</i> (olive)
HopF4	HopF3_PsvICMP13519	<i>savastanoi</i>	<i>Olea europaea</i> (olive)
	HopF4a_PcdICMP12341 (3)	<i>coriandricola</i>	<i>Coriandrum sativum</i> (coriander)
	HopF4a_PcdICMP12471	<i>coriandricola</i>	<i>Coriandrum sativum</i> (coriander)
	HopF4a_Pci0788_9_2	<i>cilantro</i>	<i>Coriandrum sativum</i> (coriander)
	HopF4a_Pci0788_9_1	<i>cilantro</i>	<i>Coriandrum sativum</i> (coriander)
	HopF4b_PmplCMP4983	<i>morsprunorum</i>	<i>Prunus avium</i> (sweet cherry)
	HopF4b_Pan302091 (13)	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP18801	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP18886	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP19068	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP19070	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP19071	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP19072	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP19079	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP19097	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP19102	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP19103	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)

Table 1 Continued

Subfamily	Member	Pathovar	Plant host of isolation
AvrRpm2	HopF4b_PanICMP19104	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PanICMP9617	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopF4b_PthNCPB2598 (2)	<i>theae</i>	<i>Camellia sinensis</i> (tea)
	HopF4b_Pae3681	<i>aesculi</i>	<i>Aesculus indica</i> (horse chestnut)
	HopF4b_Pae0893_23	<i>aesculi</i>	<i>Aesculus indica</i> (horse chestnut)
	HopF4b_Pae2250	<i>aesculi</i>	<i>Aesculus indica</i> (horse chestnut)
	AvrRpm2_PdalCMP9757	<i>daphniphylli</i>	<i>Daphniphyllum teijsmannii</i> (evergreen shrub)
	AvrRpm2_PmyICMP7118	<i>myricae</i>	<i>Myrica rubra</i> (Chinese bayberry)
	AvrRpm2_Psv3335_NCB1	<i>savastanoi</i>	<i>Olea europaea</i> (olive)
	HopBB1_PanICMP18708_1 (21)	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
HopBB1	HopBB1_PanICMP18744_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP18800_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP18801_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP18884_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP18886_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19070_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19079_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19097_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19101	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19104	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19439_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19455_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP9617_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP9853_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanNCPB3871	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanPA459_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanShaanxi_M228_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanShaanxi_M7_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanTP1_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanTP6_1_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19102	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PcbICMP2821	<i>cannabina</i>	<i>Cannabis sativa</i> (hemp/marijuana)
	HopBB1_PheICMP4531	<i>helianthi</i>	<i>Helianthus annuus</i> (sunflower)
	HopBB1_PpdiCMP8902	<i>philadelphii</i>	<i>Philadelphus coronarius</i> (mock orange)
	HopBB1_PviiCMP3963	<i>viburni</i>	<i>Viburnum</i> sp.
	HopBB1_PanICMP18708_2 (18)	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP18744_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP18800_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP18801_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP18884_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP18886_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19070_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19079_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19103	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19439_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19455_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP9617_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP9853_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanPA459_1	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanShaanxi_M228_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanShaanxi_M7_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanTP1_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanTP6_1_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PanICMP19068	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PculCMP11899	<i>cunninghamiae</i>	<i>Cunninghamia lanceolata</i> (Chinese fir)
	HopBB1_PppiCMP4986 (4)	<i>papulans</i>	<i>Malus</i> × <i>domestica</i> (apple)
	HopBB1_PppiCMP4040	<i>papulans</i>	<i>Malus</i> × <i>domestica</i> (apple)
	HopBB1_PppiCMP4048	<i>papulans</i>	<i>Malus</i> × <i>domestica</i> (apple)
	HopBB1_PppiCMP4050	<i>papulans</i>	<i>Malus</i> × <i>domestica</i> (apple)

Table 1 Continued

Subfamily	Member	Pathovar	Plant host of isolation
	HopBB1_Pan302091	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_PbrlCMP13684	<i>broussonetiae</i>	<i>Broussonetia kazinoki</i> (paper mulberry)
	HopBB1_PdelCMP9150	<i>dendropanacis</i>	<i>Dendropanax trifidus</i> (tree ivy)
	HopBB1_PseHC_1 (2)	<i>sesami</i>	<i>Sesamum indicum</i> (sesame)
	HopBB1_PbrlCMP13685	<i>broussonetiae</i>	<i>Broussonetia kazinoki</i> (paper mulberry)
	HopBB1_PcelCMP13927 (2)	<i>cerasicola</i>	<i>Prunus x yedoensis</i> (Yoshino cherry)
	HopBB1_PcelCMP13928	<i>cerasicola</i>	<i>Prunus x yedoensis</i> (Yoshino cherry)
	HopBB1_PcelCMP17524	<i>cerasicola</i>	<i>Prunus x yedoensis</i> (Yoshino cherry)
	HopBB1_PmpFTRS_U7	<i>morsprunorum</i>	<i>Prunus avium</i> (sweet cherry)
	HopBB1_Pmp302280PT (5)	<i>morsprunorum</i>	<i>Prunus avium</i> (sweet cherry)
	HopBB1_PmplCMP3897	<i>morsprunorum</i>	<i>Prunus domestica</i> (European plum)
	HopBB1_PmplCMP4983	<i>morsprunorum</i>	<i>Prunus avium</i> (sweet cherry)
	HopBB1_PmplCMP568	<i>morsprunorum</i>	<i>Prunus avium</i> (sweet cherry)
	HopBB1_PpelCMP5786	<i>persicae</i>	<i>Prunus persica</i> (nectarine)
	HopBB1_PmolCMP535 (3)	<i>mori</i>	<i>Morus</i> sp. (mulberry)
	HopBB1_PmolCMP4468	<i>mori</i>	<i>Morus alba</i> (white mulberry)
	HopBB1_PmolCMP6941	<i>mori</i>	<i>Morus alba</i> (white mulberry)
	HopBB1_PsplCMP16929	Unknown	Unknown
	HopBB1_PanlCMP19097_2	<i>actinidiae</i>	<i>Actinidia deliciosa</i> (kiwifruit)
	HopBB1_Pav631	<i>avellanae</i>	<i>Corylus avellana</i> (common hazel)
	HopBB1_Pmo301020_2 (5)	<i>mori</i>	<i>Morus</i> sp. (mulberry)
	HopBB1_Pmo301020_1	<i>mori</i>	<i>Morus</i> sp. (mulberry)
	HopBB1_PmolCMP4331	<i>mori</i>	<i>Morus alba</i> (white mulberry)
	HopBB1_PholCMP7840	<i>photinae</i>	<i>Photinia glabra</i> (Japanese photinia)
	HopBB1_PholCMP7847	<i>photinae</i>	<i>Photinia glabra</i> (Japanese photinia)
	HopBB1_PlalCMP826 (4)	<i>lachrymans</i>	<i>Cucumis sativus</i> (cucumber)
	HopBB1_PlalCMP3402	<i>lachrymans</i>	<i>Cucumis sativus</i> (cucumber)
	HopBB1_PlaYM7902	<i>lachrymans</i>	<i>Cucumis sativus</i> (cucumber)
	HopBB1_PlaYM8003	<i>lachrymans</i>	<i>Cucumis sativus</i> (cucumber)

The plant host of isolation from which the *Pseudomonas syringae* strain containing the HopF allele was obtained is shown. Members in bold were used for the phylogeny in Fig. 2. Identical HopF alleles are indented and listed below the corresponding bold member. The number of identical alleles including the bold member is shown in parentheses.

lettuce, tomato, pepper and *Arabidopsis* genotypes, and no visible reactions were observed (Wroblewski *et al.*, 2009). Although this may reflect limited HopF3 functions in these hosts, issues of poor transient expression cannot be ruled out.

HopF2 is the most genetically diverse of the HopF subfamilies with two distinct monophyletic lineages (HopF2b and HopF2c) and a third group consisting of a small number of basally branching strains (HopF2a). HopF2b contains the ADP-RT HopF2_{PtoDC3000}. A distinguishing feature of the HopF2c subgroups is the lack of a myristoylation sequence, a motif shared by all other HopF subfamilies, and which has been demonstrated to play an important role in HopF2_{PtoDC3000} function. The lack of a myristoylation sequence in members of the HopF2c subgroup suggests that there are HopF functions independent of membrane localization.

The HopF4 T3SEs form a clade that can be divided into the subgroups HopF4a and HopF4b – each of which has given rise to a chimeric T3SE subfamily. HopF4a members from *P. syringae* strains isolated from cilantro gave rise to AvrRpm2, whereas HopF4b members from *P. syringae* strains isolated from horse

chestnut gave rise to HopBB1. AvrRpm2 was formerly named HopF4 because of its evolutionary relationship to the HopF family (Rodriguez-Palenzuela *et al.*, 2010); however, as these alleles are chimeras with strong similarity to AvrRpm1 (~38% of AvrRpm2 similar to HopF and ~62% similar to AvrRpm1), they are more commonly referred to as AvrRpm2. AvrRpm1 is well characterized and elicits a strong ETI response in *Arabidopsis* via the phosphorylation of RIN4 (Mackey *et al.*, 2002). Coincidentally, AvrRpm1 is predicted to share a fold similar to the poly-ADP-RT polymerase (PARP) superfamily of enzymes (Cherkis *et al.*, 2012). AvrRpm2 contains the catalytic triad predicted for the AvrRpm1 family and, as such, the predicted enzymatic function of AvrRpm2 would be a PARP (Cherkis *et al.*, 2012). With HopF2_{PtoDC3000} and AvrRpm1 sharing similar enzymatic functions (i.e. transfer of ADP-ribose), and both families shown to target RIN4, it is perhaps functional commonalities that contributed to the creation of this chimeric group of T3SEs.

The HopBB1 group has expanded to over 24 unique members and contains the first ADP-RT catalytic residue Arg72, but no residue equivalent to Asp174 from the primary amino acid sequence.

Table 2 *Pseudomonas syringae* pathovars with multiple HopF members. Pathovars are listed alphabetically.

Strain	Effectors present	Count
Pan302091	HopF4b, HopBB1	2
PanICMP18708	HopBB1, HopBB1	2
PanICMP18744	HopBB1, HopBB1	2
PanICMP18800	HopBB1, HopBB1	2
PanICMP18801	HopF4b, HopBB1, HopBB1	3
PanICMP18807	HopF1, HopF2c	2
PanICMP18884	HopBB1, HopBB1	2
PanICMP18886	HopF4b, HopBB1, HopBB1	3
PanICMP19068	HopF4b, HopBB1	2
PanICMP19070	HopF4b, HopBB1, HopBB1	3
PanICMP19079	HopF4b, HopBB1, HopBB1	3
PanICMP19097	HopF4b, HopBB1, HopBB1	3
PanICMP19102	HopF4b, HopBB1	2
PanICMP19103	HopF4b, HopBB1	2
PanICMP19104	HopF4b, HopBB2	2
PanICMP19439	HopBB1, HopBB1	2
PanICMP19455	HopBB1, HopBB1	2
PanICMP9617	HopF4b, HopBB1, HopBB1	3
PanICMP9853	HopBB1, HopBB1	2
PanPA459	HopBB1, HopBB1	2
PanShaanxi:M228	HopBB1, HopBB1	2
PanShaanxi:M7	HopBB1, HopBB1	2
PanTP1	HopBB1, HopBB1	2
PanTP6	HopBB1, HopBB1	2
Pap1089	HopF2b, HopF2c	2
PapICMP1657	HopF2b, HopF2c	2
Pav631	HopF3, HopBB1	2
PcdICMP12341	HopF1, HopF4a	2
PcdICMP12471	HopF1, HopF4a	2
PcelCMP13928	HopF3, HopBB1	2
PcelCMP17524	HopF3, HopBB1	2
Pci0788	HopF1, HopF4a, HopF4a	3
PdalCMP9757	HopF3, AvrRpm2	2
PdelCMP9150	HopF1, HopF3, HopBB1	3
PgylCMP4324	HopF1, HopF3	2
PgylCMP4332	HopF1, HopF3	2
PgylCMP807	HopF1, HopF3	2
PhoICMP7840	HopF1, HopBB1	2
PhoICMP7847	HopF1, HopBB1	2
Pmo301020	HopBB1, HopBB1	2
Pmp302280PT	HopF2c, HopBB1	2
PmplCMP3897	HopF3, HopBB1	2
PmplCMP4983	HopF3, HopF4b, HopBB1	3
PmplCMP568	HopF3, HopBB1	2
PpelCMP5786	HopF2c, HopBB1	2
PphiCMP2740	HopF1, HopF3	2
PphiCMP637	HopF1, HopF3	2
PphK4	HopF1, HopF3	2
PphKN86	HopF1, HopF3	2
PphY5	HopF1, HopF3	2
PsplCMP16929	HopF2c, HopBB1	2
Pta11528	HopF1, HopF1	2
PtgiCMP6369	HopF1, HopF2b	2
PthNCPPB2598	HopF1, HopF4b	2

Interestingly, many pathovars that contain HopBB1 were isolated from woody plant hosts, which may reflect a host specialized role. However, the C-terminus of the HopBB1 group does not resemble

any T3SE family or known protein domain, making functional predictions difficult.

Overall, these chimeras represent excellent examples of terminal reassortment (Stavrinos *et al.*, 2006), but it remains to be determined what selective advantages have allowed the maintenance of these two families. As the N-terminus of T3SEs contains the myristoylation sequence and is also mainly responsible for secretion and T3SE chaperone binding, it is likely that this region confers advantages associated with SchF-mediated secretion and membrane localization.

PERSPECTIVE AND FUTURE DIRECTIONS

HopF is a highly diverse T3SE family distributed among numerous *P. syringae* strains that can infect a wide range of plant hosts, thereby providing an excellent opportunity to study the functional diversification of T3SEs. HopF2_{PtoDC3000} is the most extensively studied HopF family member and highlights interesting features that provide a framework for studies of this T3SE family.

HopF2_{PtoDC3000} is an ADP-RT as predicted from the crystal structure of HopF1_{Pph1449B} (Singer *et al.*, 2004; Wang *et al.*, 2010), and all non-chimeric HopF family members are predicted to be ADP-RTs based on the conservation of the catalytic residues required for this enzymatic activity. However, the observation that HopF2_{PtoDC3000} can inhibit stomatal immunity independent of its ADP-RT activity raises the possibility that it may represent a multi-functional T3SE (Hurley *et al.*, 2014). It will be interesting to determine whether HopF2_{PtoDC3000} suppresses stomatal immunity using a second unknown enzymatic activity, or whether this function is non-enzymatic. One possibility is that the ADP-RT activity is localized to the 'head' domain of HopF proteins, whereas the 'stalk' domain harbours a distinct function (Fig. 1B). This would be reminiscent of the multi-domain T3SE HopAB2_{PtoDC3000}. The N-terminal domain of HopAB2_{PtoDC3000} elicits ETI in resistance tomato cultivars, promotes ethylene biosynthesis and interacts with multiple RLKs to suppress plant immunity (Abramovitch and Martin, 2005; Shan *et al.*, 2008; Xiao *et al.*, 2007), whereas the C-terminal domain is an E3 ubiquitin ligase that suppresses ETI by targeting the tomato kinase Fen for degradation (Janjusevic *et al.*, 2006).

HopF2_{PtoDC3000} targets multiple immunity-related proteins, including MKK5 and RIN4 (Wang *et al.*, 2010; Wilton *et al.*, 2010). HopF2_{PtoDC3000} is able to suppress the ETI elicited by the unrelated T3SE AvrRpt2 in *Arabidopsis* by preventing the cleavage of RIN4 (Wilton *et al.*, 2010). As the ADP-RT catalytic residues of HopF2_{PtoDC3000} are required for this activity, it is likely that ADP-ribosylation of RIN4 interferes with AvrRpt2-mediated RIN4 cleavage (Wilton *et al.*, 2010). Interestingly, HopF1_{Pph1449B} can also suppress the RIN4-dependent immunity induced by AvrB1 in common bean, suggestive of a similar mode of action (Hou *et al.*, 2011). Identification of the HopF ADP-ribosylation sites on RIN4

will be critical to understanding HopF-mediated virulence functions, including ETI suppression. Will these sites overlap with AvrRpt2 cleavage sites and how will they influence the ability of RIN4 to modulate plant immunity?

Finally, although the HopF family is widely distributed among *P. syringae* and contains over 200 members, its representation amongst other phytopathogens is unknown. However, predicted T3SEs with limited sequence similarity to HopF are present in *Erwinia mallotivora*, *Ralstonia solanacearum* and *Acidovorax citrulli*, which suggests that the HopF family contributes to pathogenesis outside of *P. syringae* (GenBank accession: CP000512.1; Redzuan *et al.*, 2014; Salanoubat *et al.*, 2002). Interestingly, these T3SEs share homologous ADP-RT catalytic residues R72 and D174, which may indicate a common enzymatic function (Fig. 1C). A glycine residue at position 2, which is predictive of a myristoylation site, is conserved in non-*syringae* homologues, suggestive of membrane-localized functions (Fig. 1C).

There are still many important questions to be addressed for the HopF family. For example, what NLRs and associated immune sensors are used to recognize the HopF family (Khan *et al.*, 2015)? Although the bean *R1* resistance gene recognizes HopF1, it has yet to be cloned and characterized (Jackson *et al.*, 1999; Tsiamis *et al.*, 2000). Is there an *Arabidopsis* ETI response against any member of the HopF family? How many host targets does the HopF family have, and are these targets conserved across different hosts? How has host target diversification contributed to HopF functional diversification, and do different subgroups target different host proteins?

Moreover, many *P. syringae* pathovars contain more than one HopF family member in their effector repertoire (Table 2). The combination of HopF family members that exists in a given pathovar also varies, making it hard to determine which subgroups may have redundant functions. This may suggest that, although a common enzymatic function links the entire family, each subgroup may target different types of plant host protein to suppress plant immunity, thus giving a selective pressure to maintain more than one effector from a single T3SE family. Addressing these questions will be crucial to understanding the functional diversification of T3SE families.

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REFERENCES

- Abramovitch, R.B. and Martin, G.B. (2005) AvrPtoB: a bacterial type III effector that both elicits and suppresses programmed cell death associated with plant immunity. *FEMS Microbiol. Lett.* **245**, 1–8.
- Arnold, D.L., Lovell, H.C., Jackson, R.W. and Mansfield, J.W. (2011) *Pseudomonas syringae* pv. *phaseolicola*: from 'has bean' to supermodel. *Mol. Plant Pathol.* **12**, 617–627.
- Asai, T., Tena, G., Plotnikova, J., Willmann, M.R., Chiu, W.L., Gomez-Gomez, L., Boller, T., Ausubel, F.M., and Sheen, J. (2002) MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature*, **415**, 977–983.
- Axtell, M.J. and Staskawicz, B.J. (2003) Initiation of RPS2-specified disease resistance in *Arabidopsis* is coupled to the AvrRpt2-directed elimination of RIN4. *Cell*, **112**, 369–377.
- Cherkis, K.A., Temple, B.R., Chung, E.H., Sondek, J. and Dangl, J.L. (2012) AvrRpm1 missense mutations weakly activate RPS2-mediated immune response in *Arabidopsis thaliana*. *PLoS One*, **7**, e42633.
- Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J.D., Felix, G., and Boller, T. (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature*, **448**, 497–500.
- Chisholm, S.T., Dahlbeck, D., Krishnamurthy, N., Day, B., Sjolander, K. and Staskawicz, B.J. (2005) Molecular characterization of proteolytic cleavage sites of the *Pseudomonas syringae* effector AvrRpt2. *Proc. Natl. Acad. Sci. USA*, **102**, 2087–2092.
- Dangl, J.L. and Jones, J.D. (2001) Plant pathogens and integrated defence responses to infection. *Nature*, **411**, 826–833.
- Day, B., Dahlbeck, D., Huang, J., Chisholm, S.T., Li, D. and Staskawicz, B.J. (2005) Molecular basis for the RIN4 negative regulation of RPS2 disease resistance. *Plant Cell*, **17**, 1292–1305.
- Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., Somssich I., Genin S., and Marco Y. (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proc. Natl. Acad. Sci. USA*, **100**, 8024–8029.
- Dodds, P.N. and Rathjen, J.P. (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. *Nat. Rev. Genet.* **11**, 539–548.
- Guo, M., Tian, F., Wamboldt, Y. and Alfano, J.R. (2009) The majority of the type III effector inventory of *Pseudomonas syringae* pv. *tomato* DC3000 can suppress plant immunity. *Mol. Plant–Microbe Interact.* **22**, 1069–1080.
- Heath, M.C. (2000) Hypersensitive response-related death. *Plant Mol. Biol.* **44**, 321–334.
- Heese, A., Hann, D.R., Gimenez-Ibanez, S., Jones, A.M., He, K., Li, J., Schroeder, J.I., Peck, S.C., and Rathjen, J.P. (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proc. Natl. Acad. Sci. USA*, **104**, 12 217–12 222.
- Hou, S., Mu, R., Ma, G., Xu, X., Zhang, C., Yang, Y., and Wu, D. (2011) *Pseudomonas syringae* pv. *phaseolicola* effector HopF1 inhibits pathogen-associated molecular pattern-triggered immunity in a RIN4-independent manner in common bean (*Phaseolus vulgaris*). *FEMS Microbiol. Lett.* **323**, 35–43.
- Hurley, B., Lee, D., Mott, A., Wilton, M., Liu, J., Liu, Y.C., Angers, S., Coaker, G., Guttman, D.S., and Desveaux, D. (2014) The *Pseudomonas syringae* type III effector HopF2 suppresses *Arabidopsis* stomatal immunity. *PLoS One*, **9**, e114921.
- Jackson, R.W., Athanassopoulos, E., Tsiamis, G., Mansfield, J.W., Sesma, A., Arnold, D.L., Gibbon, M.J., Murillo, J., Taylor, J.D., and Vivian, A. (1999) Identification of a pathogenicity island, which contains genes for virulence and avirulence, on a large native plasmid in the bean pathogen *Pseudomonas syringae* pathovar *phaseolicola*. *Proc. Natl. Acad. Sci. USA*, **96**, 10 875–10 880.
- Janjusevic, R., Abramovitch, R.B., Martin, G.B. and Stebbins, C.E. (2006) A bacterial inhibitor of host programmed cell death defenses is an E3 ubiquitin ligase. *Science*, **311**, 222–226.
- Jiang, S., Yao, J., Ma, K.W., Zhou, H., Song, J., He, S.Y., and Ma, W. (2013) Bacterial effector activates jasmonate signaling by directly targeting JAZ transcriptional repressors. *PLoS Pathog.* **9**, e1003715.
- Khan, M., Subramaniam, R. and Desveaux, D. (2015) Of guards, decoys, baits and traps: pathogen perception in plants by type III effector sensors. *Curr. Opin. Microbiol.* **29**, 49–55.
- Lee, A.H., Hurley, B., Felsensteiner, C., Yea, C., Kukurshumova, W., Bartetzko, V., Wang, P.W., Quach, V., Lewis, J.D., Liu, Y.C., Bornke, F., Angers, S., Wilde, A., Guttman, D.S., and Desveaux, D. (2012) A bacterial acetyltransferase destroys plant microtubule networks and blocks secretion. *PLoS Pathog.* **8**, e1002523.
- Lee, J., Manning, A.J., Wolfgeher, D., Jelenska, J., Cavanaugh, K.A., Xu, H., Fernandez, S.M., Micheltore, R.W., Kron, S.J., Greenberg, J.T. (2015)

- Acetylation of an NB-LRR plant immune-effector complex suppresses immunity. *Cell Rep.* **13**, 1670–1682.
- Lewis, J.D., Wu, R., Guttman, D.S. and Desveaux, D. (2010) Allele-specific virulence attenuation of the *Pseudomonas syringae* HopZ1a type III effector via the Arabidopsis ZAR1 resistance protein. *PLoS Genet.* **6**, e1000894.
- Lewis, J.D., Lee, A., Ma, W., Zhou, H., Guttman, D.S. and Desveaux, D. (2011) The YopJ superfamily in plant-associated bacteria. *Mol. Plant. Pathol.* **12**, 928–937.
- Lewis, J.D., Lee, A.H., Hassan, J.A., Wan, J., Hurley, B., Jhingree, J.R., Wang, P.W., Lo, T., Youn, J.Y., Guttman, D.S., and Desveaux, D. (2013) The Arabidopsis ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the *Pseudomonas syringae* type III effector HopZ1a. *Proc. Natl. Acad. Sci. USA*, **110**, 18 722–18 727.
- Ma, W., Dong, F.F., Stavrinides, J. and Guttman, D.S. (2006) Type III effector diversification via both pathoadaptation and horizontal transfer in response to a coevolutionary arms race. *PLoS Genet.* **2**, e209.
- Mackey, D., Holt, B.F., III, Wiig, A. and Dangl, J.L. (2002) RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. *Cell*, **108**, 743–754.
- Mackey, D., Belkhadir, Y., Alonso, J.M., Ecker, J.R. and Dangl, J.L. (2003) Arabidopsis RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. *Cell*, **112**, 379–389.
- Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., Machado, M.A., Toth, I., Salmond, G., and Foster, G.D. (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol. Plant Pathol.* **13**, 614–629.
- McCann, H.C. and Guttman, D.S. (2008) Evolution of the type III secretion system and its effectors in plant–microbe interactions. *New Phytol.* **177**, 33–47.
- O'Brien, H.E., Thakur, S. and Guttman, D.S. (2011) Evolution of plant pathogenesis in *Pseudomonas syringae*: a genomics perspective. *Annu. Rev. Phytopathol.* **49**, 269–289.
- Postel, S., Kufner, I., Beuter, C., Mazzotta, S., Schwedt, A., Borlotti, A., Halter, T., Kemmerling, B., and Nurnberger, T. (2010) The multifunctional leucine-rich repeat receptor kinase BAK1 is implicated in Arabidopsis development and immunity. *Eur. J. Cell. Biol.* **89**, 169–174.
- Redzuan, R.A., Abu Bakar, N., Rozano, L., Badrun, R., Mat Amin, N. and Mohd Raih, M.F. (2014) Draft genome sequence of *Erwinia mallotivora* BT-MARDI, causative agent of papaya dieback disease. *Genome Announc.* **2**, e00375.
- Robert-Seilaniantz, A., Shan, L., Zhou, J.M. and Tang, X. (2006) The *Pseudomonas syringae* pv. *tomato* DC3000 type III effector HopF2 has a putative myristoylation site required for its avirulence and virulence functions. *Mol. Plant–Microbe Interact.* **19**, 130–138.
- Rodriguez-Palenzuela, P., Matas, I.M., Murillo, J., Lopez-Solanilla, E., Bardaji, L., Perez-Martinez, I., Rodriguez-Mosquera, M.E., Penyalver, R., Lopez, M.M., Quesada, J.M., Biehl, B.S., Perna, N.T., Glasner, J.D., Cabot, E.L., Neeno-Eckwall, E., and Ramos, C. (2010) Annotation and overview of the *Pseudomonas savastanoi* pv. *savastanoi* NCPPB 3335 draft genome reveals the virulence gene complement of a tumour-inducing pathogen of woody hosts. *Environ. Microbiol.* **12**, 1604–1620.
- Roux, M., Schwessinger, B., Albrecht, C., Chinchilla, D., Jones, A., Holton, N., Malinovsky, F.G., Tor, M., de Vries, S., and Zipfel, C. (2011) The Arabidopsis leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. *Plant Cell*, **23**, 2440–2455.
- Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottier, P., Camus, J.C., Cattolico, L., Chandler, M., Choise, N., Claudel-Renard, C., Cunac, S., Demange, N., Gaspin, C., Lavie, M., Moisan, A., Robert, C., Saurin, W., Schiex, T., Siguier, P., Thebault, P., Whalen, M., Wincker, P., Levy, M., Weissenbach, J., and Boucher C.A. (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature*, **415**, 497–502.
- Sarris, P.F., Gao, S., Karademiris, K., Jin, H., Kalantidis, K. and Panopoulos, N.J. (2011) Phyto bacterial type III effectors HopX1, HopAB1 and HopF2 enhance sense-post-transcriptional gene silencing independently of plant R gene-effector recognition. *Mol. Plant–Microbe Interact.* **24**, 907–917.
- Shan, L., Oh, H.S., Chen, J., Guo, M., Zhou, J., Alfano, J.R., Collmer, A., Jia, X., Tang, X. (2004) The HopPtoF locus of *Pseudomonas syringae* pv. *tomato* DC3000 encodes a type III chaperone and a cognate effector. *Mol. Plant–Microbe Interact.* **17**, 447–455.
- Shan, L., He, P., Li, J., Heese, A., Peck, S.C., Nurnberger, T., Martin, G.B., and Sheen, J. (2008) Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. *Cell Host Microbe*, **4**, 17–27.
- Singer, A.U., Desveaux, D., Betts, L., Chang, J.H., Nimchuk, Z., Grant, S.R., Dangl, J.L., and Soudak, J. (2004) Crystal structures of the type III effector protein AvrPphF and its chaperone reveal residues required for plant pathogenesis. *Structure*, **12**, 1669–1681.
- Stavrinides, J., Ma, W. and Guttman, D.S. (2006) Terminal reassortment drives the quantum evolution of type III effectors in bacterial pathogens. *PLoS Pathog.* **2**, e104.
- Tsiamis, G., Mansfield, J.W., Hockenhull, R., Jackson, R.W., Sesma, A., Athanassopoulos, E., Bennett, M.A., Stevens, C., Vivian, A., Taylor, J.D., Murillo, J. (2000) Cultivar-specific avirulence and virulence functions assigned to avrPphF in *Pseudomonas syringae* pv. *phaseolicola*, the cause of bean halo-blight disease. *EMBO J.* **19**, 3204–3214.
- Üstün, S., König, P., Guttman, D.S. and Börnke, F. (2014) HopZ4 from *Pseudomonas syringae*, a member of the HopZ Type III effector family from the YopJ superfamily, inhibits the proteasome in plants. *Mol. Plant–Microbe Interact.* **27**, 13.
- Wang, Y., Li, J., Hou, S., Wang, X., Li, Y., Ren, D., Chen, S., Tang, X., and Zhou, J.M. (2010) A *Pseudomonas syringae* ADP-ribosyltransferase inhibits Arabidopsis mitogen-activated protein kinase kinases. *Plant Cell*, **22**, 2033–2044.
- Wilton, M., Subramaniam, R., Elmore, J., Felsensteiner, C., Coaker, G. and Desveaux, D. (2010) The type III effector HopF2Pto targets Arabidopsis RIN4 protein to promote *Pseudomonas syringae* virulence. *Proc. Natl. Acad. Sci. USA*, **107**, 2349–2354.
- Wroblewski, T., Caldwell, K.S., Piskurewicz, U., Cavanaugh, K.A., Xu, H., Kozik, A., Ochoa, O., McHale, L.K., Lahre, K., Jelenska, J., Castillo, J.A., Blumenthal, D., Vinatzer, B.A., Greenberg, J.T., and Michelmore, R.W. (2009) Comparative large-scale analysis of interactions between several crop species and the effector repertoires from multiple pathovars of *Pseudomonas* and *Ralstonia*. *Plant Physiol.* **150**, 1733–1749.
- Wu, S., Lu, D., Kabbage, M., Wei, H.L., Swingle, B., Records, A.R., Dickman, M., He, P., and Shan, L. (2011) Bacterial effector HopF2 suppresses Arabidopsis innate immunity at the plasma membrane. *Mol. Plant–Microbe Interact.* **24**, 585–593.
- Xiao, F., He, P., Abramovitch, R.B., Dawson, J.E., Nicholson, L.K., Sheen, J., and Martin, G.B. (2007) The N-terminal region of *Pseudomonas* type III effector AvrPtoB elicits Pto-dependent immunity and has two distinct virulence determinants. *Plant J.* **52**, 595–614.
- Zhou, J., Wu, S., Chen, X., Liu, C., Sheen, J., Shan, L., and He, P. (2014) The *Pseudomonas syringae* effector HopF2 suppresses Arabidopsis immunity by targeting BAK1. *Plant J.* **77**, 235–245.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supporting Information 1 HopF nucleotide sequences in FASTA format.

Supporting Information 2 HopF amino acid sequences in FASTA format.

Supporting Information 3 HopF sequences from *Erwinia mal-lotivora*, *Acidovorax citrulli* and *Ralstonia solanacearum*.