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REVIEW ARTICLE

Behind the lines–actions of bacterial type III effector proteins in plant cells

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One sentence summary: Translocated type III effector proteins from Gram-negative plant-pathogenic bacteria promote bacterial virulence by interfering with defense responses, signaling pathways, protein degradation, gene expression or the formation of the cytoskeleton.

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ABSTRACT

Pathogenicity of most Gram-negative plant-pathogenic bacteria depends on the type III secretion (T3S) system, which translocates bacterial effector proteins into plant cells. Type III effectors modulate plant cellular pathways to the benefit of the pathogen and promote bacterial multiplication. One major virulence function of type III effectors is the suppression of plant innate immunity, which is triggered upon recognition of pathogen-derived molecular patterns by plant receptor proteins. Type III effectors also interfere with additional plant cellular processes including proteasome-dependent protein degradation, phytohormone signaling, the formation of the cytoskeleton, vesicle transport and gene expression. This review summarizes our current knowledge on the molecular functions of type III effector proteins with known plant target molecules. Furthermore, plant defense strategies for the detection of effector protein activities or effector-triggered alterations in plant targets are discussed.

Keywords: type III effector; plant immunity; MAPK signaling; proteasome; cytoskeleton; phytohormones

INTRODUCTION

Gram-negative plant-pathogenic bacteria cause a variety of diseases in economically important crop plants and thus often lead to major yield losses. The bacterial ability to infect plants and to multiply inside the plant tissue depends on secreted proteins such as adhesins, toxins and degradative enzymes. Furthermore, bacterial pathogens often inject effector proteins directly into plant cells. Bacterial virulence factors are delivered by specialized protein secretion systems, which are grouped into at least six different classes, designated type I to type VI (Hayes, Aoki and Low 2010; Costa *et al.* 2015). Virulence-associated proteins can also be secreted via outer membrane vesicles, which allow the transport of large quantities of proteins from the bacterial periplasm to the extracellular milieu (Ellis and Kuehn 2010; Bonnington and Kuehn 2013).

Most Gram-negative plant-pathogenic bacteria depend on a type III secretion (T3S) system to conquer their host plants and to multiply inside the plant tissue. T3S systems translocate bacterial effector proteins directly into eukaryotic cells and thus allow the manipulation of host cellular pathways to the benefit of the pathogen (He, Nomura and Whittam 2004; Dean 2011; Lee *et al.* 2013). T3S systems are present in plant- and animal-pathogenic bacteria as well as in several non-pathogenic bacteria and species (spp.) of the symbiotic bacterium *Rhizobium*, suggesting that T3S is not exclusively linked to pathogenicity (Troisfontaines and Cornelis 2005; Tampakaki 2014). According to phylogenetic analyses, T3S systems from plant-pathogenic bacteria have been grouped into different families including Hrp1 (hypersensitive response and pathogenicity 1; in spp. of *Pseudomonas syringae* and *Erwinia*) and Hrp2 T3S systems (in *Ralstonia solanacearum*, spp. of *Xanthomonas*, *Acidovorax* and

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Burkholderia) (Alfano and Collmer 1997; Troisfontaines and Cornelis 2005). In addition to the Hrp1 T3S system, some *P. syringae* strains also contain a rhizobial-like T3S system, designated Hrp3 (Gazi *et al.* 2012). Interestingly, gene clusters with homologies to T3S gene clusters from plant-pathogenic bacteria are also present in several animal-pathogenic bacteria including *Vibrio parahaemolyticus* strains isolated from patients, a clinical strain of *Pantoea*, and *Burkholderia pseudomallei* (Troisfontaines and Cornelis 2005; Kirzinger, Butz and Stavrinides 2015). *Pantoea* and *Burkholderia* spp. are cross-kingdom pathogens, which infect humans and plants (Kirzinger, Nadarasah and Stavrinides 2011). Several plant-pathogenic bacteria including *Xanthomonas albilineans* and *X. axonopodis* pv. *phaseoli* also contain a SPI-1 (*Salmonella* pathogenicity island 1) T3S gene cluster, which is usually present in animal-pathogenic bacteria (Alavi *et al.* 2008; Pieretti *et al.* 2015).

T3S systems are complex membrane-spanning nanomachines and contain an extracellular pilus-like appendage, which provides a transport channel for secreted proteins to the plant–pathogen interface (Jin and He 2001; Li *et al.* 2002; Blocker *et al.* 2008; Büttner 2012). The translocation of effector proteins into the plant cell is mediated by a bacterial T3S translocon, which presumably inserts as a pore-forming complex into the plant plasma membrane (Mattei *et al.* 2011; Guignot and Tran Van Nhieu 2016). According to the final destination of T3S substrates, protein transport into the extracellular milieu is hereafter referred to as 'secretion', and transport into the eukaryotic cell cytosol as 'translocation'. Secreted substrates of the T3S system mostly include extracellular components of the secretion apparatus such as T3S pilus and translocon proteins, whereas effectors are directly translocated into eukaryotic cells. Type III-dependent secretion and translocation depends on a specific export signal, which is often located in the N-terminal region of T3S substrates. T3S signals are not conserved on the amino acid level but are often associated with specific amino acid patterns or compositions and are structurally disordered. The lack of tertiary structures in the T3S signal might facilitate the binding of interaction partners such as components of the T3S system, which are involved in the recognition of secreted proteins (Arnold *et al.* 2009; Löwer and Schneider 2009; Samudrala, Heffron, McDermott 2009; Buchko *et al.* 2010; Schechter *et al.* 2012; Wang *et al.* 2013). In addition to the T3S signal, the efficient targeting of many secreted proteins to the T3S system also depends on cytoplasmic T3S chaperones, which bind to and often stabilize T3S substrates and presumably promote the recognition of secreted proteins by the secretion apparatus (e.g. Menard *et al.* 1994; Frithz-Lindsten *et al.* 1995; Tucker and Galan 2000; Gaudriault, Paulin and Barny 2002; Feldman and Cornelis 2003).

Given the essential contribution of pilus and translocon proteins to effector protein translocation, T3S is presumably a hierarchical process, suggesting that the T3S substrate specificity switches from the secretion of extracellular components of the secretion apparatus to effector proteins (e.g. Magdalena *et al.* 2002; Edqvist *et al.* 2003; Journet *et al.* 2003; Lara-Tejero *et al.* 2011). Furthermore, it is assumed that different effector proteins are secreted at different timepoints after the assembly of the T3S system. While experimental evidence for a secretion hierarchy of effector proteins in plant-pathogenic bacteria is still missing, differences in the timing of translocation were reported for effector proteins with antagonistic activities from animal pathogens (Enninga *et al.* 2005; Schlumberger *et al.* 2005; Mills *et al.* 2008; Van Engelenburg and Palmer 2008; Winnen *et al.* 2008). A hierarchy in effector protein translocation might help to avoid possible functional interferences between different effectors and could also prevent the clogging of the T3S channel by the simultaneous transport of multiple effector molecules.

Plant-pathogenic bacteria usually possess a large pool of different effector proteins. Genome sequence analyses of *P. syringae* strains revealed a meta-repertoire of 94 effector families with variable numbers of nine up to 39 effectors in individual strains (Baltrus *et al.* 2011; Lindeberg, Cunnac and Collmer 2012). *R. solanacearum* strains contain 60 to 75 effectors, which belong to 57 families including 32 core effectors, which are present in most of the strains (Peeters *et al.* 2013; Deslandes and Genin 2014). In *Xanthomonas* spp., the core effector set is limited to 3 out of 32 known effectors as was recently revealed by comparative genome sequence analysis (Roux *et al.* 2015). In several bacteria, deletion of single effector genes often has little influence on virulence, suggesting that effectors share redundant functions. The generation of multi-effector mutant strains in *P. syringae* revealed that the deletion of 18 effector genes from six genomic clusters is required to impair the *in planta* bacterial growth (Kvitko *et al.* 2009). A minimal set of eight effectors promotes bacterial virulence and suppresses plant defense responses (Cunnac *et al.* 2011; Wei *et al.* 2015).

Plants usually defend themselves against microbial attacks by two levels of defense responses, which are referred to as PAMP (pathogen-associated molecular pattern)-triggered immunity (PTI) and effector-triggered immunity (ETI). PTI is one of the first defense responses and is activated upon recognition of PAMPs by plant pattern recognition receptors (PRRs) (Zipfel 2014). PAMPs are conserved microbial molecules such as flagellin, bacterial elongation factor (EF)-Tu, peptidoglycan, chitin or cell-wall-derived molecules, which are essential for pathogen survival or fitness. PTI responses can be overcome by the actions of translocated type III effector proteins, which interfere with PTI responses and thus promote bacterial virulence. This so-called effector-triggered susceptibility is counteracted by a second line of plant defense responses, designated ETI, which is activated by the products of plant resistance (*R*) genes upon detection of individual effector proteins (Wu *et al.* 2014; Cui, Tsuda and Parker 2015) (Fig. 1A). Plant *R* genes often encode NB (nucleotide binding, also termed NB ARC [nucleotide-binding adaptor shared by Apaf1])-LRR (leucine-rich repeat) receptors (NLRs; see below) (Wu *et al.* 2014; Cui, Tsuda and Parker 2015). Plant PTI and ETI responses are discussed in more detail below.

In the past years, significant progress has been made in the functional characterization of effector proteins from plant-pathogenic bacteria. The results of numerous studies revealed that type III effectors interfere with multiple plant cellular pathways (Fig. 1B). The aim of this review is to summarize the known molecular functions of type III effectors from plant-pathogenic bacteria and their influence on plant target molecules. The interference of type III effectors with PTI responses, signal transduction pathways, proteasome-dependent protein degradation, phytohormone signaling, plant gene expression and the plant cytoskeleton is discussed in six separate sections. The last section describes known mechanisms underlying effector-mediated activation of plant defense responses. All sections are focused on interactions between type III effectors and plant targets, which have been confirmed by several independent experimental approaches. Predicted functions of type III effectors and known interaction partners are also summarized in Table 1. All abbreviations are listed in Table S1 (Supporting Information). For the description of general virulence functions of type III effectors, the reader is referred to excellent recent review articles (Dean 2011; Canonne and Rivas 2012; Deslandes and Rivas 2012; Feng and Zhou 2012; Lindeberg, Cunnac and

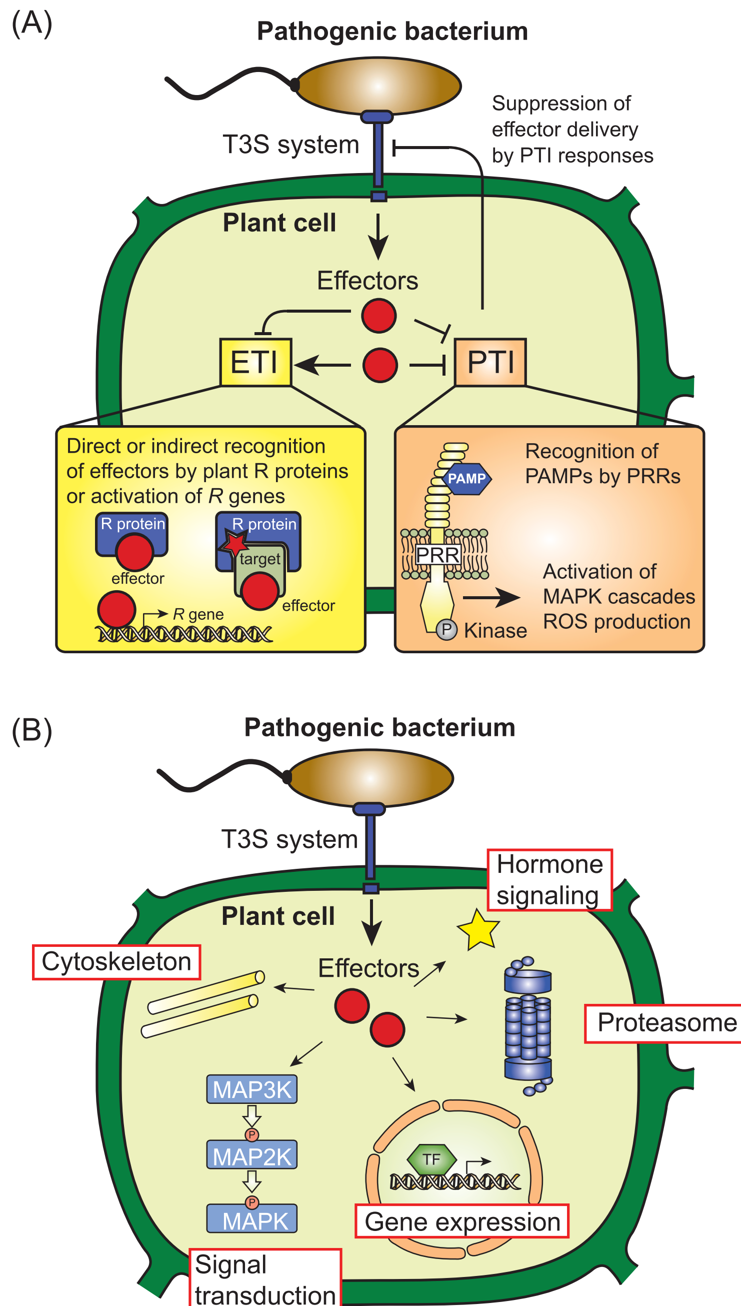

Figure 1. Interference of type III effector proteins with plant cellular pathways. **(A)** Type III effectors elicit and suppress plant defense reponses. Plant-pathogenic bacteria translocate effector proteins via the T3S system into plant cells. In resistant plants, individual effectors are directly or indirectly recognized by corresponding plant R proteins or activate plant R genes, and elicit defense responses, which are designated ETI. The indirect recognition of effector proteins by plant R proteins depends on effector-mediated modifications of plant target proteins, which are sensed by matching R proteins. Several type III effectors suppress ETI and/or PTI responses and thus promote bacterial virulence. PTI responses are triggered upon recognition of bacterial PAMPs by plant PRRs (see the text for details), and do not only interfere with pathogen survival but can also affect T3S-mediated delivery of effector proteins. **(B)** Overview on known plant targets of type III effectors. Translocated effector proteins interfere with the assembly of the cytoskeleton, MAPK cascades, gene expression, proteasome-dependent protein degradation or hormone signaling pathways (see the text for details).

Collmer 2012; Lee *et al.* 2013; Kazan and Lyons 2014; Macho and Zipfel 2015).

INTERFERENCE OF TYPE III EFFECTORS WITH BASAL PLANT DEFENSE RESPONSES

PTI responses include the production of reactive oxygen species (ROS), callose deposition in the plant cell wall, stomatal closure and the activation of defense-related genes, and interfere with the survival and multiplication of non-adapted microbial invaders (Wu, Shan and He 2014b; Bigeard, Colcombet and Hirt 2015). PTI can also lead to a reduction in type III-dependent effector protein translocation, suggesting that plants actively interfere with the expression of T3S genes and/or T3S-dependent protein delivery (Crabill *et al.* 2010; Oh, Park and Collmer 2010; Anderson *et al.* 2014).

Table 1. Targets and biochemical functions of type III effector proteins.

Effector[a]	Known or predicted biochemical function	Plant targets (organism)[b]	Effector-triggered modifications and/or functions of plant targets[c]	References
Pseudomonas syringae				
AvrB	n.d.[d]	RAR1 (*Arabidopsis*)	RAR1 mediates the interaction of AvrB with Hsp90	Cui *et al.* (2010)
		RIN4 (tomato, *Arabidopsis*)	Phosphorylation of RIN4 in NOI domains and activation of the R protein RPM1; induction of AHA1-mediated stomatal opening; increased interaction between COI1 and JAZ9	Mackey *et al.* (2002); Liu *et al.* (2009); Cui *et al.* (2010); Zhou *et al.* (2015)
		MPK4 (*Arabidopsis*)	Phosphorylation and activation of MPK4; the interaction with AvrB is increased in the presence of RAR1	Cui *et al.* (2010)
		RIPK (*Arabidopsis*)	Disruption of the RIPK-RIN4 complex; phosphorylation of RIN4; phosphorylation of AvrB by RIPK	Liu *et al.* (2011)
AvrPphB (HopAR1)	Cysteine protease	PBS1, PBS1-like kinases, RIPK (*Arabidopsis*)	Cleavage of PBS1 and PBS1-like proteins including RIPK; activation of the R protein RPS5, which guards PBS1	Shao *et al.* (2003); Zhang *et al.* (2010); Russell, Ashfield and Innes (2015)
		BIK1 (*Arabidopsis*)	Cleavage of BIK1; interference with PTI	Zhang *et al.* (2010)
AvrPto	n.d.	Pto (tomato)	Phosphorylation of Pto; activation of Prf-Pto-mediated ETI responses	Scofield *et al.* (1996); Tang *et al.* (1996); Kim, Lin and Martin (2002); Ntoukakis *et al.* (2013)
		FLS2 kinase domain (*Arabidopsis*, tomato)	Inhibition of autophosphorylation of FLS2; suppression of BIK1 phosphorylation	Xiang *et al.* (2008, 2011)
		EFR kinase domain (*Arabidopsis*)	Inhibition of autophosphorylation of EFR	Xiang *et al.* (2008)
		BAK1 kinase domain (*Arabidopsis*)	Disruption of FLS2-BAK1 complex[e]	Shan *et al.* (2008); Xiang *et al.* (2008); Zhou *et al.* (2014)
		RIN4 (tomato)	Degradation of RIN4 (dependent on Pto, Prf and the proteasome)	Luo *et al.* (2009)
		Kinases At2g23200, BSK1, BSK3, CDG1 (*Arabidopsis*)	n.d.	Xiang *et al.* (2008, 2011)
AvrPtoB (HopAB1)	E3 ubiquitin ligase	EFR (*Arabidopsis*)	Ubiquitination of EFR	Göhre *et al.* (2008)
		Kinase Bti9 (tomato)	Suppression of Bti9 kinase activity	Zeng *et al.* (2012)
		CERK1 (*Arabidopsis*)	Degradation of CERK1	Gimenez-Ibanez *et al.* (2009)
		Pto (tomato)	Phosphorylation of Pto and AvrPtoB; activation of Prf-Pto-mediated ETI responses	Kim, Lin and Martin (2002); Ntoukakis *et al.* (2009)
		Kinase Fen (tomato)	Degradation of Fen; suppression of Fen-triggered ETI	Rosebrock *et al.* (2007)
		FLS2 (*Arabidopsis*)	Dissociation of FLS2-BAK1 complex; degradation of FLS2	Göhre *et al.* (2008); Shan *et al.* (2008)
		BAK1 (*Arabidopsis*)	Dissociation of FLS2-BAK1 complex; inhibition of BAK1 kinase activity	Göhre *et al.* (2008); Shan *et al.* (2008); Cheng *et al.* (2011)
		RIN4 (tomato)	Degradation of RIN4 (dependent on Pto and Prf)	Luo *et al.* (2009)
AvrRpm1	n.d.	RIN4 (*Arabidopsis*)	Phosphorylation of RIN4 in NOI domains; activation of RPM1	Mackey *et al.* (2002)
AvrRps4	n.d.	Lipase/esterase-like protein EDS1 (*Arabidopsis*)	Disruption of the complex between EDS1 and the R protein RPS4[f]	Bhattacharjee *et al.* (2011)
		WRKY domains of RRS1-R and RRS1-S, WRKY41, WRKY70, WRKY33, WRKY60	Activation of RPS4-dependent ETI	Sarris *et al.* (2015)

Table 1. (*Continued*).

Effector[a]	Known or predicted biochemical function	Plant targets (organism)[b]	Effector-triggered modifications and/or functions of plant targets[c]	References
AvrRpt2	Cysteine protease	RIN4 (*Arabidopsis*)	Cleavage of RIN4 and activation of RPS2	Axtell *et al.* (2003b); Mackey *et al.* (2003); Kim *et al.* (2005a)
		ROC1 (*Arabidopsis*)	Activation of AvrRpt2 protease activity	Coaker, Falick and Staskawicz (2005); Coaker *et al.* (2006)
		Unknown	Degradation of the Aux/IAA repressor protein AXR2 (depends on protease activity of AvrRpt2 and proteasome; no direct cleavage)	Cui *et al.* (2013)
HopA1 (HopPsyA)	n.d.	EDS1 (*Arabidopsis*)	Disruption of the complex between EDS1 and RPS4[f]	Bhattacharjee *et al.* (2011)
HopAF1	n.d.	Methylthioadenosine nucleosidase proteins MTN1 and MTN2 (*Arabidopsis*)	MTN1 and MTN2 are involved in the methionine recycling pathway and required for PAMP-induced ethylene production; HopAF1 inhibits MTN1 activity *in vitro* and displays structural homology to deamidases	Washington *et al.* (2016)
HopAI1 (HolPtoAI)	Phosphothreonine lyase	MPK3 (*Arabidopsis*)	Dephosphorylation and inactivation of MPK3	Zhang *et al.* (2007)
		MPK4 (*Arabidopsis*)	Reduced kinase activity of MPK4	Zhang *et al.* (2012)
		MPK6 (*Arabidopsis*, tomato)	Dephosphorylation and inactivation of MPK6	Singh *et al.* (2014); Zhang *et al.* (2007)
		Kinase BSK7 (tomato)	n.d.	Singh *et al.* (2014)
HopAO1 (HopPtoD2)	Tyrosine phosphatase	FLS2 kinase domain (*Arabidopsis*)	Suppression of FLS2-mediated PTI responses	Macho *et al.* (2014)
		EFR kinase domain (*Arabidopsis*)	Reduced phosphorylation of EFR	Macho *et al.* (2014)
HopD1 (AvrPphD)	n.d.	Transcription factor NTL9 (*Arabidopsis*)	Suppression of NTL9-regulated gene expression during ETI	Block *et al.* (2014)
HopE1	n.d.	Calmodulin (*Arabidopsis*)	Dissociation of MAP65 from microtubules in the presence of calmodulin	Guo *et al.* (2016)
		MAP65 (*Arabidopsis*)		
HopF2	ADP-RT	MKK5, MAP2Ks (*Arabidopsis*)	ADP-ribosylation of MKK5 at R313, which presumably leads to the inactivation of MKK5	Wang *et al.* (2010); Singh *et al.* (2014)
		MPK6 (tomato)	n.d.	Singh *et al.* (2014)
		RIN4 (*Arabidopsis*)	ADP-ribosylation of RIN4; inhibition of RIN4 degradation by AvrRpt2	Wilton *et al.* (2010); Hurley *et al.* (2014)
		BAK1 (*Arabidopsis*)	Interference with BIK1 phosphorylation and MAPK signaling	Wang *et al.* (2010); Wu *et al.* (2011); Zhou *et al.* (2014)
		Kinase BSK7 (tomato)	n.d.	Singh *et al.* (2014)
HopG1	n.d.	Mitochondrial-localized kinesin motor protein (*Arabidopsis*)	HopG1 associates with actin presumably via its interaction with kinesin and induces actin filament bundling	Shimono *et al.* (2016)
HopI1 (HopPmaI)	n.d.	Hsp70 (*Arabidopsis*)	Increased ATPase activity of Hsp70 *in vitro*; recruitment of cytosolic Hsp70 to chloroplasts	Jelenska, van Hal and Greenberg (2010)
HopM1	n.d.	ARF-GEF MIN7 (*Arabidopsis*)	Degradation of MIN7; inhibition of vesicle trafficking	Nomura *et al.* (2006, 2011)
		14-3-3 protein GRF8/MIN10 (*Arabidopsis*)	GRF8/MIN10 retains TF BZR1 in the cytoplasm; HopM1 presumably alters the activity of GRF8/MIN10 and leads to increased accumulation of TF BZR1 in the nucleus and downregulation of BZR1 target genes (the ortholog of MIN10 is TFT1 which is targeted by XopN)	Nomura *et al.* (2006); Lozano-Duran *et al.* (2014)

Table 1. (*Continued*).

Effector[a]	Known or predicted biochemical function	Plant targets (organism)[b]	Effector-triggered modifications and/or functions of plant targets[c]	References
HopN1	Cysteine protease	Photosystem II component PsbQ (tomato)	Destabilization of PsbQ; reduced activity of PSII; suppression of ROS production	Rodriguez-Herva *et al.* (2012)
HopQ1	n.d.	14-3-3 proteins (*N. benthamiana*, tomato)	n.d.	Giska *et al.* (2013); Li *et al.* (2013b); Hann *et al.* (2014)
HopU1 (HopPtoS2)	ADP-RT	RNA-binding proteins including GRP7 and GRP8 (*Arabidopsis*)	ADP-ribosylation of GRP7; reduced binding of GRP7 to PRR transcripts; reduced FLS2 protein levels; no influence on *FLS2* transcript levels	Fu *et al.* (2007); Jeong *et al.* (2011); Nicaise *et al.* (2013)
HopW1	n.d.	Actin (*Arabidopsis*)	Disruption of actin cytoskeleton; inhibition of protein trafficking and interference with endocytosis	Kang *et al.* (2014)
		Acetylornithine transaminase WIN1, protein phosphatase WIN2, firefly luciferase superfamily protein WIN3 (*Arabidopsis*)	Overexpression of *WIN1* in *Arabidopsis* increases growth of *P. syringae* pv. *tomato* DC3000 delivering HopW1	Lee, Jelenska and Greenberg (2008)
HopX1 (AvrPphE)	Cysteine protease	JAZ proteins (*Arabidopsis*)	Proteaseome-dependent degradation of JAZ proteins	Gimenez-Ibanez *et al.* (2014)
HopZ1a	Acetyltransferase	JAZ proteins (Soybean, *Arabidopsis*)	Acetylation and degradation of JAZ proteins; induction of JA-responsive genes; Hopz1a activity depends on phytate	Jiang *et al.* (2013)
		Tubulin (*Arabidopsis*)	Acetylation of tubulin and destruction of MT; interference with vesicle trafficking	Lee *et al.* (2012)
		GmHID1 (Soybean)	Degradation of GmHID1	Zhou *et al.* (2011)
		Pseudokinase ZED1 (*Arabidopsis*)	Acetylation of ZED1 and activation of the R protein ZAR1	Lewis *et al.* (2013)
HopZ4	Protease/ acetyltransferase	ATPase of proteasome RPT6 (*Arabidopsis*)	Inhibition of proteasome activity	Üstün *et al.* (2014)
Xanthomonas spp.				
AvrAC ($XopAC_{Xcc}$)	Uridylyltransferase	BIK1 (*Arabidopsis*)	Uridylylation of BIK1; inhibition of BIK1 phosphorylation	Feng *et al.* (2012)
		RIPK (*Arabidopsis*)	Uridylylation of RIPK	Feng *et al.* (2012); Guy *et al.* (2013)
		RLCKs of family VII including PBL2 (*Arabidopsis*)	Uridylylation of RLCKs; activation of R protein ZAR1, which guards PBL2 interaction partner RKS1	Guy *et al.* (2013); Wang *et al.* (2015)
$AvrBsT_{Xcv}$	Acetyltransferase	Tubulin-binding protein ACIP1 (*Arabidopsis*)	Acetylation of ACIP1; formation of ACIP1 aggregates	Cheong *et al.* (2014)
		Arginine decarboxylase CaADC1 (pepper)	n.d.	Kim, Kim and Hwang (2013)
		SGT1 (pepper)	Inhibition of SGT1 phosphorylation	Kim *et al.* (2014)
		Hsp70 (pepper)	n.d.	Kim and Hwang (2015b)
		SNF1-related kinase SnRK1 (pepper)	n.d.	Szczesny *et al.* (2010)
		19S subunit of proteasome RPN8 (pepper)	n.d.	Szczesny *et al.* (2010)
		Aldehyde dehydrogenase ALDH1 (pepper)	n.d.	Kim and Hwang (2015a)
$AvrGf2_{Xfa}$ (XopAG family)	n.d.	Cyclophilin (grapefruit)	The interaction depends on the predicted cyclophilin binding domain of AvrGf2	Gochez *et al.* (2016)

Table 1. (*Continued*).

Effector[a]	Known or predicted biochemical function	Plant targets (organism)[b]	Effector-triggered modifications and/or functions of plant targets[c]	References
AvrXccB$_{XccB186}$	Cysteine protease/ acetyltransferase (YopJ family)	S-Adenosyl-L-methionine-dependent methyltransferases (SAM-MT1 and SAM-MT2) (*Arabidopsis*)	The interaction with SAM-MT2 was only observed *in vivo* but not detectable by *in vitro* approaches.	Liu *et al.* (2016)
XopD$_{Xcv}$	SUMO protease	Transcription factor MYB30 (*Arabidopsis*)	Reduced MYB30 activity; relocalization of MYB30 to subnuclear foci	Canonne *et al.* (2011)
		Transcription factor ERF4 (tomato)	DeSUMOylation of ERF4; destabilization of ERF4 and reduced ethylene levels	Kim, Stork and Mudgett (2013)
XopD$_{XccB100}$	SUMO protease	Transcription factor MYB30 (*Arabidopsis*)	Reduced MYB30 activity; relocalization of MYB30 from nucleus to subnuclear foci; reduced expression of MYB30 target genes	Canonne *et al.* (2011)
XopD$_{Xcc8004}$	SUMO protease	DELLA proteins (*Arabidopsis*)	Delay of the GA-induced degradation of the DELLA protein RGA; no effect on transcription of GA-responsive genes	Tan *et al.* (2014)
		Transcription factor HFR1 (*Arabidopsis*)	DeSUMOylation of HFR1	Tan *et al.* (2015)
XopJ$_{Xcv}$	Cysteine protease/ acetyltransferase	ATPase RPT6 of the proteasome (*Arabidopsis*, tobacco)	Degradation of RPT6; inhibition of proteasome activity	Üstün, Bartetzko and Börnke (2013); Üstün and Börnke (2015)
XopL$_{Xcv}$	E3 ubiquitin ligase	n.d.	Ubiquitination of *N. benthamiana* proteins	Singer *et al.* (2013)
XopN$_{Xcv}$	n.d.	Tomato atypical receptor-like kinase (TARK) 1 (tomato)	Stabilization of a TARK1/TFT1 complex; suppression of PTI responses	Kim *et al.* (2009a); Taylor *et al.* (2012)
		14-3-3 proteins TFT1, TFT3, TFT5, TFT6 (tomato)	Interaction was observed in yeast	Kim *et al.* (2009a)
XopN$_{Xoo}$	n.d.	Putative zinc finger protein OsVOZ2 (rice)	n.d.	Cheong *et al.* (2013)
		Putative thiamine synthase OsXNP (rice)	n.d.	Cheong *et al.* (2013)
XopP$_{Xoo}$	n.d.	U box domain of E3 ubiquitin ligase OsPUB44 (rice)	Inhibition of the E3 ubiquitin ligase activity of OsPUB44	Ishikawa *et al.* (2014).
XopQ$_{Xcv}$	n.d.	14-3-3 protein TFT4 (tomato, pepper, *N. benthamiana*)	n.d.	Teper *et al.* (2014)
		14-3-3 proteins (tomato)	n.d.	Teper *et al.* (2014)
XopQ$_{Xoo}$	n.d.	n.d.	XopQ$_{Xoo}$ is present in complex with adenosine diphosphate ribose	Yu, Hwang and Rhee (2014)
Xoo2875	n.d.	BAK1 (rice)	n.d.	Yamaguchi *et al.* (2013a)
Xoo1488 (XopY)	n.d.	Kinase RLCK55 (rice)	n.d.	Yamaguchi *et al.* (2013b)
		Kinase RLCK185 (rice)	Suppression of CERK1-mediated phosphorylation of RLCK185	Yamaguchi *et al.* (2013b)
TAL effectors[g]				
AvrBs3$_{Xcv}$		Importin alpha (pepper)		Szurek *et al.* (2001)
PthA4$_{X.citri}$		DNA- and RNA-binding proteins (*Citrus sinensis*)		de Souza *et al.* (2012)
		Putative chromatin-associated protein HMG (*Citrus sinensis*)	PthA4 binds to poly(U) RNA and forms higher molecular weight complexes with poly(U) RNA in the presence of CsHMG	de Souza *et al.* (2012)
		RNA polymerase III repressor MAF1 (*Citrus sinensis*)	PthA4 presumably counteracts MAF1 activity to increase transcription of host genes	Soprano *et al.* (2013)

Table 1. (*Continued*).

Effector[a]	Known or predicted biochemical function	Plant targets (organism)[b]	Effector-triggered modifications and/or functions of plant targets[c]	References
PthA1, PthA2, PthA3, PthA4$_{Xac306}$		Importin alpha, cyclophilin CsCyp, thioredoxin, ubiquitin-conjugating enzyme complex (*Citrus sinensis*)	Inhibition of the peptidyl-prolyl *cis–trans* isomerase activity of cyclophilin	Domingues *et al.* (2010, 2012)
		C-terminal domain of RNA polymerase II (*Citrus sinensis*)		Domingues *et al.* (2012)
Ralstonia solanacearum				
GALA proteins (RipG family)	F box proteins	SKP1-like proteins (*Arabidopsis*)	n.d.	Remigi *et al.* (2011)
PopP2 (RipP2)	Acetyltransferase	WRKY domains of RRS1-R and RRS1-S (*Arabidopsis*)	Stabilization of RRS1-R and RRS1-S; acetylation of WRKY domains and reduced DNA binding of RRS1-R and RRS1-S; elicitation of RRS1-R-dependent ETI responses	Deslandes *et al.* (2003); Tasset *et al.* (2010); Le Roux *et al.* (2015); Sarris *et al.* (2015)
		WRKY41, WRKY70, WRKY60, WRKY33	Acetylation of WRKY41, WRKY70 and WRKY33	Sarris *et al.* (2015)
		Protease RD19 (*Arabidopsis*)	Relocalization of RD19 from vacuole-associated cellular compartments to the nucleus; RD19 is not acetylated by PopP2 but contributes to the RRS1-R-triggered ETI	Bernoux *et al.* (2008); Tasset *et al.* (2010)
RipTps	Trehalose phosphate synthase	n.d.	Synthesis of trehalose-6-phosphate	Poueymiro *et al.* (2014)
RipAY	γ-Glutamyl cyclo-transferase	Thioredoxins (*Arabidopsis*)	RipAY presumably reduces glutathione levels in eggplant; activity of RipAY is stimulated by thioredoxin AtTrx-h5 from *Arabidopsis*	Fujiwara *et al.* (2016)[h]
			Degradation of glutathione; RipAY is activated by the cytosolic thioredoxins AtTrx-h1, -h2, -h4 and -h5, and the mitochondrial thioredoxin AtTrx-o1 from *Arabidopsis*	Mukaihara *et al.* (2016)[i]

[a]Alternative names of effectors are given in brackets. *Xac*, *Xanthomonas axonopodis* pv. *citri*; *Xcc*, *X. campestris* pv. *campestris*; *Xcv*, *X. campestris* pv. *vesicatoria*; *Xfa*, *Xanthomonas fuscans* subsp. *aurantifolii*; *Xoo*, *X. oryzae* pv. *oryzae*.
[b]The plants, in which the effector targets were identified and/or studied, are given in brackets.
[c]For details, see also the text.
[d]n.d., not determined.
[e]This result could not be reproduced in an independent study (Xiang *et al.* 2011) (see the text for details).
[f]EDS1 (enhanced disease susceptibility 1) contributes to both ETI and PTI responses and was shown to interact with the NLRs RPS4 and RPS6 (Bhattacharjee *et al.* 2011; Heidrich *et al.* 2011). The interaction of EDS1 with RPS4 and RPS6 was reduced in the presence of the effector proteins AvrRps4 and HopA1 from *P. syringae*, which were detected in a complex with EDS1 (Bhattacharjee *et al.* 2011; Heidrich *et al.* 2011). EDS1 was therefore proposed to be a guarded effector target. Notably, however, the *in planta* interaction of EDS1 with AvrRps4 could not be reproduced in an independent study (Sohn *et al.* 2012).
[g]TAL effectors induce the expression of plant target genes, which are summarized in Table S2.
[h]First published on 1 February 2016.
[i]Published on 12 April 2016.

PAMP perception depends on PRRs, which often contain an extracellular domain with the PAMP-binding site, a transmembrane domain and an intracellular kinase domain (Dodds and Rathjen 2010; Schwessinger and Ronald 2012; Böhm *et al.* 2014; Zipfel 2014). Upon PAMP binding, PRRs activate downstream signaling components such as receptor-like cytoplasmic kinases (RLCKs) and mitogen-activated protein kinase (MAPK) cascades (Fig. 2A). Among the well-studied plant PRRs are EFR (EF-Tu receptor), the chitin receptor CERK1 (chitin elicitor receptor kinase 1) and the flagellin receptor FLS2 (flagellin sensitive 2) (Gómez-Gómez and Boller 2000; Schwessinger and Ronald 2012; Wu, Shan and He 2014). PTI elicitors, which are frequently used for the activation of FLS2 and EFR, are peptides of flagellin (flg22) or EF-Tu (elf18). FLS2 belongs to the group of non-RD receptor-like kinases (RLK), which lack a conserved arginine residue (R) next to the catalytic aspartate (D) in the activation loop. FLS2 is presumably autophosphorylated at Ser-938 and associates with the RLCK BIK1 (*Botrytis*-induced

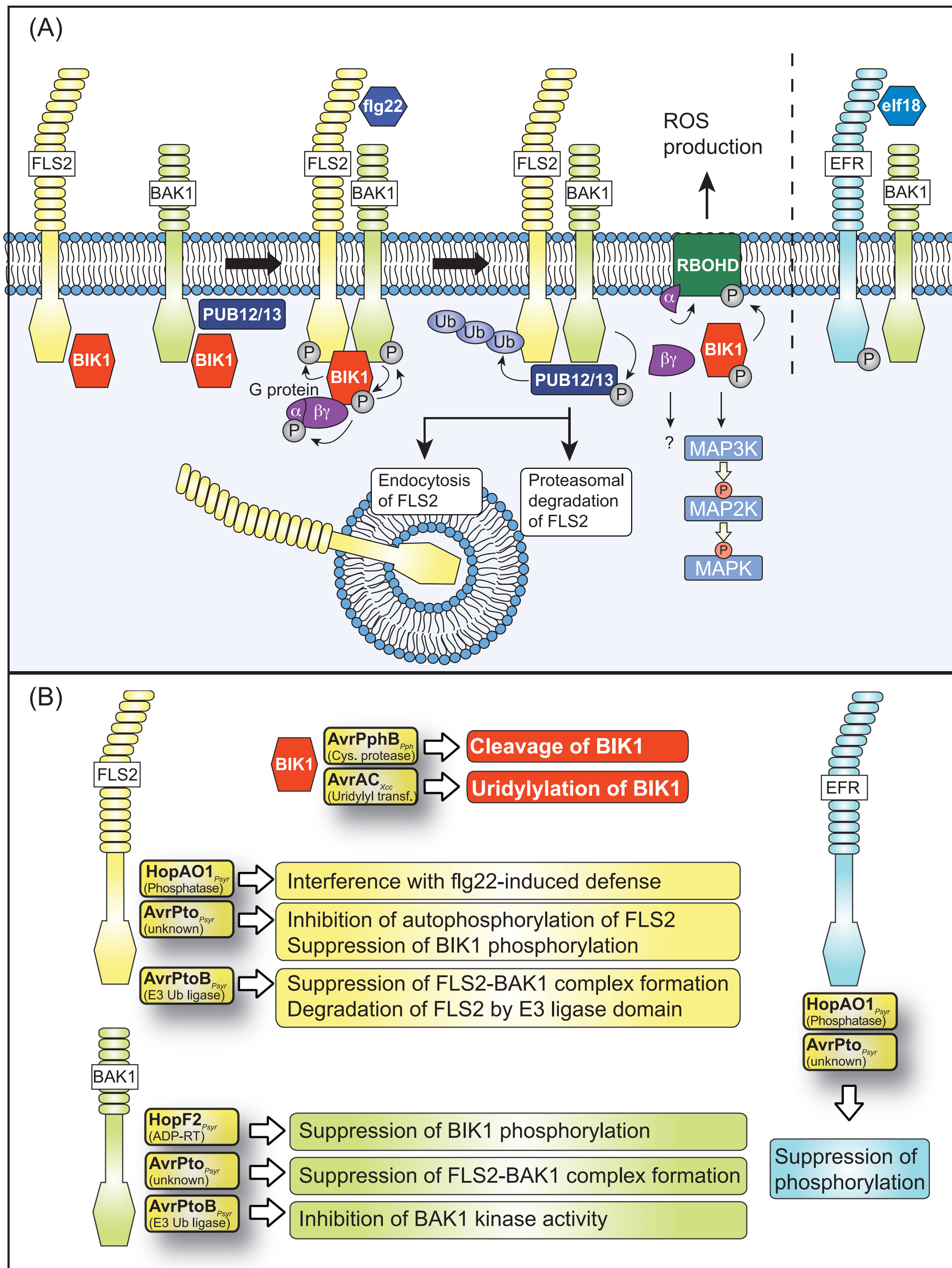

Figure 2. Interference of type III effectors with FLS2- and EFR-dependent signaling pathways during PTI. (**A**) Schematic model of signaling pathways triggered by the flagellin receptor FLS2 and the EF-Tu receptor EFR. FLS2 and EFR consist of a cytoplasmic kinase, a transmembrane and an extracellular LRR domain and insert into the plant plasma membrane. Signaling by FLS2 and EFR involves the transmembrane kinase BAK1, which associates with FLS2 and EFR upon binding of their corresponding ligands. FLS2 and BAK1 are associated with the kinase BIK1, which is phosphorylated by BAK1 upon flg22 perception by FLS2 and subsequently phosphorylates FLS2 and BAK1. BIK1 also phosphorylates an associated heterotrimeric G protein, which stabilizes BIK1 and dissociates after flg22 perception. The Gα subunit interacts with the NADPH oxidase RBOHD, which is involved in ROS production. flg22 perception also leads to the dissociation of BIK1 from the FLS2/BAK1 complex. BIK1 subsequently phosphorylates RBOHD and activates MAPK signaling pathways. FLS2-associated BAK1 phosphorylates the E3 ubiquitin ligases PUB12 and PUB13, which interact with and ubiquitinate FLS2. The proteasomal degradation of FLS2 and the endocytosis of FLS2 after flg22 perception presumably prevent the constitutive activation of FLS2-mediated PTI responses. flg22, peptide of flagellin; elf18, peptide of elongation factor EF-Tu. (**B**) As indicated, several type III effectors target FLS2, BAK1, BIK1 and EFR. Known effector-triggered alterations in PRRs, BAK1 and BIK1 are listed in boxes (see the text for details). Contradictory data were published about the interaction of AvrPto with BAK1 (see the text for details).

kinase 1) prior to ligand perception (Gómez-Gómez, Bauer and Boller 2001; Lu *et al.* 2010; Cao *et al.* 2013). Upon binding of flg22, FLS2 forms a complex with the membrane-associated RLK BAK1 (BRI1 [brassinosteroid-insensitive 1]-associated receptor kinase 1), which also interacts with BIK1 (Chinchilla *et al.* 2007). BAK1 phosphorylates BIK1, which subsequently phosphorylates FLS2 and BAK1, dissociates from the FLS2-BAK1 complex and activates downstream signaling pathways including MAPK cascades (Lu *et al.* 2010; Kim, Kim and Nam 2013; Lin *et al.* 2014) (Fig. 2A). BIK1 also phosphorylates the NADPH oxidase RBOHD (respiratory burst oxidase homolog D), which contributes to ROS production (Kadota *et al.* 2014; Li *et al.* 2014a) (Fig. 2A). In addition to BIK1, FLS2-mediated signaling events involve heterotrimeric G proteins. The Gα subunit XLG2, the Gβ subunit AGB1 and the Gγ subunit AGG1/2 from *Arabidopsis* were recently shown to suppress proteasome-dependent degradation of BIK1 (Liang *et al.* 2016). BIK1 interacts with and phosphorylates XLG2, which dissociates from BIK1 after flg22 perception and interacts with RBOHD, suggesting that it is involved in the regulation of the ROS burst (Liang *et al.* 2016).

A possible constitutive activation of FLS2-mediated PTI responses is counteracted by the endocytosis of FLS2 after ligand perception (Robatzek, Chinchilla and Boller 2006; Ben Khaled, Postma and Robatzek 2015) (Fig. 2A). Furthermore, FLS2-mediated signaling is suppressed by the proteasomal degradation of FLS2, which is controlled by the E3-ubiquitin ligases PUB12 and PUB13. Both E3 ligases interact with BAK1 and are recruited to FLS2 upon ligand perception (Li, Lu and Shan 2014). Recently, it was shown that FLS2-mediated PTI responses are also suppressed by the MAP kinase kinase kinase MKKK7, which interacts with FLS2 and is phosphorylated upon flg22 perception (Mithoe *et al.* 2016).

Given the inhibitory effect of PTI on bacterial pathogenicity, successful pathogens have developed skills to counteract basal defense responses to establish themselves in the plant tissue (Figs 1A and 2B). Several effectors were shown to target PRRs and associated proteins. Known examples include AvrPto, AvrPtoB and HopAO1 (Hrp-dependent outer protein AO1) from *Pseudomonas syringae* as is outlined below. AvrPto presumably inhibits the kinase activities of FLS2 and EFR, whereas the E3 ubiquitin ligase AvrPtoB degrades PRRs including FLS2 and CERK1. The tyrosine phosphatase HopAO1 was shown to interfere with the phosphorylation of the PRR EFR (see below). Additional effectors from *P. syringae* and *Xanthomonas campestris* pv. *campestris* including the mono-ADP-ribosyltransferase (mADP-RT) HopF2, the cysteine protease AvrPphB and the uridylyl transferase AvrAC target the PRR-associated proteins BAK1 and BIK1 (see below). Several effectors also modulate PTI responses by interfering with PTI-associated downstream MAPK signaling cascades. These effectors and their specific mode of action will be detailed in the section 'Modulation of MAPK cascades by type III effectors' below.

AvrPto from *P. syringae* targets the PRRs FLS2 and EFR and presumably interacts with BAK1

AvrPto from *P. syringae* interacts with the kinase domains of the PRRs FLS2 and EFR, and leads to the suppression of PTI responses including MAPK signaling pathways (Xiang *et al.* 2008) (Fig. 2B). Given that AvrPto inhibits the autophosphorylation of FLS2 and EFR, it likely acts as a kinase inhibitor. In agreement with this hypothesis, a point mutation in AvrPto (Y89D), which abolishes the interaction of AvrPto with FLS2 and EFR, interferes with the AvrPto-mediated suppression of MAPK activity (Xiang *et al.* 2008). The interaction between AvrPto and FLS2 presumably suppresses BIK1 phosphorylation (Xiang *et al.* 2011).

AvrPto was also reported to bind to the RLK BAK1 and to prevent the formation of the FLS2-BAK1 complex (Shan *et al.* 2008; Zhou *et al.* 2014). Experimental evidence for the interaction between AvrPto and BAK1 was provided by the results of coimmunprecipitation studies in protoplasts and transgenic *Arabidopsis* seedlings, bimolecular fluorescence complementation (BiFC) studies and *in vitro* pull-down assays (Shan *et al.* 2008; Zhou *et al.* 2014). No interaction with BAK1 was observed for an AvrPto$_{S46P}$ point mutant derivative, which does not suppress PAMP-triggered activation of MAPK signaling cascades (He *et al.* 2006). This suggests that the observed interaction with BAK1 was specific for AvrPto. In an independent study, however, similar interaction experiments did not reveal an interaction between AvrPto and BAK1 whereas the interaction between AvrPto and FLS2 was detected (Xiang *et al.* 2011). It is possible that the observed lack of interaction between AvrPto and BAK1 was caused by differences in the experimental conditions. Notably, Xiang *et al.* (2011) also did not detect the postulated AvrPto-induced dissociation of the FLS2-BAK1 complex in the presence of an AvrPto-nYFP (N-terminal region of yellow fluorescent protein) fusion protein. However, it cannot be excluded that the presence of the nYFP fusion partner interfered with the ability of AvrPto to dissociate the FLS2-BAK1 complex.

The E3 ubiquitin ligase AvrPtoB from *P. syringae* degrades the PRRs FLS2 and CERK1 and inhibits the kinase activity of BAK1

In addition to AvrPto, the distantly related effector AvrPtoB suppresses PTI responses (Fig. 2B). AvrPtoB is presumably activated *in planta* by phosphorylation of the serine residue at position 258, suggesting that it mimics a substrate of a plant kinase (Xiao, Giavalisco and Martin 2007). Given that the exchange of S258 to alanine leads to a loss of the virulence activity of AvrPtoB, phosphorylation of AvrPtoB is presumably required for protein function (Xiao, Giavalisco and Martin 2007).

AvrPtoB contains a C-terminal E3 ubiquitin-ligase domain, which leads to the proteasomal degradation of most of its plant targets (Abramovitch *et al.* 2006; Janjusevic *et al.* 2006; Göhre *et al.* 2008; Gimenez-Ibanez *et al.* 2009). Interaction partners of AvrPtoB include FLS2, BAK1 and additional receptor kinases such as the chitin receptor CERK1 (Göhre *et al.* 2008; Shan *et al.* 2008; Gimenez-Ibanez *et al.* 2009) (Table 1). AvrPtoB degrades FLS2 and CERK1 and inhibits the kinase activity of BAK1, thus suppressing PTI responses (Göhre *et al.* 2008; Gimenez-Ibanez *et al.* 2009; Cheng *et al.* 2011). The E3 ubiquitin ligase activity of AvrPtoB is described in more detail in the section 'Interference of type III effectors with the 26S proteasome'.

The tyrosine phosphatase HopAO1 interferes with EFR phosphorylation and FLS2-mediated signaling pathways

The tyrosine phosphatase HopAO1 (formerly known as HopPtoD2) from *P. syringae* interacts with the kinase domain of the PRR EFR (Macho *et al.* 2014). HopAO1 leads to reduced tyrosine phosphorylation of EFR after ligand binding, suggesting that the interaction of HopAO1 with EFR suppresses EFR autophosphorylation (Macho *et al.* 2014). Reduced phosphorylation of EFR presumably interferes with downstream

signaling pathways which involve BAK1. The targeted mutagenesis of tyrosine residues in the cytoplasmic domain of EFR revealed that tyrosine residue Y836 is essential for EFR-mediated downstream signaling but dispensable for the kinase activity of EFR (Macho *et al.* 2014). It remains to be investigated whether HopAO1 suppresses EFR-mediated signaling by interfering with the phosphorylation of Y836 of EFR. The suppression of EFR phosphorylation by HopAO1 presumably does not solely depend on its tyrosine phosphatase activity because a catalytically inactive HopAO1 derivative led to 20% reduction in EFR phosphorylation (Macho *et al.* 2014). Notably, in addition to EFR, HopAO1 also interacts with the kinase and cytoplasmic domain of FLS2 and interferes with FLS2-mediated defense signaling by a yet unknown mechanism (Macho *et al.* 2014) (Fig. 2B).

The mADP-RT HopF2 from *P. syringae* interacts with BAK1 and interferes with BIK1 phosphorylation

An additional effector from *P. syringae* pv. *tomato* DC3000, which suppresses PTI responses, is the mADP-RT HopF2 (Wu *et al.* 2011; Zhou *et al.* 2014; Lo *et al.* 2016). ADP-RTs hydrolyze NAD^+ to transfer ADP ribose to their cognate substrate molecules (Deng and Barbieri 2008). HopF2 interacts with BAK1 (Zhou *et al.* 2014) and interferes with flg22-induced BIK1 phosphorylation (Wu *et al.* 2011) (Fig. 2B). It is still unknown whether BAK1 is a substrate of the HopF2 ADP-RT activity. Given that HopF2 interacts with and ADP-ribosylates the MAP kinase kinase (MAP2K) MKK5 as well as the immune regulator RIN4 (RPM1-interacting protein 4, see below), BAK1 is not the only target of HopF2 (Wang *et al.* 2010; Wilton *et al.* 2010). HopF2 was also found in a complex with the autoinhibited plasma membrane H(+) ATPase AHA2, which associates with RIN4 and mediates stomatal closure (Liu *et al.* 2009; Hurley *et al.* 2014) (see below). HopF2 presumably interferes with stomatal immunity independently of its ADP-RT activity because transgenic *Arabidopsis* plants expressing HopF2 or the catalytically inactive $HopF2^{D175A}$ derivative were impaired in stomatal closure upon treatment with *P. syringae* pv. *tomato* DC3000 (Hurley *et al.* 2014).

The cysteine protease AvrPphB and the uridylyl transferase AvrAC target BIK1

AvrPphB from *P. syringae* pv. *phaseolicola* and AvrAC (also known as Xop [*Xanthomonas* outer protein] AC) from *X. campestris* pv. *campestris* both target BIK1. AvrPphB is a cysteine protease, which is secreted by the T3S system as a preprotease and is autocatalytically processed after the tripeptide Gly-Asp-Lys, which is the recognition site of AvrPphB (Puri *et al.* 1997; Shao *et al.* 2002). The mature AvrPphB is myristoylated inside the plant cell and associates with the plant plasma membrane (Dowen *et al.* 2009). AvrPphB interacts with and cleaves BIK1 and thus presumably interferes with PTI (Zhang *et al.* 2010) (Fig. 2B). Additional substrates of AvrPphB include the membrane-associated serine/threonine kinase PBS1 and PBS1-like (PBL) proteins, which all contain the Gly-Asp-Lys recognition site (Shao *et al.* 2003; Zhang *et al.* 2010) (Table 1). Cleavage of PBS1 by AvrPphB leads to the activation of ETI in the presence of the R protein RPS5 (Shao *et al.* 2003) (see below). AvrPphB also cleaves the kinase RIPK (RPM1-induced protein kinase), which is required for the phosphorylation of the immune regulator RIN4 (Russell, Ashfield and Innes 2015) (see below).

AvrAC from *X. campestris* pv. *campestris* contains an N-terminal LRR domain and a C-terminal Fic (filamentation-induced by c-AMP) domain and acts as uridylyl transferase, i.e. it transfers uridine 5′-monophosphate (UMP) to plant target proteins (Feng *et al.* 2012). The results of *in vitro* and *in vivo* assays suggest that AvrAC interacts with and uridylylates BIK1 (Feng *et al.* 2012) (Fig. 2B). UMP modification of BIK1 depends on amino acid residues S236 and T237 of BIK1, which are conserved phosphorylation sites in the activation loop (Lu *et al.* 2010; Zhang *et al.* 2010; Laluk *et al.* 2011). It was, therefore, suggested that the AvrAC-mediated transfer of UMP inhibits BIK1 activity (Feng *et al.* 2012). In addition to BIK1, AvrAC interacts with and uridylates other RLCKs of the family VII, which is the largest family of RLCKs and includes several RLCKs involved in plant immune responses. Among the AvrAC targets are RIPK and PBL2 (Feng *et al.* 2012; Guy *et al.* 2013; Wang *et al.* 2015). Uridylylation of PBL2 by AvrAC triggers ETI responses, which depend on the pseudokinase RKS1 and the R protein ZAR1 (see below).

MODULATION OF MAPK CASCADES BY TYPE III EFFECTORS

PRR-mediated immune responses often involve the activation of MAPK cascades. These signaling pathways are attractive targets for type III effectors because they contribute to various cellular pathways. MAPK signaling is triggered by MAP kinase kinase kinases (also termed MAP3K or MEKK), which are directly or indirectly activated by receptor proteins including PRRs (Rodriguez, Petersen and Mundy 2010; Rasmussen *et al.* 2012). MAP3Ks are serine or threonine kinases and activate MAP2Ks (also designated MEK) via phosphorylation (Rodriguez, Petersen and Mundy 2010). MAP2Ks subsequently phosphorylate threonine or tyrosine residues of MAPKs, leading to their activation (Rodriguez, Petersen and Mundy 2010; Rasmussen *et al.* 2012). Among the well-studied MAPKs from plants are MPK3, MPK4 and MPK6, which are involved in defense responses. MPK3 and MPK6 are part of a signaling cascade, which is activated by the MAP3K MEKK1 and the two MAP2Ks MKK4 and MKK5 (Meng and Zhang 2013) (Fig. 3A). A second signaling cascade involves the MAP3K MEKK1, the two MAP2Ks MKK1 and MKK2 as well as MPK4 (Meng and Zhang 2013). Known substrates of MPK4 are the MPK4 interaction partner RIN4 (Cui *et al.* 2010), the MAP3K MEKK2 (also designated SUMM1 [suppressor of *mkk1 mkk2* 1]) (Kong *et al.* 2012) and MKS1 (MPK4 substrate 1), which forms a complex with MPK4 and the transcription factor WRKY33 in the nucleus (Rasmussen *et al.* 2012). Phosphorylation of MKS1 by MPK4 leads to the release of WRKY33, which in turn induces the expression of its target genes and initiates PTI responses (Rasmussen *et al.* 2012) (Fig. 3A).

Notably, MPK4 was initially identified as a negative regulator of plant immunity because mutations in *MPK4* lead to the activation of defense responses (Meng and Zhang 2013). Genetic screens for suppressor mutations in *mkk1mkk2* plants led to the identification of SUMM1 and the R protein SUMM2. Mutations in *SUMM1* or *SUMM2* abolish the constitutive activation of plant defense responses in *mkk1mkk2* plants, suggesting that SUMM1 and SUMM2 are both involved in the activation of defense responses in the absence of MPK4 (Kong *et al.* 2012; Zhang *et al.* 2012) (Fig. 3A). The R protein SUMM2 has probably evolved to sense changes in the MEKK1-MKK1/MKK2-MPK4 signaling cascade and likely guards SUMM1, which interacts with and is phosphorylated by MPK4 (Kong *et al.* 2012; Zhang *et al.* 2012). Given the finding that overexpression of *SUMM1*

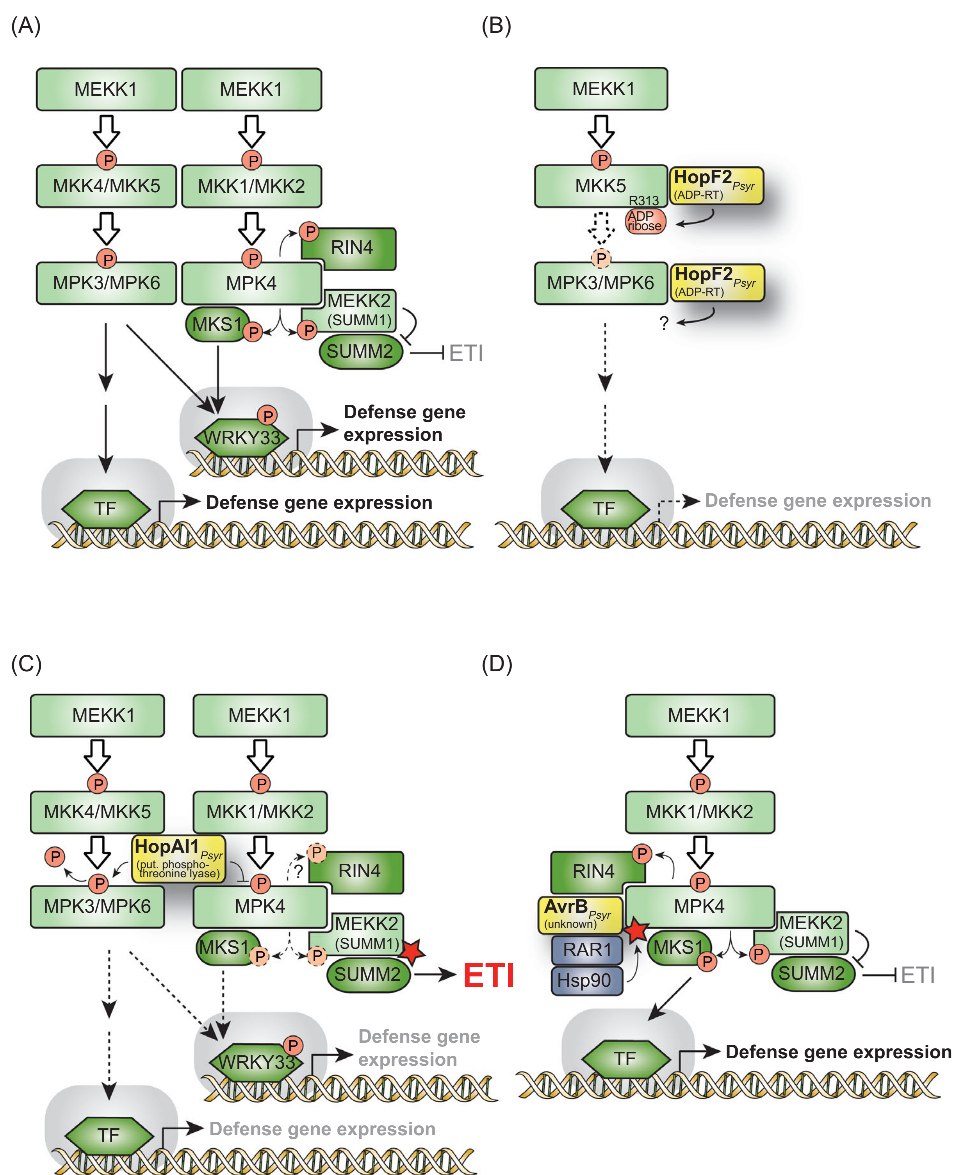

Figure 3. Influence of type III effectors on MAPK signaling pathways. (**A**) Schematic overview on MAPK signaling pathways involved in plant defense responses. During plant defense responses, two MAPK signaling pathways are activated which involve (i) MPK3/MPK6 and MKK4/MKK5 and (ii) MPK4 and MKK1/MKK2, respectively. The MP2Ks of both pathways are activated by the MP3K MEKK1, however, MPK3 and MPK6 can also be activated independently of MEKK1 (Suarez-Rodriguez *et al*. 2007). The activation of MAPKs directly or indirectly leads to the release of transcription factors (TFs), which trigger the expression of defense genes. Known substrates of MPK4 are MKS1, RIN4 and the MP3K MEKK2 (also designated SUMM1). Phosphorylation of MKS1 by MPK4 leads to the release of the MKS1-bound TF WRKY33, which subsequently activates gene expression. MPK4 also phosphorylates the MAP3K MEKK2 and presumably results in its inactivation. MPK4-mediated inactivation of MEKK2 leads to the suppression of ETI responses triggered by the CC-NB-LRR R protein SUMM2, which likely guards MEKK2 (see the text for details). (**B**) HopF2 from *P. syringae* interferes with MPK3/MPK6 signaling pathway. The mono-ADP-RT HopF2 ADP-ribosylates and thus inactivates MKK5 and suppresses the MPK3/MPK6-mediated signaling pathway (indicated by dashed arrows). HopF2 also interacts with MPK6, yet, the outcome of this interaction is unknown. (**C**) The putative phosphothreonine lyase HopAI1 from *P. syringae* inhibits the activity of MAPKs. HopAI1 dephosphorylates MPK3 and MPK6 and thus interferes with the MPK3/MPK6 signaling pathway. Furthermore, HopAI1 suppresses the kinase activity of MPK4 and thus the phosphorylation of the MPK4 substrates MKS1 and MEKK2. It has not yet been shown whether HopAI1 also interferes with the MPK4-mediated phosphorylation of RIN4 (indicated by a dashed arrow and a questionmark). The loss of MPK4 activity leads to the activation of MEKK2 (indicated by a red asterisk), which in turn triggers SUMM2-mediated ETI (see the text for details). (**D**) AvrB from *P. syringae* activates MPK4. AvrB interacts with MPK4 and leads to its phosphorylation and thus activation. The efficient interaction between AvrB and MPK4 depends on RAR1, which presumably acts as a linker between AvrB and Hsp90. Hsp90 promotes the activity of MPK4 as is indicated by a red asterisk (see the text for details).

activates SUMM2-dependent defense responses, it was proposed that SUMM1 is negatively regulated by MPK4. Thus, activation of SUMM1 in the absence of MEKK1-MKK1/MKK2-MPK4 signaling triggers SUMM2-dependent defense responses (Kong *et al*. 2012).

Effectors, which interfere with MAPK signaling pathways and SUMM2-mediated defense, include HopF2, HopAI1 and AvrB from *P. syringae*. As is detailed below, the ADP-RT HopF2 presumably inactivates the MP2K MKK5, whereas HopAI1 suppresses the activities of MAPKs. AvrB, however, activates the MAPK MPK4, suggesting that effector proteins from *P. syringae* have opposing functions with regard to the interference with MAPK signaling cascades. The functions of HopF2, HopAI1 and AvrB are also summarized in Fig. 3B-D.

HopF2 from *P. syringae* ADP-ribosylates MKK5

The ADP-RT HopF2 from *P. syringae* does not only interfere with BIK1 phosphorylation (see above) but also with MAPK signaling. HopF2 interacts with MPK6 (Singh *et al.* 2014) as well as with several MAP2Ks including MKK5. HopF2 ADP-ribosylates the arginine residue at position 313 (R313) of MKK5, which is important for MKK5 function (Wang *et al.* 2010). It was, therefore, proposed that HopF2 inactivates MKK5 and thus interferes with the MKK5-dependent signaling cascade (Wang *et al.* 2010) (Fig. 3B). This hypothesis is supported by the finding that the phosphorylation of MPK6 by a constitutively active MKK5^{DD} derivative is suppressed by HopF2 *in vitro* (Wang *et al.* 2010).

HopAI1 from *Pseudomonas syringae* suppresses MAPK activities

An additional effector, which suppresses MAPK activities, is HopAI1 from *P. syringae* pv. *tomato* (Zhang *et al.* 2007, 2012). HopAI1 interacts with several MAPKs including MPK3, MPK4 and MPK6 (Zhang *et al.* 2007, 2012; Singh *et al.* 2014) (Fig. 3C). The analysis of *hopAI1*-transgenic *Arabidopsis* plants revealed that HopAI1 suppresses the kinase activities of MPK3, MPK4 and MPK6 and thus interferes with plant defense responses, e.g. flg22-induced gene expression, ROS production and callose deposition (Zhang *et al.* 2007, 2012). In agreement with the HopAI1-mediated suppression of MPK4 activity, the *in planta* expression of *hopAI1* leads to the induction of SUMM2-dependent ETI (Zhang *et al.* 2012) (see above; Fig. 3C). HopAI1 presumably directly targets MAPKs because the HopAI1-mediated suppression of MPK3 and MPK6 kinase activities was also observed in the presence of the constitutively active MAP2K derivative MKK5^{DD} (Zhang *et al.* 2007). It was shown that HopAI1 inactivates MPK3 and MPK6 via dephosphorylation of phosphothreonine residues *in vitro*, suggesting that HopAI1 acts as a phosphothreonine lyase (Zhang *et al.* 2007).

AvrB from *Pseudomonas syringae* activates MPK4

In contrast to the HopAI1-mediated dephosphorylation and inactivation of MPK4, AvrB from *P. syringae* leads to the phosphorylation and activation of MPK4 (Cui *et al.* 2010) (Fig. 3D). Thus, HopAI1 and AvrB have opposing activities with regard to the phosphorylation of MPK4. Coimmunprecipitation studies revealed that the interaction of AvrB with MPK4 is increased in the presence of RAR1, which is a cochaperone of the heat shock protein 90 (HSP90) and interacts with AvrB (Cui *et al.* 2010). It was, therefore, suggested that the interaction between AvrB and RAR1 promotes the association of AvrB with MPK4 (Cui *et al.* 2010). Similarly to AvrB, HSP90 positively regulates the activity of MPK4 (Cui *et al.* 2010). RAR1 and MPK4 are presumably not the only virulence targets of AvrB because AvrB also interacts with RIN4 and triggers its phosphorylation (Mackey *et al.* 2002; Cui *et al.* 2010) (see below). As RIN4 was found in a complex with MPK4 and AvrB and presumably acts downstream of MPK4, it was speculated that the AvrB-mediated phosphorylation of RIN4 involves MPK4 (Cui *et al.* 2010) (Fig. 3D).

INTERFERENCE OF TYPE III EFFECTORS WITH THE 26S PROTEASOME

The eukaryotic 26S proteasome plays a central role in many cellular processes including hormone signaling and defense responses and is a virulence target of several type III effectors (Price and Kwaik 2010; Dudler 2014; Duplan and Rivas 2014; Banfield 2015). The proteasome is composed of a 20S core particle and two 19S regulatory subunits, which recognize ubiquitinated proteins. Proteins destined for degradation are covalently attached via the ε-amino group of a lysine residue to at least four ubiquitin molecules. Ubiquitin is a highly conserved 76-amino acid polypeptide and is often linked to other ubiquitin molecules via the lysine residue at position 48 (Pickart 2001). Ubiquitin is adenylated by the ubiquitin-activating enzyme (E1), transferred to a conjugating E2 enzyme and subsequently bound to a lysine residue of the target protein by an E3 ubiquitin ligase (Sadanandom *et al.* 2012) (Fig. 4A). Plants possess only one or two E1 proteins but a significantly higher number of E2 and E3 proteins (e.g. 1500 E3 ligases in *Arabidopsis*) (Chen and Hellmann 2013). E3 ubiquitin ligases are single or multisubunit proteins, which interact with E2 enzymes via a HECT (homologous to E6-associated protein C-terminus), a RING (really interesting new gene) or a U-box domain (Chen and Hellmann 2013). Very well studied are SCF (SKP1 [S-phase kinase-associated protein 1]-like-cullin 1-F-box) complexes, which are multimeric RING-finger E3 ligases and play a central role in phytohormone signaling (Shabek and Zheng 2014). SCF complexes consist of a cullin protein as a central scaffold, which associates via its C-terminal region with the RING protein RBX1 (RING box 1). The N-terminal part of the cullin protein is connected via a SKP1-like protein with a member of the F-box protein family, which provides the binding sites for the substrates of the SCF complex (Vierstra 2009; Chen and Hellmann 2013) (Fig. 4A). F-box proteins contain the F-box motif, i.e. a short motif of approximately 50 amino acids, which mediates the interaction of F-box proteins with SKP1-like proteins of the SCF complex (Schulman *et al.* 2000).

Some effectors bind to E3 ligases or act themselves as E3 ligases and exploit the proteasome for the degradation of specific plant proteins. Examples are AvrPtoB and HopM1 from *P. syringae*, XopL from *X. campestris pv. vesicatoria* and GALA proteins from *R. solanacearum*, which are discussed below. Other effectors such as XopD from *Xanthomonas* spp. can indirectly destabilize plant proteins by cleaving small ubiquitin-related modifier (SUMO) from SUMOylated proteins. SUMO is structurally related to ubiquitin and can reversibly modify proteins, thus leading to alterations in protein localization, stability and/or activity (Gill 2004; Park and Yun 2013). The functions of XopD family members are discussed in the section ‘Modulation of phytohormone signaling by type III effectors’.

In contrast to effectors that stimulate protein degradation, type III effectors of the YopJ (*Yersinia* outer protein J) family of predicted cysteine proteases and/or acetyltransferases such as XopJ from *X. campestris pv. vesicatoria* and HopZ4 from *P. syringae* suppress proteasome activity (Hotson and Mudgett 2004; Lewis *et al.* 2011). A similar effect is achieved by the bacterial tripeptide derivative syringolin, which is produced by several strains of *P. syringae* (Groll *et al.* 2008; Dudler 2014). Furthermore, the effector XopP from *Xanthomonas oryzae* pv. *oryzae* was shown to interfere with the activity of an E3 ubiquitin ligase as is discussed below. The apparent contradictory activities of bacterial virulence factors, which suppress or promote the activity of the proteasome, might be caused by different spatial distributions of effectors in the plant cell or temporal differences in their synthesis or translocation. The interference of single effector proteins with the proteasome and with proteasome-dependent protein degradation is summarized below and in Fig. 4B-G.

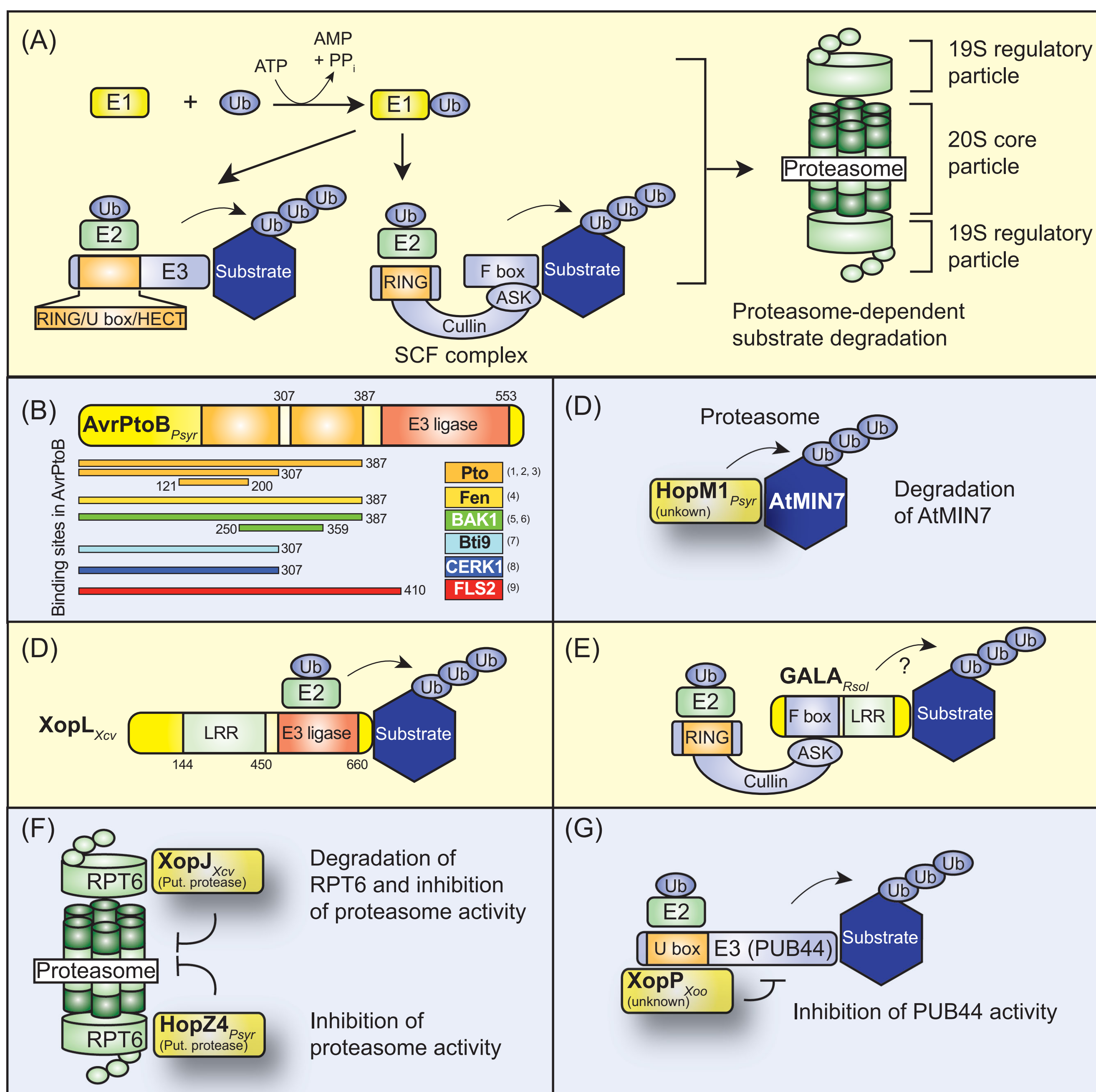

Figure 4. Contribution of type III effectors to proteasome-dependent protein degradation. **(A)** Model of the proteasome-dependent protein degradation pathway. Ubiquitin (Ub) is activated by the ubiquitin-activating enzyme (E1) and transferred to the ubiquitin-conjugating enzyme (E2), which interacts with the ubiquitin ligase (E3). E3 ubiquitin ligases are divided into several classes according to the presence of a HECT, RING or U-box domain. RING domain-containing E3 ubiquitin ligases can be part of a multimeric protein complex such as the SCF complex, which consists of a RING-box protein, the molecular scaffold protein cullin, an *Arabidopsis* SKP1-like protein (ASK1) and an F-box protein, which binds the substrate of the E3 ligase. E3 enzymes mediate the transfer of ubiquitin molecules to the substrate, thus leading to the formation of poly-ubiquitin chains, which allow the targeting of proteins to the proteasome. The proteasome consists of two 19S regulatory and a 20S core particle and catalyzes the unfolding and degradation of polyubiquitinated proteins. **(B)** Domain structure of the effector protein AvrPtoB from *P. syringae*. The regions of AvrPtoB, which provide binding sites for the AvrPtoB interaction partners Pto, Bti9, Fen, FLS2 and BAK1, are indicated. References: (1) Dong *et al.* 2009; (2) Xiao *et al.* 2007; (3) Mathieu, Schwizer and Martin 2014; (4) Rosebrock *et al.* 2007; (5) Shan *et al.* 2008; (6) Cheng *et al.* 2011; (7) Zeng *et al.* 2012; (8) Gimenez-Ibanez *et al.* 2009; (9) Göhre *et al.* 2008. Experimental evidence for the presence of two Pto-binding sites in AvrPtoB (indicated as orange boxes) was reported by Mathieu, Schwizer and Martin (2014). Numbers refer to amino acid positions in AvrPtoB. **(C)** HopM1 from *P. syringae* induces the degradation of its interaction partner AtMIN7. The HopM1-mediated degradation of AtMIN7 depends on the activity of the proteasome. **(D)** The effector protein XopL from *X. campestris pv. vesicatoria* triggers the ubiquitination of plant proteins. XopL contains an N-terminal LRR and a C-terminal E3 ubiquitin ligase domain. The plant targets of XopL are unknown. Numbers refer to amino acid positions in XopL. **(E)** GALA proteins from *R. solancearum* contain an F-box domain and were proposed to associate with components of the SCF complex. In agreement with this hypothesis, an interaction between GALA proteins and ASK proteins has been shown. A contribution of GALA proteins to the ubiquitination of substrates of the SCF complex remains to be demonstrated. **(F)** XopJ from *Xanthomonas* spp. and HopZ4 from *P. syringae* interact with the proteasome subunit RPT6 and suppress the activity of the proteasome. XopJ leads to the degradation of RPT6. **(G)** XopP from *X. oryzae* pv. *oryzae* interacts with the U box E3 ubiquitin ligase PUB44 from rice and inhibits its activity.

AvrPtoB from *P. syringae* contains an E3 ubiquitin ligase domain

As mentioned above, AvrPtoB from *P. syringae* contains a C-terminal E3 ubiquitin ligase domain and degrades interaction partners including CERK1, FLS2 and RIN4 (Fig. 4B; Table 1). An additional substrate of the E3 ubiquitin ligase activity of AvrPtoB is the kinase Fen, which is a member of the Pto (resistance to *P. syringae* pv. *tomato*) kinase family and binds to the N-terminal region of AvrPtoB (Martin *et al.* 1994; Rosebrock *et al.* 2007). Fen is ubiquitinated and degraded by AvrPtoB (Rosebrock *et al.* 2007). In the presence of AvrPtoB$_{1-387}$, however, which lacks the E3 ubiquitin ligase domain, Fen triggers defense responses in tomato. It was, therefore, suggested that AvrPtoB evades its own Fen-mediated recognition by acquiring E3 ubiquitin ligase activity (Rosebrock *et al.* 2007). Fen-dependent defense responses elicited by E3 ubiquitin ligase-inactive AvrPtoB derivatives were previously referred to as Rsb (resistance suppressed by AvrPtoB C terminus) (Abramovitch *et al.* 2003) and depend on the R protein Prf (Rosebrock *et al.* 2007). As is described below, Prf also detects full-length AvrPtoB and AvrPto and elicits ETI responses, which depend on the kinase Pto (Oh and Martin 2011).

In addition to its role in suppression of Fen-mediated defense responses, the C-terminal E3 ubiquitin ligase domain of AvrPtoB might also contribute to other virulence activities of AvrPtoB. Thus, AvrPtoB derivatives without E3 ubiquitin ligase activity did not suppress ROS production after chitin but after flg22 perception (Gimenez-Ibanez *et al.* 2009). Furthermore, AvrPtoB$_{1-387}$ failed to restore *in planta* growth of a *P. syringae avrPtoB* deletion mutant, suggesting that the E3 ubiquitin ligase domain contributes to the virulence function of AvrPtoB (Göhre *et al.* 2008). Complementation of the *avrPtoB* mutant phenotype by full-length AvrPtoB, however, was not analyzed (Göhre *et al.* 2008). Notably, a virulence function of the E3 ligase domain of AvrPtoB was not observed in additional studies, in which AvrPtoB$_{1-387}$ and catalytically inactive AvrPtoB derivatives were shown to promote virulence of *P. syringae avrPtoB* mutants on susceptible tomato lines and *Nicotiana benthamiana* plants (Xiao, Giavalisco and Martin 2007; Xiao *et al.* 2007; Zeng *et al.* 2012; Wei *et al.* 2015).

HopM1 leads to the proteasome-dependent degradation of its interaction partner AtMIN7

The effector HopM1 from *P. syringae* pv. *tomato* triggers the degradation of its plant interaction partners including AtMIN7 (*Arabidopsis* HopM interactor 7), which is an ADP ribosylation factor-guanine nucleotide exchange factor (ARF-GEF) involved in vesicle trafficking (Nomura *et al.* 2006) (Fig. 4C). AtMIN7 colocalizes with HopM1 to the trans-Golgi network/early endosome (Nomura *et al.* 2011). This is in agreement with the role of ARF-GEF proteins in vesicle trafficking. The analysis of *Arabidopsis AtMIN7* mutants revealed that AtMIN7 contributes to PTI and ETI responses but is dispensable for ROS production and stomatal closure upon flg22 treatment (Nomura *et al.* 2011; Lozano-Duran *et al.* 2014). In agreement with its role in plant defense, AtMIN7 levels increase upon activation of PTI and are stabilized during ETI (Nomura *et al.* 2011). HopM1 leads to the destabilization of AtMIN7 when *hopM1* and *AtMIN7* are coexpressed in leaves of *N. benthamiana* or when HopM1 is delivered by the T3S system of *P. syringae* pv. *tomato* DC3000 into leaves of *Arabidopsis* (Nomura *et al.* 2006). The HopM1-mediated destabilization of AtMIN7 in *N. benthamiana* was reduced in the presence of proteasome inhibitor, suggesting that HopM1 exploits the proteasome for the degradation of AtMIN7 (Nomura *et al.* 2006) (Fig. 4C). Given the contribution of AtMIN7 to plant defense, HopM1 was suggested to suppress plant defense responses via degradation of AtMIN7. HopM1 failed to trigger the degradation of AtMIN7 when delivered as heterologous protein by *P. syringae* pv. *phaseolicola*, which lacks a native *hopM1* gene (Gangadharan *et al.* 2013). However, delivery of HopM1 by *P. syringae* pv. *phaseolicola* led to the suppression of PTI responses, suggesting that AtMIN7 is not the only virulence target of HopM1 (Gangadharan *et al.* 2013). This hypothesis was supported by the finding that HopM1 suppresses PTI in *Arabidopsis atmin7* mutant plants (Gangadharan *et al.* 2013; Lozano-Duran *et al.* 2014). One additional interaction partner, which is degraded by HopM1, is the 14-3-3 protein GRF8 (general regulatory factor 8) (Nomura *et al.* 2006). GRF8 interacts with and controls the transcriptional repressor BZR1 (brassinazole resistant 1), which is involved in brassinosteroid signaling (Nomura *et al.* 2006; Gampala *et al.* 2007; Ryu *et al.* 2007). As is discussed below, HopM1 presumably interferes with the function of GRF8 and leads to the nuclear accumulation of BZR1 (Lozano-Duran *et al.* 2014).

XopL from *X. campestris* pv. *vesicatoria* acts as an E3 ubiquitin ligase

The type III effector XopL from *X. campestris* pv. *vesicatoria* contains a C-terminal E3 ubiquitin ligase domain with a novel fold and likely acts as a RING/U-box E3 ligase (Singer *et al.* 2013) (Fig. 4D). XopL leads to the ubiquitination of plant proteins, yet the plant substrates of the XopL E3 ubiquitin ligase activity remain to be identified (Singer *et al.* 2013). The analysis of different XopL protein regions revealed that the C-terminal E3 ubiquitin ligase domain is required for the XopL-mediated elicitation of plant cell death but dispensable for the suppression of PTI, which was observed in the presence of XopL. It was, therefore, speculated that the virulence function of XopL does not solely depend on its E3 ubiquitin ligase activity but also on its N-terminal LRR domain (Singer *et al.* 2013).

GALA proteins from *R. solanacearum* contain an F box motif

GALA proteins (also designated Rip [*Ralstonia* protein injected into plant cells] G family) from *R. solanacearum* are effector proteins, which contain a conserved GAxALA amino acid motif in the C-terminal LRR region and an F-box motif in the N-terminal region (Cunnac *et al.* 2004) (Fig. 4E). At least four GALA proteins from *R. solanacearum* including GALA6 were shown to interact with SKP1-like proteins from *Arabidopsis* (Angot *et al.* 2006). However, it is yet unknown whether GALA proteins contribute to protein degradation. Notably, effector proteins with F-box motifs have also been identified as substrates of the type IV secretion systems from *Agrobacterium tumefaciens* and *Legionella* spp. (Price and Kwaik 2010).

XopJ from X. *campestris* pv. *vesicatoria* and HopZ4 from *P. syringae* interact with RPT6 and interfere with proteasome activity

Several members of the YopJ family of putative proteases and acetyltransferases including XopJ from *X. campestris* pv. *vesicatoria* strain 85-10 and HopZ4 from *P. syringae* interfere with the activity of the proteasome. The analysis of fluorogenic peptide substrates revealed that the proteasome activity in

N. benthamiana was reduced in the presence of XopJ but not of a derivative thereof with a mutation in the catalytic cysteine residue (Üstün, Bartetzko and Börnke 2013). This suggests that the enzymatic activity of XopJ is required for the suppression of the proteasome. In agreement with the observed influence of XopJ on the proteasome, infection of pepper leaves with an *X. campestris* pv. *vesicatoria xopJ* deletion mutant led to increased proteasome activity when compared with leaves infected with the wild-type strain (Üstün, Bartetzko and Börnke 2013). XopJ interacts with and degrades the ATPase RPT6 (regulatory particle ATPase 6) of the 19S regulatory particle of the 26S proteasome at the plant plasma membrane (Üstün, Bartetzko and Börnke 2013; Üstün and Börnke 2015) (Fig. 4F). It was, therefore, proposed that XopJ acts as a protease and interferes with the activity of the proteasome by targeting RPT6. Similarly to XopJ, the homologous HopZ4 protein from *P. syringae* pv. *lacrymans* binds to RPT6 and interferes with proteasome activity (Üstün *et al.* 2014) (Fig. 4F). Furthermore, the related effector protein AvrBsT from *X. campestris* pv. *vesicatoria* interacts with the 19S subunit RPN8 of the proteasome in yeast (Szczesny *et al.* 2010), suggesting that the proteasome is a virulence target of several YopJ family members.

XopP from *X. oryzae* pv. *oryzae* inhibits the activity of the E3 ubiquitin ligase OsPUB44 from rice

The effector protein XopP from *X. oryzae* pv. *oryzae* interacts with the E3 ubiquitin ligase OsPUB44 from rice (Ishikawa *et al.* 2014) (Fig. 4G). OsPUB44 presumably contributes to basal plant defense responses because rice *OsPUB44* RNAi lines promote growth of *X. oryzae* pv. *oryzae* wild-type and non-pathogenic mutant strains (Ishikawa *et al.* 2014). The results of interaction studies and *in vitro* activity assays revealed that XopP binds to the U-box domain of OsPUB44 and inhibits its E3 ubiquitin ligase activity (Ishikawa *et al.* 2014). XopP was, therefore, suggested to suppress plant defense by interfering with the activity of OsPUB44.

MODULATION OF PHYTOHORMONE SIGNALING BY TYPE III EFFECTORS

Phytohormones are chemical messengers, which initiate signaling responses during various cellular processes such as plant growth, development, reproduction and responses to biotic and abiotic stress. Phytohormones usually do not function independently of each other but are often controlled by a regulatory network, which links different hormone responses (Robert-Seilaniantz, Grant and Jones 2011; Gimenez-Ibanez and Solano 2013). Signaling by several hormones such as auxin, jasmonic acid (JA), ethylene (ET) and salicylic acid (SA) involves the proteasomal degradation of transcriptional repressors and the release or activation of transcription factors, which lead to hormone-induced gene expression. Research in the model plant *Arabidopsis* revealed that JA, SA and ET are key players in plant defense against microbial pathogens. While SA is usually involved in resistance against biotrophic and hemibiotrophic pathogens, JA and ET can act antagonistically to SA and promote resistance against necrotrophic pathogens (Glazebrook 2005) (Fig. 5). Due to the antagonistic interplay between JA and SA, the activation of JA-dependent defense responses often represses SA-induced signaling pathways, which are usually mounted upon infection by biotrophic pathogens (Gimenez-Ibanez and Solano 2013; Kazan and Lyons 2014). In addition to JA, SA and ET, recent studies revealed a role of other phytohormones including auxin, cytokinins, brassinosteroids, abscisic acid and gibberellins in plant–pathogen interactions (Gimenez-Ibanez and Solano 2013; Kazan and Lyons 2014).

Plant-pathogenic bacteria produce phytohormone mimics to interfere with hormone signaling pathways. One prominent example is the phytotoxin coronatine, which is synthesized by a few pathovars of *P. syringae* and mimicks the action of bioactive JA-isoleucine (JA-Ile) (Katsir *et al.* 2008). In addition to phytohormone mimics, plant-pathogenic bacteria deliver type III effector proteins to interfere with hormone signaling pathways. Effectors, which interfere with JA signaling pathways, include the cysteine protease HopX1, the acetyltransferase HopZ1a and AvrB from *P. syringae* (see below). Furthermore, the cysteine protease AvrRpt2 from *P. syringae* was shown to interfere with auxin signaling, whereas XopD from *X. campestris* pv. *campestris* modulates gibberellic acid (GA) and ET pathways. The functions of HopX1, HopZ1a, AvrB, AvrRpt2 and XopD are desribed below and summarized in Figs 5 and 6.

The *P. syringae* effectors HopX1 and HopZ1a promote the degradation of JAZ proteins

HopX1 and HopZ1a from *P. syringae* target JAZ (jasmonate ZIM-domain) proteins, which are involved in JA signaling. JAZ proteins act as transcriptional repressors and interact with and inhibit transcription factors. JAZ proteins are degraded by the proteasome in the presence of JA-Ile, which is perceived by the JA receptor and F-box protein COI1 (coronatine insensitive 1). COI1 is a component of the SCF complex (Shabek and Zheng 2014) and associates upon binding of JA-Ile with JAZ proteins to promote their proteasome-dependent degradation (Chini *et al.* 2007; Thines *et al.* 2007; Sheard *et al.* 2010). This leads to the release of JAZ-interacting transcription factors and thus to the activation of JA-responsive gene expression (Robert-Seilaniantz, Grant and Jones 2011) (Fig. 6A).

The effector protein HopX1 is a cysteine protease and is delivered by *P. syringae* pv. *tabaci* strain 11528, which does not produce coronatine (Gimenez-Ibanez *et al.* 2014). Transient coexpression studies in *N. benthamiana* revealed that HopX1 directly or indirectly degrades at least eight out of 12 JAZ protein family members (Gimenez-Ibanez *et al.* 2014). The HopX1-mediated degradation of JAZ proteins occurs independently of the JA receptor COI1 and leads to the activation of JA-responsive genes as well as to the repression of SA-induced signaling pathways. It was, therefore, assumed that HopX1 directly or indirectly degrades JAZ proteins independently of the SCF complex (Gimenez-Ibanez *et al.* 2014) (Fig. 6A).

JAZ proteins are also targeted by HopZ1a from *P. syringae*. Yeast two-hybrid, pull-down and BiFC studies revealed that HopZ1a interacts with JAZ proteins from soybean and *Arabidopsis* at the plasma membrane and in the nucleus (Jiang *et al.* 2013). HopZ1a leads to the degradation of JAZ proteins and the induction of JA-responsive genes in *Arabidopsis* when delivered by a coronatine-deficient mutant derivative of *P. syringae* pv. *tomato* DC3000 (Jiang *et al.* 2013) (Fig. 6A). No effect of HopZ1a on JAZ proteins was observed in *Arabidopsis coi1* mutants, suggesting that HopZ1a exploits the proteasome to induce the degradation of JAZ proteins (Jiang *et al.* 2013). A transferase assay with ^{14}C-labeled acetyl-CoA revealed that HopZ1a acetylates JAZ proteins as well as additional plant targets including tubulin (see below) and the pseudokinase ZED1 (*hopZ1*-ETI deficient) (Lee *et al.* 2012; Jiang *et al.* 2013; Lewis *et al.* 2013). The activity of HopZ1a depends on the presence of phytic acid (also known as phytate or

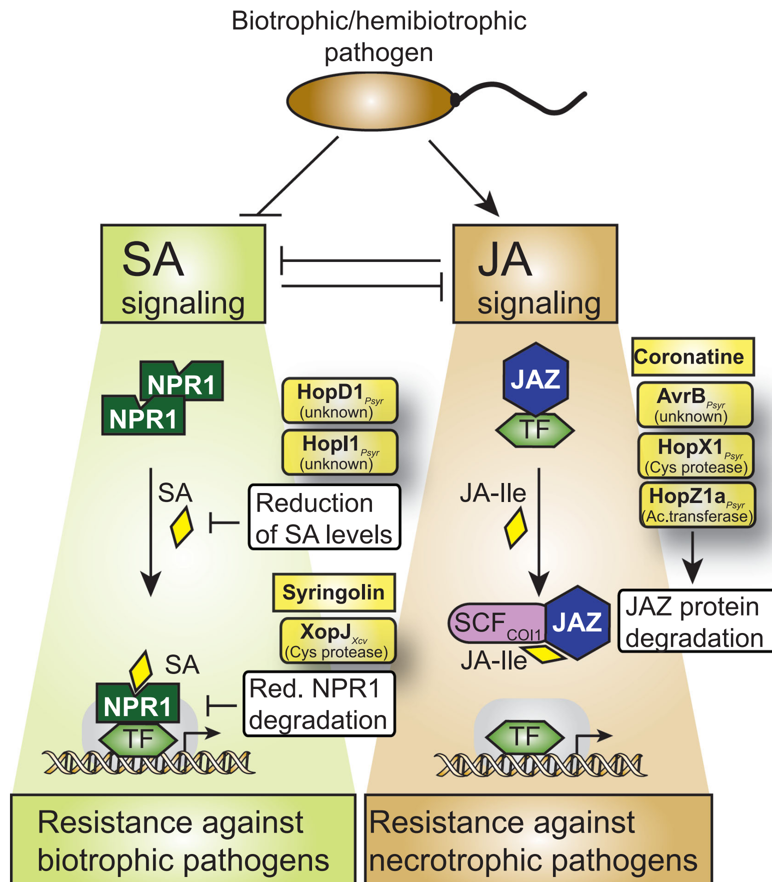

Figure 5. Interference of type III effectors with SA and JA signaling pathways. SA-dependent defense responses are required for plant resistance against biotrophic pathogens whereas JA-dependent defense is mounted against necrotrophic pathogens. SA and JA pathways thus act antagonistically and can suppress each other. Type III effectors from biotrophic or hemibiotrophic pathogens activate JA signaling pathways and suppress SA-mediated defense by the actions of translocated type III effectors. SA-dependent defense responses depend on NPR1 (non-expressor of PR genes), which is present in an oligomeric inactive state in the absence of SA. Upon binding of SA, monomeric NPR1 binds to and activates transcription factors (TF) and thus induces the expression of SA-dependent genes (Gimenez-Ibanez and Solano 2013). The effectors HopD1 and HopI1 from *P. syringae* lead to reduced SA levels whereas the bacterial toxin syringolin and the effector XopJ from *X. campestris* pv. *vesicatoria* interfere with the degradation of NPR1. The turnover of NPR1 is required for the expression of SA-responsive genes. Stabilization of NPR1, therefore, suppresses SA signaling (Robert-Seilaniantz, Grant and Jones 2011). JA signaling pathways involve JAZ proteins and the SCF complex. The bacterial toxin coronatine and the effector proteins AvrB, HopX1 and HopZ1 from *P. syringae* lead to the degradation of JAZ proteins and thus activate the expression of JA-responsive genes (see the text for details).

IP6), which is a cofactor of YopJ family members, and induces a conformational change in HopZ1a (Lee *et al.* 2012; Ma *et al.* 2015). It remains to be investigated whether the acetylation of JAZ proteins by HopZ1a facilitates their COI1-dependent degradation.

AvrB promotes the interaction between JAZ proteins and COI1 through RIN4 and AHA1

The type III effector AvrB from *P. syringae* can substitute coronatine for the induction of JA-responsive genes and stomatal opening (He *et al.* 2004; Zhou *et al.* 2015). The regulation of stomatal opening involves the autoinhibited plasma membrane H(+) ATPases AHA1 and AHA2, which pump protons from the cytosol into the apoplast. This leads to the establishment of a proton electrochemical gradient, which is utilized by channel and carrier proteins to mediate the uptake of charged solutes into cells (Sondergaard, Schulz and Palmgren 2004; Liu *et al.* 2009; Elmore and Coaker 2011). Increased concentrations of charged solutes in the guard cells result in a water uptake and elevated turgor, thus leading to stomatal opening. The induction of stomatal opening by AvrB depends on the F-box protein COI1 and the immune regulator RIN4, which interacts with AvrB (Mackey *et al.* 2002; He *et al.* 2004; Cui *et al.* 2010; Zhou *et al.* 2015) (see above). RIN4 also interacts with AHA1 and promotes its activity (Liu, Elmore and Coaker 2009) (see below). Transient expression studies in *N. benthamiana* revealed that AHA1, RIN4 and AvrB trigger the degradation of JAZ proteins, suggesting a link between JA signaling, the RIN4-AvrB interaction and stomatal opening (Zhou *et al.* 2015). Furthermore, coimmunprecipitation experiments showed that AvrB and AHA1 promote the interaction between COI1 and the JAZ protein JAZ9 (Zhou *et al.* 2015). Given that AvrB induces the phosphorylation of RIN4 and that RIN4 positively regulates AHA proteins (Liu *et al.* 2009), it was suggested that AvrB promotes the COI1-JAZ interaction through RIN4 and AHA1 and thus leads to the degradation of JAZ proteins (Zhou *et al.* 2015) (Fig. 6A). The biochemical mechanism underlying the AvrB-RIN4-AHA1-mediated induction of COI1-JAZ interactions is yet unknown.

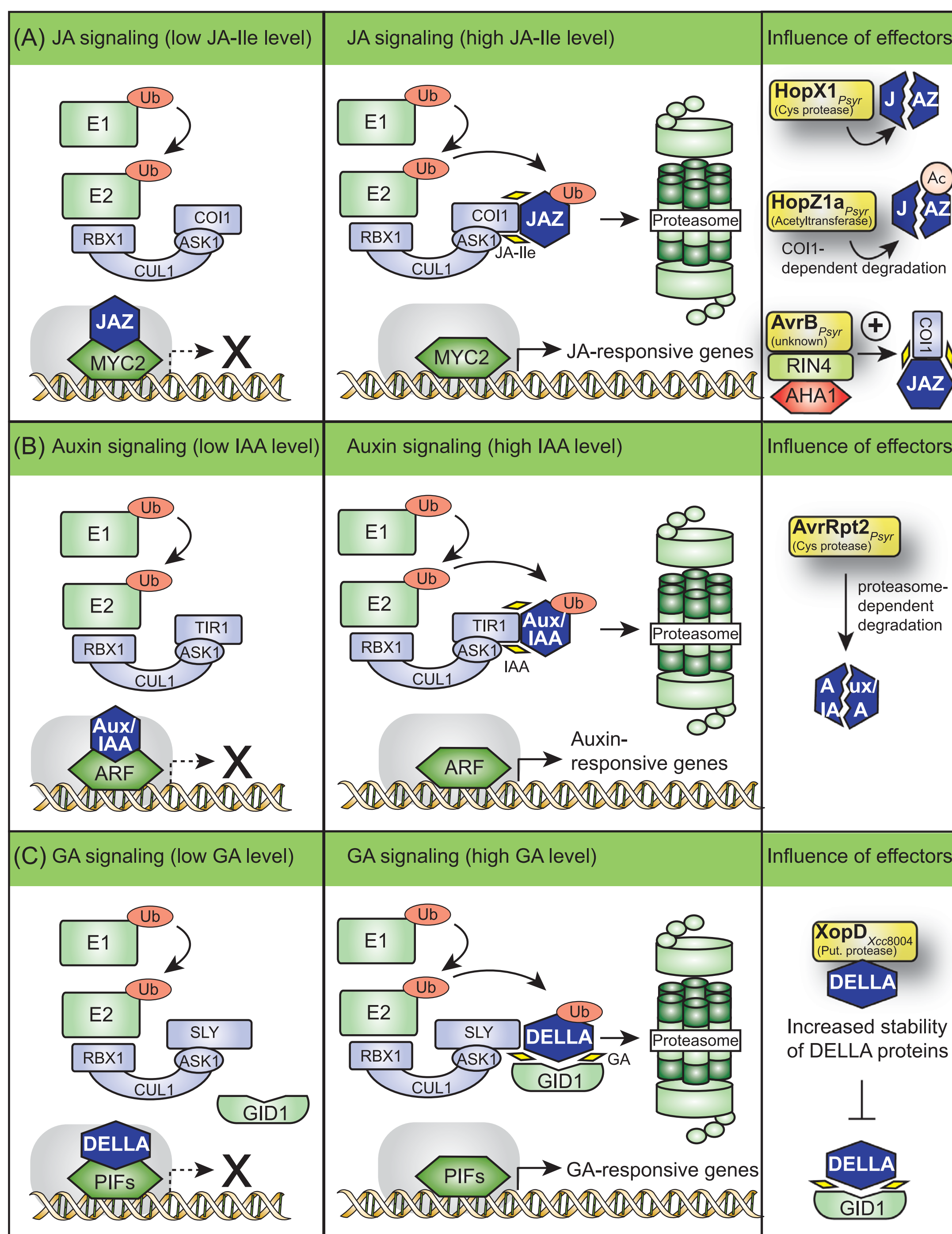

Figure 6. Modulation of JA, auxin and GA signaling pathways by type III effectors. (**A**) HopX1, HopZ1a and AvrB from *P. syringae* interfere with JA signaling pathways. Bioactive JA-Ile promotes the interaction between JAZ proteins and the F-box protein COI1, which is a component of the SCF complex. The subsequent degradation of JAZ proteins leads to the release of JAZ-interacting transcription factors (e.g. MYC2), which activate the expression of JA-responsive genes. The cysteine protease HopX1 directly or indirectly degrades several JAZ proteins independently of the JA receptor COI1 and thus activates the expression of JA-responsive genes. The acetyltransferase HopZ1a acetylates JAZ proteins and leads to their proteasome-dependent degradation. The effector protein AvrB from *P. syringae* interacts with RIN4, which is a negative regulator of PTI and associates with the H^+ ATPase AHA1. The interaction of AvrB with the RIN4-AHA1 complex promotes the interaction between JAZ proteins and COI1 and leads to the activation of JA-responsive genes. (**B**) Auxin signaling pathways are targeted by the cysteine protease AvrRpt2 from *P. syringae*. IAA promotes the interaction between Aux/IAA proteins and the F-box protein TIR1. This leads to the proteasome-dependent degradation of Aux/IAA proteins and to the release and activation of ARFs. ARFs subsequently activate the expression of auxin-responsive genes. The cysteine protease AvrRpt2 directly or indirectly induces the degradation of Aux/IAA proteins by the proteasome and thus activates the expression of auxin-responsive genes. (**C**) XopD from *X. campestris* pv. *campestris* strain 8004 interferes with the stability of DELLA proteins, which are negative regulators of GA-responsive genes. GA-dependent signaling is controlled by DELLA proteins, which inactivate PIF (phytochrome interacting factors) transcription factors. Binding of GA to its receptor GID1 leads to a conformational change in GID1, which subsequently binds to DELLA proteins. The formation of a GID1-DELLA complex promotes the interaction between DELLA proteins and the F-box protein SLY and thus the proteasome-dependent degradation of DELLA proteins. This leads to the release of PIF transcription factors, which activate the expression of GA-responsive genes. $XopD_{Xcc8004}$ presumably interferes with the binding of GID1 to DELLA proteins and delays the GA-induced degradation of the DELLA protein RGA. Notably, however, an influence of $XopD_{Xcc8004}$ on the transcription of GA-responsive genes has not yet been detected.

The cysteine protease AvrRpt2 promotes the degradation of Aux/IAA proteins and leads to the activation of auxin-responsive genes

The cysteine protease AvrRpt2 from *P. syringae* interferes with auxin signaling and was shown to induce the expression of auxin-responsive genes (Chen *et al.* 2007). Expression studies in *Arabidopsis* protoplasts and transgenic plants revealed that AvrRpt2 enhances the degradation of the Aux/IAA (auxin/indole acetic acid) transcription repressor protein AXR2 and promotes the effect of auxin (Cui *et al.* 2013). Members of the Aux/IAA family of transcription suppressors bind to and inactivate auxin response factors (ARFs) and thus suppress the expression of auxin-responsive genes (Fu and Wang 2011). In the presence of auxin, Aux/IAA proteins interact with the auxin receptor and F-box protein TIR1 (transport inhibitor response 1), which is a component of the SCF complex (Fig. 6B). This promotes the degradation of Aux/IAA proteins by the proteasome and leads to the release of ARFs, which activate the expression of auxin-responsive genes (Fu and Wang 2011).

The AvrRpt2-induced degradation of the Aux/IAA protein AXR2 was abolished in the presence of proteasome inhibitor or upon mutation of catalytic residues in AvrRpt2. This suggests that the proteasome and the cysteine protease activity of AvrRpt2 are required to induce the degradation of Aux/IAA proteins (Cui *et al.* 2013). Given that a direct cleavage of AXR2 by AvrRpt2 was not observed *in vitro*, the AvrRpt2-triggered degradation of Aux/IAA proteins likely involves additional plant proteins (Cui *et al.* 2013). In addition to the interference with auxin-regulated gene expression, AvrRpt2 was recently shown to promote bacterial growth in a COI1-dependent manner (Geng *et al.* 2016). It was, therefore, proposed that AvrRpt2 also interferes with JA signaling pathways and thus suppresses SA-mediated defense (Geng *et al.* 2016).

XopD proteins from *Xanthomonas* modify the stability of an ET-responsive transcription factor (ERF) and DELLA proteins

Members of the XopD family of nuclear-localized effector proteins from *Xanthomonas* spp. contain a C-terminal cysteine protease domain and ERF-associated amphiphilic repression (EAR) motifs, which were previously described for plant transcriptional regulators (Kazan 2006). Sequence comparisons revealed variations in the domain organization of XopD family members. While XopD from *X. campestris* pv. *vesicatoria* ($XopD_{Xcv}$) and *X. campestris* pv. *campestris* strain B100 ($XopD_{XccB100}$) contains N-terminal extensions and a central putative DNA-binding helix-loop-helix (HLH) domain, these regions are absent in XopD from *X. campestris* pv. *campestris* strain 8004 ($XopD_{Xcc8004}$) (Canonne *et al.* 2010; Kim, Taylor and Mudgett 2011) (Fig. 7A). The XopD cysteine protease domain shares structural similarity with the yeast ubiquitin-like protease ULP1 and was shown to cleave tomato SUMO from SUMOylated proteins (Hotson *et al.* 2003; Chosed *et al.* 2007). $XopD_{Xcv}$ deSUMOylates and thus destabilizes SlERF4 from *Solanum lycopersicum* (Kim, Stork and Mudgett 2013). As the presence of proteasome inhibitor interferes with the XopD-induced destabilization of SlERF4, it was suggested that XopD facilitates the degradation of SlERF4 by the proteasome (Kim, Stork and Mudgett 2013). SlERF4 is presumably involved in the regulation of ET biosynthesis and colocalizes with XopD to subnuclear foci (Kim, Stork and Mudgett 2013). In agreement with the observed XopD-mediated destabilization of SlERF4, $XopD_{Xcv}$ leads to reduced ET levels in infected plant tissue and suppresses the expression of genes involved in ET production (Kim, Stork and Mudgett 2013). Given that ET production is required for plant immunity, $XopD_{Xcv}$ likely deSUMOylates SlERF4 to suppress plant defense responses (Kim, Stork and Mudgett 2013). Notably, however, $XopD_{Xcv}$ also suppresses SA- and JA-induced gene transcription (Kim *et al.* 2008a).

Interference with hormone signaling was also shown for the XopD family member $XopD_{Xcc8004}$, which interacts with DELLA proteins (Tan *et al.* 2014) (Figs 6C and 7A). DELLA proteins are negative regulators of GA response activators and colocalize with $XopD_{Xcc8004}$ to the plant nucleus. The degradation of DELLA proteins by the proteasome is stimulated in the presence of GA, which binds to its receptor GID1 (gibberellin insensitive dwarf 1) (Hauvermale, Ariizumi and Steber 2012). GA promotes the interaction between GID1 and DELLA proteins, which are subsequently targeted to the F-box protein SLY and degraded by the proteasome. The degradation of DELLA proteins leads to the activation of GA response activators, which induce the expression of GA-responsive genes (Hauvermale, Ariizumi and Steber 2012) (Fig. 6C). $XopD_{Xcc8004}$ delays the GA-induced degradation of the DELLA protein RGA (Tan *et al.* 2014). The N-terminal EAR domain of $XopD_{Xcc8004}$ interacts with the DELLA domain of RGA, which contains the conserved DELLA motif and is required for the GA-induced degradation of DELLA proteins (Sun and Gubler 2004; Tan *et al.* 2014). It is speculated that the N-terminal EAR domain of $XopD_{Xcc8004}$ interferes with the binding of GID1 to DELLA proteins (Tan *et al.* 2014). Notably, however, despite the observed effect of $XopD_{Xcc8004}$ on DELLA protein stability, no influence on the transcription of GA-responsive genes was detected (Tan *et al.* 2014).

MODULATION OF PLANT GENE EXPRESSION BY TYPE III EFFECTORS

One effective strategy employed by type III effectors to interfere with plant cellular processes is the manipulation of gene expression on the transcriptional or posttranscriptional level. Effector proteins, which are directly imported into the nucleus and either bind to DNA or to components of the plant transcription machinery, are transcription activator-like (TAL) effectors from *Xanthomonas* spp. and the effector protein HsvG from *Pantoea agglomerans* (see below). Type III effectors, which target plant transcription factors and RNA-binding proteins, include XopD proteins from *Xanthomonas* spp., PopP2 from *R. solanacearum* as well as HopU1, HopD1 and HopM1 from *P. syringae*. Known mechanisms underlying type III effector-mediated modulation of plant gene expression are summarized below and in Fig. 7.

TAL effectors from *Xanthomonas* spp. bind to sequence-specific promoter elements of plant target genes

Members of the TAL effector family were mainly isolated from *Xanthomonas* spp. However, related proteins are also present in *R. solanacearum* and *Burkholderia rhizoxinica* (de Lange *et al.* 2013, 2014; Li *et al.* 2013a; Juillerat *et al.* 2014). Characteristic features of TAL effectors include a C-terminal acidic activation domain and nuclear localization signals (NLSs), which are required for the import of TAL effectors into the plant nucleus (Boch and Bonas 2010). DNA binding is mediated by the central region of TAL effectors, which consists of 1.5 to 33.5 repeats with an average of approximately 17 repeats (Boch and Bonas 2010) (Fig. 7B). A minimum of 6.5 repeats is required to induce target gene

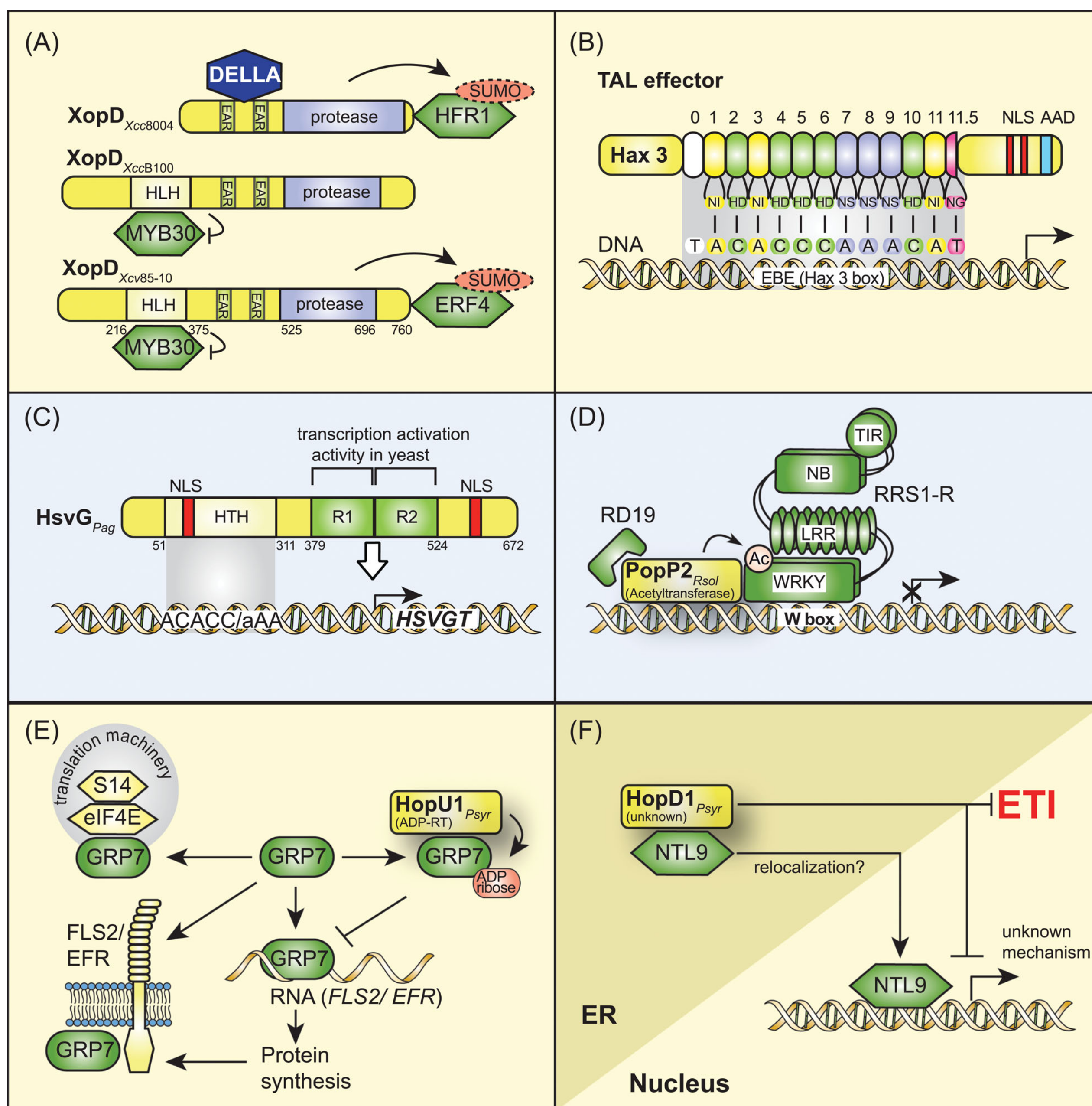

Figure 7. Interference of type III effector proteins with plant gene expression. (**A**) Domain organization of XopD proteins from *Xanthomonas* spp. XopD family members consist of a C-terminal cysteine protease domain and N-terminal EAR motifs. Additionally, XopD from *X. campestris* pv. *vesicatoria* strain 85-10 (XopD$_{Xcv85\text{-}10}$) and *X. campestris* pv. *campestris* strain B100 (XopD$_{XccB100}$) contain N-terminal extensions and a central putative DNA-binding HLH domain. XopD$_{Xcc8004}$ was shown to interact with and stabilize DELLA proteins via the EAR motif-containing region. Furthermore, XopD$_{Xcc8004}$ interacts with and deSUMOylates the transcription factor HFR1. XopD$_{XccB100}$ and XopD$_{Xcv85\text{-}10}$ bind to the transcription factor MYB30 via the HLH domain and suppress its transcriptional activity. XopD$_{Xcv}$ deSUMOylates and thus destabilizes the transcription factor ERF4. Numbers refer to amino acid positions in XopD$_{Xcv85\text{-}10}$. (**B**) Domain organization and DNA-binding specificity of the TAL effector Hax (homolog of AvrBs3 in *Xanthomonas*) 3 from *X. campestris* pv. *armoraciae*. TAL effectors contain a C-terminal acidic activation domain (AAD), two NLSs and a central protein region with repeats. The RVDs of Hax3 and the matching bases in the EBE in the promoter regions of Hax3-induced genes are indicated. (**C**) Domain organization of HsvG from *P. agglomerans* (*Pag*). HsvG contains N- and C-terminal NLSs, an N-terminal HTH region and two repeats of 71 and 74 amino acids (R1 and R2), which confer transcription activation activity in yeast. The repeats determine the specificity of plant gene activation (indicated by a white arrow) but are dispensable for DNA binding of HsvG, which depends on the N-terminal region. Numbers refer to amino acid positions in HsvG. (**D**) Modification of RRS1-R by the effector protein PopP2 from *R. solanacearum*. The TIR-NB-LRR protein RRS1-R forms a dimer and binds via its WRKY domain to a DNA motif (W box) present in promoters of target genes of WRKY transcription factors. PopP2 interacts with and acetylates the WRKY domain of RRS1-R and thus interferes with its DNA binding. The additional PopP2 interaction partner RD19, which is a predicted protease, is presumably not acetylated by PopP2. RRS1-R also interacts with the R protein RPS4, which is required for the induction of ETI and is not shown in this figure (see the text for details). (**E**) The mono-ADP-RT HopU1 from *P. syringae* pv. *tomato* DC3000 ADP-ribosylates the RNA-binding protein GRP7, which interacts with components of the translational machinery including the cap-binding protein eIF4E and the ribosomal subunit S14. GRP7 also interacts with the PRRs FLS2 and EFR and with *FLS2* and *EFR* transcripts, and was, therefore, assumed to promote PRR translation. ADP-ribosylation of GRP7 by HopU1 reduces the ability of GRP7 to bind to RNA and might suppress FLS2 and EFR protein synthesis. (**F**) HopD1 from *P. syringae* pv. *tomato* DC3000 interacts with the transcription factor NTL9 at the ER and leads to reduced expression of NTL9-induced genes during ETI. Furthermore, HopD1 suppresses ETI responses. The mechanisms underlying the HopD1-mediated inhibition of NTL9-dependent gene expression are unknown.

expression (Boch *et al.* 2009). The repeats are nearly amino acid sequence identical and usually 33 to 35 amino acids long, but longer and shorter repeats have also been described (Boch and Bonas 2010).

Sequence-specific binding to DNA bases depends on the polymorphic amino acids at positions 12 and 13 of each repeat of the TAL effector. These amino acids are referred to as RVDs (repeat variable diresidues) and are exposed on a short loop located between two nearly identical alpha helices (Deng *et al.* 2012; Mak *et al.* 2012). Direct DNA contact is mediated by the RVD at position 13 (also referred to as base-specifying residue), whereas the RVD at position 12 stabilizes the loop (Deng *et al.* 2012; Mak *et al.* 2012). The RVDs determine the binding specificity of TAL effectors to DNA. Common RVDs are HD, NG, NN and NI (letters refer to amino acid residues), which bind to cytosine (HD), thymine (NG), guanine or adenine (NN), and adenine (NI), respectively (Boch *et al.* 2009; Moscou and Bogdanove 2009).

In the past years, numerous studies have focused on the analysis of the binding specificity of TAL effectors to the effector-binding elements (EBEs) in the promoter regions of plant target genes. Repeat number and RVDs determine the number and nature of DNA bases, which are bound by the TAL effectors. Furthermore, different RVDs differ in DNA-binding affinity and thus contribute differently to TAL effector activity (Streubel *et al.* 2012; Meckler *et al.* 2013). Replacement of the natural RVDs of specific repeats by all possible 400 RVD combinations revealed that not all artificial RVDs are functional. Optimal reporter gene activation was observed with TAL effectors containing naturally occurring RVD combinations (Yang *et al.* 2014). In addition to the RVD composition, the length of the repeats affects the binding to DNA bases. Experimental evidence suggests that shorter or longer repeats bind to matching nucleotides of the EBE or are excluded from binding (Richter *et al.* 2014). The looping-out of repeats allows a shift of the following repeats by one nucleotide position in the EBE. This provides flexibility in the DNA-binding activities of TAL effectors and enables TAL effectors to bind to promoter elements in which the EBEs have been modified by insertion/deletion mutations (Richter *et al.* 2014). Notably, however, DNA binding of TAL effectors to EBEs is not only determined by the RVDs but also by N-terminal non-canonical repeats as well as C-terminal protein regions and can be affected by epigenetic modifications of the DNA (Boch, Bonas and Lahaye 2014; Schreiber *et al.* 2015).

The groundbreaking discovery of the TAL-DNA-binding code has marked the beginning of a new era in genome engineering because it has led to the design of various genome editing tools (e.g. TAL effector nucleases), which allow sequence-specific binding of DNA-modifying enzymes by the use of TAL effector repeats as fusion partners (Scharenberg, Duchateau and Smith 2013; Mahfouz, Piatek and Stewart 2014). The mechanisms leading to transcriptional activation of plant genes by TAL effectors are not yet understood. The results of interaction studies suggest that TAL effectors interact with RNA polymerase II (Domingues *et al.* 2012), and with negative regulators of RNA polymerase II and III, respectively (de Souza *et al.* 2012; Soprano *et al.* 2013) (Table 1). It is, therefore, assumed that TAL effectors do not only bind to DNA but also associate with components of the plant transcription machinery to activate gene expression.

Among the plant genes targeted by TAL effectors are those encoding transcription factors and proteins involved in senescence, development, stress response and sugar transport (Table S2, Supporting Information). Examples are the *SWEET* genes from rice, which are involved in sucrose or fructose transport and are induced by TAL effectors from the systemic rice pathogen *X. oryzae* pv. *oryzae* (Boch, Bonas and Lahaye 2014; Chen 2014) (Table S2). TAL target genes, which contribute to virulence, are also referred to as plant susceptibility genes. Notably, however, TAL effectors can also induce the expression of plant resistance (*R*) genes and thus trigger ETI responses (Boch, Bonas and Lahaye 2014). TAL effector-responsive *R* genes have been categorized into different groups including recessive and dominant *R* genes. Recessive *R* genes have evolved from *S* genes and contain mutations in the EBE, which abolish TAL effector binding (Hutin *et al.* 2015). In this case, resistance is the result of the loss of induction of an *S* gene. Dominant *R* genes such as *Bs4* from tomato, which elicits ETI in response to the TAL effector AvrBs4 (Schornack *et al.* 2004), confer non-transcriptional-based resistance upon recognition of corresponding TAL effectors. The third group of TAL effector-responsive *R* genes are executor *R* genes, which contain EBEs in their promoter regions and are specifically activated by matching TAL effectors (Zhang, Yin and White 2015). Examples are *Bs3* from pepper, and *Xa10*, *Xa23* and *Xa27* from rice (Table S2). The engineering of executor *R* gene promoters allows gene induction by various TAL effectors and might help to improve strategies for plant resistance and disease control (Boch, Bonas and Lahaye 2014).

The effector protein HsvG from *P. agglomerans* binds to DNA and activates plant gene expression

DNA binding has also been described for the effector protein HsvG, which is an important pathogenicity factor of the gall-forming plant-pathogenic bacterium *P. agglomerans* pv. *gypsophilae* (Valinsky *et al.* 1998; Nissan *et al.* 2006). HsvG is homologous to the type III effector HsvB, which is required for pathogenicity of *Pa. agglomerans* pv. *betae* on beet (Nissan *et al.* 2006). Both effectors localize to the plant cell nucleus and harbor N- and C-terminal NLSs as well as an N-terminal helix-turn-helix (HTH) domain (Nissan *et al.* 2006; Weinthal *et al.* 2011) (Fig. 7C). HsvG and HsvB mainly differ in the C-terminal region, which contains two tandem direct repeats of 71 and 74 amino acids in HsvG whereas one repeat is missing in HsvB (Nissan *et al.* 2006). The analysis of deletion derivatives of HsvG revealed that the presence of two repeats is required for the contribution of HsvG to bacterial pathogenicity on *gypsophila* (Nissan *et al.* 2006).

The results of yeast one-hybrid assays suggest that HsvG acts as a transcriptional activator. One repeat of HsvG is sufficient for the transcriptional activation of reporter genes in yeast (Nissan *et al.* 2006). Notably, in contrast to TAL effectors, DNA binding of HsvG does not depend on the repeats but on an N-terminal protein region consisting of 14 predicted alpha helices and one beta strand (Nissan *et al.* 2012). A random binding-site selection procedure revealed that HsvG binds to DNA sequences with the consensus motif ACACC/aAA (Nissan *et al.* 2006), which is present in the promoter of the HsvG target gene *HSVGT* from *gypsophila* (Nissan *et al.* 2012). *HSVGT* encodes a protein with homology to the chaperone DnaJ and contains predicted NLSs as well as DNA-binding motifs, suggesting that it acts as a transcription factor (Nissan *et al.* 2012). Expression of *HSVGT* is specifically induced by HsvG as well as by a modified HsvB derivative containing one additional repeat from HsvG (Nissan *et al.* 2012). These findings suggest that the repeats and not the DNA-binding region of HsvG and HsvB determine the specificity in target gene activation.

XopD proteins from *Xanthomonas* spp. target plant transcription factors

Among the nuclear localized effectors, which presumably interfere with plant gene expression, are members of the XopD family from *Xanthomonas* spp. As described above, XopD family members interfere with hormone signaling and cleave SUMO from SUMOylated proteins. Furthermore, $XopD_{Xcc8004}$ binds to and deSUMOylates the basic HLH transcription factor HFR1 in subnuclear foci (Tan *et al.* 2015) (Fig. 7A), suggesting that XopD family members target plant transcription regulators to promote bacterial virulence. This hypothesis is supported by the finding that $XopD_{Xcv}$ and $XopD_{XccB100}$ interact with the transcription factor MYB30, which acts as a positive regulator of hypersensitive cell death responses and resistance to avirulent pathogens in *Arabidopsis* (Canonne *et al.* 2011; Raffaele and Rivas 2013) (Fig. 7A). No interaction with AtMYB30 was observed for $XopD_{Xcc8004}$, which lacks the HLH domain (Canonne *et al.* 2011). Domain swapping experiments with $XopD_{Xcv}$ and $XopD_{Xcc8004}$ suggest that the central HLH domain of $XopD_{Xcv}$ is required for the interaction with AtMYB30 (Canonne *et al.* 2011).

$XopD_{Xcv}$ and $XopD_{XccB100}$ induce the relocalization of MYB30 from the nucleus to subnuclear foci and suppress its transcriptional activity (Canonne *et al.* 2011). In agreement with this finding, $XopD_{XccB100}$ reduces the expression of AtMYB30 target genes in *Arabidopsis* during the natural infection (Canonne *et al.* 2011). It was, therefore, suggested that XopD family members suppress plant defense responses by targeting MYB30.

PopP2 from *R. solanacearum* acetylates RRS1-R and WRKY transcription factors and interferes with the transcription of plant genes

Another effector protein, which modulates plant gene expression, is the YopJ family member and acetyltransferase PopP2 from *R. solanacearum*. PopP2 localizes to the plant cell nucleus and acetylates lysine residues of WRKY transcription factors (Deslandes *et al.* 2003; Le Roux *et al.* 2015; Sarris *et al.* 2015). WRKY transcriptional regulators contain an N-terminal WRKY amino acid motif and a zinc finger structure, and bind to a conserved sequence motif (W box; TTGACC/T) in the promoter of target genes (Rushton *et al.* 2010; Bakshi and Oelmuller 2014; Llorca, Potschin and Zentgraf 2014). Acetylation by PopP2 suppresses the DNA binding of WRKY transcription factors and the transcriptional activation of their target genes (Le Roux *et al.* 2015; Sarris *et al.* 2015). In addition to WRKY transcription factors, PopP2 binds to and acetylates lysine residues in the C-terminal WRKY domain of the R protein RRS1-R from *Arabidopsis* (Deslandes *et al.* 2003; Le Roux *et al.* 2015; Sarris *et al.* 2015) (Fig. 7D). Acetylation of RRS1-R by PopP2 interferes with the binding of RRS1-R to W box-containing DNA sequences and thus with the RRS1-R-mediated activation of gene expression (Le Roux *et al.* 2015; Sarris *et al.* 2015) (see below). Notably, PopP2 does not only acetylate but also stabilizes RRS1-R. Stabilization of RRS1-R by PopP2 is independent of the acetyltransferase activity of PopP2 (Tasset *et al.* 2010).

The mADP-RT HopU1 from *P. syringae* targets RNA-binding proteins and reduces transcript levels of PRR genes during PTI

Type III effectors from plant-pathogenic bacteria do not only bind to DNA or transcription factors but can also interact with RNA-binding proteins. One example is the mADP-RT HopU1 from *P. syringae* pv. *tomato* DC3000, which targets several plant RNA-binding proteins (Fu *et al.* 2007). Among the HopU1 targets are the glycine-rich protein (GRP) 7 and GRP8, which colocalize with HopU1 to the cytoplasm and possibly to the nucleus (Fu *et al.* 2007; Jeong *et al.* 2011). GRP7 is abundantly expressed in guard cells, affects stomatal opening and closing, and contributes to PTI responses in *Arabidopsis* (Fu *et al.* 2007; Kim *et al.* 2008b; Jeong *et al.* 2011). In line with its contribution to PTI, GRP7 interacts with the PRRs FLS2 and EFR at the plant plasma membrane (Nicaise *et al.* 2013). Furthermore, GRP7 binds to RNAs including its own transcript as well as *FLS2* and *EFR* transcripts (Nicaise *et al.* 2013). Given that GRP7 was also found in a complex containing components of the translational machinery such as the cap-binding protein eIF4E and the ribosomal subunit S14, it was assumed that GRP7 promotes PRR translation (Nicaise *et al.* 2013) (Fig. 7E).

The results of coimmunprecipitation experiments suggest that HopU1 interferes with the association of GRP7 with PRR transcripts (Nicaise *et al.* 2013). A site-directed mutagenesis approach revealed that the arginine residue at position 49 of GRP7 is mono-ADP-ribosylated by HopU1 (Jeong *et al.* 2011). R49 is located in the conserved ribonucleoprotein consensus sequence 1 (RNP-1) motif of the RNA recognition motif of GRP7 and is essential for the ability of GRP7 to bind to RNA and to contribute to plant immunity (Fu *et al.* 2007; Jeong *et al.* 2011). ADP-ribosylation of R49 reduces the ability of GRP7 to bind to RNA *in vitro* and therefore likely blocks complex formation between GRP7 and the 3′-untranslated region of *FLS2* (Jeong *et al.* 2011) (Fig. 7E). Taken together, these findings suggest that HopU1 suppresses the GRP7-induced accumulation of FLS2 by ADP-ribosylation of GRP7.

HopD1 from *P. syringae* targets the transcription factor NTL9 at the ER and inhibits ETI responses

HopD1 from *P. syringae* pv. *tomato* DC3000 is an effector protein with unknown enzymatic function, which interacts with the transcription factor NTL9 from *Arabidopsis* (Block *et al.* 2014). NTL9 is a member of the NTLM1 (NAC with transmembrane motif 1) family of transcription factors. This family is one of the largest families of plant transcription factors and is involved in various processes including developmental and stress-related signaling (Nuruzzaman, Sharoni and Kikuchi 2013). At least 13 out of more than 110 NAC transcription family members contain C-terminal transmembrane domains, suggesting that they do not exclusively localize to the nucleus (Kim *et al.* 2007). NTL9 contains an N-terminal DNA-binding domain and a C-terminal transmembrane motif and colocalizes with HopD1 to the endoplasmic reticulum (ER) (Block *et al.* 2014) (Fig. 7F).

NTL9 is preferentially expressed in guard cells (Yoon *et al.* 2008) and activates the isochorismate synthase 1 gene *ICS1*, which encodes a key enzyme in the biosynthesis of SA (Wildermuth *et al.* 2001; Zheng *et al.* 2015). SA is involved in various cellular processes including plant stomatal immunity, i.e. the closure of stomata upon pathogen attack. Stomatal immunity is abolished in *Arabidopsis ntl9* mutant plants and this phenotype is suppressed upon application of SA. It was, therefore, proposed that the contribution of NTL9 to SA synthesis is required for stomatal immunity (Zheng *et al.* 2015). NTL9 was also shown to contribute to ETI responses in *Arabidopsis* (Block *et al.* 2014). A search for genes with a putative DNA-binding site for NTL9 led to the identification of genes, which are induced by NTL9 during ETI (Block *et al.* 2014). Expression of NTL9-induced genes during

ETI is reduced in the presence of HopD1. Several NTL9-regulated genes were also induced upon treatment of plants with flg22; however, no effect of HopD1 on NTL9-regulated gene expression was observed, suggesting that HopD1 specifically interferes with NTL9-regulated gene expression during ETI (Block *et al.* 2014). This is in agreement with the finding that HopD1 specifically suppresses ETI responses (Jamir *et al.* 2004; Guo *et al.* 2009). It was therefore suggested that HopD1 targets NTL9 at the ER to inhibit NTL9-mediated gene expression during ETI (Block *et al.* 2014) (Fig. 7F). Given that HopD1 did not alter the localization or activity of NTL9 (Block *et al.* 2014), the mechanisms underlying the HopD1-mediated inhibition of NTL9-dependent gene expression are still unknown.

HopM1 indirectly targets the transcription factor BZR1

As mentioned above, HopM1 from *P. syringae* triggers the degradation of its interaction partners including the ARF/GEF AtMIN7 and the 14-3-3 protein GRF8/AtMIN10 (Nomura *et al.* 2006, 2011). GRF8/AtMIN10 interacts with BZR1, a major transcription factor in brassinosteroid signaling, and retains BZR1 in the cytoplasm (Gampala *et al.* 2007; Ryu *et al.* 2007). HopM1 leads to an increased accumulation of BZR1 in the nucleus and thus likely interferes with the function of GRF8/AtMIN10 (Lozano-Duran *et al.* 2014). A similar phenomenon was observed upon treatment of plants with AICAR (5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside), which inhibits the interaction of 14-3-3 proteins with their targets (Lozano-Duran *et al.* 2014). The HopM1-induced nuclear accumulation of BZR1 results in the downregulation of BZR1 target genes (Lozano-Duran *et al.* 2014). Taken together, these results suggest that HopM1 targets a 14-3-3 protein to interfere with the activity of a transcriptional repressor.

INTERFERENCE OF TYPE III EFFECTORS WITH THE PLANT CYTOSKELETON

The plant cytoskeleton mainly consists of actin filaments and microtubules and contributes to many processes including cell division and growth, organelle movement, vesicle trafficking, endocytosis, opening of stomata and plant defense responses (Day *et al.* 2011; Henty-Ridilla *et al.* 2013a). The analysis of actin filaments using a GFP (green fluorescent protein)-fABD2 (filamentous actin-binding domain 2) reporter fusion in *Arabidopsis* epidermis cells revealed a transient increase in actin filaments upon infection with *Pseudomonas syringae* pv. *tomato* DC3000 wild-type or T3S mutant strains (Henty-Ridilla *et al.* 2013b) (Fig. 8A). A similar formation of actin filaments was observed upon treatment of plants with PAMPs and was shown to depend on FLS2, BAK1 and BIK1. It was, therefore, suggested that the increase in actin filaments is part of the PTI response (Henty-Ridilla *et al.* 2013b). Twenty-four hours after infection with the *P. syringae* wild-type strain, a reduced number of actin filments and an increase in bundled actin structures were observed (Fig. 8A). No changes were induced by *P. syringae* strains lacking a functional T3S system or multiple effectors, suggesting that the delivery of type III effectors leads to a decrease in actin filaments (Henty-Ridilla *et al.* 2013b; Shimono *et al.* 2016). Infiltration of latrunculin B, which inhibits actin polymerization, promotes susceptibility of *Arabidopsis* leaves to bacterial infections and leads to an increased growth of *P. syringae* pv. *tomato* DC3000 wild-type or T3S mutant strains (Henty-Ridilla *et al.* 2013b). This is in agreement with the predicted contribution of actin filaments to PTI and the observed effector-mediated decrease in actin filaments (Henty-Ridilla *et al.* 2013b; Shimono *et al.* 2016). The actin filament network might also be involved in ETI responses because the actin-depolymerizing factor 4 (ADF4), which is required to sever and disassemble F-actin, contributes to the expression of the *R* gene *RPS5* (Porter *et al.* 2012; Henty-Ridilla *et al.* 2014).

In addition to actin filaments, plant defense against bacterial infections also involves microtubules. Treatment of *Arabidopsis* plants with oryzalin, which disrupts microtubules, enhances the growth of *P. syringae* pv. *tomato* DC3000 but not of a T3S-deficient strain (Lee *et al.* 2012). It was, therefore, suggested that effector proteins from *P. syringae* interfere with microtubule formation to promote bacterial virulence (Lee *et al.* 2012). Given that oryzalin did not enhance the growth of a T3S mutant strain, the disruption of microtubules is presumably not sufficient to counteract PTI responses (Lee *et al.* 2012). The influence of type III effector proteins including HopW1, HopG1 and HopE1 from *P. syringae* and members of the YopJ family on the plant cytoskeleton is described below and summarized in Fig. 8.

HopW1 from *P. syringae* disrupts the actin cytoskeleton

HopW1 from *P. syringae* pv. *maculicola* was found in a complex with actin after transient expression in *N. benthamiana* (Kang *et al.* 2014). Confocal microscopy using the reporter protein Lifeact-GFP, which binds to filamentous actin, showed that the delivery of HopW1 by *P. syringae* pv. *tomato* DC3000 leads to the disruption of the actin cytoskeleton (Kang *et al.* 2014) (Fig. 8B). A disruption of actin filaments was also observed when *hopW1* was transiently expressed in *N. benthamiana* or *Arabidopsis* protoplasts (Kang *et al.* 2014). Furthermore, recombinant HopW1 disassembles filamentous actin *in vitro* (Kang *et al.* 2014). In agreement with the HopW1-mediated destabilization of actin filaments and the contribution of the actin cytoskeleton to protein trafficking, HopW1 inhibits protein trafficking to the ER or the vacuole (Kang *et al.* 2014). As expected, a similar effect on protein trafficking was observed for latrunculin B (Kang *et al.* 2014). HopW1 also interferes with endocytosis as was shown in *Arabidopsis* cotyledons, which were stained with the lipophilic dye FM4-64 (Kang *et al.* 2014). Thus, the delivery of HopW1 by *P. syringae* pv. *tomato* DC3000 leads to a reduction in the number of endosomes during the early stages of infection (Kang *et al.* 2014). The mechanisms by which HopW1 destabilizes actin remain to be elucidated.

HopG1 from *P. syringae* induces actin filament bundling

HopG1 from *P. syringae* pv. *tomato* DC3000 is targeted to the mitochondria of infected plant cells and was shown to interact with a mitochondrial-localized kinesin motor protein (Mukhtar *et al.* 2011; Shimono *et al.* 2016). Kinesin motor proteins are known as microtubule-associated proteins but can also interact with actin filaments and might be involved in the crosstalk and crosslinking between microtubules and actin filaments (Schneider and Persson 2015). The analysis of a *hopG1* deletion mutant revealed that HopG1 induces actin bundling and promotes disease symptom development (Shimono *et al.* 2016). The results of coimmunprecipitation experiments suggest that HopG1 and kinesin associate with actin. No interaction between actin and HopG1 was observed, suggesting that the interaction between HopG1 and actin is indirect and requires the HopG1-interacting kinesin motor protein (Shimono *et al.* 2016). Given the predicted role of kinesin motor proteins in cytoskeleton assembly, it was proposed that HopG1 targets kinesin to induce the formation of actin bundles and thus to promote disease (Shimono *et al.* 2016) (Fig. 8C).

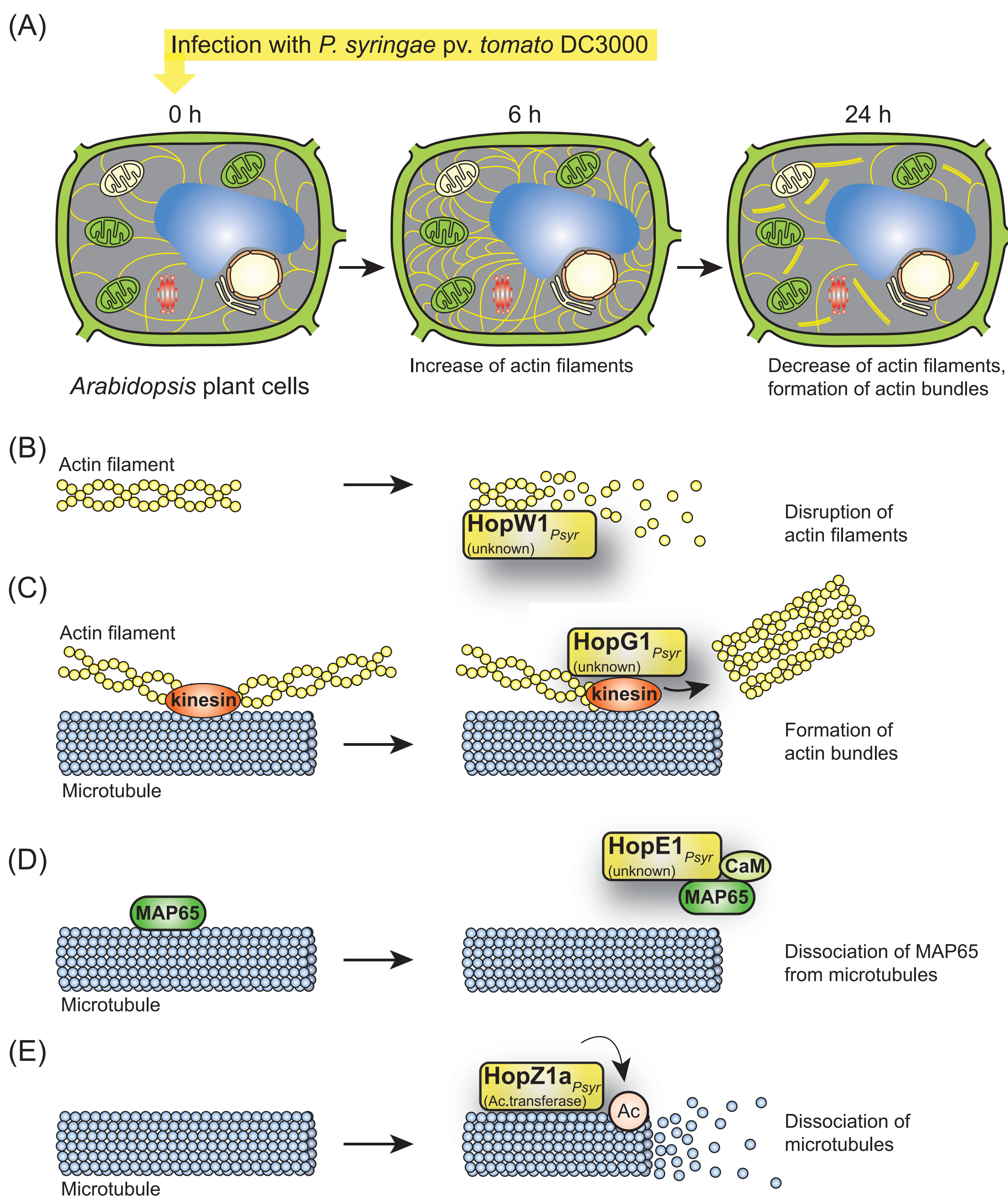

Figure 8. Influence of type III effectors on actin filaments and microtubules. (**A**) Infection of *Arabidopsis* cells with *P. syringae* pv. *tomato* DC3000 leads to alterations in the actin cytoskeleton. The infection with wild-type or T3S mutant strains leads to an increase in actin filaments 6 hours post infection. Twenty-four hours post infection, the wild-type strain induces the formation of actin bundles and leads to a reduced number of actin filaments. Actin filaments and bundles are indicated as yellow dashes. The plant cell wall is represented in green. The following cell organelles are shown: chloroplasts (green), mitochondria (beige), vacuole (blue), nucleus (beige), ER (light brown) and Golgi apparatus (red). (**B**) HopW1 leads to the disruption of actin filaments. The effector protein HopW1 binds to filamentous actin and leads to the disruption of actin filaments. (**C**) HopG1 induces the formation of actin bundles. HopG1 binds to a mitochondrial-localized kinesin motor protein, which associates with microtubules and presumably links microtubules to actin filaments. HopG1 induces the formation of actin bundles, presumably via its interaction with kinesin. (**D**) HopE1 leads to the dissociation of MAP65 from microtubules. HopE1 interacts with calmodulin (CaM) and the microtubule-associated protein MAP65 and leads to the dissociation of MAP65 from microtubules. No effect of HopE1 on the microtubule network was observed. (**E**) HopZ1a from *P. syringae* dissociates microtubules. The acetyltransferase HopZ1a binds to and acetylates tubulin and disrupts microtubules.

HopE1 from *P. syringae* interacts with calmodulin and the microtubule-associated protein MAP65

The effector HopE1 from *P. syringae* pv. *tomato* DC3000 contributes to virulence and suppresses PTI responses when transiently expressed in *Arabidopsis* (Guo *et al.* 2016). Interaction studies revealed that HopE1 interacts with calmodulin and the microtubule-associated protein MAP65, which is required for the crosslinking of anti-parallel microtubule bundles (Guo *et al.* 2016). The results of pull-down experiments and BiFC assays suggest that HopE1, calmodulin and MAP65 are present in the same complex and that calmodulin is required for the inter-

action between HopE1 and MAP65 (Guo *et al.* 2016). HopE1 induces the dissociation of a MAP65-GFP fusion protein from microtubules and inhibits secretion of secGFP, which is a fusion protein between GFP and the secretion signal of the *Arabidopsis* PR-3 protein (Guo *et al.* 2016). No effects were observed for HopE1 derivatives with a mutated calmodulin-binding site, which is located between amino acids 171 and 190 of HopE1 and required for the contribution of HopE1 to *in planta* growth of *P. syringae* pv. *tomato* DC3000 (Guo *et al.* 2016). It was, therefore, proposed that HopE1 in association with calmodulin binds to MAP65 and dissociates MAP65 from microtubules to inhibit protein secretion (Guo *et al.* 2016) (Fig. 8C). *Arabidopsis map65* mutants are impaired in PTI responses and more susceptible to infections with *P. syringae* pv. *tomato* DC3000. Reduced growth of *hopE1* mutants was observed in wild-type but not in *map65* mutant plants, suggesting that MAP65 is an important virulence target of HopE1 (Guo *et al.* 2016). Notably, however, the microtubule network was not significantly altered in the presence of HopE1 (Guo *et al.* 2016).

Members of the YopJ effector family target tubulin or tubulin-binding proteins

The YopJ effector family members HopZ1a from *P. syringae* and AvrBsT from *Xanthomonas* spp. were shown to interfere with microtubule formation. As mentioned above, HopZ1a acts as an acetyltransferase and acetylates plant targets including JAZ proteins and tubulin in the presence of phytic acid (Lee *et al.* 2012; Jiang *et al.* 2013). Fluorescence microscopy of *Arabidopsis* seedlings containing GFP-labeled microtubule markers revealed that delivery of HopZ1a by *P. syringae* pv. *tomato* DC3000 leads to the destruction of microtubules 16 hours post infection (Fig. 8D). In contrast, no effect of HopZ1a on the actin cytoskeleton was observed (Lee *et al.* 2012). Twenty-two hours post infection, microtubules were destroyed even in the absence of HopZ1a, suggesting that microtubules are also targeted by additional effectors from *P. syringae* pv. *tomato* DC3000 (Lee *et al.* 2012). In agreement with the HopZ1a-mediated destruction of microtubules and the role of microtubules in vesicle trafficking, the transient expression of HopZ1a in *N. benthamiana* interferes with the secretion of the secGFP reporter protein to the apoplast (Boutte, Vernhettes, Satiat-Jeunemaitre 2007; Lee *et al.* 2012). An interference with secretion of secGFP was also reported for the YopJ homolog XopJ from *X. campestris* pv. *vesicatoria* (Bartetzko *et al.* 2009).

AvrBsT from *X. campestris* pv. *vesicatoria* is an additional member of the YopJ effector family, which might indirectly interfere with microtubule formation. AvrBsT interacts with and acetylates the putative tubulin-binding protein ACIP1 (acetylated interacting protein 1), which is presumably involved in plant immunity (Cheong *et al.* 2014). Infection experiments revealed that the *in planta* growth of virulent and avirulent strains of *P. syringae* pv. *tomato* DC3000 was increased in *ACIP1*-silenced *Arabidopsis* Pi-0 plants, suggesting that ACIP1 and ACIP family members contribute to PTI and ETI responses (Cheong *et al.* 2014). The analysis of a GFP-ACIP1 fusion revealed that ACIP1 colocalizes with microtubules. In the presence of AvrBsT, GFP-ACIP1 forms aggregates, suggesting that AvrBsT alters the localization of ACIP1 (Cheong *et al.* 2014). It remains to be investigated whether AvrBsT acetylates ACIP1 to interfere with plant defense responses and to alter microtubule formation. Notably, ACIP1 is presumably not the only plant target of AvrBsT because an arginine decarboxylase, the defense-related protein SGT1, Hsp70, the aldehyde dehydrogenase ALDH1 and the SNF1-related kinase 1 (see Table 1) were also reported as AvrBsT interaction partners. The relevance of most of these interactions for the virulence function of AvrBsT has not yet been adressed and should be in the focus of future studies.

EFFECTOR PROTEINS AND PLANT DEFENSE RESPONSES

Type III effectors do not always act as virulence factors but can also trigger defense responses in plants, which possess corresponding *R* genes and can thus recognize individual effector proteins. *R* genes often encode NLR proteins and are divided into TIR (toll/interleukin 1 receptor-like)-NB-LRR or CC (coiled-coil)-NB-LRR proteins according to their N-terminal domains (Takken and Goverse 2012; Wu *et al.* 2014; Cui, Tsuda and Parker 2015). NLR protein activity is tightly regulated to avoid unnecessary and harmful activation of defense responses. On the posttranscriptional level, intramolecular interactions between the LRR and the NB domain of the R protein might prevent NB-mediated nucleotide exchange and thus NLR activation (Bonardi and Dangl 2012; Takken and Goverse 2012). Activation of R proteins upon direct or indirect recognition of effectors is triggered by molecular rearrangements, which allow nucleotide exchanges and/or the interaction of the NLR protein with other signaling molecules (Fig. 9A).

Direct recognition of a bacterial type III effector was reported for the R protein RRS1-R from *Arabidopsis* which interacts with the type III effector PopP2 from *R. solanacearum* as is described below. Most other known NLR proteins recognize their cognate effectors presumably upon detection of effector-triggered changes in plant target molecules. According to the so-called guard model, a small repertoire of R proteins can detect a wide variety of effector proteins by guarding common effector targets (Van der Biezen and Jones 1998) (Fig. 9A). Alternatively, plant R proteins were proposed to detect effector-mediated changes in a non-functional effector target mimic, which acts as a decoy to trap the effector (Van der Hoorn and Kamoun 2008) (Fig. 9A). Decoys might have evolved from effector targets by gene duplication and do not contribute to the pathogen's fitness. In an evolutionary point of view, the presence of decoys guarantees the persistence of NLR-mediated effector recognition, even upon changes in the virulence targets of effector proteins. Known examples of guarded effector targets are detailed below and include (i) the Pto kinase, which interacts with AvrPto and AvrPtoB; (ii) RIN4, which is a negative regulator of plant immunity; (iii) the RLCK PBS1, which is cleaved by AvrPphB; and (iv) the pseudokinase ZED1, which mediates the recognition of HopZ1. Effector-R protein interactions and guarded plant targets or decoys of effector proteins are also summarized in Figs 9–12.

PopP2 from *R. solanacearum* is recognized by the R proteins RRS1-R and RPS4

One example for a putative plant decoy, which mimics an effector target, is the WRKY domain of the R protein RRS1-R, which is bound and acetylated by PopP2 from *R. solancearum* (Figs 7D and 9B). Acetylation of RRS1-R interferes with its DNA binding and thus with the RRS1-R-mediated activation of gene expression (Le Roux *et al.* 2015; Sarris *et al.* 2015). Given that PopP2 acetylates multiple WRKY transcription factors, it was suggested that the WRKY domain of RRS1-R acts as an integrated decoy, which

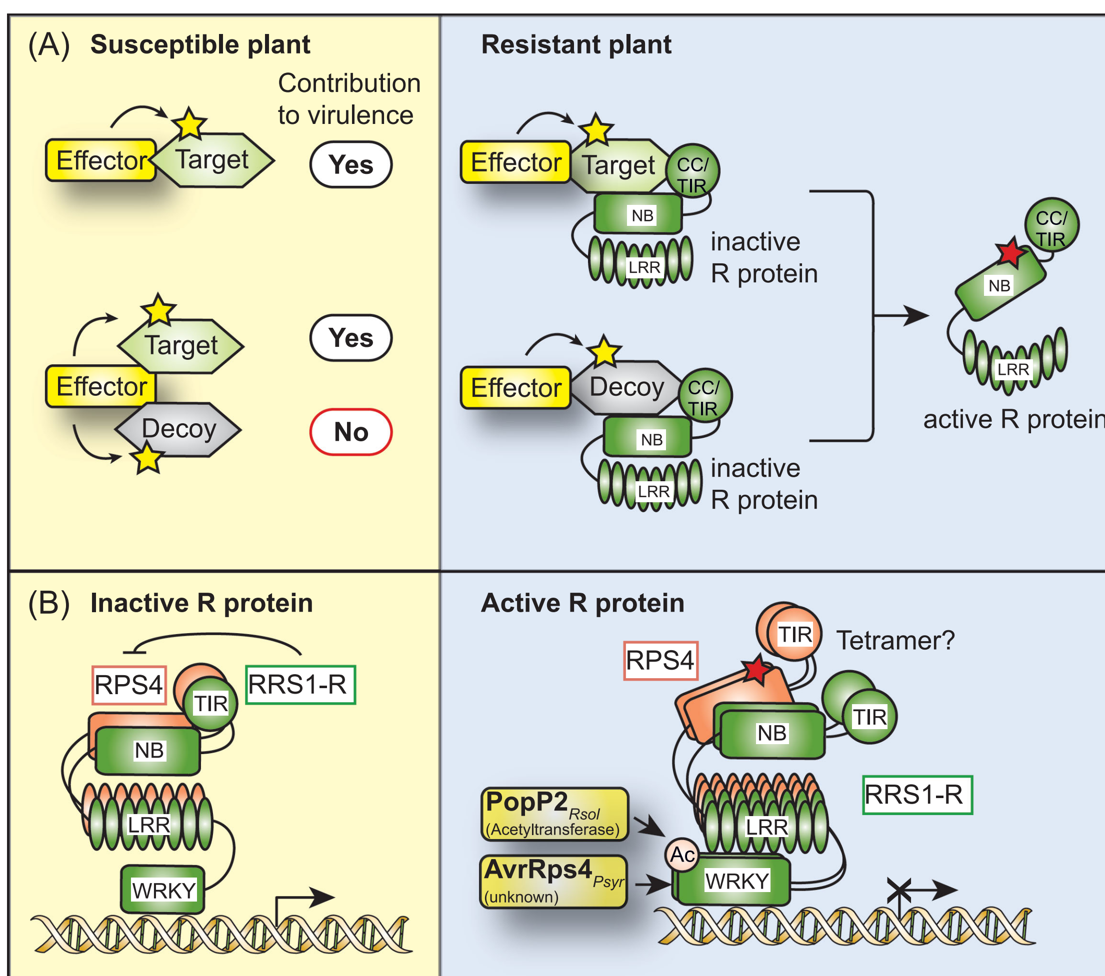

Figure 9. Activation of R protein-mediated defense responses by type III effectors. (**A**) Detection of effector protein-triggered modifications in plant target proteins by R proteins. Type III effectors interact with and modify plant target proteins to promote bacterial virulence. According to the guard model, plant R proteins in resistant plants detect effector-triggered modifications (indicated by yellow asterisks) in plant target molecules. Many R proteins consist of CC/TIR, NB and LRR domains. The activity of the NB domain is often regulated by intramolecular interactions with the LRR domain. The detection of effector-triggered modifications in plant targets leads to intramolecular rearrangements in the R proteins and thus to the activation of the NB domain (indicated by a red asterisk). According to an alternative model, plants have evolved decoy molecules, which resemble virulence targets of effectors but do not contribute to bacterial virulence. Effector-mediated modifications in plant decoys can also lead to the activation of R protein-mediated resistance. (**B**) Model of the activation of RRS1-R/RPS4-triggered plant defense responses. The TIR-NB-LRR proteins RPS4 and RRS1-R form a heterodimer in the plant nucleus, and RRS1-R negatively regulates RPS4. The WRKY domain of RRS1-R binds to DNA and presumably triggers the RRS1-R-dependent expression of plant genes. The effector proteins PopP2 from *R. solanacearum* and AvrRps4 from *P. syringae* interact with the WRKY domain of RRS1-R, which is acetylated by PopP2. Interactions with effectors and/or acetylation by PopP2 lead to molecular rearrangements in the RPS4/RRS1-R immune receptor complex and thus to the activation of RPS4 (indicated by a red asterisk). It has been suggested that the WRKY domain of RRS1-R acts as an integrated decoy, which allows the elicitation of AvrRps4- and PopP2-triggered ETI responses. The activated complex of RRS1-R and RPS4 was proposed to be a tetramer.

allows the detection of PopP2-mediated modifications of WRKY domains (Le Roux *et al.* 2015; Sarris *et al.* 2015). In agreement with this model, the exchange of the lysine residue at position 1221 of RRS1-R by glutamine, which mimics the acetylation of K1221, interferes with DNA binding of RRS1-R and leads to the activation of plant defense responses (Le Roux *et al.* 2015).

The recognition of PopP2 in *Arabidopsis* does not only depend on RRS1-R but also on the TIR-NB-LRR R protein RPS4 (Gassmann, Hinsch and Staskawicz 1999; Sohn *et al.* 2014; Williams *et al.* 2014). RRS1-R and RPS4 form a functional immune receptor complex via their TIR domains and trigger defense responses upon recognition of PopP2 (Narusaka *et al.* 2009; Williams *et al.* 2014; Sarris *et al.* 2015) (Fig. 9B). Transient expression studies in tobacco revealed that the TIR domain of RPS4 triggers an effector-independent cell death, which is suppressed upon coexpression of the TIR domain of RRS1-R (Williams *et al.* 2014). It was, therefore, suggested that RRS1-R negatively regulates RPS4 via the TIR domain (Cesari *et al.* 2014; Williams *et al.* 2014). The RRS1-R/RPS4 immune receptor pair also detects AvrRps4 from *P. syringae*. Similarly to PopP2, AvrRps4 interacts with the WRKY domain of RRS1-R and activates defense responses in the presence of RPS4 (Sarris *et al.* 2015) (Fig. 9B). Mutations in the P loop (phosphate-binding loop) of RPS4 but not of RRS1-R abolish AvrRps4- and PopP2-triggered ETI, suggesting that the function of RRS1-R is independent of NB (Williams *et al.* 2014). It was proposed that RRS1-R acts as a sensor R protein, which binds to the effector proteins PopP2 and AvrRps4 and triggers plant defense via the executor R protein RPS4 (Delga, Le Roux and Deslandes 2015).

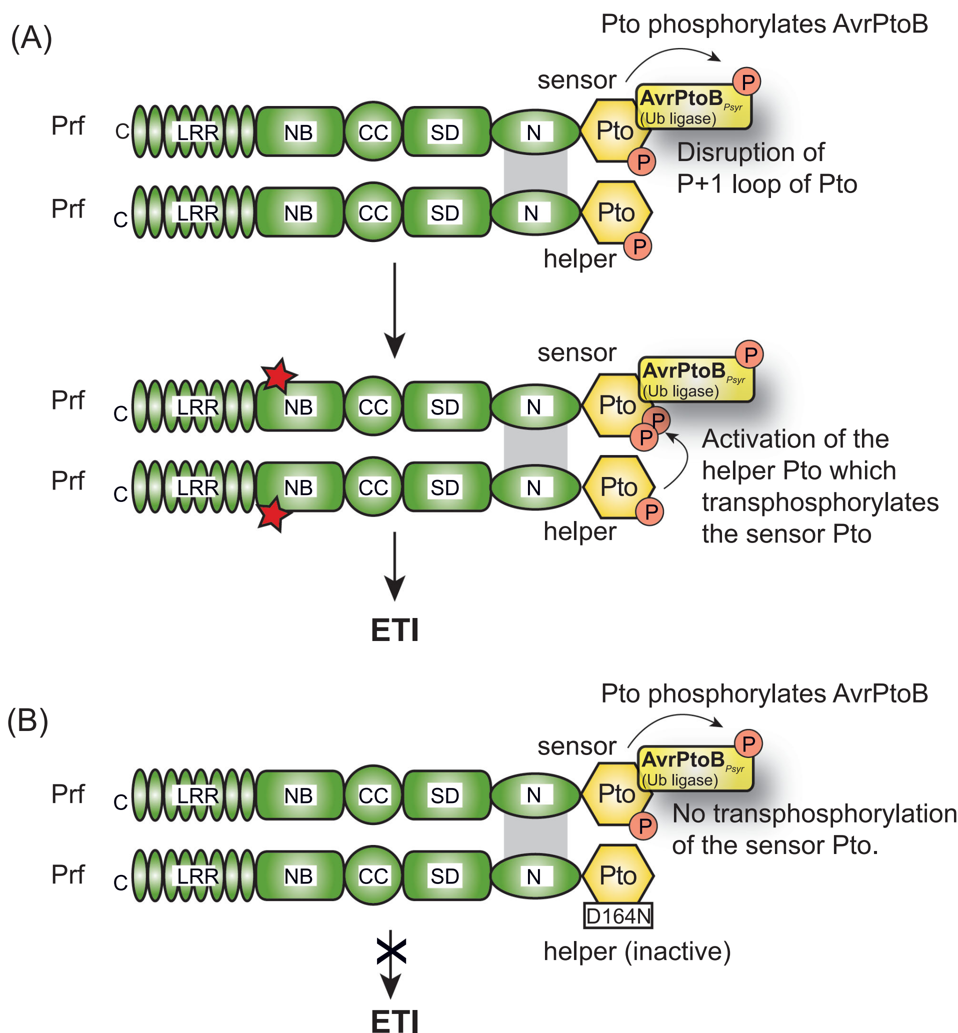

Figure 10. Model of Prf/Pto-triggered activation of plant defense responses. **(A)** AvrPtoB triggers Prf/Pto-dependent ETI responses. The CC-NB-LRR protein Prf contains an SD (*Solanaceae* domain) domain with weak homology to other solanaceous R proteins and an N-terminal domain (N), which interacts with the Pto kinase and mediates the Pto-independent dimerization of Prf (Gutierrez *et al.* 2010). The Prf/Pto complex contains at least two molecules of each protein. Pto is autophosphorylated at S198 and required to maintain Prf in an inactive conformation. The sensor Pto molecule interacts with and phosphorylates the E3 ubiquitin ligase AvrPtoB from *P. syringae* and evades AvrPtoB-mediated degradation. Effector binding to the P+1 loop of the Pto sensor presumably triggers a conformational change in this loop, which activates the Pto helper protein. The Pto helper molecule transphosphorylates the sensor at amino acid residue T199. Transphosphorylation of the sensor Pto leads to the activation of Prf (indicated by a red asterisk) and thus to Prf/Pto-triggered ETI responses. **(B)** A kinase-inactive Pto helper molecule (Pto_{D164N}) does not transphosphorylate the kinase-active Pto sensor and therefore does not activate Prf/Pto-dependent ETI responses.

In addition to RRS1-R, AvrRps4 is also recognized by RRS1-S, which is a derivative of RRS1-R and lacks the C-terminal 96 amino acids of RRS1-R (Deslandes *et al.* 1998, 2002; Sarris *et al.* 2015). The RRS1-S-mediated recognition of AvrRps4 depends on the WRKY domain, which provides the binding site for AvrRps4 and is also acetylated by PopP2 (Sarris *et al.* 2015). PopP2-mediated acetylation of the WRKY domain of RRS1-S does not trigger ETI but leads to reduced binding of RRS1-S to DNA and interferes with the RRS1-S-mediated recognition of AvrRps4 (Sarris *et al.* 2015). Notably, the WRKY domains of RRS1-R and RRS1-S are not the only targets of PopP2 and AvrRps4 because coimmunprecipitation experiments revealed that both effectors associate with additional WRKY proteins (Le Roux *et al.* 2015; Sarris *et al.* 2015). The contribution of these interactions to the virulence functions of PopP2 and AvrRps4 remains to be investigated.

The Pto kinase is guarded by the CC-NB-LRR protein Prf

The guard model was initially proposed for the recognition of the effector proteins AvrPto and AvrPtoB from *P. syringae*. AvrPto and AvrPtoB are two unrelated effectors, which differ significantly in their tertiary structures (Xing *et al.* 2007; Dong *et al.* 2009). Both effectors interact with the serine/threonine kinase Pto from tomato, which elicits ETI responses upon recognition of AvrPto or AvrPtoB in concert with its interaction partner Prf (Oh and Martin 2011). Prf is a CC-NB-LRR protein, which is encoded in the *PTO* locus (Martin *et al.* 1993; Salmeron *et al.* 1996; Pedley and Martin 2003). Prf contains a large N-terminal region, which is required for dimerization and interacts with Pto as well as with the kinase Fen (Mucyn *et al.* 2006; Gutierrez *et al.* 2010) (Fig. 10A). Fen shares 80% amino acid identity with Pto but does not confer resistance to AvrPto or AvrPtoB (Martin *et al.* 1994). As mentioned above, Fen is degraded by AvrPtoB and only elicits a

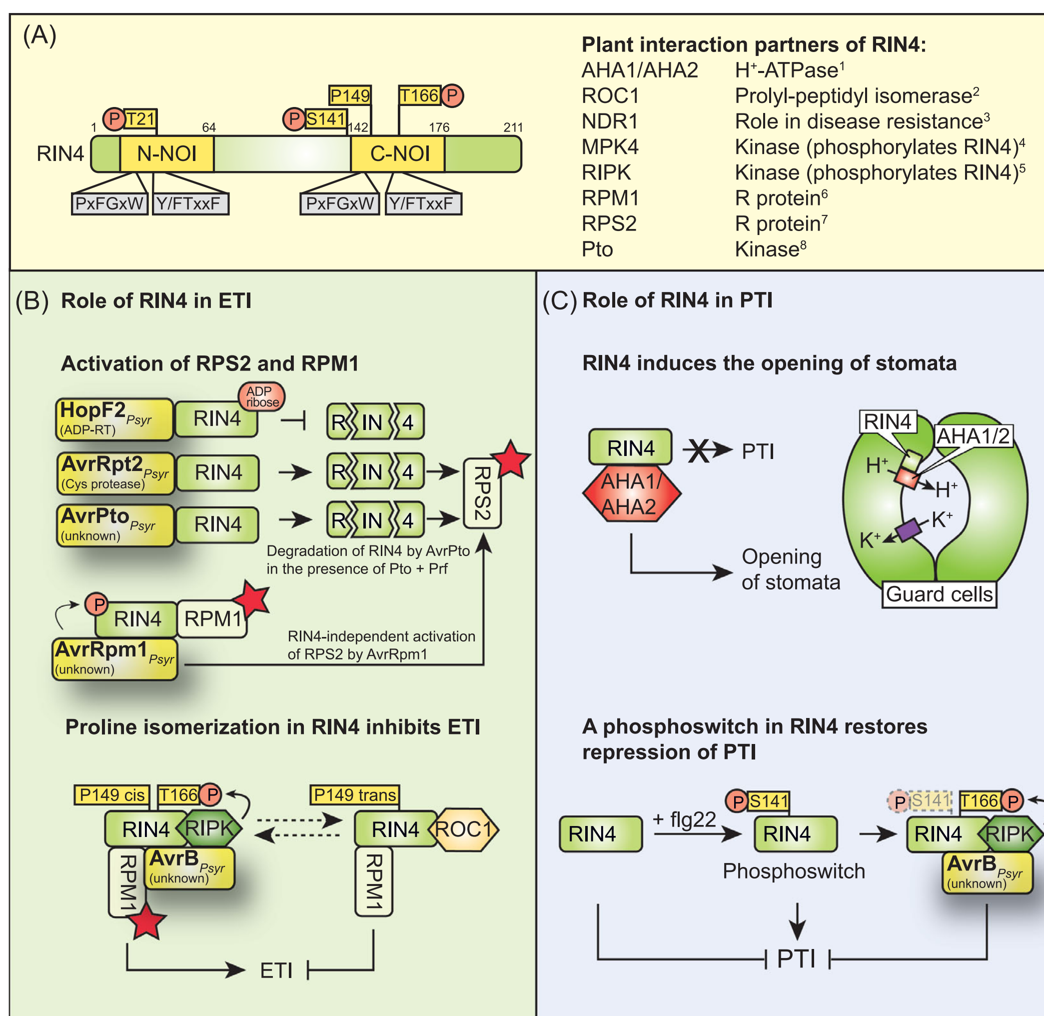

Figure 11. Effector-triggered modifications of RIN4 and their contributions to PTI and ETI responses. (A) Domain organization of RIN4 and list of known plant interaction partners of RIN4. RIN4 contains N- and C-terminal NOI domains with conserved PxFGxW and Y/FTxxF amino acid motifs. The PxFGxW motif is the cleavage site of the effector protein AvrRpt2 from *P. syringae*. The Y/FTxxF motif contains a conserved threonine residue, which is phosphorylated by the effector proteins AvrB and AvrRpm1 from *P. syringae*. Additional important amino acids are indicated (see the text for details). Known plant interaction partners of RIN4 and their predicted functions are listed. References: 1, Liu *et al.* (2009); 2, Li *et al.* (2014b); 3, Day, Dahlbeck and Staskawicz (2006); 4, Cui *et al.* (2010); 5, Liu *et al.* (2011); 6, Mackey *et al.* (2002); 7, Axtell and Staskawicz (2003); 8, Luo *et al.* (2009). (B) Contribution of RIN4 to RPS2- and RPM1-triggered ETI responses. The ADP-RT HopF2 from *P. syringae* ADP-ribosylates RIN4 and suppresses the degradation of RIN4 by the cysteine protease AvrRpt2 from *P. syringae*. RIN4 is also degraded by AvrPto from *P. syringae* in the presence of Pto and Prf. The cleavage products of RIN4 are detected by the R protein RPS2, which triggers ETI. The effector proteins AvrRpm1 and AvrB from *P. syringae* lead to the phosphorylation of RIN4 and thus to the activation of the R protein RPM1 (indicated by a red asterisk). Effector-triggered phosphorylation of RIN4 presumably depends on the kinase RIPK, which interacts with RIN4 and AvrB and phosphorylates RIN4 at several amino acid residues including T166. The phosphorylation of T166 of RIN4 interferes with the interaction of RIN4 with the cyclophilin ROC1, which catalyzes the *cis/trans* isomerization of the proline residue at position 149 of RIN4. The *cis/trans* isomerization of P149 presumably leads to a specific conformational change in RIN4 and thus inhibits the activation of RPM1 and RPS2. Phosphorylation of RIN4 in the presence of AvrB likely induces conformational changes, which interfere with ROC1-mediated isomerization of RIN4 and lead to the activation of RPM1 (see the text for details). (C) Model of the role of RIN4 during PTI. RIN4 is a suppressor of PTI responses and interacts with and activates the H^+ ATPases AHA1 and AHA2, which pump protons from the cytosol of guard cells into the apoplast. The activity of AHA1/2 leads to the establishment of a proton electrochemical gradient, which is used by channel and carrier proteins to mediate the uptake of ions into guard cells, thus leading to stomatal opening. Upon detection of flg22 during PTI, RIN4 is phosphorylated at amino acid S141, which leads to the derepression (= activation) of PTI. The phosphorylation of RIN4 at amino acid T166 by AvrB and AvrRpm1 from *P. syringae* presumably counteracts the effect of the S141 phosphorylation and restores the repression of PTI (see the text for details).

cell death reaction in the presence of a C-terminally truncated AvrPtoB derivative, which lacks the C-terminal E3 ubiquitin ligase domain (Rosebrock *et al.* 2007).

Pto is probably not the main virulence target of AvrPto and AvrPtoB because both effectors also interact with other kinase domains of plant proteins including kinase domains of PRRs (see above; Table 1). Given the assumed roles of AvrPto and AvrPtoB in the suppression of PTI, Pto might have evolved as a decoy to detect the activities of AvrPto and AvrPtoB inside the plant cell. Binding of AvrPto or AvrPtoB to Pto likely disturbs the Pto-mediated negative regulation of the R protein Prf and thus leads to ETI. Both effectors interact with the catalytic region of Pto including the P+1 loop and can act as kinase inhibitors (Xing *et al.* 2007; Dong *et al.* 2009; Cheng *et al.* 2011; Ntoukakis *et al.* 2014). The P+1 loop of kinases is involved in substrate binding and is located adjacent to the activation loop (Huse and Kuriyan 2002). The P+1 loop and the activation loop of kinases are part of the activation segment, which controls kinase activity (Nolen, Taylor and Ghosh 2004). In many kinases, phosphorylation events within the activation segment lead to

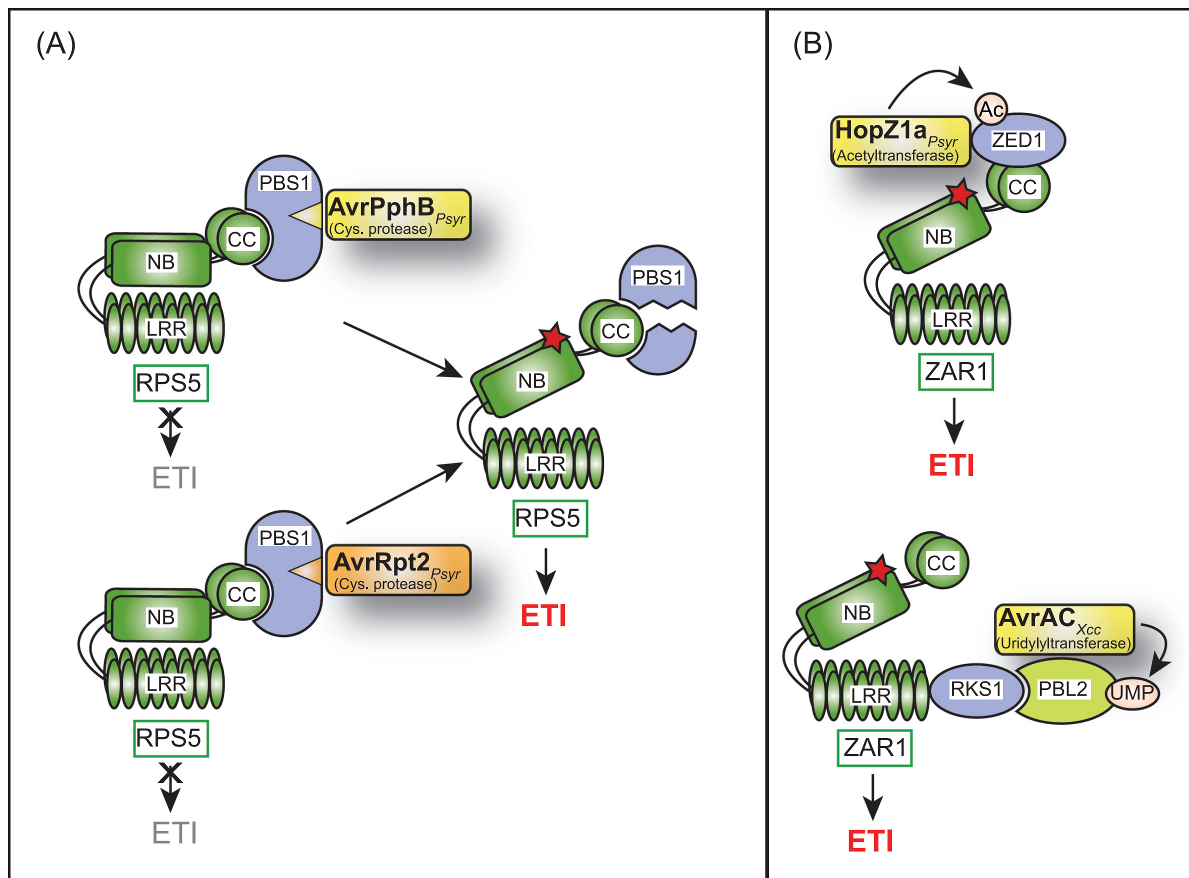

Figure 12. Detection of effector-triggered modifications in plant target molecules by the R proteins RPS5 and ZAR1. (**A**) RPS5 activates ETI upon cleavage of PBS1. The CC-NB-LRR protein RPS5 interacts via the CC domain with the kinase PBS1. Cleavage of PBS1 by the effector protein and cysteine protease AvrPphB leads to the activation of RPS5. Exchange of the AvrPphB cleavage site (indicated by a yellow triangle) against the recognition site of the cysteine protease AvrRpt2 (indicated by an orange triangle) leads to cleavage of PBS1 in the presence of AvrRpt2 and thus to the activation of RPS5. (**B**) The R protein ZAR1 detects modifications in ZED1 and PBL2. The CC-NB-LRR protein ZAR1 interacts via the CC domain with the pseudokinase ZED1. Acetylation of ZED1 by the effector protein HopZ1a leads to the activation of ZAR1 (indicated by a red asterisk) and thus to ETI responses. ZAR1 also interacts via the LRR domain with the ZED1-related pseudokinase RKS1. The ZED1-RKS1 complex detects modifications in PBL2, which is a target of the uridylyltransferase AvrAC from *X. campestris* pv. *campestris* (see the text for details). Uridylylation of PBL2 by AvrAC leads to the activation of ZAR1-dependent ETI responses.

conformational changes and thus alterations in the kinase activity (Nolen, Taylor and Ghosh 2004). In agreement with this model, mutations within the P+1 loop of Pto interfere with the kinase activity of Pto, suggesting that the P+1 loop is involved in the regulation of the Pto kinase activity (Xing *et al.* 2007). Furthermore, the P+1 loop also interferes with the Pto-mediated regulation of Prf-dependent ETI because mutations within this region lead to constitutive gain-of-function Pto mutant derivatives, which trigger a ligand-independent HR in *Nicotiana benthamiana* (Rathjen *et al.* 1999; Wu *et al.* 2004; Mucyn *et al.* 2006; Xing *et al.* 2007; Dong *et al.* 2009) (see Table 2). Similar findings were observed for a kinase-inactive Pto derivative with a mutation in the P+1 loop (Wu *et al.* 2004). This suggests that the conformation of the P+1 loop rather than the kinase activity of Pto is important for the regulation of Prf activity by Pto. Some of the results of the numerous coexpression studies with *Prf*, *Pto* and *avrPto/avrPtoB* in *N. benthamiana* are summarized in Table 2. According to a current model, effector binding to the P+1 loop of Pto presumably triggers a conformational change in this loop, which derepresses Prf activity and thus leads to the activation of ETI (Ntoukakis *et al.* 2014).

The role of the Pto kinase activity in the elicitation of ETI has been intensively studied by several research groups. With regard to the recognition of AvrPtoB, the Pto kinase activity was proposed to be required for the phosphorylation of AvrPtoB and thus for the inhibition of its E3 ligase activity (Ntoukakis *et al.* 2009). In agreement with this model, the transient expression of the kinase-inactive Pto derivative Pto_{D164N} did not trigger the HR in the presence of AvrPtoB but of an E3 ubiquitin ligase mutant derivative thereof (Mucyn *et al.* 2009; Ntoukakis *et al.* 2009) (Table 2). According to an alternative hypothesis, however, Pto evades the degradation by AvrPtoB by binding to two distinct binding sites in the N-terminal and central region of AvrPtoB (Mathieu, Schwizer and Martin 2014). It was suggested that binding of Pto to the central region of AvrPtoB adjacent to the E3 ubiquitin ligase domain leads to the degradation of Pto. In contrast, Pto bound to the N-terminal-binding site in AvrPtoB was reported to be stable (Mathieu, Schwizer and Martin 2014). This model was mainly based on the results of yeast two-hybrid studies, which revealed that Pto does not interact with an AvrPtoB derivative containing the N-terminal Pto-binding site in fusion with the E3 ubiquitin ligase domain (Mathieu, Schwizer and Martin 2014). The authors concluded from this observation that the

Table 2. Results of selected transient expression assays with *Pto*, *Prf* and *avrPto(B)* in *N. benthamiana*.

Prf (tomato)[a]	Pto (tomato)[a]	Effector[a]	Plant reaction[b]	References	Comments[c]
Prf and Pto trigger a ligand-independent HR					
Prf$_{35S}$	Pto$_{35S}$	–	HR	Mucyn *et al.* (2006)	Overexpression of *Pto* and *Prf* under control of the 35S promoter leads to a ligand-independent HR, which occurs in the absence of AvrPto or AvrPtoB. Overexpression of *Prf* under control of the DEX-inducible promoter induces a Pto-independent HR, suggesting that Prf alone can activate downstream signaling. The Prf$_{DEX}$-induced HR is presumably independent of Pto because it is not abolished upon silencing of the *Pto* homolog *Pth1* from *N. benthamiana* with a tobacco rattle virus (TRV) silencing vector (Mucyn *et al.* 2006).
Prf$_{35S}$	Pto$_{Pnat}$	–	HR	Mucyn *et al.* (2006)	
	Pto$_{35S}$	–	–	Mucyn *et al.* (2006)	
Prf$_{35S}$	–	–	–	Mucyn *et al.* (2006)	
Prf$_{DEX}$	–	–	HR	Mucyn *et al.* (2006)	
Prf$_{DEX}$	Pto$_{35S}$	–	HR	Mucyn *et al.* (2006)	
Prf$_{DEX}$	TRV:*Pth1*	–	HR	Mucyn *et al.* (2006)	
The Prf/Pto-triggered ligand-independent HR depends on the kinase activity of Pto					
Prf$_{35S}$	Pto$_{D164N/35S}$	–	–	Mucyn *et al.* (2006)	The ligand-independent HR induced by Prf and Pto depends on the kinase activity of Pto. The Prf$_{DEX}$-induced HR is suppressed by the kinase-inactive Pto$_{D164N}$ derivative, but not by Pto$_{G50S}$, which has residual kinase activity.
Prf$_{35S}$	Pto$_{G50S/35S}$	–	–	Mucyn *et al.* (2006)	
Prf$_{DEX}$	Pto$_{D164N/35S}$	–	–	Mucyn *et al.* (2006)	
Prf$_{DEX}$	Pto$_{G50S/35S}$	–	HR	Mucyn *et al.* (2006)	
Pto and AvrPto trigger an HR in N. benthamiana					
–	–	AvrPto$_{35S}$	–	Rathjen *et al.* (1999)	Expression of *Pto* and *avrPto* in *N. benthamiana* induces the HR. Silencing of the *Prf* homolog from *N. benthamiana* abolishes the HR, suggesting that the elicitation of ETI by Pto and AvrPto depends on Prf.
–	Pto (transgene)	AvrPto$_{35S}$	HR	Scofield *et al.* (1996)	
–	Pto$_{35S}$	AvrPto$_{35S}$	HR	Rathjen *et al.* (1999)	
TRV:*Prf*	Pto$_{35S}$	AvrPto$_{35S}$	–	Wu *et al.* (2004)	
Recognition of AvrPtoB depends on both Pto and Prf					
–	Pto$_{35S}$ (transgene)	AvrPtoB$_{35S}$	–	Kim, Lin and Martin (2002); Mucyn *et al.* (2006)	Coexpression of *Pto* and *avrPtoB* does not trigger the HR, suggesting that the intrinisic *Prf* gene from *N. benthamiana* is not sufficient for the recognition of AvrPtoB. Coexpression of Prf, Pto and AvrPtoB triggers the HR, which appears earlier than the ligand-independent HR induced by Prf and Pto (see above).
Prf$_{35S}$	–	AvrPtoB$_{35S}$	–	Mucyn *et al.* (2006)	
Prf$_{35S}$	Pto$_{35S}$ (transgene)	AvrPtoB$_{35S}$	HR	Mucyn *et al.* (2006)	
AvrPtoB deleted in the E3 ubiquitin ligase domain triggers Prf-dependent defense reactions					
–	Pto$_{35S}$	AvrPtoB$_{35S}$	–	Abramovitch *et al.* (2003)	AvrPtoB$_{1\text{-}387}$ lacks the C-terminal E3 ubiquitin ligase domain and triggers a Prf-dependent defense reaction, designated Rsb (resistance suppressed by AvrPtoB C terminus) (Abramovitch *et al.* 2003). The C-terminal E3 ubiquitin ligase domain of AvrPtoB presumably suppresses the Rsb phenotype and also the recognition of AvrPtoB by Pto (Abramovitch *et al.* 2003). Note that AvrPtoB$_{1\text{-}308}$ is recognized by Pto but is not sufficient to elicit the Rsb phenotype (Abramovitch *et al.* 2003).
–	Pto$_{35S}$	AvrPtoB$_{1\text{-}387/35S}$	HR	Abramovitch *et al.* (2003)	
–	–	AvrPtoB$_{1\text{-}387/35S}$	HR	Abramovitch *et al.* (2003); Rosebrock *et al.* (2007)	
TRV:*Prf*	–	AvrPtoB$_{1\text{-}387/35S}$	–	Rosebrock *et al.* (2007)	
–	Pto$_{35S}$	AvrPtoB$_{1\text{-}308/35S}$	HR	Abramovitch *et al.* (2003)	
–	–	AvrPtoB$_{1\text{-}308/35S}$	–	Abramovitch *et al.* (2003)	
The kinase activity of Pto is required for ETI					
–	Pto$_{G50S/35S}$	AvrPto$_{35S}$	HR	Mucyn *et al.* (2006)	Pto$_{G50S}$, which has residual kinase activity (Mucyn *et al.* 2006), induces the HR in the presence of AvrPto as well as of Prf and AvrPtoB. It was, therefore, proposed that the kinase activity of Pto is dispensable for the activation of ETI and for the suppression of the AvrPtoB-mediated degradation of Pto (Mathieu, Schwizer and Martin 2014). This is in contrast to the finding that the kinase-inactive Pto$_{D164N}$ derivative does not trigger the HR in the presence of Prf and AvrPto or AvrPtoB. In contrast, Pto$_{D164N}$ triggers the HR in the presence of the E3 ligase mutant derivative AvrPtoB$_{F479A}$, suggesting that the Pto kinase activity is required to evade AvrPtoB-mediated degradation.
–	Pto$_{D164N/35S}$	AvrPto$_{35S}$	–	Rathjen *et al.* (1999); Wu *et al.* (2004)	
Prf$_{35S}$	Pto$_{D164N/35S}$	AvrPto$_{DEX}$ (transgene)	–	Mucyn *et al.* (2009)	
Prf$_{35S}$	Pto$_{35S}$	AvrPtoB$_{35S}$	HR	Mathieu, Schwizer and Martin (2014)	
Prf$_{35S}$	Pto$_{G50S/35S}$	AvrPtoB$_{35S}$	HR	Mathieu, Schwizer and Martin (2014)	
Prf$_{pnat}$ (transgene)	Pto$_{D164N/Pro(Pto)}$[d]	AvrPtoB$_{35S}$	–	Ntoukakis *et al.* (2009)	
Prf$_{pnat}$ (transgene)	Pto$_{D164N/Pro(Pto)}$	AvrPtoB$_{F479A/35S}$	HR	Ntoukakis *et al.* (2009)	

Table 2. (*Continued*).

Prf (tomato)[a]	Pto (tomato)[a]	Effector[a]	Plant reaction[b]	References	Comments[c]
Degradation of Pto by AvrPtoB might depend on the Pto-binding site in AvrPtoB					
Prf$_{35S}$	Pto$_{35S}$	AvrPtoB$_{F173A/35S}$	–	Mathieu, Schwizer and Martin (2014)	The N-terminal Pto-binding site is mutated in AvrPtoB$_{F173A/35S}$. Based on the results of yeast two-hybrid studies, it was suggested that Pto binds adjacent to the E3 ligase domain of AvrPtoB$_{F173A}$ and is therefore degraded. In contrast, Pto is not degraded by AvrPtoB$_{F173A/E3\text{-}LOF/35S}$, which contains an inactive E3 ubiquitin ligase domain.
Prf$_{35S}$	Pto$_{35S}$	AvrPtoB$_{F173A/E3\text{-}LOF/35S}$	HR	Mathieu, Schwizer and Martin (2014)	
The kinase activity of Pto is dispensable for ETI if Pto has been made active by mutation					
–	Pto$_{L205D/35S}$	–	HR	Wu *et al.* (2004); de Vries *et al.* (2006)	The Pto derivative Pto$_{L205D}$ with a mutation in the P+1 loop induces the HR in the absence of AvrPto. Similar results were observed with the kinase-inactive Pto$_{L205D/D164N}$, suggesting that the kinase activity of Pto is dispensable for the HR induction by Pto$_{L205D}$. The Pto$_{L205D}$-triggered HR depends on the *Prf* gene from *N. benthamiana* because no HR induction was observed in *Prf*-silenced plants (TRV:*Prf*).
–	Pto$_{L205D/D164N/35S}$	–	HR	Wu *et al.* (2004)	
TRV:*Prf*	Pto$_{L205D/35S}$	–	–	Wu *et al.* (2004)	
Double phosphorylation of Pto is required for signaling					
Prf$_{Pnat}$ (transgene)	Pto$_{35S}$	AvrPtoB$_{F479A/35S}$	HR	Ntoukakis *et al.* (2013)	The Pto derivative Pto$_{S198A/T199A}$ with mutations in both phosphorylation sites is an active kinase but does not trigger the HR in the presence of the E3 ubiquitin ligase-deficient AvrPtoB$_{F479A}$. This suggests that both phosphorylation sites of Pto rather than the kinase activity per se are required for the induction of ETI. The phosphomimic Pto$_{S198D/T199D}$ derivative induces cell death in the presence of AvrPto or AvrPtoB. AvrPto also induces the HR in the presence of the kinase-inactive Pto$_{S198D/T199D/D164N}$, suggesting that the kinase activity of Pto is dispensable after phosphorylation of S198 and T199. AvrPtoB is not recognized by the kinase-inactive Pto$_{S198D/T199D/D164N}$ derivative, which is presumably degraded by AvrPtoB. The L205D mutation in the P+1 loop of Pto leads to the induction of an AvrPto/B-independent cell death (see above), which is abolished in the presence of S198A/T199A mutations in Pto. This suggests that the phosphorylation of Pto at S198 and T199 is required for the ETI induction by the gain-of-function derivative Pto$_{L205D}$.
Prf$_{Pnat}$ (transgene)	Pto$_{S198A/T199A/35S}$	AvrPtoB$_{F479A/35S}$	–	Ntoukakis *et al.* (2013)	
Prf$_{Pnat}$ (transgene)	Pto$_{S198A/35S}$	AvrPto/B$_{35S}$	Cell death	Ntoukakis *et al.* (2013)	
Prf$_{Pnat}$ (transgene)	Pto$_{T199A/35S}$	AvrPto/B$_{35S}$	Cell death	Ntoukakis *et al.* (2013)	
Prf$_{Pnat}$ (transgene)	Pto$_{S198A/T199A/35S}$	AvrPto/B$_{35S}$	–	Ntoukakis *et al.* (2013)	
Prf$_{Pnat}$ (transgene)	Pto$_{S198D/T199D/35S}$	AvrPto/B$_{35S}$	Cell death	Ntoukakis *et al.* (2013)	
Prf$_{Pnat}$ (transgene)	Pto$_{S198D/T199D/D164N/35S}$	AvrPto$_{35S}$	Cell death	Ntoukakis *et al.* (2013)	
Prf$_{Pnat}$ (transgene)	Pto$_{S198D/T199D/D164N/35S}$	AvrPtoB$_{35S}$	–	Ntoukakis *et al.* (2013)	
Prf$_{Pnat}$ (transgene)	Pto$_{L205D/S198A/T199A/35S}$	–	–	Ntoukakis *et al.* (2013)	
Pto is transphosphorylated after effector binding					
Prf$_{Pnat}$ (transgene)	Pto$_{35S}$ + Pto$_{D164N/35S}$	AvrPto$_{35S}$	–	Ntoukakis *et al.* (2013)	AvrPto binds to autophosphorylated Pto, which cannot be transphosphorylated by Pto$_{D164N}$ and does not trigger ETI.
Prf$_{Pnat}$ (transgene)	Pto$_{D164N/35S}$ + Pto$_{S198D/T199D/35S}$	AvrPto$_{35S}$	Cell death	Ntoukakis *et al.* (2013)	AvrPto triggers ETI in the presence of the kinase-inactive Pto$_{D164N}$ upon coexpression of the phosphomimic Pto derivative Pto$_{S198D/T199D}$.
Prf$_{Pnat}$ (transgene)	Pto$_{S198D/T199D/35S}$	–	–	Ntoukakis *et al.* (2013)	The phosphomimic Pto$_{S198D/T199D}$ derivative does not induce the ligand-independent HR, suggesting that phosphorylation of Pto is not sufficient and that the induction of Pto-dependent defense responses depends on the disruption of the P+1 loop of Pto.

Table 2. (*Continued*).

Prf (tomato)[a]	Pto (tomato)[a]	Effector[a]	Plant reaction[b]	References	Comments[c]
Prf_{Pnat} (transgene)	$Pto_{L205D/35S}$	–	Cell death	Ntoukakis *et al.* (2013)	Pto_{L205D} induces a cell death because of the disruption of the P+1 loop. The authors speculate that Pto_{L205D} is transphosphorylated by a Pto homolog from *N. benthamiana*. The presence of Pto_{D164N} prevents transphopshorylation of Pto_{L205D} and, therefore, suppresses the induction of ETI.
Prf_{Pnat} (transgene)	$Pto_{L205D/35S}$ + $Pto_{D164N/35S}$	–	–	Ntoukakis *et al.* (2013)	
Prf_{Pnat} (transgene)	$Pto_{L205D/35S}$ + $Pto_{S198D/T199D/35S}$	–	Cell death	Ntoukakis *et al.* (2013)	Pto_{L205D} is transphosphorylated by the kinase-active derivative $Pto_{S198D/T199D}$ or $Pto_{S198A/T199A}$ and induces ETI.
Prf_{Pnat} (transgene)	$Pto_{L205D/35S}$ + $Pto_{S198A/T199A/35S}$	–	Cell death	Ntoukakis *et al.* (2013)	

[a]The promoters, which were used for transient expression or the expression of transgenes, are indicated in all cases for which this information was provided in the publications. *Pnat*, native promoter of *Prf*; *35S*, 35S promoter; *DEX*, DEX-inducible promoter; transgene, integration of the gene into the genome of *N. benthamiana*.
[b]HR, hypersensitive response; -, no visible plant reactions.
[c]For the better understanding of some of the results of the selected transient expression studies, conclusions provided by the authors of the indicated publications are shortly summarized. See also the text for details.
[d]Pto_{D164N} is kinase deficient but binds to AvrPto in yeast (Scofield *et al.* 1996; Tang *et al.* 1996).

binding of Pto adjacent to the E3 ubiquitin ligase domain of AvrPtoB leads to its AvrPtoB-dependent degradation. Biochemical evidence for this hypothesis is still missing. Mathieu, Schwizer and Martin (2014) also observed that a Pto derivative with a G50S mutation and significantly reduced kinase activity triggers ETI in the presence of AvrPtoB and Prf (Table 2). Based on this finding, it was proposed that the Pto kinase activity is dispensable for the recognition of AvrPtoB and the elicitation of ETI. This hypothesis is in contrast to the finding that the kinase-inactive Pto derivative Pto_{D164N} fails to trigger the HR in the presence of AvrPtoB (see above; Table 2).

The activation of Prf-mediated defense signaling correlates with a phosphorylation of serine and threonine residues at positions 198 and 199 in Pto (Ntoukakis *et al.* 2013) (Fig. 10A). Mutation of both S198 and T199 did not interfere with the kinase activity of Pto but with the elicitation of ETI in the presence of AvrPto or an E3 ligase mutant derivative of AvrPtoB (Ntoukakis *et al.* 2013) (Table 2). However, a phosphomimetic derivative of Pto ($Pto_{S198D/T199D}$) did not trigger ligand-independent defense, suggesting that the phosphorylation of Pto per se is not sufficient for the signaling (Ntoukakis *et al.* 2013). Thus, the activation of Prf by Pto presumably depends on the additional disruption of the P+1 loop after binding of AvrPto or AvrPtoB (Ntoukakis *et al.* 2013). This hypothesis is supported by the finding that the coexpression of AvrPto or AvrPtoB with $Pto_{S198D/T199D}$ and Prf triggers a cell death (Table 2) (Ntoukakis *et al.* 2013). An additional mutation leading to the kinase-inactive $Pto_{S198D/T199D/D164N}$ abolished cell death induction by AvrPtoB but not by AvrPto (Table 2) (Ntoukakis *et al.* 2013). This is in agreement with the model that the kinase activity of Pto is dispensable for signaling per se but required for Pto to evade degradation by AvrPtoB (see above).

The double phosphorylation of Pto is presumably the result of a transphosphorylation event which might be stimulated upon disruption of the P+1 loop of one Pto molecule (the sensor) upon binding of AvrPto or AvrPtoB (Ntoukakis *et al.* 2013). It was proposed that the sensor Pto molecule is initially autophosphorylated at S198 and subsequently transphosphorylated by a second Pto molecule (the helper) upon binding of AvrPto or AvrPtoB (Ntoukakis *et al.* 2014). This model is supported by the finding that the constitutively active Pto_{L205D} (with a mutation in the P+1 loop) does not trigger cell death upon coexpression of the kinase-inactive Pto_{D164N} because Pto_{L205D} likely needs to be transphosphorylated by a kinase-active Pto derivative (Table 2; Fig. 10B) (Ntoukakis *et al.* 2013). Taken together, binding of AvrPto or AvrPtoB is assumed to trigger a conformational change in the P+1 loop of an autophosphorylated sensor Pto molecule and leads to the transphosphorylation by a second helper Pto molecule. The double phosphorylation of Pto finally activates Prf, which triggers plant defense responses.

The effector target RIN4 is guarded by the R proteins RPS2 and RPM1

RIN4 was identified as a regulator of both PTI and ETI, and as interaction partner of at least two R proteins, RPS2 and RPM1 (Mackey *et al.* 2002; Axtell and Staskawicz 2003). RIN4 contains N- and C-terminal NOI (NO_3-induced) domains, a C-terminal cysteine-rich membrane anchoring motif and was shown to be acylated and anchored in the plasma membrane (Mackey *et al.* 2002; Kim *et al.* 2005b; Afzal, Kim and Mackey 2013) (Fig. 11A). Intrinsically disordered protein regions in RIN4 presumably allow the specific binding of RIN4 to various interaction partners (Sun *et al.* 2014; Lee *et al.* 2015) including at least four different effector proteins from *P. syringae* (see below). RIN4 also interacts with and activates the plant plasma membrane H(+) ATPases AHA1 and AHA2 and thus presumably promotes stomatal opening (Liu *et al.* 2009). Plant interaction partners, effector-triggered modifications of RIN4 and their effect on plant defense responses are summarized below and in Fig. 11.

Effector-triggered cleavage of RIN4 and activation of the R protein RPS2

RIN4 is cleaved by the effector protein AvrRpt2 from *P. syringae*, which is a cysteine protease and is self-processed inside the plant cell (Axtell and Staskawicz 2003; Axtell *et al.* 2003; Mackey *et al.* 2003). The self-cleavage of AvrRpt2 requires the plant cyclophilin ROC1, which catalyzes the isomerization between *cis* and *trans* isoforms of X-prolyl peptide bonds (Coaker, Falick and Staskawicz 2005; Coaker *et al.* 2006; Aumüller *et al.* 2010; Kumari *et al.* 2013). AvrRpt2 cleaves RIN4 within the NOI domains at cleavage sites (consensus sequence: PXFGXW), which

are similar to the AvrRpt2 processing site (Chisholm *et al*. 2005; Kim *et al*. 2005a) (Fig. 11A). At first glance, the cleavage of a PTI suppressor such as RIN4 by AvrRpt2 does not appear to be favorable for the pathogen. However, RIN4 cleavage products were shown to be even more active PTI suppressors than the uncleaved protein (Afzal, da Cunha and Mackey 2011). RIN4 is presumably not the only target of AvrRpt2 because predicted AvrRpt2 cleavage sites are also present in other plant proteins. Furthermore, RIN4 is not essential for the virulence function of AvrRpt2 as was shown by infection studies with *Arabidopsis rin4 rps2* plants (Belkhadir *et al*. 2004; Lim and Kunkel 2004; Chisholm *et al*. 2005).

The cleavage of RIN4 by AvrRpt2 is detected by the R protein RPS2, which interacts with the C-terminal nine amino acids of RIN4 and is negatively regulated by RIN4 (Mackey *et al*. 2003; Day *et al*. 2005) (Fig. 11B). The cleavage of RIN4 abolishes the interaction between RIN4 and RPS2 and results in the activation of the RPS2-dependent ETI (Mackey *et al*. 2003; Day *et al*. 2005). The AvrRpt2-mediated ETI is compromised by the ADP-RT HopF2 (see above), which interacts with and ADP-ribosylates RIN4 and thus attenuates the AvrRpt2-induced cleavage of RIN4 (Wilton *et al*. 2010). Interestingly, RPS2 can also be activated by the effector protein AvrRpm1 from *P. syringae*, which interacts with and phosphorylates RIN4 (Mackey *et al*. 2002; Chung *et al*. 2011) (Fig. 11B). As described below, the AvrRpm1-mediated phosphorylation of RIN4 is detected by the R protein RPM1. Notably, however, AvrRpm1 also triggers RPS2-dependent defense, which is independent of RIN4, suggesting that RPS2 guards additional plant proteins (Kim *et al*. 2009b; Cherkis *et al*. 2012).

In tomato, RIN4 is also cleaved in the presence of AvrPto, which interacts with RIN4, Pto and Prf (Luo *et al*. 2009). Furthermore, RIN4 degradation is induced in the absence of AvrPto by the Pto derivative Pto_{L205D}, which contains a mutation in the P+1 loop and triggers a ligand-independent HR (see above, Table 2). This suggests that AvrPto-triggered RIN4 degradation depends on Pto and on a plant-specific protease (Luo *et al*. 2009). In the absence of Pto, AvrPto interacts with RIN4 without triggering RIN4 degradation (Luo *et al*. 2009). Experimental evidence suggests that RIN4 is also degraded in the presence of additional effectors including HopQ1 and HopAM1 (Luo *et al*. 2009).

Phosphorylation of RIN4 and activation of the R protein RPM1

The type III effectors AvrRpm1 and AvrB from *P. syringae* phosphorylate the threonine residue of the conserved Y/FTXXF motif within the NOI domains of RIN4 (Chung *et al*. 2011; Liu *et al*. 2011). The AvrB/AvrRpm1-mediated phosphorylation of RIN4 leads to the activation of the R protein RPM1, which guards RIN4 (Mackey *et al*. 2002) (Fig. 11B). Phosphomimic mutations in RIN4 cause an effector-independent activation of RPM1 (Chung *et al*. 2011; Liu *et al*. 2011) as well as increased disease susceptibility and stomatal opening in the absence of RPM1 (Chung *et al*. 2014; Lee *et al*. 2015). The latter finding suggests that AvrB and AvrRpm1 induce the phosphorylation of RIN4 to suppress PTI responses. The RPM1-mediated recognition of AvrB and AvrRpm1 is suppressed by AvrRpt2, which cleaves RIN4 (Mackey *et al*. 2002) (see above).

Phosphorylation of RIN4 by AvrB and AvrRpm1 presumably depends on the plant kinase RIPK, which interacts with AvrB and RIN4, and phosphorylates RIN4 at the amino acid residues T21, S160 and T166 (Chung *et al*. 2011; Liu *et al*. 2011). RIPK is also targeted by the cysteine protease AvrPphB from *P. syringae*, which cleaves RIPK (Russell, Ashfield and Innes 2015). Notably, AvrPphB suppresses the AvrB- but not the AvrRpm1-mediated activation of RPM1, suggesting that RIPK is dispensable for the RPM1-dependent recognition of AvrRpm1 (Liu *et al*. 2011; Russell, Ashfield and Innes 2015). AvrB does not only target RIPK but also interacts with MPK4, which is an additional RIN4 interaction partner (see above). As MPK4 can phosphorylate RIN4, AvrB might interact with different plant kinases to induce the phosphorylation of RIN4 (Cui *et al*. 2010).

The biochemical mechanisms leading to the AvrRpm1- and AvrB-induced phosphorylation of RIN4 and their impact on ETI and PTI are not yet completely understood. Experimental evidence suggests that the phosphorylation of T166 of RIN4 by AvrRpm1 interferes with the interaction of RIN4 with the cyclophilin ROC1, which was identified as RIN4 interaction partner (Li *et al*. 2014b). ROC1 catalyzes the isomerization between *cis* and trans isoforms of X-prolyl peptide bonds and promotes the cleavage of RIN4 (Coaker *et al*. 2006; Li *et al*. 2014b). Thus, a gain-of-function derivative of ROC1 ($ROC1_{S58F}$) leads to enhanced RIN4 cleavage (Ma *et al*. 2013; Li *et al*. 2014b). Notably, however, the increased RIN4 cleavage in the presence of $ROC1_{S58F}$ does not promote the RPS2-specific HR. On the contrary, $ROC1_{S58F}$ interferes with RPM1 and RPS2 activation and this inhibitory effect on ETI responses is dependent on the catalytic activity of ROC1 (Li *et al*. 2014b).

Experimental evidence suggests that ROC1 interacts with the C-terminal region of RIN4 and catalyzes the *cis/trans* isomerization of P149. This presumably induces a conformational change in RIN4, which interferes with the AvrB-induced phosphorylation of RIN4 as well as with RPM1 and RPS2 activation (Li *et al*. 2014b) (Fig. 11B). A targeted mutagenesis approach led to the identification of P149 as essential residue for RPM1 activation. Thus, the mutant derivative $RIN4_{P149V}$ did not trigger the RPM1-dependent HR (Li *et al*. 2014b). Furthermore, the introduction of the P149V mutation into the phosphomimic RIN4 derivative $RIN4_{T166D}$ abolished the RPM1-dependent HR induced by $RIN4_{T166D}$ (Li *et al*. 2014b). It was, therefore, suggested that the activation of RPM1 does not only depend on the phosphorylation of RIN4 at T166 but also on P149.

Deletion of P149 leads to a constitutive phosphorylation of RIN4 and thus to the activation of RPM1, suggesting that the phosphorylation of RIN4 is regulated by protein conformation. The HR triggered by $RIN4_{\Delta P149}$ was not suppressed by the constitutively active $ROC1_{S58F}$ and was not affected upon introduction of an additional T166A mutation into $RIN4_{\Delta P149}$ (Li *et al*. 2014b). It was, therefore, concluded that $RIN4_{\Delta P149}$ is recognized by RPM1 even in the absence of T166 phosphorylation and that RPM1 senses a specific conformation of RIN4, which is adopted by $RIN4_{\Delta P149}$ (Li *et al*. 2014b). Notably, phosphorylation of RIN4 at T166 leads to a reduced interaction of RIN4 with ROC1 and could thus interfere with the ROC1-mediated isomerization of P149. The authors of this study proposed that ROC1 induces a specific conformational change in RIN4, which interferes with the activation of RPM1 and RPS2. Phosphorylation of RIN4 in the presence of AvrB likely leads to conformational changes that suppress the ROC1-mediated isomerization of RIN4. Thus, the AvrB-mediated phosphorylation of T166 of RIN4 presumably leads to a conformation similar to that of $RIN4_{\Delta P149}$ and is sensed by RPM1 (Li *et al*. 2014b) (Fig. 11B).

Notably, the AvrB- and AvrRpm1-dependent phosphorylation of RIN4 also affects its role in PTI. RIN4 is phosphorylated at the conserved serine residue at position 141 upon perception of flg22 (Chung *et al*. 2014). Phosphorylation of S141 requires BIK1 and/or the BIK1 paralog PBL1 and leads to the derepression (i.e. activation) of PTI (Chung *et al*. 2014). Phosphorylation of S141 is reduced in the phosphomimic RIN4 derivative $RIN4_{T166D}$, suggesting that the T166 phosphorylation is epistatic to S141 phosphorylation (Fig. 11C). Thus, the AvrB/AvrRpm1-induced

phosphorylation of T166 of RIN4 presumably counteracts the effect of the S141 phosphorylation and restores the repression of PTI (Chung *et al.* 2014).

The RLCK PBS1 acts as a decoy for AvrPphB and is guarded by the R protein RPS5

The cysteine protease AvrPphB from *P. syringae* was shown to cleave several plant kinases including BIK1 and RIPK as well as the membrane-associated serine/threonine kinase PBS1 and PBL proteins (Puri *et al.* 1997; Shao *et al.* 2003; Zhang *et al.* 2010; Russell, Ashfield and Innes 2015) (see above). The cleavage of PBS1 by AvrPphB leads to the activation of the CC-NB-LRR R protein RPS5 from *Arabidopsis*, which interacts with PBS1 via the N-terminal CC domain (Swiderski and Innes 2001; Shao *et al.* 2003; Ade *et al.* 2007). RPS5 also interacts with kinase-inactive PBS1 derivatives, suggesting that the kinase activity of PBS1 is not required for the interaction with RPS5 (DeYoung *et al.* 2012). *In planta* expression and interaction studies revealed that both cleavage products of PBS1 interact with RPS5 and are required for HR induction (DeYoung *et al.* 2012). It is assumed that RPS5 detects a conformational change in PBS1 and thus triggers ETI (Fig. 12A). This model is based on the observation that the insertion of five amino acids at the cleavage site of PBS1 interferes with the cleavage of PBS1 and leads to the activation of RPS5 in the absence of AvrPphB (DeYoung *et al.* 2012). RPS5 presumably senses the movement of a loop-exposed five-amino acid motif in the kinase domain of PBS1 (Qi *et al.* 2014). As this motif is polymorphic in different PBL proteins, the AvrPphB-induced cleavage of PBL proteins other than PBS1 does not trigger the RPS5-dependent ETI (Qi *et al.* 2014).

In the absence of PBS1 cleavage, the activity of RPS5 is presumably suppressed by intramolecular interactions between the C-terminal LRR domain and the NB domain (Ade *et al.* 2007). Cleavage of PBS1 by AvrPphB presumably leads to conformational changes in RPS5, which relieves the negative regulation of the NB by the LRR domain and thus leads to the activation of RPS5. Notably, PBS1 is not the main virulence target of AvrPphB because *pbs1* mutants are only marginally affected in PTI defense (Zhang *et al.* 2010). It was, therefore, suggested that PBS1 acts as a decoy, which is guarded by RPS5 and mimicks true virulence targets of AvrPphB such as BIK1 and RIPK (Zhang *et al.* 2010) (see above). A recent study showed that PBS1 can be engineered to mediate recognition of bacterial effectors with protease activity other than AvrPphB. Thus, the exchange of the AvrPphB cleavage site against the cleavage site of AvrRpt2 led to a PBS1 derivative, which is cleaved in the presence of AvrRpt2 and subsequently triggers the RPS5-dependent HR (Kim *et al.* 2016) (Fig. 12A).

The pseudokinase ZED1 acts as a decoy and mediates recognition of HopZ1a by the R protein ZAR1

Another plant decoy is the pseudokinase ZED1 from *Arabidopsis*, which interacts with and is acetylated by the YopJ family member and acetyltransferase HopZ1a from *P. syringae*. Acetylation of ZED1 is presumably recognized by the CC-NB-LRR protein ZAR1, which guards ZED1, and leads to ETI (Lewis *et al.* 2010, 2013) (Fig. 12B). Given that ZED1 is dispensable for the virulence function of HopZ1a, it has probably evolved as a decoy to trigger ZAR1-dependent defense in response to the acetylation by HopZ1a (Lewis *et al.* 2013). As mentioned above, HopZ1a likely targets additional plant proteins including kinases, JAZ proteins and GmHID1, which is involved in isoflavone biosynthesis (Zhou *et al.* 2011; Jiang *et al.* 2013) (Table 1).

In addition to ZED1, ZAR1 interacts with the ZED1-related pseudokinase RKS1. ZAR1 and RKS1 mediate the recognition of the effector protein and uridylyltransferase AvrAC from *Xanthomonas* (see above) (Feng *et al.* 2012; Wang *et al.* 2015). AvrAC uridylylates several RLCKs including BIK1 and RIPK as well as the BIK1 paralog PBL2, which interacts with AvrAC (Feng *et al.* 2012; Guy *et al.* 2013; Wang *et al.* 2015). Uridylylated PBL2 is recruited to the ZAR1-RKS1 complex and triggers ZAR1-dependent ETI (Fig. 12B). As uridylylation of PBL2 is required for AvrAC recognition but dispensable for the virulence activity of AvrAC, PBL2 presumably acts as a decoy for the detection of the enzymatic activity of AvrAC (Wang *et al.* 2015).

CONCLUDING REMARKS

In the past years, significant progress has been made in the identification and molecular characterization of type III effector proteins from plant-pathogenic bacteria. The increasing number of available genome sequences paved the way for comparative studies of type III effectomes and led to the identification of core sets of effectors. The analysis of single- and multi-effector mutants revealed that individual effector proteins do not always significantly contribute to bacterial virulence, presumably because they share functional redundancies. Key for the understanding of the molecular functions of type III effectors in plant cells is the identification of plant targets and the characterization of effector-triggered modifications in plant cellular processes. To date, effectors from plant-pathogenic bacteria were shown to interfere with PTI and ETI responses, MAPK signaling, proteasome-dependent protein degradation, phytohormone signaling, photosynthesis, plant gene expression and the formation of the cytoskeleton. The results of various experimental approaches revealed that effectors from different pathogens employ common as well as pathogen-specific strategies to subvert plant immunity and to promote pathogen survival. It is interesting to note that several effectors appear to have multiple plant targets and thus presumably interfere with different cellular pathways. Examples are HopM1, which interferes with vesicle trafficking and gene expression, and HopZ1a, which targets JAZ proteins, tubulin and the isoflavone biosynthesis enzyme GmHID1. Given the multiple functions of some effectors and the large size of the effectome in different bacterial species, the analysis of effector-triggered alterations in plant cellular pathways and their contribution to bacterial virulence remains a major challenge for ongoing and future research projects.

Several effectors do not only target different plant proteins but also have antagonistic activities as was for instance reported for effectors that suppress or activate the proteasome or MAPK signaling pathways. It is yet largely unknown how effector activities inside the plant cell are regulated. Are all effectors simultaneously translocated after the assembly of the T3S system or is there a hierarchy in type III-dependent effector protein delivery as was reported for animal-pathogenic bacteria? It also remains to be investigated whether effector delivery is not only regulated by the bacterium but also by translocated effectors, which possibly interfere with the insertion of the translocon of the T3S system into the plant plasma membrane. Notably, translocated effectors from animal-pathogenic bacteria were shown to control effector protein translocation from inside the host cell (Dewoody *et al.* 2011; Gaus *et al.* 2011; Berger *et al.* 2012). The efficient translocation of effector proteins into animal cells

depends on host actin filaments, which are one of the targets of type III effectors (Viboud and Bliska 2001; Mejia, Bliska and Viboud 2008; Verove *et al*. 2012). Similar findings have not yet been described for effectors from plant-pathogenic bacteria. Intriguingly, however, it was reported that the recognition of AvrBs2 from *X. campestris* pv. *vesicatoria* in plants possessing the *R* gene *Bs2* suppresses the translocation of additional type III effectors into the plant cell (Zhao *et al*. 2011). It remains to be investigated whether the Bs2-triggered ETI affects the insertion of the translocon or the expression of T3S genes.

In conclusion, we are just beginning to understand the highly complex interplay between type III effectors and plant cellular processes. The characterization of effector activities in plant cells and the analysis of mechanisms underlying the control of effector translocation will, therefore, remain major projects for future research.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSRE online.

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