CURRENT REVIEW

14-3-3 Proteins in Plant-Pathogen Interactions

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14-3-3 proteins define a eukaryotic-specific protein family with a general role in signal transduction. Primarily, 14-3-3 proteins act as phosphosensors, binding phosphorylated client proteins and modulating their functions. Since phosphorylation regulates a plethora of different physiological responses in plants, 14-3-3 proteins play roles in multiple signaling pathways, including those controlling metabolism, hormone signaling, cell division, and responses to abiotic and biotic stimuli. Increasing evidence supports a prominent role of 14-3-3 proteins in regulating plant immunity against pathogens at various levels. In this review, potential links between 14-3-3 function and the regulation of plant-pathogen interactions are discussed, with a special focus on the regulation of 14-3-3 proteins in response to pathogen perception, interactions between 14-3-3 proteins and defense-related proteins, and 14-3-3 proteins as targets of pathogen effectors.

Since protein-protein interactions are fundamental drivers and regulators of cellular functions, spatial and timely control of these molecular interactions is essential for survival of organisms. A major way to regulate protein-protein interactions is through posttranslational modifications that provide high proteomic plasticity in a dynamic and cost-efficient manner. Posttranslational modifications seem to play a prominent role in plant biology and are key regulators of plant growth and development and of responses to environmental cues (Vierstra 2012). The remarkable expansion and relevance of posttranslational modification in plants could be an effect of the sessile nature of this kingdom, which makes it vital to integrate cues and adjust plant physiology appropriately, as opposed to behavioral changes in animals.

Phosphorylation is an essential posttranslational modification, fast and reversible, that affects thousands of proteins and regulates a plethora of different processes in the plant, including signal transduction, cell cycle regulation, metabolism, and transcriptional and translational control. Protein phosphorylation occurs mainly on serine (pS), threonine (pT), and tyrosine (pY) residues. To monitor these posttranslational changes, cells contain protein modules acting as phosphosensors with the capacity to sense the phosphostatus of a specific site and translate that information into a physiological change. One such module is formed by homo- or hetero-dimers of the so-called 14-3-3 proteins, which define a eukaryotic-specific protein family with a primary role in signal transduction.

14-3-3 proteins: Features, properties, and modes of action.

14-3-3 proteins were originally isolated from mammalian brain tissue and were named according to their elution and migration pattern on DEAD-cellulose chromatography and starch-gel electrophoresis (14-3-3 proteins eluted in the 14th fraction and were found on position 3.3 in the following electrophoresis) (Moore and Perez, 1967). Subsequent studies demonstrated that 14-3-3 proteins are eukaryotic-specific evolutionary conserved proteins encoded by multigene families. Yeast has two genes encoding 14-3-3 proteins, and the double mutant is lethal, indicating that the function of this protein family is essential (van Heusden et al. 1995). Animals typically have seven 14-3-3 genes, while plants have more: 13 genes have been found in *Arabidopsis* (Rosenquist et al. 2001) (Table 1), 12 in tomato (Roberts and Bowles 1999; Xu and Shi 2006) (Table 2), and 17 in tobacco (Konagaya et al. 2004) (Table 2). Interestingly, monocot plants appear to have lower 14-3-3 numbers, with eight members in rice (Yao et al. 2007) (Table 2) and five in barley (Schoonheim et al. 2007). In *Arabidopsis*, 14-3-3 proteins are designated according to two different nomenclatures, both of which are used in the literature. The first one uses Greek letters, in analogy with the animal 14-3-3 proteins. More recently however, *Arabidopsis* 14-3-3 proteins were termed general regulatory factors (GRF1-13) (Table 1).

The protein sequence of 14-3-3 proteins can be divided into three regions: a variable N-terminus, a conserved core region, and a variable C-terminus. Based on their gene structure, plant 14-3-3 proteins can be divided into two distinct groups, namely epsilon and non-epsilon, the latter being plant-specific (Chung et al. 1999; DeLille et al. 2001; Ferl et al. 2002). Crystal structures of 14-3-3 proteins, both from animal and plant origin, have been resolved, revealing that 14-3-3 proteins form homo- or hetero-dimers, with a characteristic flattened horseshoe structure containing a central cavity and two amphipathic grooves that are the phosphopeptide-binding pockets (Wurtele et al. 2003) (Fig. 1A).

The structure of 14-3-3 proteins underlies their activity, since they act as sensors for the phosphostatus of specific phosphosites, in turn translating this status into a change in the activity of the target (client) protein. To date, no enzymatic activity has been ascribed to 14-3-3 proteins, thus indicating that their function is most likely based solely on protein-protein interactions. Most of the 14-3-3 client proteins are phosphorylated on serine or threonine residues, often present in conserved binding motifs. Three canonical motifs have been described so far: mode I (RSx[pS/pT]xP); mode II (Rx Φx[pS/pT]xP) (where Φ stands for an aromatic or aliphatic amino acid and x for any amino acid) (Muslin et al. 1996; Yaffe et al. 1997); and the C-terminal mode III ([pS/pT]x₁₋₂-COOH) (Coblitz et al. 2005; Ganguly et al. 2005). The latter mode III is best characterized from the

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plant plasma membrane H⁺-ATPase pump (YpTV-COOH) (Maudoux et al. 2000; Ottmann et al. 2007). Additionally, there are also proteins that bind 14-3-3 proteins through non-canonical or even nonphosphorylated motifs (Aitken et al. 2002). The functional mechanism of 14-3-3 proteins can be grouped into five modes of action. 14-3-3 proteins can (i) interfere with the ability of the client protein to bind other interaction partners, (ii) alter the subcellular localization of the client protein, (iii) bridge two client proteins together, (iv) modify the catalytic activity of the client protein, or (v) protect the client protein from either posttranslational modifications, proteolysis, or both (Fig. 1C).

In plants, large-scale interactomics and mass-spectrometry-based studies have revealed more than 300 potential 14-3-3 target proteins, making 14-3-3 proteins one of the most highly connected nodes in the plant interactome (de Boer et al. 2013). However, only around 40 of these protein-protein interactions have been further characterized. Despite the structural conservation of 14-3-3 proteins, and although redundancy exists between closely related members of the family, there is functional specificity within the 14-3-3 family. This functional specificity can be determined at different levels: (i) regulation of gene expression in specific cell types or upon selected stimuli; (ii) differences in protein structure, which could determine binding partners and distinct subcellular localization; and (iii) differential phosphorylation of 14-3-3 proteins themselves, which occurs in an isoform-specific manner (Aitken 2011).

A growing body of work indicates that 14-3-3 proteins regulate plant responses to pathogens, and may play roles in different

interactions and at multiple levels. Some 14-3-3 proteins change their transcriptional expression, their protein abundance or their properties in response to pathogen detection or infection, and some 14-3-3 proteins interact or associate with known regulators of plant defense. Significantly, some 14-3-3 proteins are targeted by pathogen effector proteins, which strongly suggest a function of these 14-3-3 proteins in plant resistance. In the following sections, we review the potential involvement of 14-3-3 proteins in plant-pathogen interactions.

Changes in 14-3-3 expression, abundance, or properties in response to pathogens.

14-3-3 proteins from different plant species have been shown to display changes in transcript levels or, in some instances, in protein abundance or properties, or both, upon pathogen infection or perception (Table 3). For example, the tomato *14-3-3 TFT1* gene is repressed in the interaction between tolerant tomato and *Verticillium dahliae* (Robb et al. 2007). Most reported expression changes during plant-pathogen interactions, however, involve an induction of *14-3-3* genes. These include tomato *TFT1*, *TFT4*, and *TFT6* genes, which are up-regulated in the Cf-9-mediated hypersensitive response (HR) (Roberts and Bowles 1999). Similarly, *14-3-3* genes are expressed during a race-specific HR of soybean inoculated with *Pseudomonas syringae* (Seehaus and Tenhaken 1998) and upon a resistant reaction to the soybean cyst nematode (Klink et al. 2009). The tobacco *14-3-3* isoform *h* is induced after inoculation with *Tobacco mosaic virus* (TMV) (Konagaya et al. 2004). A *Gossypium hirsutum 14-3-3* is also rapidly expressed in response to *Verticillium dahliae* (Hill et al. 1999) in a cultivar with enhanced wilt resistance, which may suggest a specific role for 14-3-3 in this process.

In a few cases, experimental data provides a functional link between the detected abundance changes and a subsequent impact on plant defense responses. In barley, a *14-3-3* is expressed in leaves after inoculation with *Blumeria graminis* (Smedegaard-Petersen et al. 1992). The transcript for this *14-3-3* accumulates early, though weakly, in the defense response, concomitantly with a number of other defense-related transcripts (encoding pathogenesis-related proteins and peroxidases, among others). In contrast to other interactions, no difference in the accumulation of *14-3-3* transcripts is noted between compatible and incompatible interactions with the barley powdery mildew fungus (*Blumeria graminis* f. sp. *hordei*), which prompted the idea that increased transcript accumulation is associated with the penetration stage. Supporting this notion, transcript and protein accumulation was most marked in the epidermis of the infected leaves, while levels in the mesophyll were essentially unaffected (Finnie et al. 2002; Gregersen et al. 1997). A *14-3-3* (homolog to the barley *Hv14-3-3b*) is specifically expressed in wheat resistant to *Puccinia triticina* upon interaction with the fungus (Manickavelu et al. 2010) and, interestingly, a *14-3-3* protein-encoding gene has been proven to be associated with a quantitative trait locus for stem rust resistance in this plant species (Faris et al. 1999).

In maize, the *14-3-3 ZmGF14-6* is up-regulated in response to *Fusarium verticilloides*. However, transgenic expression of this gene in rice leads to increased susceptibility to the fungal pathogens *F. verticilloides* and *Magaporthae oryzae* and reduced expression of pathogen-regulated defense genes (Campo et al. 2012). Since rice expressing *ZmGF14-6* displays enhanced tolerance to drought stress, which is accompanied by stronger induction of drought-associated genes, *ZmGF14-6* seems to inversely regulate plant responses to biotic and abiotic stresses (Campo et al. 2012).

In rice, several genes encoding 14-3-3 proteins are induced in response to compatible or incompatible interactions with

Table 1. Nomenclature of 14-3-3 proteins in *Arabidopsis*

AGI number ^a	Greek letter	General regulatory factor
At4g09000	Chi (χ)	GRF1
At1g78300	Omega (ω)	GRF2
At5g38480	Psi (ψ)	GRF3
At1g35160	Phi (φ)	GRF4
At5g16050	Upsilon (υ)	GRF5
At5g10450	Lambda (λ)	GRF6
At3g02520	Nu (ν)	GRF7
At5g65430	Kappa (κ)	GRF8
At2g42590	Mu (μ)	GRF9
At1g22300	Epsilon (ε)	GRF10
At1g34760	Omicron (ο)	GRF11
At1g26480	Iota (ι)	GRF12
At1g78220	Pi (π)	GRF13

^a *Arabidopsis* Genome Initiative identification number.

Table 2. 14-3-3 proteins in tomato, tobacco, and rice

Tomato		Tobacco		Rice	
Isoform	GenBank	Isoform	GenBank	Isoform	GenBank
TFT1	X95900	14-3-3a-1	AB119466	GF14a	AK101599
TFT2	X95901	14-3-3b-1	AB119467	GF14b	AK071822
TFT3	X95902	14-3-3b-2	AB119468	GF14c	AK070230
TFT4	AJ504807	14-3-3c-1	AB119468	GF14d	AK101580
TFT5	X95903	14-3-3c-2	AB119470	GF14e	AK068248
TFT6	X95904	14-3-3d-1	AB119471	GF14f	AK103065
TFT7	X95905	14-3-3d-2	AB119472	GF14g	AK103145
TFT8	X98864	14-3-3d-2-AS	AB119473	GF14h	AK072443
TFT9	X98865	14-3-3e-1	AB119474		
TFT10	X98866	14-3-3e-2	AB119475		
TFT11	–	14-3-3f-1	AB119476		
TFT12	–	14-3-3f-2	AB120322		
		14-3-3g-1	AB119477		
		14-3-3h-1	AB119478		
		14-3-3h-2	AB119479		
		14-3-3i-1	AB119480		
		14-3-3i-2	AB119481		

Fig. 1. Structure and modes of action of 14-3-3 proteins. **A**, Structure of a 14-3-3c homodimer from *Nicotiana tabacum*. The image was generated using the Protein Data Bank (PDB) viewer, with PDB ID 1O9C; monomers are indicated with different shades. Asterisks indicate the phosphopeptide-binding pockets. **B**, Legend of symbols used in C. **C**, General modes of action of 14-3-3 proteins. 14-3-3 proteins can (i) interfere with the ability of the client protein to bind other interaction partners, (ii) alter the subcellular localization of the client protein, (iii) bridge two client proteins together, (iv) modify the catalytic activity of the client protein, or (v) protect the client protein from either posttranslational modifications, proteolysis, or both. 14-3-3 dimers are shown in white and gray, dark gray circles represent phosphate groups, and light gray circles represent ubiquitin moieties.

Magnaporthe grisea (GF14b, GF14c, GF14e, GF14f) or *Xanthomonas oryzae* pv. *oryzae* (GF14b, GF14c, GF14e) (Chen et al. 2006). GF14b is also induced during effector-triggered immunity (ETI) against *X. oryzae* and negatively affects cell death and disease resistance in rice (Manosalva et al. 2011). This suggests that 14-3-3 proteins may control a negative feedback loop to prevent harmful overactivation of defense responses. Additionally, rice GF14a has been linked to quantitative blast resistance (Liu et al. 2004).

Recent reports indicate that the protein levels of 14-3-3 proteins can also vary upon interaction with pathogens. In *Medicago* roots, mixed infections with symbiotic soil microorganisms (*Glomus intraradices* or *Sinorhizobium meliloti*) and the pathogenic oomycete *Aphanomyces euteiches* result in the accumulation of 14-3-3 proteins (four isoforms, representing two different proteins) (Schenkluhn et al. 2010). Although these

proteins were induced during mixed infections, they were predominant in the symbiotic interactions, raising the possibility that they may contribute to pretriggering the plant's defense potential, leading to subsequent increased pathogen resistance.

In *Arabidopsis*, potyvirus infection results in a general decrease of GRF6/14-3-3 λ . *GRF6* is transcriptionally repressed in response to the viral infection. Additionally, this interaction activates mitogen-activated protein kinase 11, which in turn phosphorylates GRF6, increasing its ubiquitination and turnover (Carrasco et al. 2014). Given that a *grf6* mutant is more resistant to potyvirus infection, GRF6 can be considered a susceptibility factor toward potyviruses (Carrasco et al. 2014).

The detection of pathogen-associated molecular patterns (PAMPs), which are perceived by the plant as nonself, is the basis of the first layer of active plant immunity, termed

Table 3. Plant 14-3-3 proteins transcriptionally regulated in response to pathogen perception

Species/Isoform	Stimulus	Effect on 14-3-3	Outcome	Reference
Barley	<i>Blumeria graminis</i> f. sp. <i>tritici</i> (nonhost)	Gene induction	?	Smedegaard-Petersen et al. 1992; Brandt et al. 1992
Maize/ZmGF14 to GF146	<i>Fusarium verticilloides</i>	Gene induction	Negative regulation of defense	Campo et al. 2012
Rice/GF14b, GF14c, GF14e, GF14f	<i>Magnaporthe grisea</i> (compatible or incompatible interaction)	Gene induction	?	Chen et al. 2006
Rice/GF14b, GF14c, GF14e	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> (compatible or incompatible interaction)	Gene induction	?	Chen et al. 2006
Rice/GF14b	Effector-triggered immunity (ETI) triggered by <i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Gene induction	Negative regulation of cell death, defense and resistance.	Manosalva et al. 2011
Soybean	ETI triggered by <i>Pseudomonas syringae</i> pv. <i>glycinea</i>	Gene induction	U	Seehaus and Tenhaken 1998
Soybean/GRF2	Soybean cyst nematode (resistance reaction)	Gene Induction	?	Klink et al. 2009
<i>Arabidopsis</i> /GRF6	Potyvirus infection	Increased protein phosphorylation and turnover and repressed gene expression	Susceptibility factor/negative regulation of defense	Carrasco et al. 2014
<i>Gossypium hirsutum</i>	<i>Verticillium dahliae</i>	Gene induction	?	Hill et al. 1999
<i>Nicotiana tabacum</i> /14-3-3h	<i>Tobacco mosaic virus</i>	Gene induction	?	Konagaya et al. 2004
<i>Nicotiana tabacum</i>	Lipopolysaccharide	Protein phosphorylation	?	Gerber et al. 2006
<i>Nicotiana tabacum</i> /14-3-3c	Cryptogein	Enrichment in detergent-resistant membranes	?	Stanislas et al. 2009
Poplar	Chitosan	Gene induction	?	Lapointe et al. 2001
Sycamore	Chitosan	Protein accumulation	?	Malerba et al. 2012
Tomato/TFT1, 4, 6	Cf-9-mediated hypersensitive response	Gene induction	?	Roberts and Bowles 1999
Tomato/TFT1	<i>Verticillium dahliae</i>	Gene repression	?	Robb et al. 2007
Wheat/Hv14-3-3b	<i>Puccinia trititica</i>	Expressed in resistant sample specifically	?	Manickavelu et al. 2010

Table 4. Plant 14-3-3 proteins shown to interact with defense-related proteins

Species/isoform	Interactors	In vivo interaction	Effect	Reference
<i>Arabidopsis</i> /14-3-3 ω , 14-3-3 ψ , 14-3-3 ϕ	CPK1	No	Positive regulation of defense?	Camoni et al. 1998
<i>Arabidopsis</i> /14-3-3 λ	AKR2	No	?	Yan et al., 2002
<i>Arabidopsis</i> /14-3-3 ω	CPK1, BAK1, WRKYs, RPP13-like 1 and 2	Yes	?	Chang et al. 2009
<i>Arabidopsis</i> /14-3-3 λ	RPW8.2	No	Increased resistance	Yang et al. 2009
<i>Nicotiana tabacum</i> /14-3-3a-1, b-1, b-2, c-1, c-2, d-1, d-2, d-2-AS, e-1, e-2, f-1, g-1, h-1, h-2, i-1, i-2.	N protein	No	?	Konagaya et al. 2004
<i>Nicotiana tabacum</i> /14-3-3h	NtrbohD	No	Negative regulation of ROS production?	Elmayan et al. 2007
Tomato/TFT7	SIMAPKKK α	Yes	Positive regulation of immunity-associated programmed cell death (PCD)	Oh et al. 2010
Tomato/TFT7	SIMKK2	Yes	Positive regulation of immunity-associated PCD	Oh and Martin 2011

PAMP-triggered immunity (PTI) (Boller and Felix 2009). PTI is sufficient to fend off most potentially infectious pathogens, therefore providing evident fitness benefits. As could be expected from their role in plant-pathogen interactions, 14-3-3 proteins undergo significant changes following PAMP perception (Table 2). For example, *14-3-3* genes are induced in poplar in response to the fungal and insect PAMP chitosan (Lapointe et al. 2001). This PAMP also induces accumulation of 14-3-3 proteins in sycamore cell cultures (Malerba et al. 2012). In tobacco, four different 14-3-3 proteins have been shown to undergo phosphorylation in response to the bacterial PAMP lipopolysaccharide from *Burkholderia cepacia* (Gerber et al. 2006). Moreover, another 14-3-3 is enriched in detergent-resistant membranes (DRM) upon treatment with the *Phytophthora* elicitor cryptogein (Stanislas et al. 2009), suggesting that 14-3-3 proteins are recruited to function in defense signaling. In line with this idea, cryptogein

treatment induces the expression of the *14-3-3* isoform *h* (Elmayan et al. 2007).

14-3-3 proteins interacting with defense-related proteins.

Several 14-3-3 proteins have been found to interact with components of the plant immune system (Table 4). In *Arabidopsis*, proteomic profiling of tandem affinity-purified 14-3-3 protein complexes has shown that these proteins associate with several defense-related proteins in planta, including BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), which is a co-receptor kinase of PAMP receptors, several WRKY transcription factors, and two disease resistance (R) proteins (Chang et al. 2009). In a far-Western screen searching for interactors of the tobacco *N* resistance gene, which recognizes the helicase (HEL) domain of the TMV replicase, nearly 92% of positive clones were related to the 14-3-3 gene family (Konagaya et al. 2004). Remarkably,

Table 5. Plant 14-3-3 proteins shown to interact with pathogen effectors

Species/isoform	Pathogen/effector	Effect of the interaction	Reference
<i>Arabidopsis</i> /14-3-3κ	<i>Pseudomonas syringae</i> /HopM1	Promotion of virulence	Nomura et al. 2006
<i>Nicotiana benthamiana</i> /14-3-3a-i	<i>Pseudomonas syringae</i> /HopQ1	?	Giska et al. 2013
Tomato/TFT9	<i>Xanthomonas campestris</i> /AvrRxv	Promotion of virulence	Whalen et al. 2008
Tomato/TFT1	<i>Xanthomonas campestris</i> /XopN	Promotion of virulence	Kim et al. 2009; Taylor et al. 2012
Tomato/TFT1, TFT5, TFT7, TFT9, TFT10	<i>Pseudomonas syringae</i> /HopQ1	?	Li et al. 2013
Tomato and pepper/tomato TFT4 (also TFT2, TFT5, TFT11 and, weakly, TFT3, TFT6, TFT7, TFT8, and TFT9) and pepper TFT4	<i>Xanthomonas euvesicatoria</i> /XopQ	Suppression of effector-triggered immunity	Teper et al. 2014

Fig. 2. Phylogenetic tree of *Arabidopsis* and tomato 14-3-3 proteins. 14-3-3 proteins known to interact with pathogen effectors are highlighted by shaded boxes. The tree was generated with phylogeny.fr (Dereeper et al. 2008).

subsequent *in vitro* binding assays revealed a degree of isoform specificity: only 14-3-3 isoforms of groups ω and ψ bound to both the toll/interleukin-1 receptor (TIR) and leucine-rich repeat (LRR) domains of the N protein and to the HEL domain of the TMV replicase. The 14-3-3 isoform *h*, whose expression is induced in response to TMV infection, showed significant binding to the LRR domain of the N protein and to the viral HEL domain but only ancillary to the TIR domain in N. These results raise the possibility that 14-3-3 proteins may act as scaffolds, bridging the N protein and its target, the viral replicase, together to promote pathogen recognition and resistance (Konagaya et al. 2004).

Interestingly, tobacco 14-3-3 isoform *h*, whose expression is induced upon cryptogeiin treatment, binds the C terminus of the tobacco NADPH oxidase NtrbohD in yeast, and expression of an antisense construct of this 14-3-3 abolishes the RBOHD-mediated cryptogeiin-triggered burst of reactive oxygen species (ROS) (Elmayan et al. 2007). However, the direct or indirect nature of this functional link as well as the isoform specificity of both silencing construct and interaction remain to be determined. A closely related member of the 14-3-3 family associates to DRM in response to cryptogeiin treatment (Stanislas et al. 2009). Since NtrbohD has been shown to associate to DRM in a sterol-dependent manner (Roche et al. 2008), the enrichment of 14-3-3 proteins in this fraction upon PAMP perception and the concomitant interaction with NtrbohD, which would promote the activity of the NADPH oxidase, could potentially be relevant for the activation of immunity.

The tomato 14-3-3 protein TFT7 positively regulates programmed cell death (PCD) associated with immunity upon recognition of several pathogen virulence proteins by the cognate plant R proteins (Oh et al. 2010). TFT7 interacts with MAPKKK α and the downstream SIMKK2 *in vivo* (Oh and Martin 2011). Since TFT7 homodimerizes, it has been suggested to act as a scaffold, recruiting and bridging these client proteins for optimal signal transfer, which would, in turn, result in PCD.

The *RPW8* locus from *Arabidopsis* Ms-0 confers salicylic acid-dependent resistance to powdery mildew (*Golovinomyces* spp.) (Xiao et al. 2001). In a yeast two-hybrid screen searching for *RPW8* regulators, *Arabidopsis* 14-3-3 λ was identified (Yang et al. 2009). Consequently, a T-DNA insertional mutant of 14-3-3 λ displays compromised resistance, whereas a respective overexpressing line shows HR-like cell death and enhanced resistance (Yang et al. 2009), illustrating a functional role for this 14-3-3 protein in *RPW8*-mediated resistance.

14-3-3 proteins as pathogen targets.

Plant pathogens can secrete effector proteins into the host cell as a virulence strategy to promote infection. Since an effective suppression of immunity is a requisite for pathogens to establish a successful infection, the primary targets of many of these effectors are defense-related components. This effector-mediated suppression of immunity favors pathogen fitness and proliferation (Dou and Zhou 2012). Because effector proteins have evolved to efficiently target specific processes in the host cell, they can be used as molecular probes to further our understanding of eukaryotic cell biology. This approach has been particularly fruitful in the case of the plant immune system, since pathogen effectors target many defense components and regulators (Lee et al. 2013; Xin and He 2013).

Of particular interest is the fact that, in recent years, 14-3-3 proteins have consistently appeared as targets of bacterial effectors (Table 5). AvrRxv from *Xanthomonas campestris* pv. *vesicatoria* functions by interacting with tomato TFT9 (Whalen et al. 2008). However, an alternative hypothesis is that the AvrRxv-TFT9 interaction results in ETI, the plant immune response derived from recognition of pathogen effectors by disease resistance proteins (Taylor et al. 2012). Interestingly, another

effector from the same bacterial species, XopN, binds tomato TFT1, which functions in PTI, to promote bacterial virulence (Kim et al. 2009; Taylor et al. 2012). XopN forms a complex with TFT1 and the atypical LRR receptor-like kinase TARK1, also required to inhibit *Xanthomonas* growth (Kim et al. 2009), potentially promoting or stabilizing the interaction between these two host proteins (Taylor et al. 2012). Given that TFT1 and TARK1 have not been found to interact in the absence of XopN, this virulence strategy may lead to the sequestration of positive regulators of immunity (namely TFT1 and TARK1) in inactive complexes, hence suppressing downstream immune responses (Taylor et al. 2012).

A third 14-3-3 from tomato, TFT4, seems to be required for ETI-associated cell death (Teper et al. 2014). This 14-3-3 is targeted by the effector XopQ from *Xanthomonas euvesicatoria*, and this interaction results in suppression of ETI (Teper et al. 2014). Intriguingly, XopQ also interacts with TFT2, TFT5, and TFT11 in yeast and, more weakly, with TFT3, TFT6, TFT7, TFT8, and TFT9 (Teper et al. 2014). In a similar fashion, two different effectors from *Pseudomonas syringae* associate with a number of host 14-3-3 proteins. HopQ1 interacts with several 14-3-3 proteins in a phosphorylation-dependent manner in both *Nicotiana benthamiana* and tomato (Giska et al. 2013; Li et al. 2013). The interactions between HopQ1 and the 14-3-3 proteins seem to influence the effector's subcellular localization and virulence-promoting activity (Li et al. 2013). Altogether, it is noteworthy that 11 of 12 tomato 14-3-3 proteins have been shown to be bound by bacterial effectors (Fig. 2) (Kim et al. 2009; Li et al. 2013; Teper et al. 2014; Whalen et al. 2008).

The conserved *Pseudomonas syringae* effector HopM1, which significantly contributes to bacterial pathogenesis, binds *Arabidopsis* 14-3-3 κ in addition to its well-known target AtMIN7/BEN1/BIG5 and promotes its proteasomal degradation (Nomura et al. 2006). Notably, *Arabidopsis* 14-3-3 κ is the closest homolog of tomato TFT1, which is required for PAMP-triggered production of ROS, a response suppressed by HopM1 (Lozano-Duran et al. 2014). Therefore, it has been speculated that HopM1 suppresses early PTI responses, namely PAMP-triggered production of ROS and stomatal closure, through destabilization of 14-3-3 κ /TFT1, although a direct link remains to be established (Lozano-Duran et al. 2014).

Perspectives.

The involvement of 14-3-3 proteins in plant-pathogen interactions is an emerging theme in this field. A growing body of reports show differential regulation of 14-3-3 proteins in response to pathogen perception and interaction of 14-3-3 proteins with known regulators of plant immunity and unveil 14-3-3 proteins as targets of pathogen effectors. Functional data support a role of 14-3-3 proteins in the regulation of the outcome of plant-pathogen interactions. Together with evidence obtained from different plant species and pathosystems, this suggests a prominent role for 14-3-3 proteins in plant defense and immunity. However, the nature and full extent of such a role, with special focus on the exact underlying molecular mechanisms and considering isoform specificity, still needs to be explored.

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