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Review

14-3-3 proteins as a major hub for plant immunity

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14-3-3 proteins, ubiquitously present in eukaryotic cells, are regulatory proteins involved in a plethora of cellular processes. In plants, they have been studied in the context of metabolism, development, and stress responses. Recent studies have highlighted the pivotal role of 14-3-3 proteins in regulating plant immunity. The ability of 14-3-3 proteins to modulate immune responses is primarily attributed to their function as interaction hubs, mediating protein–protein interactions and thereby regulating the activity and overall function of their binding partners. Here, we shed light on how 14-3-3 proteins contribute to plant defense mechanisms, the implications of their interactions with components of plant immunity cascades, and the potential for leveraging this knowledge for crop improvement strategies.

Highlights

At the cellular level, plant immunity is orchestrated by a complex network of proteins, among which the 14-3-3 proteins have emerged as pivotal players.

Both microbe-associated molecular pattern (MAMP)-triggered immunity (MTI) and effector-triggered immunity (ETI) are critical for plant survival against microbial threats, and the 14-3-3 proteins have been identified as central regulators of these processes.

Due to their multifunctionality in many plant pathways and conserved nature, 14-3-3 proteins often serve as targets for viral, bacterial, and fungal elicitors/effectors. This highlights their significance as crucial targets to enhance crop resilience and disease resistance.

14-3-3 proteins in plants

14-3-3 proteins were discovered in 1967 by Moore and Perez in bovine brain tissue, earning their name based on their unique elution and migration characteristics during DEAD-cellulose **chromatography** (see Glossary) and starch-gel **electrophoresis**. Later research uncovered that these proteins are exclusive to eukaryotes and consistently maintained through evolutionary changes [1]. For instance, yeast possesses two such genes, while animals typically contain seven 14-3-3 genes. Interestingly, dicot plants have more members of the 14-3-3 proteins than monocot plants, for example, arabidopsis (*Arabidopsis thaliana*) has 13, tomato (*Solanum lycopersicum*) 12, and tobacco (*Nicotiana tabacum*) 17, compared with rice (*Oryza sativa*) eight and barley (*Hordeum vulgare*) five [2]. However, the maize (*Zea mays*) genome encodes 28 14-3-3 proteins, which is almost four times more than other Gramineae species like sorghum, rice, and *Brachypodium* [3]. The comparison between **monocot and dicot** 14-3-3s suggests a conserved evolution of 14-3-3 in monocots, while 14-3-3s are more distantly related between monocots and dicots [3,4]. Segmental gene duplication has been suggested to cause the gene family expansion in many species, like grapevine and soybean [5,6]. In plants, the different 14-3-3 isoforms are denoted by Greek letters or named G-box factor 14-3-3s (GF14s), general regulatory factors (GRFs), and tomato fourteen-three-threes (TFTs) [7].

Structural features

14-3-3s are small acidic proteins (27–32 kDa) that have a dynamic N terminus, a stable core, and a versatile C terminus. Gene structures help to categorize plant 14-3-3 proteins into epsilon and non-epsilon groups, with the non-epsilon group found exclusively in plants. Crystal structures of 14-3-3 proteins of both animals and plants revealed that 14-3-3 proteins form stable homo- or heterodimers with each monomer comprising nine tightly packed antiparallel α-helices forming a characteristic horseshoe or cup-like structure approximately 35 Å broad, 35 Å wide, and 20 Å deep [8]. This structure creates an **amphipathic** ligand-binding groove able to recognize the positively charged pSer or pThr of their interacting protein partners. However, 14-3-3 dimers can also be destabilized by phosphorylation on specific residues, such as Ser58 at the dimer

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interface [9]. The C-terminal helix I was shown to be flexible and could be involved in recognition of target proteins [10]. Large-scale protein interactome mapping and mass spectrometry-based studies have identified more than 300 putative interactors of 14-3-3 proteins [11]. Most of the interactors are phosphoproteins and therefore 14-3-3 proteins are primarily considered phospho-serine/threonine sensors. Interestingly, no enzymatic activity has been attributed to 14-3-3 proteins, suggesting that their predominant role is mediating protein–protein interactions. Three canonical binding motifs are usually associated with 14-3-3 interaction: mode I (R/K)SX(S/T)PXP, mode II (R/R)XΦX(S/T)PXP (with Φ representing an aromatic or aliphatic amino acid and X representing any amino acid), and the C-terminal mode III motif (S/T)P X 1–2–COOH [12]. Recent high-resolution structural analyses have uncovered noncanonical binding sites for the small regulatory molecules associated with plant 14-3-3 proteins like fusicoccin (FC) and S4 [10,13,14]. Despite their observed structural consistency, members of the 14-3-3 protein family exhibit functional specificity. Overlapping functionalities are found among closely related proteins, while unique roles become evident based on their unique expression profiles and phosphorylation patterns under different developmental and stress conditions [15].

Diverse functions

Based on the biochemical characteristics of their phosphorylated target, 14-3-3 proteins can assume a wide array of regulatory roles (Box 1 and see Figure I in Box 1). Functionally, 14-3-3 proteins operate by modifying the interaction ability, catalytic activity, or cellular localization of their target proteins. Additionally, 14-3-3 proteins can act as a bridges/scaffolds linking target proteins and regulating their post-translational modifications and stability [2]. Since Michael Roberts summarized the expanding interaction network of plant 14-3-3 proteins two decades ago, numerous studies have highlighted their strengthened interactions and increased functional capabilities [16]. For example, 14-3-3 play pivotal roles in various cellular processes, such as metabolism, transport, signal transduction, and plant adaptation to abiotic and biotic stresses [7]. In this review, we focus on the role of 14-3-3 proteins in plant immunity and their potential as targets for disease prevention in crops.

The role of 14-3-3 in pattern-triggered immunity (PTI)

Plant cells activate an immune response termed PTI by identifying conserved microbial patterns, known as pathogen-associated molecular patterns (PAMPs) through cell-surface receptors called pattern recognition receptors (PRRs), [17]. A variety of PRRs have been discovered orchestrating PTI in response to bacterial, fungal, oomycete, nematode, and insect pathogens. PRRs mainly comprise receptor kinases and receptor-like proteins. Examples include: FLAGELLIN SENSITIVE2 (FLS2), which recognizes the bacterial flagellin epitope flg22; EF-TU RECEPTOR, which binds to the bacterial translation elongation factor EF-Tu epitope elf18; LYSIN MOTIF RECEPTOR KINASE5, which detects the fungal cell wall component chitin; and PLANT ELICITOR PEPTIDE RECEPTORs (PEPRs) that are specific for plant elicitor peptides (Peps) [18]. The recognition of MAMP elicitors by their corresponding PRRs triggers a cascade of downstream cellular signalling activities. This includes the production of reactive oxygen species (ROS), changes in calcium ion fluxes, the activation of mitogen-activated protein kinase (MAPK) pathways, the deposition of callose, and extensive **chromatin** remodeling leading to transcriptional reprogramming [19–22].

Receptor-like cytoplasmic kinases (RLCKs) are instrumental in bridging the activated PRRs with the activation of these essential downstream components [23]. For example, multiple members of RLCK subfamily VII, like PBL19, 20, 37, 38, and 39, and PBL40 and BSK1 of subfamily XII are shown to regulate both antifungal and antibacterial immunity by phosphorylating the N or C terminus of MAPKKK5 or MPK15 [24–26].

Glossary

Amphipathic: amphipathic molecules are chemical compounds that have both polar and nonpolar regions, giving them both hydrophilic (water loving) and lipophilic (fat loving) properties.
Chromatin: primarily the complex of genomic DNA with proteins called histones that forms the chromosomes in the nucleus of a eukaryotic cell.
Chromatography: a laboratory technique for the separation of a mixture into individual components by passing it in solution or suspension through a medium in which the components move at different rates.
Effectors: bacterial effectors are proteins secreted by pathogenic bacteria into the cells of their host, usually using a type 3 secretion system (TTSS/T3SS). Effectors can target different host proteins and consequently alter signaling pathways to modulate host responses, which is critical for the establishment of a successful infection by the pathogen.
Electrophoresis: a laboratory technique used to separate DNA, RNA, or protein molecules based on their size and electrical charge, where electric current moves the molecules through a gel or other matrix.
Monocot and dicot: a monocot (monocotyledons) is a flowering plant (angiosperm) with an embryo that bears a single cotyledon (seed leaf), while dicots possess two cotyledons. Monocots typically have elongated, stalkless leaves with parallel veins (e.g., grasses, lilies, palms) while dicots typically have reticulate venation and floral organs usually arranged in multiples of four or five.

Box 1. 14-3-3 signalling mechanisms and regulation

Owing to their rigid structure, 14-3-3 proteins are often considered 'molecular anvils', exhibiting minimal conformational changes on target binding. Instead, this interaction compels target proteins to undergo structural changes, subsequently modifying their functional attributes, localization, or protein–protein interactions. For proteins whose activity hinges on their specific subcellular positioning, 14-3-3s can be crucial in masking or exposing their localization motifs. Moreover, when bound to 14-3-3 proteins, target proteins might not successfully engage with their subsequent interacting partners. Based on their capacity to concurrently interact with multiple target proteins via their bivalent docking sites, 14-3-3s can function as scaffold proteins thereby creating central hubs fostering productive interactions among protein partners (Figure I). Although 14-3-3 proteins are posited to function as molecular chaperones in animal systems, participating in the regulation of protein folding and aggregation, such activity has yet to be documented in plant systems [56].

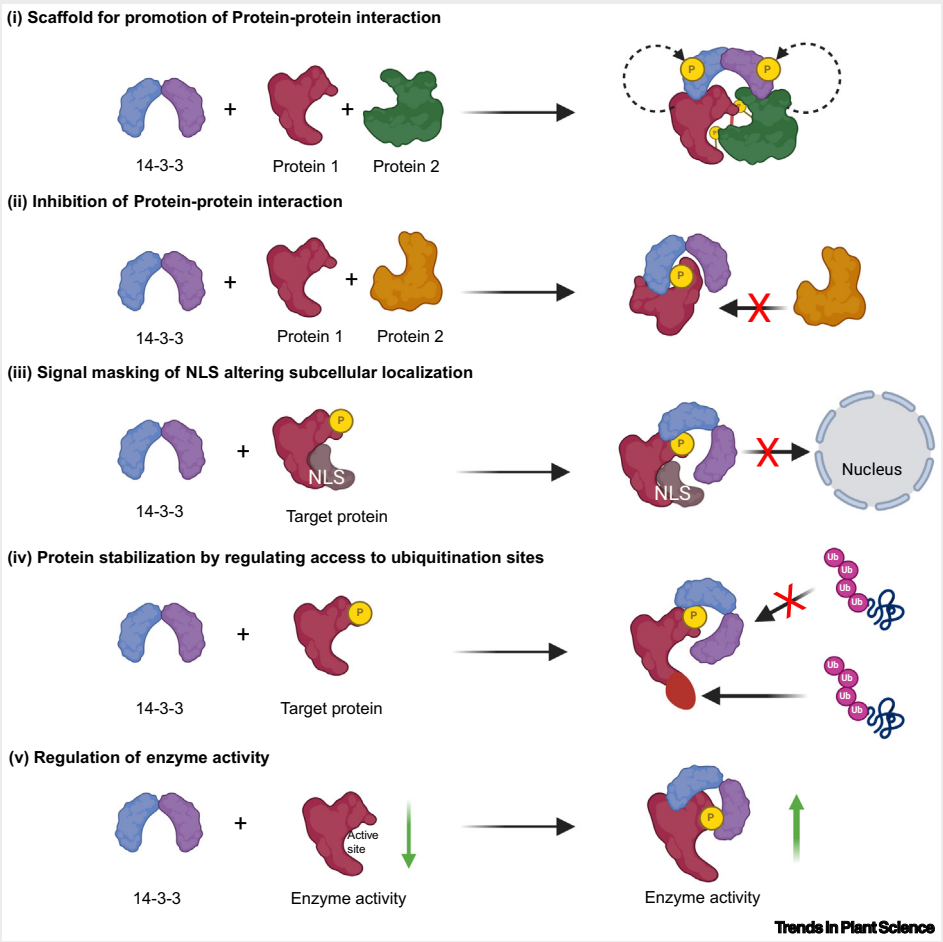

Figure I. Modes of action of 14-3-3 proteins. 14-3-3 proteins have the capacity to: (i) act as a bridge between two client proteins and promote their protein–protein interaction; (ii) disrupt the client protein's ability to engage with other interaction partners; (iii) alter/mask the subcellular localization signals of the client protein thereby regulating distinct cytosolic and organellar (e.g., nuclear) interactions; (iv) shield or expose the client protein from post-translational modifications like ubiquitination and proteasome degradation; and (v) modulate the catalytic activity of the client protein. The figure was generated using BioRender (www.biorender.com). Abbreviation: NLS, nuclear localization signal.

While the activation of RLCKs post MAMP elicitation is well documented, the subsequent molecular players responsible for downstream signal activation remain underexplored. Recently, immunoprecipitation and mass spectrometry analyses demonstrated that multiple 14-3-3 proteins interact with RLCKs like PBL19, PBL20, and BIK1 [27]. In this study, GRF6 was found to

interact with FLS2 in a flg22-independent manner suggesting that 14-3-3s are part of the PRR receptor complex. Furthermore, GRF6 directly interacted with the C terminus of MAPKKK5 to promote its accessibility by the activated PBL19, thereby potentiating MAPK activation. Interestingly, GRF8, another closely related isoform of GRF6, displayed a similar interaction pattern with MAPKKK5, suggesting a redundant function for GRF6. The double mutant *grf6grf8* shows reduced MAPK activation post-flg22 or chitin elicitation and is subsequently more susceptible to *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 and *Botrytis cinerea*. Based on these data, the authors proposed that GRF6/8 acts as a scaffold, assembling PBL19 and MAPKKK5 into a complex that facilitates the phosphorylation of MAPKKK5's C-terminal tail by PBL19, which is otherwise inhibited by the N terminus of MAPKKK5 [27]. Despite their role in PTI, both GRF6 and GRF8 were dispensable for **effector-triggered immunity (**ETI) responses, suggesting that other 14-3-3 isoforms may be involved in ETI (Figure 1).

Similarly, tomato TFT1 was found to be a PTI-induced gene in tomato, playing a crucial role in inhibiting the growth of *Xanthomonas campestris* pathovar *vesicatoria* (*Xcv*) [28]. In addition to TFT1, numerous other 14-3-3 are transcriptionally regulated in response to pathogen perception in both monocots and dicots [2].

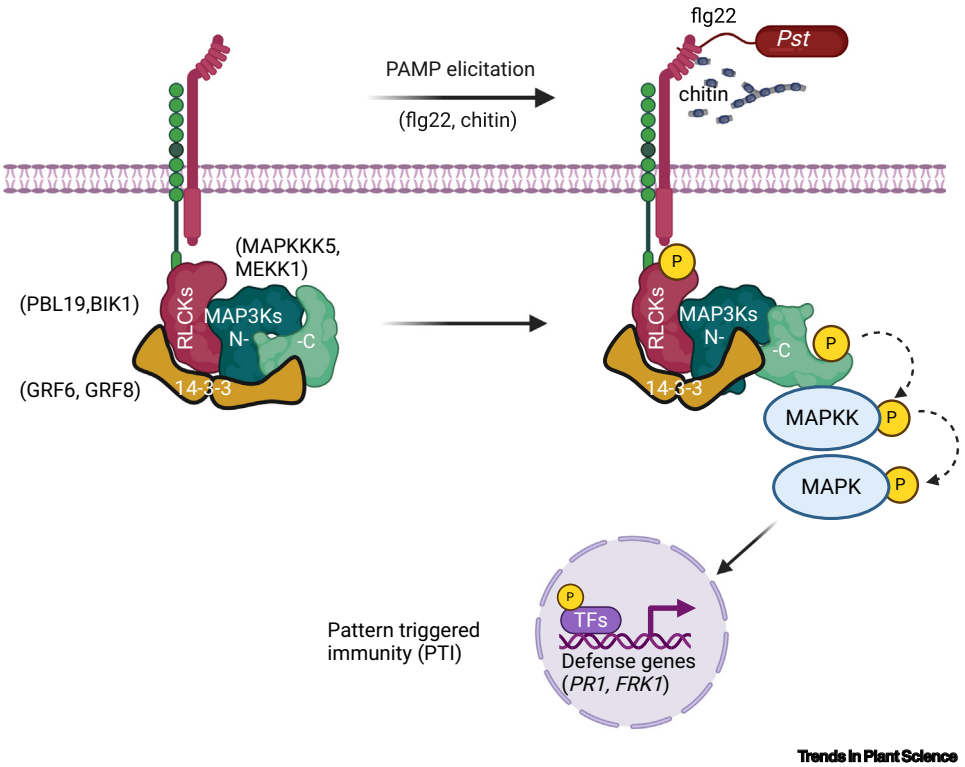

Figure 1. 14-3-3s facilitate mitogen-activated protein kinase (MAPK) activation during pattern-triggered immunity (PTI). 14-3-3 proteins regulate the phosphorylation of MAPKKK5 during PTI. The 14-3-3 dimer, formed through general regulatory factor (GRF) 6 and/or GRF8, functions as a scaffold for receptor-like cytoplasmic kinases (RLCKs) (e.g., PBL19, BIK1) and MAPKKKs (e.g., MAPKKK5, MEKK1). The interaction between GRF6/8 and the C terminus of MAPKKKs is likely to expose this domain by alleviating intramolecular inhibition imposed by the N terminus. This facilitates enhanced access to the C terminus of MAPKKKs by the RLCKs, ultimately promoting the phosphorylation of MAPKKKs and the activation of downstream MAPK cascades. The figure was generated using BioRender (www.biorender.com). Abbreviation: PAMP, pathogen-associated molecular pattern.

The role of 14-3-3 in ETS

Host-adapted pathogens utilize a wide variety effector proteins to interfere with and overcome various plant immune signals, thereby causing effector-triggered susceptibility (ETS) and subsequent disease [29]. Given that 14-3-3 proteins are ubiquitous and conserved eukaryotic signalling components, they have emerged as virulence targets of various plant pathogens including viruses, bacteria, and oomycetes [2] (Figure 2). For example, the viral coat protein (CP) of beet black scorch virus (BBSV) counteracts the MAPKKKα-mediated antiviral defense of the host plant by competitively binding to the 14-3-3a protein. This leads to the destabilization of MAPKKKα and the undermining of MAPKKKα-mediated antiviral defense [30]. Similarly, bacterial effectors like *Xanthomonas* XopQ can suppress plant immunity by targeting 14-3-3 proteins in tomato, pepper, and rice [31–33]. Another effector, XopX, interacts with two of the eight rice 14-3-3 proteins. Notably, mutants of XopX, impaired in their ability to bind to 14-3-3s, exhibit defects in suppressing immune responses, suggesting that its interaction with 14-3-3 proteins is crucial for the effective suppression of the host's innate immunity [34]. Tomato TFT1 was found to be a *bona fide* target of XopN1, as mutations disrupting XopN1/TFT1 binding not only impede the interaction but also

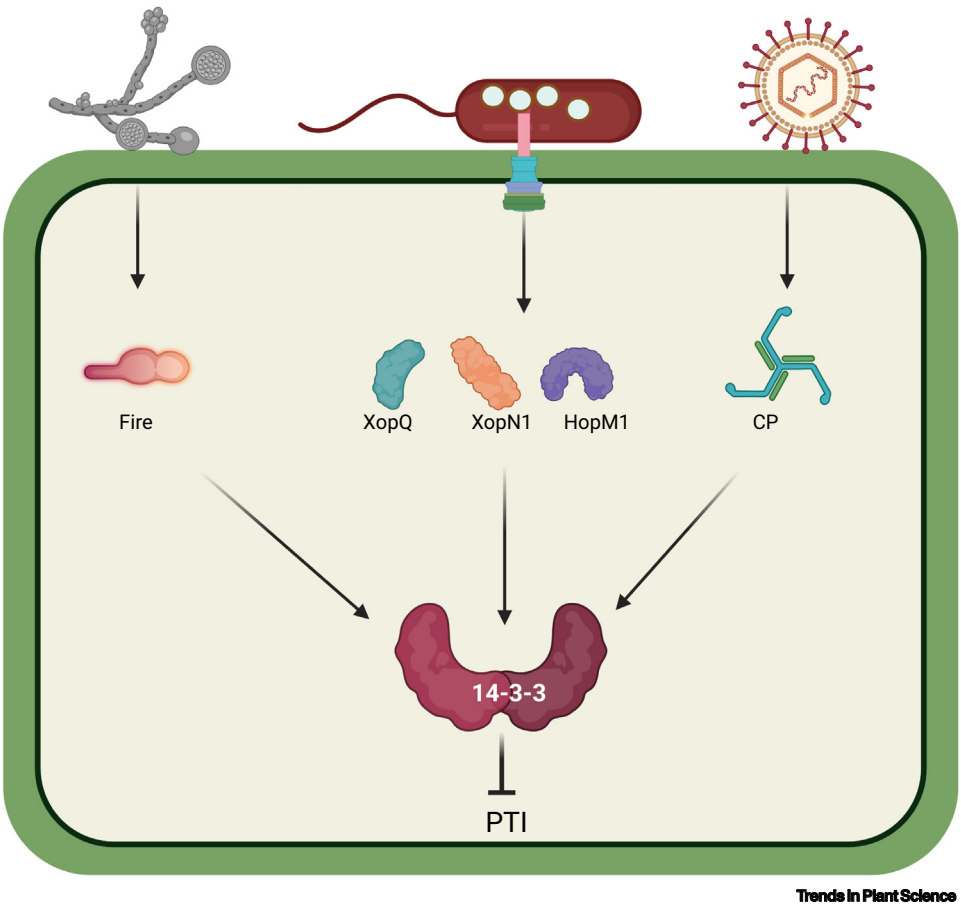

Figure 2. 14-3-3s as common targets of pathogen effectors. 14-3-3s, being ubiquitous yet conserved proteins in eukaryotes, have emerged as targets of pathogens like viruses, bacteria and fungi. For example, viral coat protein (CP), bacterial effectors like XopQ, XopX, XopN1, HopQ1, and HopM1, and the fungal effectors PITG06478 and FIRE interact with multiple plant 14-3-3 proteins. Substrate mimicry targeting 14-3-3 proteins could represent a cross-kingdom effector strategy employed by numerous plant pathogens to subvert host immunity. The figure was generated using BioRender (www.biorender.com). Abbreviation: PTI, pattern-triggered immunity.

abolish XopN1-dependent virulence in tomato [28]. *P. syringae* effectors HopM1 and HopQ1 are also known to bind and modulate 14-3-3 proteins in arabidopsis, *Nicotiana benthamiana*, and tomato [35–37]. 14-3-3s are well known to regulate the function of proton pump H^+-ATPases (AHAs) under growth, development, and stress conditions [8,38]. The oomycete *Phytophthora infestans* RxLR effector PITG06478 induces necrotrophic cell death in *N. benthamiana* by interacting with and impeding the Nb14-3-3a/b mediated regulation of plasma membrane H^+-ATPases (AHAs) [39]. Another effector of *Phytophthora palmivora* called FIRE (PLTG_13996) interacts with multiple plant 14-3-3 proteins, suggesting that substrate mimicry targeting 14-3-3 proteins represents a cross-kingdom effector strategy employed by numerous plant pathogens to subvert host immunity [40]. The different approaches to study the modulation of 14-3-3 protein–protein interactions are discussed in Box 2.

The role of 14-3-3 in ETI

In response to ETS, plants have evolved NOD-like receptors (NLRs) that detect pathogen effectors either directly or through modifications induced by effectors on host structures, mounting ETI [41]. NLR signalling modules usually comprise sensor and helper (hNLR) NLR proteins. Sensor NLRs are categorized into either coiled-coil (CC) or TOLL–INTERLEUKIN-1 receptor (TIR) NLRs based on the distinct structure of their N-terminal domains. In solanaceous plant species, sensor NLRs depend on hNLRs termed NRCs (NLRs required for cell death) for downstream signal transduction, while in arabidopsis ADR1 and NRG1 proteins serve as helper NLRs. ETI responses include the generation of ROS, the formation of high-molecular-weight NLR complexes (resistosomes) [42–44], transcriptional reprogramming, and, ultimately, programmed cell death (PCD) restricting pathogen growth at the infection site. Furthermore, ETI manifests a robust and prolonged activation of MAPK cascades, for which the activation mechanism is not fully understood [45]. Using the well-studied *Pst* effectors AvrPto/B and the Prf/Pto tomato NLR complex, 14-3-3 proteins were shown to be involved in ETI MAPK activation [46]. Two tomato 14-3-3 proteins, TFT1 and TFT3, along with hNLRs NRC1/2/3 were shown to be integral parts of the Prf/Pto complex [46]. Upon AvrPto/B recognition, the complex dissociates into two distinct ETI signalling modules. The TFT3-dependent branch is crucial for full activation of the MAPK cascade and also partially

Box 2. Modulators of 14-3-3 interactions

The family of 14-3-3 proteins, known for having several hundred identified protein interacting partners in eukaryotic cells, present a particularly intriguing case for small-molecule modulation of protein–protein interactions. These small molecules and peptides could be employed for numerous plant immunity biological pathways, presenting distinct opportunities for intervention through inhibition and stabilization [57].

Inhibitors

A highly effective inhibitor of 14-3-3 is the 20-amino-acid peptide R18, which binds 14-3-3 through salt-bridge and hydrophobic interactions. The central sequence (WLDLE) of R18 binds to the amphipathic binding groove of 14-3-3 thereby competing for both phosphorylation-dependent and -independent 14-3-3 protein interactions [58,59]. Additional peptide-based inhibitors have been created, including macrocyclic peptides derived from the structure of the virulence factor of the pathogenic bacterium *Pseudomonas aeruginosa* exoenzyme S (ExoS) [60]. The structure of the Tau peptide has led to the development of another potent inhibitor by targeting the highly conserved pocket in the amphipathic groove of 14-3-3 [61]. With the advancement of computational drug design, many small molecules such as BV101, BV02, FOBISIN, as well as phosphate-containing inhibitors called molecular tweezers like CLR01, have been developed [57].

Stabilizers

FC is a natural diterpene glycoside toxin derived from the phytopathogenic fungus *Phomopsis amygdali* (formerly *Fusicoccum amygdali*) that binds to the protein complex formed by the H^+ pump C terminus and 14-3-3 proteins and strongly stabilizes the interaction of the two partners [8,14]. Synthetic small molecules like epibestatin, pyrrolidone1, and pyrazole34 have two distinct binding pockets in the 14-3-3/PMA2 protein–protein interaction interface. Interestingly, AMP has been shown to enhance the stability of the complex formed between 14-3-3 and the carbohydrate-response element-binding protein (ChREBP) in a phosphorylation-independent manner [57].

contributes to PCD. Meanwhile, the NRC1/2/3-dependent branch is essential for complete PCD formation and the restriction of pathogen growth [46]. Reduced MAPK activity was observed in a CRISPR/Cas9 knockout of *tft3* compared with wild-type (WT) tomato plants. An essential role of NRC2 and NRC3 in the regulation of the TFT3-dependent MAPK activation was also recently suggested [47], implying possible crosstalk between the TFT3 and NRC-dependent branches of ETI. Furthermore, the tomato 14-3-3 protein TFT7 was found to boost Prf/Pto-mediated PCD upon recognition of AvrPto in tomato [48]. Furthermore, TFT7 interacts with MKK2, a protein that functions downstream of the MAPKKKα signalling pathway, suggesting its role as a bridge for optimal signal transmission and subsequent cell death development [49]. In addition, the arabidopsis 14-3-3 lambda interacts with the NLR RPW8 to mediate disease resistance against the biotrophic fungal pathogen *Golovinomyces* spp. [50]. The recent advances demonstrating the role of 14-3-3 in ETI are summarized in Figure 3.

Concluding remarks and future perspectives

Pathogens from different kingdoms tend to target common, conserved plant protein hubs. In this regard, 14-3-3 proteins warrant more thorough attention, given their role in mediating protein–protein interactions. Future studies deciphering the specific interacting residues or protein regions of 14-3-3 proteins that can alter the outcome of immune responses will be important for the design of plants resistant to a broad range of pathogens. Large-scale interactome/proteome

Outstanding questions

Why do different effectors from various pathogens target 14-3-3 proteins?

Which are the key 14-3-3 proteins relevant to plant immunity?

Do 14-3-3 proteins change the subcellular localization of their interacting proteins related to plant immunity?

How can we develop very specific and selective plant 14-3-3 modulators (inhibitors/stabilizers) to study plant immunity protein–protein interactions?

Do 14-3-3 proteins contribute to the maintenance of protein homeostasis during immunity?

How can 14-3-3 proteins be utilized to engineer plants resistant to pathogens?

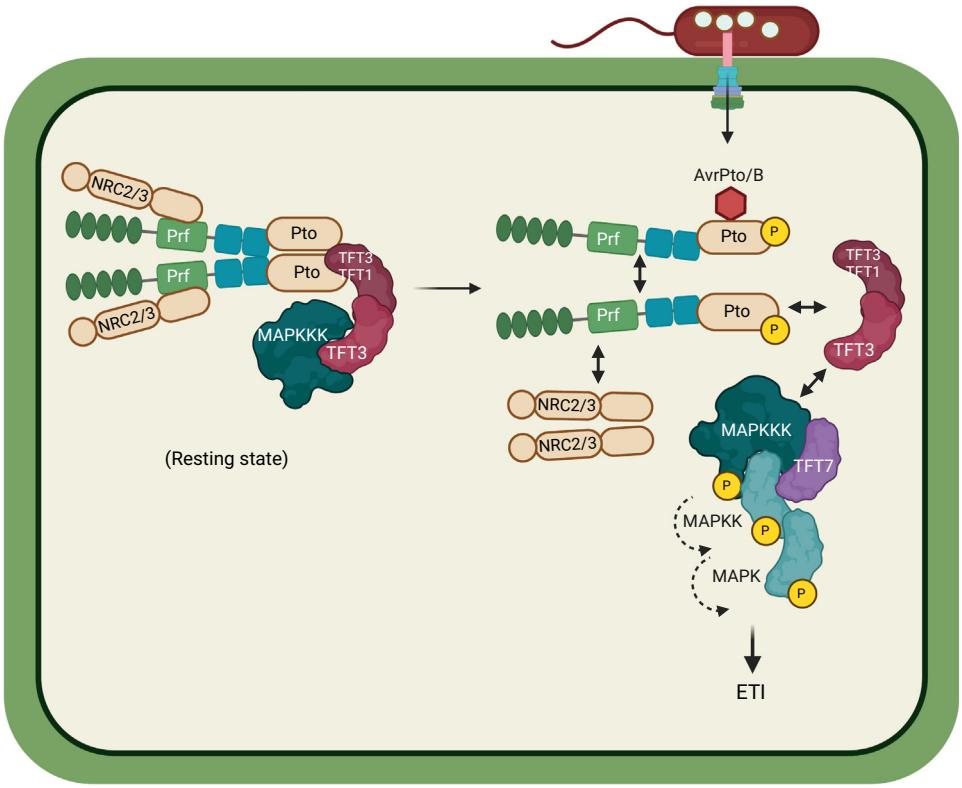

Figure 3. 14-3-3 proteins modulate effector-triggered immunity (ETI) in plants. The tomato resistance complex consist of sensor (Prf/Pto) and helper (NRC2/3) NOD-like receptors (NLRs), 14-3-3 proteins [tomato fourteen-three-three (TFT) 3 and TFT1], and mitogen-activated protein kinase kinase kinase alpha (MAPKKKα). Upon effector (AvrPto/B) recognition, the complex dissociates into individual modules and facilitates the phosphorylation and subsequent activation of robust MAPK activation. The figure was generated using BioRender (www.biorender.com).

and *in silico* prediction studies need to be undertaken to define the interaction hubs of 14-3-3 proteins. Whether the targeting of 14-3-3 proteins by pathogen effectors always leads to plant disease remains an open question. In this regard, it is worth investigating whether 14-3-3 proteins also mediate crosstalk between other signalling and biochemical pathways activated during plant immunity like autophagy, resistosome formation, and PCD [51,52]. As our knowledge of 14-3-3 proteins expands, it will enable gene editing approaches for the engineering of 14-3-3 proteins that enhance plant immune responses, escape effector manipulation, or alter the crosstalk between pathways for the benefit of plant robustness. As conserved small proteins targeted by diverse pathogens, 14-3-3 proteins are also ideal modules to incorporate into modified receptors in order to engineer plants with broad resistance [53,54]. Finally, it would be intriguing to investigate whether beneficial microbes exert their effects by influencing 14-3-3 proteins, especially considering that their expression has been demonstrated to be modulated by arbuscular mycorrhizal fungi [55] (see Outstanding questions).

Acknowledgments

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Declaration of interests

No interests are declared.

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