Characteristics of 14-3-3 Proteins and Their Role in Plant Immunity

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Phosphorylation is a major post-translational modification of proteins that regulate diverse signal transduction pathways in eukaryotic cells. 14-3-3 proteins are regulatory proteins that bind to target proteins in a phosphorylation-dependent manner and have been shown to play an important role in plant growth and development, primary metabolism, and signal transduction. Because phosphorylation plays a critical role in signal transduction pathways to trigger plant immunity, involvement of 14-3-3 proteins in plant immunity has been suggested for a long time. Recent studies have provided new evidence to support a role for 14-3-3 proteins in plant immunity. This review will briefly discuss general characteristics of 14-3-3 proteins and their involvement in plant immunity.

Keywords: disease resistance, HR, PCD, phosphopeptide binding, phosphorylation

Plants have innate immunity systems that consist of two layers like animals to defend themselves from various pathogens (Jones and Dangl, 2006). The first layer of innate immunity is critical for initial defense against most of plant pathogens and is triggered by pathogen-associated molecular patterns (PAMPs) such as flagellin, elongation factor Tu, and chitin, which are recognized by corresponding pattern recognition receptors (PRRs) such as FLS2, EFR, and CERK1 (Zipfel, 2008). This recognition turns on downstream signal transduction pathways to elicit PAMP-triggered immunity (PTI). The second layer of innate immunity is triggered by plant resistance (R) proteins when they directly or indirectly recognize corresponding pathogen’s effector proteins, and it is called effector-triggered immunity (ETI). One of typical ETI responses is the hypersensitive response (HR) that is a rapid cell death at the infection sites and is a type of programmed cell death (PCD) (van Doorn and Woltering, 2005).

Signaling components for both PTI and ETI have been comprehensively screened and identified from various plant species, including Arabidopsis, tobacco, and tomato (Asai et al., 2002; Hammond-Kosack and Parker, 2003; Lu et al., 2009). One protein family of downstream signaling components is a protein kinase. Generally a protein kinase transfers a phosphate group to its target proteins to activate or inhibit their activities in the cell. Phosphorylation is one of key post-translational modifications in proteins. For example, Pto is a serine/threonine protein kinase that can recognize AvrPto or AvrPtoB of Pseudomonas syringae pv. tomato causing a bacterial speck disease on tomato and Arabidopsis (Kim et al., 2002; Tang et al., 1996), and its kinase activity is critical for transfer of a molecular signal to downstream components such as Pti1, another serine/threonine protein kinase (Xing et al., 2007; Zhou et al., 1995). Several other protein kinases, including Adi3 (AGC kinase), Adi2 (serine/threonine protein kinase) and mitogen-activated protein kinases (MAPKs) have been shown to be involved in Pto pathway (Bogdanove and Martin, 2000; del Pozo et al., 2004; Devarenne et al., 2006). MAPKs are representative groups of protein kinases that control both PTI and ETI in plants (Chisholm et al., 2006; Pedley and Martin, 2005). In these kinases, molecular signals from protein receptors are transferred to downstream signal components to activate the first module of MAPK cascades, MAPK kinase kinases (MAPKKKs). These kinases phosphorylate MAPK kinases (MAPKKs), which phosphorylate MAPKs. These phosphorylated MAPKs activate various target proteins, including transcription factors (TFs), by phosphorylation.

Because of importance of protein kinases and phosphorylation as a key post-translational modification in signal transduction for PTI and ETI in plants, the involvement of 14-3-3 proteins as phosphopeptide binding proteins in these pathways has been suggested for a long time. However, most of initial evidence for roles of 14-3-3 proteins in plant immunity was indirect. Recently, two cases that 14-3-3 proteins play an crucial role in disease resistance or immunity-associated PCD in plants have been reported (Oh et al., 2010; Yang et al., 2009). In this review, I will briefly summarize general characteristics and modes of actions of 14-3-3 proteins and the role of 14-3-3 proteins in plant immunity.

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General characteristics of 14-3-3 proteins

14-3-3 proteins are acidic proteins of approximately 30-kDa that can bind to phosphopeptides such as phosphoserine and phosphothreonine (Bridges and Moorhead, 2005; Ferl et al., 2002; Ferl, 2004). These proteins are quite conserved in all eukaryotic organisms such as human, yeast, and plants and are known to be involved in many important biological processes such as metabolism, transcription, cell-cycle control, protein trafficking, signal transduction, programmed cell death, and stress responses (Bridges and Moorhead, 2005; Darling et al., 2005; Morrison, 2009; Roberts et al., 2002; Roberts, 2003; Sehnke et al., 2002). Two conserved 14-3-3-binding motifs have been identified in many 14-3-3 target proteins: RxxpS/TxP and RxxxpS/TxP, where pS/T and x represent a phosphoserine/phosphothreonine and any amino acid, respectively.

Multiple 14-3-3 protein isoforms exist in each organism. Human has seven isoforms and yeast has only two. Plant species have more isoforms. For examples, Arabidopsis has thirteen functional 14-3-3 protein isoforms (there are fifteen genes in total) and tomato and rice have twelve and eight, respectively (Bridges and Moorhead, 2005; Chevalier et al., 2009; Ferl et al., 2002). Based on phylogenetic analysis, plant 14-3-3 proteins fall into two major groups, which are a ε-like group and a non-ε group (Ferl et al., 2002). Fig. 1 shows the phylogenetic tree generated with 14-3-3

![Fig. 1. Alignment of 14-3-3 proteins from Arabidopsis, rice, tomato, yeast, and human at the amino acid level using ClustalW program. Eight from rice (GF14s), twelve from tomato (TFTs), thirteen from Arabidopsis (GRFs), two from yeast (BMHs), and seven from human (Greek letters) were used in this alignment. While plant 14-3-3 proteins (blue-colored) are divided into two groups (a ε-like group and a non-ε group), all human and yeast 14-3-3 proteins (red-colored) belong to a ε-like group.](image-url)
proteins from human, yeast, Arabidopsis, rice, and tomato, using ClustalW program. The tertiary structures of all seven human 14-3-3 proteins have been solved by X-ray crystallography (Yang et al., 2006). Based on these structures, each 14-3-3 protein consists of nine helices, and some are critical for making a dimer or binding to phosphopeptides. Fig. 2 shows the tertiary structure of a human 14-3-3 ε dimer with two phosphopeptide binding pockets (Yang et al., 2006). The protein structures of some plant 14-3-3 proteins also have been solved or predicted based on human 14-3-3 proteins (Ottmann et al., 2007; Oh et al., 2010). For example, the structure of tomato 14-3-3 protein 7 was predicted based on the structure of human 14-3-3 ε (Oh et al., 2010). Based on this structural similarity, amino acid residues critical for the phosphopeptide binding were determined. Mutational analysis showed that those residues were indeed necessary for binding to a target protein.

14-3-3 proteins typically make a homodimer or heterodimer for proper function and they have a distinct dimerization preference (Bridges and Moorhead, 2005). This dimerization preference was well studied with human 14-3-3 proteins. By in vivo co-immunoprecipitation experiments with the human 14-3-3 γ and 14-3-3 ε, it was shown that 14-3-3 γ makes both a homodimer and a heterodimer mainly with the 14-3-3 ε, whereas 14-3-3 ε predominantly makes a heterodimer with either the 14-3-3 β, γ, ζ, or η (Chaudhri et al., 2003). Although the crystal structure of 14-3-3 ε was derived from a homodimer, this 14-3-3 protein showed a high preference to make a heterodimer with either β, γ, and ι in vitro before virtually any homodimers were detected (Yang et al., 2006). In the two independent studies with 14-3-3 proteins from barley and Arabidopsis, their target proteins have been comprehensively screened by a yeast two-hybrid assay or by combining TAP-tag affinity protein purification with tandem mass spectrometry (Chang et al., 2009; Schoonheim et al., 2007). From these assays, over 100 target proteins including 14-3-3 proteins, were found, indicating that plant 14-3-3 proteins can form dimers.

**Modes of action of 14-3-3 proteins**

The functional mechanisms of 14-3-3 proteins have been suggested based on reported examples of how they control target proteins, and they can be divided into four modes of actions (Darling et al., 2005). Fig. 3 shows simplified modes of actions of 14-3-3 proteins. First, 14-3-3 proteins can increase stability of target proteins by preventing their accessibility to proteases and phosphatases. One of tomato 14-3-3 proteins, TFT7, has been shown to bind to the C-terminal region of MAPKKKα and increases its protein stability (Oh et al., 2010). A nitrate reductase (NR) converts nitrate to nitrite in nitrate assimilation. This protein activity is regulated by its phosphorylation status, i.e. dephosphorylated NR is an active form (Chevalier et al., 2009). If NR is phosphorylated, 14-3-3 proteins including 14-3-3 ω interact with NR to complete inactivation process because this binding prevents NR from dephosphorylation by protein phosphatase (PP2A). Second, 14-3-3 proteins can trigger a conformational change to activate or inhibit target proteins’ activities. Binding of a 14-3-3 protein to one site of a target protein causes its conformational change, and it results in exposing one or more new 14-3-3 binding sites.
soybean and race-specific HR-PCD elicited by interaction between proton pump (Ottmann et al., 2007). Secondly, during a hexamer is formed with a 14-3-3 hexamer as an active 3-3 dimer binds to two H+-ATPase, and this causes oligomerization. Based on this structural analysis, a 14-3-3 proteins combined with plant plasma membrane H+-ATPase was solved. Recent studies indicate that 14-3-3 proteins play roles in plant immunity, based on gene expression data from several independent studies. First, inoculation of barley leaves with avirulent strains of powdery mildew fungus Blumeria graminis f.sp. hordei induced expression of several 14-3-3 genes, and those expression was induced mostly in the epidermal cells of the infected leaves (Finnie et al., 2002). When the HR-PCD is induced by this interaction, this HR-PCD is more stimulated by a fungal toxin fusicoccin, which binds to H+-ATPase proton pump associated with 14-3-3 proteins (Roberts and Bowles, 1999). Recently, the structure of 14-3-3 proteins combined with plant plasma membrane H+-ATPase was solved. Based on this structural analysis, a 14-3-3 dimer binds to two H+-ATPase, and this causes oligomerization of H+-ATPases. As a result, an H+-ATPase hexamer is formed with a 14-3-3 hexamer as an active proton pump (Ottmann et al., 2007). Secondly, during a race-specific HR-PCD elicited by interaction between soybean and P. syringae pv. glycinea, expression of a 14-3-3 gene was induced (Finnie et al., 2002; Seehaus and Tenhaken, 1998). Third, in the incompatible interaction between tomato and its fungal pathogen Cladosporium fulvum carrying Avr9 elicitor, expression of three (TFT1, TFT4, and TFT6) out of ten 14-3-3 genes was induced (Roberts and Bowles, 1999), indicating that those three 14-3-3 genes are specifically induced by Cf9/Avr9-mediated HR-PCD and may play a role in HR-PCD in plants. Fourth, among over 1,000 rice genes that are screened by a large scale microarray analysis after infection with Magnaporthe grisea, a rice blast pathogen, four rice 14-3-3 genes, GF14b, GF14c, GF14e, and GF14f were found as genes differentially regulated by pathogen infection (Chen et al., 2006). Lastly, treatment of γ-aminobutyric acid (GABA), of which level is highly elevated by pathogen infection and wounding, repressed some 14-3-3 genes including GRF8 in Arabidopsis, and its repression was dependent on ethylene and ABA signaling pathways (Lancien and Roberts, 2006).

Two independent recent reports provide more direct evidence that 14-3-3 proteins play an important role in plant immunity. First, an Arabidopsis 14-3-3 protein λ encoded by AtGRF6 gene was reported to interact with the RPW8.2 protein and to play a role in defense responses mediated by the R protein (Yang et al., 2009). RPW8 locus consists of two functional paralogous genes, RPW8.1 and RPW8.2. These two genes encode small proteins with a N-terminal transmembrane domain and a coiled-coil (CC) domain. These RPW8 proteins in Arabidopsis confer broad spectrum resistance to the ascomycete fungal pathogen, Golovinomyces spp., which causes powdery mildew diseases on many plant species. The knock-out of GRF6 gene by T-DNA insertion compromised both basal and RPW8-mediated resistance to the powdery mildew fungus in Arabidopsis, indicating that this 14-3-3 gene play a critical role in disease resistance to the fungus. Interestingly, over-expression of this 14-3-3 gene in Arabidopsis caused HR-like cell death. Previously, Arabidopsis 14-3-3 λ was known to interact with a putative peroxisomal ascorbate peroxidase APX3, which is an enzyme that scavenges H2O2 to prevent cell damage from this reactive oxygen species, and AKR2, an ankyrin repeat-containing protein that is probably involved in antioxidation metabolism (Yan et al., 2002). In addition, it was shown that this 14-3-3 protein interacted with SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK1) (Rientes et al., 2005). SERK1 is a very important component in brassinosteroid signaling in combination with BAK1, another receptor-like protein kinase, which plays a critical role in PTI induction in Arabidopsis with other PRRs such as FLS2 and EFR (Zipfel, 2008). These results indicate that Arabidopsis 14-3-3 λ may be involved in not only HR-PCD, but also PTI signaling pathways.

Secondly, tomato 14-3-3 protein 7 (TFT7) was reported to bind to MAPKKKα, which is a key positive regulator of Pto-mediated HR-PCD in tomato and Nicotiana benthamiana and to positively regulate Pto-mediated HR-PCD (Oh et al., 2010). When TFT7 gene was silenced by virus-induced gene silencing (VIGS) technology in N. benthamiana, Pto- and MAPKKKα-mediated HR-PCDs were compromised. In addition, this gene silencing compromised HR-PCDs mediated by four more R/effector interactions, as summarized in Fig. 4. In contrast to Arabidopsis GRF6, over-expression of TFT7 did not cause HR-like cell death. In addition to HR-PCD, TFT7 contributed to disease resistance to the bacterial pathogen, P. syringae pv. tomato, because more severe disease symptom was developed in TFT7-silenced N. benthamiana plants, compared to the
control plants. This report provides an insight into how 14-3-3 proteins control target proteins. TFT7 enhanced protein abundance and signaling ability of MAPKKKa by direct binding to the putative phosphorylated site Ser535 of the protein and positively regulates Pto- and RPP13-mediated PCDs. In addition, TFT7 regulates PCDs mediated by other R/effector interactions such as RPS2/AvrRpt2, Rx2/CP, and Gpa2/RBP-1 through an unknown signaling component, X.

14-3-3 proteins as a host target of bacterial effector proteins?

Bacterial effector proteins have been shown to target host proteins to promote disease development. Recently, two cases have been shown that bacterial effector proteins from plant-pathogenic bacteria interacted with plant 14-3-3 proteins, based on a yeast two-hybrid assay (Kim et al., 2009; Nomura et al., 2006). HopM1 of P. syringae pv. tomato is delivered into the plant cytosol, and it interacts with immunity-associated plant protein AtMIN7, which may be important for vesicle trafficking, resulting in degradation of AtMIN7 by proteasome. HopM1 protein also interacted with the Arabidopsis 14-3-3 protein κ (GRF8), of which its function in plant immunity has not been determined. In addition to HopM1, XopN of Xanthomonas campesiris pv. vesicatoria was found to interact with four different 14-3-3 proteins (TFT1, TFT3, TFT5, and TFT6) in tomato (Kim et al., 2009). This effector protein suppresses PTI by targeting and interfering with atypical receptor-like protein kinase. So far, neither one has been shown that interactions between bacterial effector proteins and plant 14-3-3 proteins have any biological significance.

However, it will be very interesting to determine if pathogen effectors can disturb plant immunity by interfering with functions of 14-3-3 proteins in plants.

Future perspectives

Although there are many indirect indications that 14-3-3 proteins play an important role in plant immunity, there are only a few cases directly to show the role of 14-3-3 proteins in plant immunity as described above. In addition, each organism has multiple isoforms of 14-3-3 proteins, and each protein can interact with over a hundred other target proteins in plants. Due to the limited information on roles of 14-3-3 proteins in plant immunity, there are many interesting questions about 14-3-3 proteins that should be addressed in the future. First, how many 14-3-3 proteins do play a role in plant immunity in each plant species such as Arabidopsis and tomato? The availability of Arabidopsis T-DNA insertion lines and VIGS technology in N. benthamiana make it possible to address this question in a high throughput manner. Secondly, what are specific plant target proteins of 14-3-3 proteins that control plant immunity? If we can determine an important 14-3-3 protein in plant immunity, we can easily screen its interacting proteins using several molecular techniques to find known or unknown components in plant immunity. Third, do bacterial effectors target and disturb function of 14-3-3 proteins in plant immunity? So far, there has been no evidence that 14-3-3 proteins interacting with bacterial effector proteins are involved in plant immunity. Fourth, what is dimerization preference of plant 14-3-3 proteins? So far, no evidence on dimerization preference of plant 14-3-3 proteins has been reported. Because 14-3-3 proteins function as a dimer, study on dimerization preference will give us more dynamic relationships among 14-3-3 proteins in terms of their function. Lastly, why do plant species have more isoforms of 14-3-3 proteins than animals including human? Based on phylogenetic analysis, non-ε group of 14-3-3 proteins is unique to plant species. Is there evolutionary significance of this difference between plants and animals?

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References


