Protein phosphorylation in plant immunity: insights into the regulation of pattern recognition receptor-mediated signaling

Chang-Jin Park, Daniel F. Caddell and Pamela C. Ronald*

Department of Plant Pathology and the Genome Center, University of California at Davis, Davis, CA, USA

Plants are continuously challenged by pathogens including viruses, bacteria, and fungi. The plant immune system recognizes invading pathogens and responds by activating an immune response. These responses occur rapidly and often involve post-translational modifications (PTMs) within the proteome. Protein phosphorylation is a common and intensively studied form of these PTMs and regulates many plant processes including plant growth, development, and immunity. Most well-characterized pattern recognition receptors (PRRs), including Xanthomonas resistance 21, flagellin sensitive 2, and elongation factor-Tu receptor, possess intrinsic protein kinase activity and regulate downstream signaling through phosphorylation events. Here, we focus on the phosphorylation events of plant PRRs that play important roles in the immune response. We also discuss the role of phosphorylation in regulating mitogen-associated protein kinase cascades and transcription factors in plant immune signaling.

**INTRODUCTION**

Proteins can undergo various post-translational modifications (PTMs) that affect their conformation, activity, stability, and localization. These PTMs, which are often reversible, are highly specific regulators of many cellular processes (Jensen, 2004). Currently, more than 300 types of PTMs have been described including ubiquitination, sumoylation, sulfation, glycosylation, and phosphorylation (Stellemeyer and Joosten, 2008; Ghelis, 2011). Phosphorylation is one of the most prevalent PTMs and one-third of all eukaryotic proteins are thought to be phosphorylated (Jensen, 2004; Ronald and Beutler, 2010; Schwessinger and Ronald, 2012). In general, recognition of conserved microbial signatures (also called pathogen-associated molecular patterns, PAMP) by PRRs triggers mitogen-associated protein kinase (MAPK) activation, production of reactive oxygen species (ROS), Ca²⁺ burst, transcriptional reprogramming, hormone biosynthesis, and deposition of callose in the cell wall (Nurnberger et al., 2004; Ronald and Beutler, 2010; Segonzac and Zipfel, 2011). The rice PRR, Xanthomonas resistance 21 (XA21), recognizes a conserved sulfated peptide called AxtP22, derived from the Xanthomonas oryzae pv. oryzae (Xoo) protein Axx21 (activator of XA21-mediated immunity; Lee et al., 2009). In Arabidopsis, two additional plant PRRs have been well-characterized. These are flagellin sensitive 2 (FLS2) and elongation factor (EF)-Tu receptor (EFR), which recognize the flg22 peptide from flagellated bacteria and the EF-Tu-derived peptide elf18, respectively (Gomez-Gomez and Boller, 2000; Zipfel et al., 2006). These PRRs consist of an extracellular leucine-rich repeat (LRR) domain, a transmembrane (TM) domain, a juxtamembrane domain, and a cytoplasmic kinase domain.

**PATTERN RECOGNITION RECEPTORS IN RICE AND ARABIDOPSIS**

Plants use a diverse repertoire of pattern recognition receptors (PRRs) to recognize conserved microbial signatures. There are two main categories of PRRs in plants: extracellular pattern-recognition receptors (XPRRs) and intracellular nucleotide-binding leucine-rich repeat (NB-LRR) receptors. XPRRs include PAMP-associated receptors (PPRAs), nucleotide-binding site (NBS) receptors, and multi-functional proteins. In contrast, NB-LRR receptors are a single domain of plant immunity.

**Keywords:** EFR, FLS2, pattern recognition receptor, plant immunity, post-translational modifications, protein phosphorylation, XA21
(JM) domain, and an intracellular non-arginine–aspartate (non-RD) kinase domain (Figure 1; Durdick and Ronald, 2006; Schwessinger and Ronald, 2012).

Non-RD kinases typically carry a cysteine (C), or glycine (G) before the conserved catalytic aspartate (D) residue. All plant receptor kinases (RKs) characterized to date that carry the non-RD kinase motif are involved in recognition of conserved microbial signatures (Schwessinger and Ronald, 2012). In contrast, the larger group of RD kinases have an arginine (R) immediately preceding the conserved catalytic aspartate (D). RD kinases are known to perform more diverse functions and are often associated with developmental processes. RD kinases also work in partnership with non-RD kinases to transduce immune responses. In Arabidopsis, brassinosteroid insensitive 1 (BRI1)-associated kinase 1 (BAK1), an RD kinase, was initially identified as a positive regulator of brassinosteroid responses. BAK1 forms an in vivo ligand-dependent complex with the BRI1 receptor (Li et al., 2002; Nam and Li, 2002). Further research revealed that BAK1 is also involved in PRR-mediated signaling, physically interacting with the non-RD kinases FLS2 and EFR (Chinchilla et al., 2007, 2009; Schwessinger et al., 2011). BAK1 null mutants are compromised in their responsiveness to several other conserved microbial signatures including HrpZ (hypersensitive response and pathogenicity Z), lipopolysaccharides, and peptidoglycans (Heese et al., 2007; Shan et al., 2008). The rice ortholog of BAK1, XA21-associated kinase 1 (XAK1), is required for XA21-mediated immunity (Chen et al., unpublished). These results demonstrate that PRRs utilize consorgulatory receptors carrying RD kinases as signaling partners to transduce the immune response.

**PHOSPHORYLATIONS OF PATTERN RECOGNITION RECEPTORS**

In accordance with an essential role of phosphorylation in immune signaling, phosphorylation of FLS2 is the first step in the FLS2-mediated intracellular signaling events (Boller and Felix, 2009). De novo phosphorylation of a FLS2/BAK1 complex is clearly detectable in cells 15 s after the addition of flg22 using in vivo labeling with short pulses of [33P]orthophosphate (Schulze et al., 2010). Treatment with protein kinase inhibitors is able to block a broad spectrum of early defense responses (Lecourieux et al., 2002; Navazio et al., 2002; Kadota et al., 2004).

In animals, signal transduction is often regulated by phosphorylation of residues in the JM domain of RKs (Aifa et al., 2006; Thiel and Carpenter, 2007). It is now becoming clear that plant PRRs, at least XA21 and FLS2, are also phosphorylated on residues in their JM domains (Figure 1, Table 1). Targeted mutagenesis of the XA21 JM domain indicated that amino acids Ser686, Thr688, and Ser689 are autophosphorylated and required to maintain XA21 protein stability (Xu et al., 2006). Transgenic rice carrying XA21 mutants with alanine replacement of these three sites display partially compromised resistance compared to wildtype XA21 plants (Xu et al., 2006). Thr705 in the XA21 JM domain is also an important phosphorylation site and also affects the autophosphorylation activity of XA21 (Chen et al., 2010b). The XA21 mutant derivatives, XA21T705A and XA21T705E, are both unable to transduce the XA21-mediated immune response. The importance of the JM domain in XA21-mediated immunity was also demonstrated through isolation of XA21-binding proteins (XBs). For example, the protein phosphatase 2C XB15

**FIGURE 1** | Characterized Ser/Thr residues of pattern recognition receptors in plants. Top: identified and proposed autophosphorylation sites on rice XA21 and Arabidopsis FLS2 and EFR are highlighted in red. The conserved tyrosine that is essential for autophosphorylation is highlighted in green. The JM, kinase, and catalytic domains are indicated by black brackets. Center: The domain structure of rice XA21. Bottom: Alignment of the catalytic domains of XA21, FLS2, and EFR. The cysteine that replaces the R in these non-RD kinases is highlighted in blue. Putative autophosphorylation sites of FLS2 are highlighted in red. Amino acids that are conserved between XA21, FLS2, and EFR are marked as ***. LRR, leucine-rich repeats domain; TM, transmembrane domain; JM, juxtamembrane domain; non-RD kinase, non-arginine-aspartate kinase; XA21, rice Xanthomonas resistance 21; FLS2, Arabidopsis flagellin sensitive 2; EFR, Arabidopsis elongation factor Tu receptor.
no longer interacts with XA21<sup>AXYS22</sup>, indicating that Ser97 in the JM domain is critical for interaction with XB15 (Park et al., 2008). Autophosphorylated XA21 is dephosphorylated by XB15 treatment, suggesting that the function of XB15 is to attenuate the XA21-mediated innate immune response. The ATPase XB24 also associates with the XA21 JM domain and uses ATP to promote phosphorylation of certain Ser/Thr sites on XA21, keeping the XA21-mediated innate immune response in an inactive state. Upon recognition of sulfated Ax21, the XA21 kinase dissociates from XB24 and is activated, triggering downstream defense responses (Chen et al., 2010c; Figure 2).

In Arabidopsis, the FLS2 JM residue Thr<sup>867</sup> appears to be analogous to Thr<sup>867</sup> in XA21 (Figure 1; Table 1; Chen et al., 2010c) and is also essential for the function of FLS2 (Robatzek et al., 2006). FLS<sub>2</sub><sup>TMV</sup> inhibits FLS2 internalization and response to flg22, indicating that both processes are intimately connected (Robatzek et al., 2006). Although the FLS<sub>2</sub><sup>TMV</sup> mutation had no effect on flg22-binding, FLS<sub>2</sub><sup>TMV</sup> mutant lines were insensitive to flg22 treatment. Three of these mutations (FLS<sub>2</sub><sup>T867V</sup>, FLS<sub>2</sub><sup>T1040A</sup>, and FLS<sub>2</sub><sup>T1072A</sup>) also abolished flg22-induced generation of ROS (Robatzek et al., 2006). It is not known if these sites are phosphorylated or if they are required for kinase activity.

In all protein kinases, it is well known that a conserved lysine residue is responsible for a phosphotransfer reaction (Carreras et al., 1991). The importance of this lysine for kinase function has not been demonstrated for plant PRRs. For example, the Lys<sup>705</sup> residue inside the XA21 kinase domain is essential for XA21 autophosphorylation (Liu et al., 2002). However, although catalytic activity of XA21 is essential for full resistance levels, the catalytically impaired XA21 mutant maintains partial resistance activity (Andaya and

Table 1 | Summary of rice and Arabidopsis PRRs.

| Organism | PRR | PRR class | Phosphorylation site | Ligand | Interacting protein | Protein class |
|----------|-----|-----------|---------------------|--------|--------------------|---------------|
| Rice     | XA21| LRR RK, non-RD | S669<sup>1</sup>  T867<sup>13</sup> | Ax21   | XA21<sup>1</sup>  | LRR RK |
|          |     |           | S689<sup>1</sup>  T705<sup>17</sup> |         | XB<sup>3</sup>  | E3 ubiquitin ligase |
|          |     |           |                     |         | XB<sup>10</sup> | WIRKY transcription factor |
|          |     |           |                     |         | XB<sup>15</sup> | Protein phosphatase 2C |
|          |     |           |                     |         | XB24<sup>4</sup> | ATPase |
|          |     |           |                     |         | RX<sup>12</sup> | Thiamine pyrophosphokinase |
|          |     |           |                     |         | ROX<sup>29</sup> | NOL1/NOP2/sun protein |
| Arabidopsis | FLS2| LRR RK, non-RD | T867<sup>13</sup>  S689<sup>23</sup>  T1040<sup>13</sup>  T1072<sup>13</sup> | Flagellin | FLS2T867V-GFP | LysM RK, RD kinase |
| CEBP     | LyM | NA        |                     | Chitin oligosaccharide<sup>16</sup> | ONCERK<sup>15,12</sup> | LysM RK, RD kinase |
| EFR      | LRR RK, non-RD | NA | Elongation factor-Tu elf18<sup>23</sup> | | | |

References:
- Park et al. (2008)
- Peng et al. (2008)
- Robatzek et al. (2006)
- Kurasick et al. (2010)
- Lee et al. (2011)
- Frei Dit Frey et al. (2012)
- Zhang et al. (2010)
- Gomez-Gomez et al. (2001)
- Schwessinger and Ronald (2012)
- Kaku et al. (2006)
- Schwessinger and Ronald (2012)
- Chinchilla et al. (2007)
- Schwessinger and Ronald (2012)
- Kaku et al. (2006)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessinger and Ronald (2012)
- Schwessing
Park et al. Pattern recognition receptor-mediated phosphorylation

**FIGURE 2** | Models for pattern recognition receptor-mediated phosphorylation pathways in Arabidopsis and rice. **Left:** Arabidopsis FLS2 and BAK1 associate with the membrane-associated cytoplasmic kinase Botrytis-induced kinase 1 (BIK1) in vitro and in vivo (Lu et al., 2010). In the resting state, Arabidopsis FLS2 interacts with BIK1. Flg22 perception induces FLS2 and BAK1 association and phosphorylation. Activated BAK1 phosphorylates BIK1, which in turn transphosphorylates the FLS2/BAK1 complex. Phosphorylated BIK1 is released from the FLS2/BAK1 complex to activate downstream intracellular signaling. No direct phosphorylation targets of FLS2 have yet been identified. At least two MAPK cascades are initiated downstream of activated FLS2, leading to the phosphorylation of the adaptor protein MKS1 and the transcription factors, AtWRKY33 and ERF104. Kinase-associated protein phosphatase (KAPP), a PP2C, blocks the activated FLS2 signaling and attenuates the downstream immune response. EFR-mediated immunity is believed to trigger the same MAPK cascades as FLS2. **Right:** In the resting state, rice XA21 forms an in vivo complex with the ATPase, XB24, and the XA21-associated kinase, XAK1. Association of XA21 and XAK1 requires the XA21 JM and kinase domains. Binding of AxYS22 to XA21 induces dissociation of XA21 from XB24 and activates XA21, triggering autophosphorylation. Activated XA21 likely activates a MAPK cascade that includes MPK17-1, leading to the phosphorylation of the transcription factors, OsWRKY33 and EREBP1. Recruitment of XB15 to the XA21 JM domain and subsequent dephosphorylation of phosphorylated residues attenuates XA21 signaling. Cleavage of XA21 and translocalization of the intracellular kinase domain to the nucleus is required for the XA21-mediated immune response (Park and Ronald, 2012). Unlike other well-characterized PRRs, the rice receptors CEBiP and OsCERK1 contain extracellular LysM (lysine motif) domains in place of LRR domains (Kaku et al., 2006; Shimizu et al., 2010). Upon chitin perception, the CEBiP and OsCERK1 complex activates MAPK cascades. In Arabidopsis and rice, phosphorylation of transcription factors leads to large-scale transcriptional reprogramming, including the activation of WRKYs, PAD3, PAL, PRs, PDF1.2, and camalexin biosynthetic genes.

Ronald, 2003). The partial resistance is comparable to that of transgenic lines expressing XA21Δ18, an XA21 family member consisting of an LRR domain but lacking a kinase domain, indicating that XA21 catalytic activity is not absolutely required for function. In Arabidopsis, a mutation in Lys708 of FLS2, which is analogous to Lys794 in XA21, abolishes MPK3 and MPK6 activation by flg22 when transiently overexpressed in protoplasts (Asai et al., 2002). Similarly, a kinase inactive mutation at Lys776 of EFR is unable to confer elf18-triggered ROS burst when transiently expressed in Nicotiana benthamiana (Schwessinger et al., 2011).
MITOGEN-ACTIVATED PROTEIN KINASES SERVE AS INTERNODES IN PRR-MEDIATED IMMUNITY

Morgen-associated protein kinase cascades are important for transmitting signals generated by receptors into cellular responses. Multiple studies support central roles for MAPK cascades in the immunity of Arabidopsis, parsley, tobacco, tomato, and rice (Frye et al., 2001; Zhang and Klessig, 2001; Cardinale et al., 2002; del Pozo et al., 2004; Pitzschke et al., 2009; Jung et al., 2010). Generally, MAP kinase kinase cascades (MAP3Ks, also called MEKKs) are activated by Rxs. MAP3Ks activate downstream MAP kinase cascades (MAP2Ks, also called MKks or MEks) that in turn activate MAPks (also called MPks). MAPks then target various proteins, which include other kinases, enzymes, and transcription factors (Koohianlzet al., 1998; Rodriguez et al., 2010). Genomic sequencing of Arabidopsis and rice have revealed the existence of approximately 60 MAP3Ks, 10 MAP2Ks, and 20 MAPks in Arabidopsis (Goyo, 2002) and at least 75 MAP3Ks, 8 MAP2Ks, and 17 MAPks in rice (Reyna and Yang, 2006; Rao et al., 2010).

In Arabidopsis, many studies have shown that activated FLS2 triggers MAPK signaling cascades (Asai et al., 2002; Chinchilla et al., 2007; Pitzschke et al., 2009, Figure 2). Initially, MEKK1 activates MPK4 which was previously shown to negatively regulate the defense response (Andreasson et al., 2005). At the MAPK kinase level, flg22-induced activation of MPK3/4/6 is dependent on MKK1/2, while MPK3 and MPK6 are also activated by MKK4 (Morosoz et al., 2006; Guo et al., 2006). Thus, two simultaneous MAPK cascades are postulated. The first consists of an unknown MEKK–MKK4/5–MKK6/8 and acts positively on FLS2-mediated signaling. The other consists of MEKK1–MKK1/2–MPK4 and acts negatively on the pathway (Nicouer et al., 2009). A physical interaction between MEKK1 and FLS2 has not been observed. Therefore, researchers are searching for signaling intermediates that function upstream of MEKK1 that would link FLS2 with the key MAPK cascades. Elongation factor–Tu receptor-mediated signaling in Arabidopsis is thought to utilize a similar signal transduction pathway with FLS2. In-gel assays detect a rapid activation of MAPks in flg22 treatment (Zulpel et al., 2006). Treatment with both flg22 and inf1 at the same time induces the same MAPks without an additive effect, indicating that these kinases belong to the same cellular pool of enzymes. These results suggest that PRR-mediated signaling induced by the two conserved microbial signatures, inf1 and flg22, converge at a step upstream of these kinases.

The role of MAPK cascades in PRR-mediated immunity has also been investigated in rice. For example, OsMPK3 [previously named OsBWMK1 (Song and Goodman, 2002)] and OsMPK17-1 [previously named OsBIMK1 (He et al., 1999)] both interact with XBs, suggesting that these MAP kinases are components of the XA21-mediated signaling pathway (Seo et al., 2011). OsMPK3 suppressing plants display enhanced resistance to Xoo, suggesting that it serves as a negative regulator in the XA21-mediated response. In contrast, OsMPK17-1 knockouts displayed increased susceptibility to Xoo, suggesting a positive role in XA21-mediated immunity. OsMPK3/6 and OsMKK4 are activated by chitin (Kishi-Koboshi et al., 2010; Kim et al., 2012).

MAPks PHOSPHORYLATE TRANSCRIPTION FACTORS

Transcriptional reprogramming of immune responses in the nucleus is regulated by transcription factors including the WRKY and ethylene-responsive factor [ERF, also called ethylene-responsive element binding protein (EREBP)] families (Gutterson and Reuber, 2004; Ishihama and Yoshioka, 2012). In animals, MAPks are activated and then often translocate to the nucleus where MAPks will directly or indirectly phosphorylate transcription factors (Harding et al., 2005; Rodriguez et al., 2010). Examples of nuclear localization of MAPks have been reported in Arabidopsis and rice (Cheong et al., 2003; Voo et al., 2008; Koo et al., 2009). Therefore, WRKY proteins and EREBPs constitute an important link between pathogen-activated MAPK signaling pathways and downstream transcriptional reprogramming.

High-density protein microarrays, employed to identify downstream factors of MAPks in Arabidopsis, revealed that many WRKYs are directly regulated by MAPks (Popescu et al., 2009). For example, Arabidopsis WRKY33 (AWRKY33) is induced by conserved microbial signatures, such as the osmocyted-derived peptide Pep25 (Lippok et al., 2007). Subsequent experiments showed that AWRKY33 is phosphorylated by MPK3/MPK6 in vivo in response to Botrytis cinerea infection and by MPK4 at least in vitro (Mao et al., 2011). Phosphorylation of AWRKY33 inhibits the growth of pathogenic fungi and bacteria by promoting the production of camalexin, a major antimicrobial phytoalexin.

Phosphorylation of AWRKY33 at residue 1 phosphorylates the AtWRKY25 and AtWRKY33 interact with MPK4 (Andreasson et al., 2005). Mutation of MPK3/MPK6 phosphorylation sites in AWRKY33 compromises its ability to complement the camalexin induction in the AWRKY mutant. Another transcription factor, ethylene response factor (ERF104), is directly associated and phosphorylated by MPK6 but not MPK3 (Boitke et al., 2009). Perception of flg22 via FLS2 induces disruption of the MPK6/ERF104 complex, releasing ERF104 to its target promoters including PDF2.1 (plant defense 1.2).

There is an increasing body of evidence that suggests MAPks also regulate transcription factors indirectly. Two WRKY transcription factors AWRKY25 and AWRKY33 interact with MPK4 substrate 1 (MKS1) in yeast, suggesting that these WRKYs regulate gene expression downstream of MPK4 (Andreasson et al., 2005). It was later reported that AWRKY33 also forms an in vivo complex with MPK4 and MKS1 (Qu et al., 2008a). However, although MKS1 is directly associated with AWRKY33 and is phosphorylated by MPK4, no interaction has been detected between AWRKY33 and MKS4 (Andreasson et al., 2005; Qu et al., 2008a). This suggests MPK4 and AWRKY33 associate indirectly and require the adaptor protein MKS1 for their interaction (Qu et al., 2008a). Following pathogen perception, the MKS1–AWRKY33 complex binds the phytalexin deficient 3 (PAD3) promoter, which promotes camalexin synthesis.

To date, there are only a few reports suggesting that MAPks phosphorylate rice transcription factors in response to pathogen infection. For example, OsMPK17-1 phosphorylates OsWRKY33 in vitro, which binds to the W-box element in the OAP2 gene promoter (Koo et al., 2009). OsMPK17-1 also phosphorylates the transcription factor OsEREBP1 in vitro (Cheong et al., 2003). Transient co-expression of OsMPK17-1 and OsEREBP1 in Arabidopsis protoplasts elevates the expression of the β-glucuronidase reporter gene driven by the ethylene-responsive element GCC box.
in several basic PR gene promoters. Thus, OsMPK17-1 is involved in rice defense signal transduction and is responsible for the direct phosphorylation of a transcription factor(s).

Although a role for MAPK-mediated phosphorylation of WRKYs has not been demonstrated for XA21-mediated immunity, several WRKYs interact directly with XA21 in yeast. For example, WRKY32, identified as XTH9 in a yeast two-hybrid screen using the XA21 intracellular domain as bait, interacts with the XA21 kinase domain in yeast protoplasts (Park and Ronald, 2012) and negatively regulates XA21-mediated immunity (Peng et al., 2008). Transgenic rice plants overexpressing OsWRKY62 are compromised in XA21-mediated immunity and are impaired in the activation the defense-related genes OsPR1 and OsPR2. Additionally, OsWRKY76 was recently shown to negatively regulate XA21-mediated immunity when challenged with Xoo (Soo et al., 2011). Although these studies indicate a functional relationship between OsWRKYs and XA21-mediated immunity, XA21 has not been shown to directly phosphorylate the WRKYs, thus the role of phosphorylation is unknown.

CONCLUSION AND PERSPECTIVES

Recognition of conserved microbial signatures by PRRs is critical to plant survival. PR activation induces rapid autophosphorylation, leading to phosphorylation of many other proteins. Despite the importance of phosphorylation in PR-mediated immunity, only a few phosphorylation sites of PRRs have been identified. Those phosphorylation sites were initially found by targeted mutagenesis. Although recent advances in phosphoproteomic analyses using mass spectrometry have greatly expanded our capability to identify phosphopeptides (Benschop et al., 2007; Nuhse et al., 2007; Stulemeijer and Joosten, 2008; Kersten et al., 2009), this approach has not yet lead to the identification of additional in vivo PR phosphosites. This lack of success may be due to the observed rapid endocytosis and/or degradation of PRs following perception of conserved microbial signatures (Rohatok et al., 2006; Robatzek, 2007; Chen et al., 2012a), which likely serves as a barrier to identifying PRR phosphorylation sites using mass spectrometry. Progress in mass spectrometric technology to enhance sensitivity of detection of low abundance phosphopeptides is needed to overcome this limitation. Once identified, such sites can be confirmed using independent techniques such as immunoblotting with anti-phospho-specific antibodies and in vivo genetic studies.

In addition to slow progress in identifying residues phosphorylated on the PRR itself, other proteins that could serve as targets of PR phosphorylation have not yet been identified. Therefore, there is still a gap in our understanding of how precisely PRRs are able to initiate early signaling events such as activation of MAPKs, a rapid calcium influx and an oxidative burst. To answer these fundamental questions, it will be essential to identify such target proteins and to determine how these proteins regulate downstream events. Phosphoproteomic comparison is one method that can identify proteins that become phosphorylated during PRR-mediated immunity. For example, quantitative phosphoproteomic analyses performed on flg22- or xylanase-treated Arabidopsis cells successfully revealed several differentially phosphorylated proteins such as auxin efflux carriers and respiratory burst oxidase protein D (Nuhse et al., 2007; Stulemeijer and Joosten, 2008).

Another important goal is to identify the substrates of MAPKs that are phosphorylated during PRR-mediated immunity. To date, only a few transcription factors have been shown to be phosphorylated by MAPKs during PRR-mediated immune responses. Studies utilizing protein microarrays, protein complex immunoprecipitations, and phosphoproteomic analyses will continue to uncover additional transcription factors and other potential MAPK targets, further contributing to our understanding of the role of phosphorylation in plant immune responses.

ACKNOWLEDGMENTS

This work was supported by the National Institute of Health (NIH, GM55962) and the National Science Foundation (NSF, IOS-0817738).

REFERENCES

Adik, S., Frithka, F., Mikol, N., Johansson, K., Lundstrom, I., and Svensson, S. P. (2000). Phosphorylation of Thr694 but not Thr877 within the juxtamembrane domain of the EGF receptor inhibits adipocyte binding. Biochem. Biophys. Res. Commun. 272, 381–387.

Andrus, C. B., and Ronald, P. C. (2003). A catalytically impaired mutant of the rice Xa21 receptor kinase confers partial resistance to Xanthomonas oryzae pv. oryzae. Physiol. Mol. Plant Pathol. 63, 203–208.

Andresson, E., Jenkens, T., Broderick, P., Thogerrens, S., Petersen, N. H., Zhu, S., Qin, J. L., Michelsen, P., Rocher, A., Petersen, M., Newmann, M. A., Byren Nielsen, H., Hirt, H., Somsuch, I., Mattsson, O., and Svensson, I. (2002). Constitutively expressed Xa21 protein kinase kinases. Plant Cell 14, 281–290.

Balogh, G., Unthan, T., Ueng, I. F., Poohki, Y., Gant, A. A., Schud, D., and Lee, J. I. (2009). Fg22 regulates the release of an ethylene response factor substrate from MAP kinase 6 in Arabidopsis thaliana via ethylene signaling. Proc. Natl. Acad. Sci. U.S.A. 106, 8077–8082.

Boller, T., and Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. 60, 379–406.

Cardinals, F., Meikle, L., Oakeley, E., and Hirt, H. (2002). Convergent and divergent activation of two mitogen-activated protein kinase signaling pathways at the level of two distinct mitogen-activated protein kinase kinases. Plant Cell 14, 705–711.

Carroll, A. C., Alejandro, K., and Roberts, T. M. (1993). The conserved brm of the catalytic domain of protein kinase is actively involved in the phosphorylate transfer reaction and not required for anchoring ATP.
Park et al. Pattern recognition receptor-mediated phosphorylation

fips-03-00177 — 2012/8/2 — 19:38 — page 7 — #7
Nakagami, H., Sugiyama, N., Mochida, K., Park et al. Pattern recognition receptor-mediated phosphorylation 446–458. Mol. Plant 12, 421–426.

Pepperdine, S. C., Papinova, V., Buchanan, S., Zhang, Z., Geotest, M., Snedek, M., and Dimidio-Kumar, S. P. (2008). MAPK target networks in Arabidopsis thaliana revealed using functional protein microarrays. Genes Dev. 23, 80.

Qiu, J. L., Zhu, L., Yuan, B. W., Nielsen, H. B., Bik, B. E., Petersen, K., Mackeit, J., Leuke, G. I., Mundy, J., and Morris, P. C. (2008). Arabidopsis MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. EMBO J. 27, 2214–2223.

Qiu, J. L., Zhou, L., Yuan, B. W., Nielsen, H. B., Bik, B. E., Petersen, K., Mackeit, J., Leuke, G. I., Mundy, J., and Morris, P. C. (2008). Arabidopsis mitogen-activated protein kinase 4 regulates gene expression through transcription factor release in the nucleus. EMBO J. 27, 2214–2223.

Rao, K. P., Richa, T., Kumar, K., Raghu, A., Pulli, M., and Peck, S. C. (2007). Quantitative phosphoproteomic analysis of plant plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. Plant Physiol. 145, 542–547.

Rao, K. P., Richa, T., Kumar, K., Raghu, A., Pulli, M., and Peck, S. C. (2007). Quantitative phosphoproteomic analysis of plant plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. Plant Physiol. 145, 542–547.
could be construed as a potential conflict of interest.

Received: 08 May 2012; paper pending published: 01 June 2012; accepted: 18 July 2012; published online: 09 August 2012.

Citation: Park C-J, Caddell DF and Ronald PC (2012) Protein phosphorylation in plant immunity: insights into the regulation of pattern recognition receptor-mediated signaling. Front. Plant Sci. 3:177. doi: 10.3389/fpls.2012.00177

This article was submitted to Frontiers in Plant Proteomics, a specialty of Frontiers in Plant Science.

Copyright © 2012 Park, Caddell and Ronald. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and any copyright notices concerning any third-party graphics etc.

www.frontiersin.org August 2012 | Volume 3 | Article 177 | 3