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MAP kinase cascades in Arabidopsis innate immunity

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MAP kinase cascades generally transduce extracellular stimuli into cellular responses. These stimuli include the perception of pathogen-associated molecular patterns (PAMPs) by host transmembrane pattern recognition receptors which trigger MAPK-dependent innate immune responses. In the model Arabidopsis, molecular genetic evidence implicates a number of MAPK cascade components in PAMP signaling, and in responses to immunity-related phytohormones such as ethylene, jasmonate, and salicylate. In a few cases, cascade components have been directly linked to the transcription of target genes or to the regulation of phytohormone synthesis. Thus MAPKs are obvious targets for bacterial effector proteins and are likely guardians of resistance proteins, which mediate defense signaling in response to the action of effectors, or effector-triggered immunity. This mini-review discusses recent progress in this field with a focus on the Arabidopsis MAPKs MPK3, MPK4, MPK6, and MPK11 in their apparent pathways.

Keywords: calcium signaling, hypersensitive response, MAP kinase cascade, MAP kinase substrates, pathogen effectors, pattern recognition receptors, reactive oxygen species, resistance proteins

INTRODUCTION

Plants have evolved an effective basal defense system to detect and limit the growth of pathogens. Pathogens may be recognized by the host via the perception of conserved microbial structures termed pathogen-associated molecular patterns (PAMPs). PAMPs are recognized via transmembrane pattern recognition receptors (PRRs) that bind specific PAMPs and initiate intracellular immune responses (Zipfel, 2008). These PAMP-triggered immunity (PTI) responses include the generation of reactive oxygen species (ROS), extracellular alkalization, and protein phosphorylation with associated gene regulation that ultimately restricts the growth of the microbial intruder (Cuestas-Blanes and Rathjen, 2010).

Mitogen-activated protein kinase (MAPK) signaling plays central roles in such intracellular immunity pathways. In general, MAP kinase signaling is initiated by the stimulus-triggered activation of a MAP kinase kinase kinase (MAP3K; also called MEKK). MAP3K activation, which may be directly or indirectly effected by a PRR, in turn leads to the phosphorylation and activation of downstream MAP kinase kinases (MAP2K; also called MKK or MEK). Subsequently, the MAP2K phosphorylates the downstream MAP kinase kinases (MAP3K; also called MEKK). This impairs its flg22-regulated kinase activity and inhibits phosphorylation of MPK4. However, these three MAPK cascades are differently regulated already at the PRR level. For example, the two receptor kinases BAK1 and BKK1 genetically regulate PAMP signaling through their interactions with cognate PRRs (Roux et al., 2011; Schwessinger et al., 2011). The BAK1 mutant allele bak1-5 carries a Cys408Tyr substitution adjacent to its kinase catalytic loop. This impairs its flg22-regulated kinase activity and inhibits phosphorylation of MPK4. However, the catalytic complex formed between mutant BAK1 in bak1-5 and FLS2 is still able to induce phosphorylation of MPK3/MPK6 (Roux et al., 2011; Schwessinger et al., 2011). Interestingly, MPK3/MPK6 phosphorylation was impaired in only the double bak1-5 bkk1 background and not in the individual bak1-5 and bkk1 lines (Roux et al., 2011). Asai et al. (2002) developed an elegant protoplast expression system in an attempt to identify signaling components downstream of FLS2. With this system they were able to show a complete MAPK cascade downstream of FLS2 consisting of the MAP3K MEKK1, two MAP2Ks (MKK4 and MKK5), and the MAPKs MPK3/MPK6. However, genetic evidence later showed
MAPK signaling cascades are attractive targets for bacterial effectors. The \textit{P. syringae} hopA1 effector irreversibly inactivates MPK4 to prevent immune responses. The R protein SUMM2 may guard processes downstream of MPK4 independent from MKS1, and triggers a hypersensitive response in the event of loss or inactivation of MPK4.

(A) MAPK signaling cascades are attractive targets for bacterial effectors. The \textit{P. syringae} hopA1 effector irreversibly inactivates MPK4 to prevent immune responses. The R protein SUMM2 may guard processes downstream of MPK4 independent from MKS1, and triggers a hypersensitive response in the event of loss or inactivation of MPK4.

(B) PAMP perception by PRRs instigates a signaling cascade, often via co-receptors, which causes activation of MAP3K MEKK1 and two MAP2Ks MKK1 and MKK2. These phosphorylate and activate MPK4 which then phosphorylates its substrate MKS1, releasing WRKY33 in complex with WRKY33. MKP2/MKP6 sequentially phosphorylate WRKY33 allowing it to promote PAD3 transcription, thus activating plant defense.

that MEKK1 kinase activity was dispensable for MPK3/MPK6 activation, although mkk1 plants were impaired in MPK4 activation (Rodriguez et al., 2007). Interestingly, expressing a kinase dead version of MEKK1 in mkk1 plants completely restored the activation of MPK4 upon treatment with flg22, suggesting that MEKK1 may “simply” act as a scaffold protein (Rodriguez et al., 2007). Biochemical and genetic studies further revealed that the two MAP2Ks MKK1 and MKK2 interact with both MEKK1 and with MPK4, and that flg22-induced MPK4 activation is impaired in the double mkk1 mkk2 mutant. This indicates that MKK1 and MKK2 are partially redundant in MPK4 mediated downstream signaling (Gao et al., 2008; Qiu et al., 2008b).

MPK4 was originally reported as a negative regulator of plant immunity because the \textit{mpk4} mutant accumulates high levels of salicylic acid, constitutively expresses pathogenesis-related (PR) genes, and has a severely dwarfed growth phenotype (Petersen et al., 2000). This phenotype is very similar to that of the mkk1 single and mkk1 mkk2 double mutants, further supporting their functional relationships (Rodriguez et al., 2007; Gao et al., 2008; Qiu et al., 2008b).

MAPK CASCADES IN EFFECTOR-TRIGGERED IMMUNITY

In addition to PRRs, plants also employ resistance (R) proteins as cytoplasmic receptors to directly or indirectly recognize specific pathogenic effector proteins injected into host cells as virulence factors. Effector-triggered immunity (ETI) and PTI share a number of responses, although ETI also includes varying levels of rapid, localized cell death in what is called the hypersensitive response. R protein-dependent recognition initiates immune responses in ETI. R proteins may recognize effector proteins either directly or indirectly by monitoring changes in the effector’s host target(s). This latter case gave rise to the guard hypothesis in which R proteins guard host guardees that are manipulated by pathogen effectors (Van Der Biezen and Jones, 1998).

The genetic characterization of the MEKK1/MKK1–MKK2/MPK4 cascade as a negative regulatory pathway of defense responses was at odds with the activation of the pathway by PAMPs. Instead, it was possible that the severe phenotypes of the kinase knockout mutants were caused by activation of one or more R protein(s) guarding this kinase pathway. Indeed, in an elegant screen for suppressors of the mkk1 mkk2 double mutant, Zhang et al. (2012) identified the R protein SUMM2 (suppressor of mkk1 mkk2). The T-DNA insertion line \textit{summ2-8} completely suppressed the severe mkk1 mkk2 phenotype in respect to morphology, cell death, ROS levels and PR gene expression (Zhang et al., 2012). The analogous knockout phenotype of the upstream MAP3K mkk1 is also completely suppressed in the \textit{summ2-8} background. Interestingly, although the mpk4 mutant shares a similar phenotype with the knockouts of its upstream kinase partners, the mpk4 phenotype is not fully suppressed by the \textit{summ2-8}
mutation, as double mpk4 sum2-8 mutants still retain residual cell death and low levels of ROS. This suggests that MPK4 is involved in other pathways independent of SUM2, and that MPK4 may be guarded by additional R proteins (Zhang et al., 2012; Figure 1A).

The importance of MAPK signaling in immunity is emphasized by studies reporting bacterial effector proteins targeting MAPK cascades for downregulation (Zhang et al., 2007a,b, 2012; Rasmussen et al., 2011). For example, the Pseudomonas syringae effector protein HopA1 targets and irreversibly inactivates MPK3, MPK4, and MPK6, thereby suppressing immune responses which would otherwise inhibit bacterial growth (Zhang et al., 2007a, 2012). In addition, the P. syringae effector protein AvrB has been shown to interact with and induce the phosphorylation of MPK4, although it has not been shown if this phosphorylation occurs as a direct effect of AvrB action or via recognition of AvrB by the plant immune system (Cui et al., 2010).

In plants carrying functional SUM2 alleles, immune responses are activated by bacterial effector proteins targeting the MPK4 pathway (Figure 1A). For example, inducible expression of the bacterial HopA1 effector in wild-type plants gives rise to a defense phenotype similar to that seen in mekk1, mkk2, mkk3 and mpk4 mutants including elevated levels of ROS, PR gene expression, and cell death (Zhang et al., 2012). SUMM2 apparently does not interact directly with the kinase components of the MEKK1/MKK1–MKK2/MPK4 signaling cascade, suggesting that SUMM2 most likely guards a downstream target of MPK4 activity (Zhang et al., 2012). At present, the best studied in vivo substrate of MPK4 activity is MPK4 substrate 1 (MKS1) which forms a nuclear complex with MPK4 and the WRKY33 transcription factor (Andreason et al., 2005; Qiu et al., 2008a). Phosphorylation of MKS1 follows MPK4 activation by flg22 perception and, once phosphorylated, MKS1 is released from complexes with MPK4, thereby releasing the WRKY33 transcription factor to bind to its cognate target genes (Qiu et al., 2008a). Phosphorylation of MKS1 is arrested at the cotyledon stage and is unable to initiate true leaves (Wang et al., 2007). Upstream of MPK3/MPK6 in camalexin induction, MKK4 and MKK5 are activated by the MAPK3s MEKK1 and MAPK4Ks in response to fungal pathogens (Ren et al., 2008). Yet another MAPK, MKK9, whose upstream MAPK(s) remains unidentified, is also involved in MPK3/MPK6 signaling, as plants expressing phospho-mimic MKK4DD produce even more camalexin than plants expressing MKK4DD or MKK5DD (Xu et al., 2008).

To delineate the link between MPK3/MPK6 activation and camalexin accumulation, Mao et al. (2011) elegantly introduced the phospho-mimic mutant NtMEKK2DD, an MKK4 and/or MKK5 ortholog from Nicotiana tabacum, into an array of different wrky mutants in a search for essential transcription factors involved in MPK3/MPK6 mediated camalexin induction. Interestingly, NtMEKK2DD was able to induce camalexin accumulation in all tested mutant lines except wrky33. In addition, WRKY33 proved to be a substrate of MKP3/MPK6 activity, and overexpression of non-phosphorylatable forms of WRKY33 could not fully complement the inability of wrky33 mutants to express PAD3 and accumulate camalexin (Mao et al., 2011; Figure 1B, right).

WRKY33-induced PAD3 expression therefore appears to involve both MPK4- and MPK3/MPK6-mediated signaling (Andreason et al., 2005; Qiu et al., 2008a; Mao et al., 2011). Mao et al. (2011) proposed a model in which PAD3-mediated camalexin induction occurs differentially depending on the type of pathogen causing the immune response. In this model, bacterial pathogens induce an MPK4 mediated response while fungal pathogens initiate an MPK3/MPK6 mediated response. This hypothesis is based on overexpression of the constitutively active MKK4/MKK5 ortholog NtMEKK2DD, rendering MPK3/MPK6 hyperactive and able to induce PAD3 expression (Mao et al., 2011). In support of this hypothesis, the mpk3 mpk6 double mutant is blocked in R. oryzae-induced PAD3 induction (Ren et al., 2008). Nonetheless, and as noted above, some care should be taken with experiments based on mpk3 mpk6 double mutants given their developmental lethality (Wang et al., 2007).

An alternative model may therefore be proposed which combines the MPK4 and MPK3/MPK6 pathways into a dual control of PAD3 regulation in response to pathogen perception (Figure 1B). In such a model, WRKY33 is sequestered in a nuclear complex...
comprising at least MPK4 and MKS1 in unchallenged plants, and is released following PAMP perception (Qi et al., 2008a). Phosphorylation is dispensable for WRKY33 to bind its cognate W-box cis-elements, although it does promote transcriptional activation (Mao et al., 2011). This is illustrated by the fact that 4A3D expression is induced in mpk4 plants (Qi et al., 2008a), which is probably due to the basal activity of free, non-phosphorylated WRKY33 or by free WRKY33 activated by basal MPK3 and/or MPK6 activity. In this scenario, once WRKY33 is released from its nuclear complex with MPK4 and MKS1, it is phosphorylated and hence activated by MKP3/MPK6, thereby inducing calmelexin levels through PAD3 expression. The elevated 4A3D expression induced from NMEKK2 hyper-activated MPK3/MPK6 (Mao et al., 2011) is not in conflict with this model, as it is likely that hyperactive MPK3/MPK6 are able to phosphorylate residual free WRKY33, thus bypassing other possible feedback mechanisms in PAD3 expression.

In this model, MPK4 and MPK3/MPK6 function together as a binary switch conferring dual level regulation. Clarification of the mode of action in which MPK4 and MPK3/MPK6 acts MAPK downregulation. MAP kinase phosphatase 1 (MKP1), which interacts with MPK3, MPK4, and MPK6 (Ulm et al., 2002), binds CaM in a Ca2+ dependent manner and stimulates MKP1 phosphatase activity (Lee et al., 2008). The associations between CDPKs and MAPK cascades have recently been reviewed elsewhere (Warzinger et al., 2011).

Much progress has been made in understanding how MAPK signaling functions in plant immunity. In Arabidopsis, 3 of the 60 identified MAP3Ks are involved in defense, namely MEKK1 (Asai et al., 2002), ED1 (Frye et al., 2001), and MEKKa (del Pozo et al., 2004; Ren et al., 2008). In addition, at least 6 of the 10 identified MAP2Ks (MKK1, MKK2, MKK4, MKK5, MKK7, and MKK9) are involved in defense signaling (Asai et al., 2002; Djamchi et al., 2007; Döczi et al., 2007; Zhang et al., 2007b; You et al., 2008). This situation requires tight regulation of the spatial and temporal kinase activities in order to impose specificity upon downstream signaling. To shed light on this regulation, high-throughput methods such as those used by Popescu et al. (2009) are particularly valuable and help to outline MAPK signaling cascades. While this progress may be lauded, further work needs to focus on identifying direct, in vivo kinase substrates and their respective phosphorylation sites. This may bring us closer to bridging the apparent gap between PRRs and MAPK cascades, and to understanding how specificity is achieved among MAPK pathways both spatially and temporally.

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MPK8 activity has been shown to negatively regulate the expression of OXI1 in order to maintain ROS homeostasis. Remarkably, activation of MPK8 is not limited to the upstream MAP3K MK3, as the Ca2+ binding protein calmodulin (CaM) is able to bind and activate MPK8 in an Ca2+ dependent manner (Takahashi et al., 2011). CaM-mediated MPK8 activation is interesting because it bypasses the traditional, sequential activation of MAPKs and also unequivocally links MAPK activation with the ROS burst and ion flux during stress signaling. In addition, CaM also mediates MAPK downregulation. MAP kinase phosphatase 1 (MKP1), which interacts with MPK3, MPK4, and MPK6 (Um et al., 2002), binds CaM in a Ca2+ dependent manner and stimulates MKP1 phosphatase activity (Lee et al., 2008). The associations between CDPKs and MAPK cascades have recently been reviewed elsewhere (Warzinger et al., 2011).

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