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MINI REVIEW

MAPK signaling: A key element in plant defense response to insects

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Abstract Insects have long been the most abundant herbivores, and plants have evolved sophisticated mechanisms to defend against their attack. In particular, plants can perceive specific patterns of tissue damage associated with insect herbivory. Some plant species can perceive certain elicitors in insect oral secretions (OS) that enter wounds during feeding, and rapidly activate a series of intertwined signaling pathways to orchestrate the biosynthesis of various defensive metabolites. Mitogen-activated protein kinases (MAPKs), common to all eukaryotes, are involved in the orchestration of many cellular processes, including development and stress responses. In plants, at least two MAPKs, salicylic acid-induced protein kinase (SIPK) and wound-induced protein kinase (WIPK), are rapidly activated by wounding or insect OS; importantly, genetic studies using transgenic or mutant plants impaired in MAPK signaling indicated that MAPKs play critical roles in regulating the herbivory-induced dynamics of phytohormones, such as jasmonic acid, ethylene and salicylic acid, and MAPKs are also required for transcriptional activation of herbivore defense-related genes and accumulation of defensive metabolites. In this review, we summarize recent developments in understanding the functions of MAPKs in plant resistance to insect herbivores.

Key words defense; FAC; insect; jasmonate; mitogen-activated protein kinase (MAPK); plant–insect interaction

Introduction

Insects are the most abundant and among the oldest herbivores: plants and insects have co-evolved for more than 350 million years (Gatehouse, 2002). Thus plants have evolved sophisticated response systems to defend against their insect predators. Plants are equipped with physical defenses, such as thorns, trichomes and cuticles, but they are also rich in secondary metabolites, most of which are thought to function against herbivores. Some secondary metabolites are maintained at near-constant concentrations regardless of whether plants are attacked by insects, and these can be categorized as constitutive defenses. In contrast, many metabolites are produced at low levels, or not at all, in unattacked plants, but increase after insect feeding; these are termed inducible defenses. There is evidence that the production of defensive compounds is costly, and inducible defenses may be more cost-effective (Steppuhn & Baldwin, 2007). Inducible defenses are tightly controlled by signaling systems that regulate downstream responses to herbivore-specific cues, including transcriptional activation of genes which encode enzymes for the biosynthesis of defensive compounds (Wu & Baldwin, 2010; Howe & Jander, 2008; Wu & Baldwin, 2009).

One of the earliest signaling events after herbivore attack is the activation of mitogen-activated protein kinases (MAPKs), and growing biochemical and genetic evidence has revealed that MAPKs play critical roles in plant resistance to herbivores. Following herbivore attack, plants activate MAPK signaling, which in turn alters

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levels of phytohormones, including jasmonic acid (JA) and ethylene, reshaping the transcriptome and thus the proteome in preparation to defend against attack. In this review, we summarize recent progress in elucidating the functions of MAPKs in plant–insect interactions, including SIPK (salicylic acid-induced protein kinase), WIPK (wound-induced protein kinase), and MPK4, which are the only MAPKs that have been studied in plant–insect interactions.

MAPKs

MAPKs are part of well-conserved eukaryotic signaling cascades which regulate numerous cellular responses (Herskowitz, 1995; Chang & Karin, 2001). MAPKs consist of 11 domains (I–XI) that are found in all serine/threonine protein kinases (Hanks *et al.*, 1988), and are activated by the dual phosphorylation of Thr and Tyr residues in a TxY motif located in the activation loop (T-loop) between subdomains VII and VIII. Activation occurs via MAPK kinases (MAPKKs or MEKs), which in turn are activated by MAPKK kinases (MAPKKKs or MEKKs) through phosphorylation of conserved Ser and/or Thr residues in their T-loop. Activated MAPKs phosphorylate their substrates, most of which are transcription factors and enzymes, triggering downstream stress-related responses (Hazzalin & Mahadevan, 2002). Based on the phylogenetic analysis of amino acid sequences and TxY phosphorylation motifs, plant MAPKs can be divided into four groups (A, B, C and D). Members of subfamilies A, B and C have a well conserved TEY motif, whereas MAPKs in subfamily D contain a TDY motif and a long C-terminal sequence, and also lack the evolutionarily conserved C-terminal CD domain which is consistently found in members of the other MAPK groups and functions as a docking site for MAPKKs, phosphatases and protein substrates (MAPK Group, 2002).

Genome sequencing projects revealed that the Arabidopsis, rice and poplar genomes contain 20, 15 and 21 MAPKs, respectively; in contrast, there are only 6 and 10 MAPKs in yeast and humans (Hamel *et al.*, 2006). Considering that plants are sessile and cannot relocate themselves to avoid unfavorable conditions, the expanded numbers of MAPKs in plants imply that they assist in adaptation to a variety of environmental stresses.

Insect-derived elicitors

Plants recognize microbe-associated molecular patterns (MAMPs), most of which are conserved proteins (e.g., bacterial flagellin and elongation factor EF-Tu) or cell wall components (such as fungal chitin and bacterial lipooligosaccharides). MAMPs are mostly perceived by pattern recognition receptors (Boller & Felix, 2009) and recognition of MAMPs often leads to activation of MAPKs.

In analogy to MAMPs, herbivore-associated molecular patterns (HAMPs) was proposed as a term to cover all herbivore-derived signaling compounds that can be perceived by host plants and thereby elicit defense reactions (Mithöfer & Boland, 2008; Mithöfer & Boland, 2012). Among the HAMPs, elicitors in insect OS form the largest group of substances that have been studied in plant–herbivore interactions (Bonaventure *et al.*, 2011). These elicitors play important roles in activating herbivory-specific responses after they enter plant tissues during insect feeding. The first elicitor was identified in beet armyworm (*Spodoptera exigua* Hübner) OS: *N*-(17-hydroxylinolenoyl)-L-glutamine. Commonly referred as volicitin, it is able to induce volatile compounds in corn, and these volatiles attract parasitic wasps of beet armyworm (Alborn *et al.*, 1997). Later, compounds with similar structures to volicitin, the fatty acid–amino acid conjugates (FACs), were identified in many different lepidopteran insects, such as the tobacco hornworm *Manduca sexta* (Halitschke *et al.*, 2001) and other *Spodoptera* spp. (Pohnert *et al.*, 1999; Spiteller & Boland, 2003). Thus far, FACs are the best-studied group of insect elicitors. Applying FACs to wounds, a way of mimicking caterpillar feeding, activates herbivory-specific responses in plants, such as greatly increased jasmonic acid (JA), ethylene (ET), and salicylic acid (SA) levels and transcriptome reconfigurations (Halitschke *et al.*, 2003; Halitschke *et al.*, 2001; von Dahl *et al.*, 2007). Importantly, FACs were found to be the elicitors that activate MAPK signaling (Wu *et al.*, 2007) (see below).

In addition to FACs, Schäfer *et al.* (2011) demonstrated that grasshopper (*Schistocerca gregaria*) OS activate MPK3 and MPK6 in Arabidopsis (*Arabidopsis thaliana*), although the identity of the elicitor remains unknown. When fall armyworm (*Spodoptera frugiperda*) feeds on cowpea plants (*Vigna unguiculata*), the ingested plant chloroplastic ATP synthase γ-subunit forms proteolytic fragments in the insect midgut, and one of these fragments, called inceptin, dramatically augments levels of JA, ET and SA in damaged cowpea leaves (Schmelz *et al.*, 2006). Caeliferins, sulfur-containing compounds from American bird grasshoppers (*Schistocerca americana*), induce releases of terpenoid volatiles from maize seedlings (Alborn *et al.*, 2007). Other high molecular-weight insect-derived elicitors also exist: β-glucosidase in cabbage butterfly (*Pieris brassicae*) OS elicits volatiles in Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* cv. Titurel) (Mattiacci *et al.*, 1995); and interestingly, glucose oxidase (GOX) in corn earworm (*Helicoverpa zea*) OS suppresses plant defense responses (Musser *et al.*, 2005). Schmelz *et al.* (2009) investigated the specificity and phylogenic distribution of elicitor recognition in cultivated plants and demonstrated that volicitin exhibited the widest range of phytohormone-inducing activity, whereas inceptin-related peptides only induced defense responses in a few plant species. Much work is still needed to reveal how elicitors activate plant signaling (e.g., MAPKs) and downstream defense reactions, and given the diversity of plant–insect interactions, it is expected that there are still more insect-derived elicitors to be discovered, which may activate specific defense responses in particular plant species.

Functions of SIPK and WIPK in plant–insect interactions

The first line of evidence of the involvement of MAPKs in plant–herbivore interactions was from a study on tobacco (*Nicotiana tabacum*) in which Seo *et al.* (1995) demonstrated that *WIPK*, a member of MAPK subfamily A, transcripts and total MAPK activity increased rapidly (1 min) after wounding; furthermore, antisense expression of *WIPK* led to reduced levels of JA and defense-related genes.

Later, the functions of MAPKs in plant resistance to herbivores were studied using coyote tobacco (*N. attenuata*), a wild tobacco which grows in post-fire monocultures in the deserts of western North America. Wu *et al.* (2007) found that supplementing *M. sexta* OS to wounded *N. attenuata* leaves to simulate herbivory rapidly (within 5 min) activated SIPK and WIPK, and the OS-induced activity levels of SIPK and WIPK were at least twofold greater than those induced by mechanical wounding alone; importantly, removing FACs from OS using ion-exchange columns abolished the ability of OS to amplify SIPK and WIPK activity, and adding synthetic FACs to wounds increased the activity of these MAPKs to levels similar to those induced by OS. Like WIPK, SIPK also belongs to subfamily A, and the two MAPKs are homologues (e.g., in *N. attenuata*, they share 73% identity on the amino acid sequence level). When *SIPK* or *WIPK* were silenced in *N. attenuata*, the levels of JA induced by simulated herbivory or wounding treatment were strongly reduced, demonstrating that SIPK and WIPK are both important regulators of induced JA biosynthesis (Wu *et al.*, 2007). At least eight different enzymes are involved in JA biosynthesis (Wasternack, 2007), and although the mode of action is still unknown, there is evidence that SIPK and WIPK function differently: SIPK likely regulates the lipase which releases linolenic acid (18:3) from chloroplast membranes, and WIPK probably influences the activity of allene oxide synthase (AOS) which converts 13-OOH-18:3 to the immediate precursor of 12-oxo-phytodienoic acid (OPDA) (Kallenbach *et al.*, 2010). In contrast, only SIPK, but not WIPK, is involved in *M. sexta* herbivory-induced ET production (Wu *et al.*, 2007). ET is synthesized in two consecutive steps catalyzed by 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO). In Arabidopsis, MPK6 (the homologue of SIPK), but not MPK3 (the homologue of WIPK), directly phosphorylates ACS6 and ACS2 and thus stabilizes these enzymes, and this increases the production of ET (Liu & Zhang, 2004); Arabidopsis leaves show greatly increased MPK6 and MPK3 activity after wounding (Ichimura *et al.*, 2000). Very likely, the same SIPK-regulated mechanism accounts for the herbivory-induced ET "burst" in *N. attenuata*. Given the role of JA and ET in modulating plant defense against herbivores (Paschold *et al.*, 2007; von Dahl *et al.*, 2007; O'Donnell *et al.*, 1996; Xie *et al.*, 1998), it is not surprising that many herbivore resistance-related genes and consequently many defensive metabolites were found to have decreased levels in *SIPK*- and *WIPK*-silenced plants (Meldau *et al.*, 2009; Wu *et al.*, 2007).

Herbivory-induced MAPK activation has also been demonstrated in tomato (*Solanum lycopersicum*). In transgenic tomato overexpressing the prosystemin gene, which increases plant resistance to insects, *M. sexta* feeding activates MPK3 (the tomato homologue of WIPK), MPK1 and MPK2 (two tomato homologues of SIPK) within 10 min; furthermore, co-silencing *MPK1/2* reduces induced JA levels and the expression of JA biosynthetic genes, and consistently, *M. sexta* larvae grow better on *MPK1/2*-silenced plants (Kandoth *et al.*, 2007).

Compared with what is known about the function of MAPKs in plant defense against chewing insects, the involvement of MAPK signaling in the interactions between plants and piercing and sucking insects, such as aphids and whiteflies, is not well understood. Li *et al.* (2006) demonstrated that tomato MAPKs (MPK1, MPK2 and MPK3) confer resistance to aphids, although the underlying mechanism remains unclear.

MPK4, a multi-functional MAPK having plant species-specific functions

MPK4, a member of the MAPK subfamily B, was initially found to play a role in the resistance of Arabidopsis to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) (Petersen *et al.*, 2000): the *mpk4* mutant has strongly elevated levels of SA and likely exhibits SA-dependent bacterial resistance; it furthermore displays stunted growth and is sterile, indicating that MPK4 is also important for plant development. Later, it was found that MPK4 is required for cytokinesis in Arabidopsis (Kosetsu *et al.*, 2010). Using a virus-induced gene silencing (VIGS) system, Liu *et al.* (2011) showed that the homologues of *MPK4* in soybean (*Glycine max*) have similar functions as in Arabidopsis.

However, studies in *N. tabacum* and *N. attenuata* indicated that MPK4 plays a distinct role in this genus. Gomi *et al.* (2005) showed that MPK4 is activated 10 min after tobacco is wounded, and silencing *MPK4* compromises the expression of JA-responsive genes. In contrast to SIPK and WIPK in *N. attenuata*, MPK4 did not regulate JA levels in response to wounding or OS from *Spodoptera littoralis*, but it negatively affected *M. sexta* OS-induced JA levels (Hettenhausen *et al.*, 2013a). While experiments with MPK4-deficient lines indicated that MPK4 suppresses a novel JA-independent defense pathway against the solanaceous specialist *M. sexta*, MPK4 is not required for resistance to the generalist *S. littoralis* (Hettenhausen *et al.*, 2013a). It is likely that the FAC contents of *S. littoralis* OS are too low (about 500-fold lower than those in *M. sexta* OS) to activate the MPK4 signaling pathway. Interestingly, in *N. attenuata*, MPK4 also plays a role in regulating drought- or abscisic acid-induced stomatal closure and is also required for full resistance to leaf surface-deposited *Pst* DC3000 (Hettenhausen *et al.*, 2012).

Thus, some functions of MPK4 appear specific to particular plant species and interactions. Studies of the evolution of MPK4 and how it exerts its function in different plant species will help to elucidate the processes through which plants distinguish between attackers.

MAPK signaling in systemic tissues

It is generally agreed that systemic induced responses benefit plants under insect attack, since systemic tissues can accumulate defensive metabolites before insects arrive. Systemic defense was first found in tomato, in which wounding not only induces proteinase inhibitors (small peptides which inhibit digestive proteinases in the insect midgut) in the wounded leaves (the so-called "local" leaves), but also in distal untreated leaves (the "systemic" leaves) (Green & Ryan, 1972).

Systemic activation of MAPKs is very likely an important part of the signaling events that eventually lead to proper deployment of defenses in undamaged tissues. In tobacco, Seo *et al.* (1995) found that *WIPK* mRNA levels increase just 1 min after wounding in both local and systemic leaves. The same research group demonstrated that cutting off tobacco stems rapidly induces WIPK activity in leaves adjacent to the cut position (Seo *et al.*, 1999). Similarly, in tomato, wounding treatment elevates the activity of a 48 kDa MAPK in systemic leaves within a few minutes (Stratmann & Ryan, 1997), and wounding-induced systemic MAPK activation was also found in soybean (Lee *et al.*, 2001). Using *N. attenuata* as the model, Hettenhausen *et al.* (Hettenhausen, Baldwin, and Wu, unpublished data) systematically examined local and systemic SIPK activity and found that after simulated *M. sexta* feeding, only specific (not all) systemic leaves show activation of SIPK, and unlike tomato and cultivated tobacco, *N. attenuata* systemic leaves do not have increased SIPK activity until 30 min after local elicitation. Importantly, only simulated herbivory, but not wounding alone, can trigger the elevation of SIPK activity in systemic leaves, indicating that *N. attenuata* is able to specifically recognize *M. sexta* feeding and activate systemic SIPK signaling. In addition to systemic leaves, SIPK activity can be also detected in specific unwounded regions of herbivore-attacked leaves (Wu *et al.*, 2007).

Taken together, these studies indicate that a mobile signal(s), which is produced in the attacked leaf area, is transported to specific regions of the same leaf and to systemic leaves to activate MAPK signaling. A growing body of evidence has suggested the involvement of electric signals in systemic signaling (Maffei *et al.*, 2004; Mousavi *et al.*, 2013; Zimmermann *et al.*, 2009). Recent work by Mousavi *et al.* (2013) showed that the electrical signals are generated via glutamate receptor-like genes and are sufficient to increase production of the active jasmonate, jasmonoyl-isoleucine (JA-Ile) in leaves.

MAPKKs in plant–insect interactions

MAPKKs are the immediate upstream activators of MAPKs. Arabidopsis, rice and poplar genomes contain 10, 13 and 13 canonical MAPKKs, respectively (Hamel *et al.*, 2006), in all cases fewer than the numbers of MAPKs in the corresponding genomes. Thus, it is likely that MAPKKs have multiple downstream MAPK targets. Some progress has been made in understanding the involvement of MAPKKs in plant–pathogen interactions (Kiegerl *et al.*, 2000; Gao *et al.*, 2008; Liu *et al.*, 2004); however, the roles of MAPKKs in plant–insect interactions are still not well studied.

In *N. tabacum*, overexpressing constitutively active MEK2, a MAPKK, strongly activates SIPK and WIPK

(Yang *et al.*, 2001). Similarly, the homologues of MEK2 in Arabidopsis, MKK4 and MKK5, were also found to activate MPK6 and MPK3, when constitutively activated in Arabidopsis protoplasts (Asai *et al.*, 2002). Using the VIGS approach, Heinrich *et al.* (2011a, b) examined the functions of MEK1, MEK2, MKK1, SIPKK and NPK2 in the defense of *N. attenuata* against *M. sexta*. Of these MAPKKs, only MEK2 was required for the activation of SIPK and WIPK after wounding or simulated *M. sexta* herbivory, although it is likely that in addition, one or more unknown MAPKKs contribute to the full activation of SIPK and WIPK. Interestingly, all five of these MAPKKs affect the activity levels of trypsin proteinase inhibitors, suggesting that certain MAPKKs may regulate plant resistance to herbivores via a mechanism independent of the MAPKs SIPK and WIPK, and of JA signaling.

Perspectives

Approximately 1/3 of all proteins in eukaryotic cells are phosphorylated at any given time (Zolnierowicz & Bollen, 2000). Given the known importance of MAPKs in yeast and animals, the functions of MAPKs have drawn much attention in plant research. Multiple MAPK signaling pathways, including those of MPK3, MPK6 and MPK4 in Arabidopsis, have been intensively studied for their roles in plant–pathogen interactions. In these pathways, MAPKKs, MAPKs and direct MAPK substrates (e.g., transcription factors or enzymes) have been identified (Asai *et al.*, 2002; Gao *et al.*, 2008; Kong *et al.*, 2012; Andreasson *et al.*, 2005). However, many questions remain to be answered in order to understand MAPK-mediated signaling in plant–insect interactions, including: (i) which genes encode receptors for insect-derived elicitors, such as FACs, which trigger the activation of MAPKs; (ii) what are the components of the MAPK cascades involved in plant–insect interactions and how is MAPK-substrate specificity achieved; and (iii) how do plants employ these pathways in recognition of different insect herbivores to deploy specific responses?

In addition to MAPKs, other types of kinases are also important for regulating plant defense responses against herbivores. Two *N. attenuata* calcium-dependent protein kinases (CDPKs), CDPK4 and CDPK5, negatively affect wounding- and herbivory-induced JA levels (Hettenhausen *et al.*, 2013b; Yang *et al.*, 2012). BRI1-associated kinase 1 (BAK1) has been intensively studied for its function as a co-receptor of brassinosteroids and as a regulator of immunity to pathogens, and it was found that BAK1 is also involved in plant defense against insects (Yang *et al.*, 2011). *N. attenuata* lectin receptor kinase1 (LecRK1) inhibits the accumulation of *M. sexta* feeding-induced SA and thus promotes JA-mediated defense (Gilardoni *et al.*, 2011). Given the known importance of protein phosphorylation in regulating responses to external stimuli, it is very likely that many more types of kinases also contribute to plant resistance to insects.

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Disclosure

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