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MAPK cascade signalling networks in plant defence

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The sensing of stress signals and their transduction into appropriate responses is crucial for the adaptation and survival of plants. Kinase cascades of the mitogen-activated protein kinase (MAPK) class play a remarkably important role in plant signalling of a variety of abiotic and biotic stresses. MAPK cascade-mediated signalling is an essential step in the establishment of resistance to pathogens. Here, we describe the most recent insights into MAPK-mediated pathogen defence response regulation with a particular focus on the cascades involving MPK3, MPK4 and MPK6. We also discuss the strategies developed by plant pathogens to circumvent, inactivate or even 'hijack' MAPK-mediated defence responses.

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Introduction

MAPK cascades are highly conserved modules in all eukaryotes. In plants, MAPK pathways are involved in the regulation of development, growth, programmed cell death and in responses to a diversity of environmental stimuli including cold, heat, reactive oxygen species, UV, drought and pathogen attack [1]. Via a phosphorelay mechanism these cascades, minimally composed of a MAPKKK (MAPK kinase kinase), a MAPKK (MAPK kinase) and a MAPK, link upstream receptors to downstream targets. The *Arabidopsis* genome contains about 110 genes coding for putative MAPK pathway components: 20 MAPKs, 10 MAPKKs and more than 80 MAPKKKs [2]. Scaffolding proteins or specific expression of distinct components, confer the specificity to MAPK components engaged in a module. So far, only a few MAPK cascade components have been studied in detail. The *Arabidopsis* MAPKKKs YODA, ANP2/ANP3 and MP3K6/MP3K7 function in development [3••,4–6], MEKK1 and ANP1 act in the environmental stress response [7•–9•], whereas CTR1 plays a pivotal role in ethylene signalling [10]. Among the 10 MAPKKs, MKK1/MKK2, MKK4/MKK5 as well as MKK3, MKK7 and MKK9 have been analyzed [11,12,13••,14••,15,16•,17,18••,19,20••]. Finally, 8 of the 20 MAPKs have been studied to various degrees (for review, see [1]).

The best-characterized MAPKs are MPK3, MPK4 and MPK6, all of which are activated by a diversity of stimuli including abiotic stresses, pathogens and oxidative stress. While MPK4 negatively regulates biotic stress signalling, MPK3 and MPK6 act as positive mediators of defence responses. The key role of these three MAPKs for normal plant growth and development is evidenced by the severely dwarfed phenotype of *mpk4* and the embryo lethal phenotype of *mpk3/mpk6* double mutants [14••,16•,21].

In this review, we focus on the activation of MAPK cascades involving MPK3, MPK4 and MPK6, after the perception of pathogen-associated molecular patterns (PAMPs), and on strategies developed by specific plant pathogens to deal with MAPK-mediated defence responses.

PAMP-triggered activation of MAPK cascades

In the ongoing battle between plants and pathogens, plants have adapted the capacity to recognize pathogens through PAMPs via cell surface-located pathogen-recognition receptors. The activation of these receptors induces convergent intracellular signalling pathways in plant cells, which ultimately result in the establishment of PAMP-triggered immunity [22]. PAMPs are mostly small molecules derived from different pathogen structures common to a class of pathogens. Consequently, the responses to PAMPs are not specific, but rather reflect a response to a given group of pathogens. Despite the large variety of known PAMPs, PAMP receptors in plants have so far only been identified for flagellin, the translation elongation factor EF-Tu and chitin [23,24••,25,26,27••].

PAMP-triggered immunity requires a signal transduction from receptors to downstream components via the MAPK cascade, and many of the known PAMPs were shown to activate MAP kinases. The flagellin derived peptide flg22 triggers a rapid and strong activation of MPK3, MPK4 and MPK6 [28]. MPK4 and MPK6 are also activated by harpin proteins, which are encoded by *hrp* (hypersensitive response and pathogenicity) genes in

many plant pathogenic bacteria. This activation is followed by the induction of pathogenesis-related (*PR*) genes [29], encoding for proteins with antimicrobial activities. Similarly, various NLPs (necrosis and ethylene-inducing peptide1-like proteins) trigger MAPK activation and induce defence responses [30].

MPK3/MPK6 are necessary to induce defence responses

MPK3 and MPK6 are closely related proteins that show a high level of functional redundancy. Both MAPKs are key regulators of a diverse set of processes including abscission, stomatal development, signal various abiotic stresses and defence response to bacterial and fungal pathogens (for review see [1]).

On the basis of experiments using transient expression in protoplasts, the MAPK module MEKK1-MKK4/MKK5-MPK3/MPK6 was proposed to be responsible for flg22 signal transmission [20••]. The involvement of MEKK1 in flg22-induced MKK4/MKK5-MPK3/MPK6 signalling is unlikely, since *mekk1* mutant plants are compromised in flg22-triggered activation of MPK4, but show normal activation of MPK3 and MPK6 [9•]. However, besides this uncertainty, modules involving the MAPKKs MKK1/MKK4/MKK5/MKK9 and the MAPKs MPK3/MPK6 are clearly implicated in different defence strategies.

MPK3/MPK6 are compulsory in camalexin biosynthesis

Intensive research has been attributed to plant defence responses to *Botrytis cinerea*. This fungal pathogen triggers the synthesis of camalexin, a major phytoalexin in *Arabidopsis*. Camalexin is required for resistance to *B. cinerea*, as shown by the susceptible phenotype of the *phytoalexin deficient 3* (*pad3*) mutant [32]. The key role of MPK3/MPK6 in camalexin-based fungal resistance is demonstrated by the fact that *mpk3* and *mpk6* mutants are compromised in camalexin production and consequently more susceptible to *B. cinerea* [31]. Recent data implicate a MAPK cascade composed of MKK4/MKK5 and MPK3/MPK6 in response to fungal pathogens, based on the observation that activation of MPK3/MPK6 in conditional gain-of-function (GOF) plants for MKK4/MKK5 or MEKK1/MKKKa is sufficient to induce accumulation of camalexin, even in the absence of pathogen attack [31]. However, another MAPKK, MKK9, whose upstream MAPKKK is unknown, is apparently also involved in camalexin biosynthesis via MPK3/MPK6: Conditional MKK9-GOF plants have elevated MPK3/MPK6 activity and produce even more camalexin after transgene induction than MKK4-GOF or MKK5-GOF plants [17]. In addition, the MKK9-MPK3/6 is involved in the biosynthesis of ethylene [17] a plant hormone involved in defence responses. Whether both MKK4/MKK5 and MKK9 act as upstream regulators of MPK3/MPK6 in camalexin biosynthesis will have to be verified.

MPK3 is required for stomatal immune responses

Stomata are indispensable for gas exchange and transpiration. However, in terms of pathogen attack stomata are weak points, since they serve as 'entrance gate' for various microbial invaders. A tight regulation of stomatal opening and closure, therefore, is pivotal not only to withstand unfavourable environmental conditions, such as drought and heat but also to restrict pathogen invasion. Recent studies implicate MAPK cascades in stomatal regulation in both abiotic and biotic stress responses.

MPK3/MPK6 are key players in stomatal development and stomatal dynamics [16•]. Upon drought, stomatal closure is mediated through the phytohormone ABA and involves MKK1, MPK3 and MPK6 [33,34]. Pathogen-induced stomatal closure restricts the invasion of many bacteria and is a part of the plant innate immune response. The pathogen *Xanthomonas campestris pv. Campestris* (*Xcc*) excretes a cell–cell signal-regulated virulence factor, which reverts stomatal closure induced by bacteria and ABA [33] and which can complement the infectivity of *Pseudomonas syringae pv. tomato* (*Pst*) mutants deficient in the production of coronatine, a toxin required to overcome stomatal defence. Recent findings suggest a unique function of MPK3 in the stomatal innate immunity response. Guard cell specific Arabidopsis *MPK3* antisense plants are more sensitive to *Pst* coronatine-deficient mutants. Their stomata close normally upon ABA [33], but are unresponsive to bacteria. Moreover, in these plants, *Xcc* extracts do not revert bacteria-induced or ABA-induced stomatal closure. Whether pathogen-induced and ABA-induced stomatal closures are signalled via a common MAPK cascade remains to be investigated.

Negative regulation of defence responses by the MPK4 pathway

A number of studies identified the MEKK1-MKK1/2-MPK4 cascade in pathogen signalling. Using genetic approaches, independent reports show MKK1 and MKK2 as functionally redundant activators of MPK4 [13••,14••,35••], thus confirming previous experimental evidence that MKK1 and MKK2 interact with MPK4 [36,37].

Mekk1, *mkk1/mkk2* double and *mpk4* mutants are severely dwarfed and accumulate high amounts of reactive oxygen species [7•–9•,13••,14••,38]. These abnormalities are most probably due to their drastically enhanced SA levels and can be partially reverted by expression of a bacterial SA hydrolase [38]. *Mekk1*, *mkk1/mkk2* double and *mpk4* mutants also display spontaneous cell death, upregulation of pathogenesis-related genes and enhanced resistance to pathogens. Therefore, a negative regulatory role of the MEKK1-MKK1/2-MPK4 module in SA and H_2O_2 production has been proposed.

The *mekk1*, *mkk1/2* and *mpk4* mutants have strongly overlapping expression profiles, with many defence-related genes being similarly deregulated [14••]. Interestingly, comparative transcriptome studies have implicated that a subset of the MEKK1-dependent MPK4 responses are regulated independently of MKK1 and MKK2 [35••]. In addition, based on the significant proportion of genes exclusively deregulated in *mekk1* mutants, MEKK1 seems to function in other as yet unknown pathways involving neither MKK1/MKK2 nor MPK4. The WRKY53 transcription factor may be partially responsible for the *mekk1*-specific gene set, as MEKK1 directly interacts with WRKY53 and alters the activity of this transcription factor [39•]—a unique short cut of MAPK signalling. The interaction of MKK1-MPK4 and MKK2-MPK4 was lately confirmed *in vivo*, and the complexes were located at the plasma membrane and in the nucleus [13••]. Three proteins have been identified as substrates of MPK4: WRKY33, WRKY25 and MKS1 [40]. WRKY33 was previously shown to interact with MKS1 *in yeast* and *in vivo* [38]. Although no MPK4-WRKY33 interaction was originally observed, recent results propose an MKS1-dependent MPK4-WRKY33 interaction [41••]. Immunoprecipitation from nuclear extracts revealed the existence of a ternary MKS1-MPK4-WRKY33 complex. The recruitment of WRKY33 to this complex depends on the phosphorylation state of MPK4. After activation, MPK4 multiphosphorylates MKS1, resulting in the release of WRKY33 from the ternary complex. According to this model, free WRKY33 then induces transcription of its target genes [41••], providing a molecular mechanism for the negative regulation of defence signalling by MPK4. In the absence of functional MPK4, WRKY33 (and probably other MPK4 targets) would not be sequestered in the complex any more, leading to activation of gene expression even in the absence of pathogens.

How pathogens manipulate MAPK signalling

Pathogens have evolved strategies to overcome defence responses. This can be achieved through inactivation of PAMP-induced signalling pathways by targeting the MAPK cascade components.

The pathogen *Pseudomonas syringae* injects a number of effectors into plant cells. Among these, AvrPto and AvrPtoB interact with the FLS2 receptor and its co-receptor BAK1. AvrPtoB catalyses the polyubiquitination and subsequent proteasome-dependent degradation of FLS2 [42••,43]. This process is enhanced when FLS2 binds to flg22. AvrPto can interact with BAK1 and thereby prevent its binding to FLS2 [42••]. In this way, both AvrPto and AvrPtoB interrupt signalling to the downstream MAPK module.

Pseudomonas syringae has yet another factor that can directly interact with the MAPK cascade components: HopAI1 is a phosphothreonine lyase that dephosphorylates the threonine residue at which MAPKs are activated by their upstream MAPKKs [44•]. HopAI1 (when expressed *in planta*) directly interacts with MPK3 and MPK6, thus attenuating flg22-induced MAPK activation and downstream defence responses. Strikingly, HopAI1 is also present in animal/human pathogens such as *Shigella* spp. (OspF) [45,46] and *Salmonella* spp. (SpvC) [47], where it interacts with the MAPKs ERK1/2 and p38.

HopPtoD2 is another bacterial effector from *P. syringea pv. tomato* that displays phosphatase activity [48]. Expression of HopPtoD2 in tobacco cells suppresses the cell death provoked by expression of the constitutively active MAPKK variant $NtMEK2^{DD}$. However, HopPtoD2 does not inhibit flg22-induced MAPK activation in *Arabidopsis*.

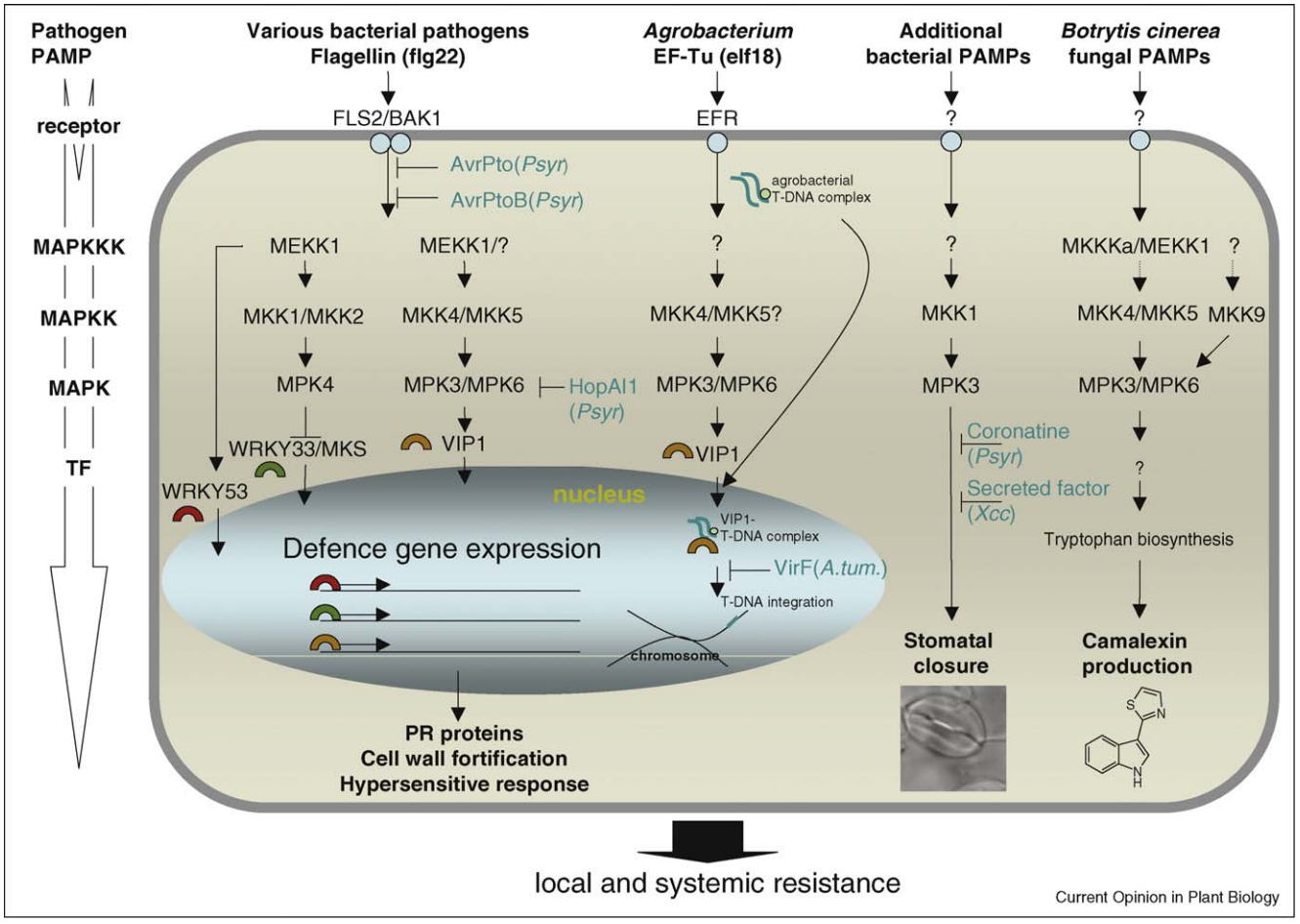
Agrobacterium hijacks the MPK3/MPK6 pathway

An entirely different mode of host MAPK signalling manipulation by pathogens has recently been unravelled. The soil-borne pathogen *Agrobacterium tumefaciens* carries flagellin variants that are non-detectable by the *Arabidopsis* FLS2 [24••,49], yet it triggers a typical innate immune response through the EF-Tu protein. The EF-Tu-derived peptide elf18 is sufficient to trigger the defence response. Interestingly, the receptors for flg22 and efl18, FLS2 and EFR, respectively, belong to the same subfamily of LRR-RLKs, LRRXII. Moreover, elf18 and flg22 induce an overlapping set of responses, including extracellular alkalisation, a rapid activation of MAPKs and the induction of similar response genes [27••]. It is tempting to speculate that also other members of the LRRXII subfamily function as PAMP receptors and it will be interesting to see whether their signalling converge in the same pathways.

Agrobacterium tumefaciens is the causal agent of crown galls. It infects plants by integrating a segment of its DNA (transfer DNA) into the host chromosomal DNA. *efr1* mutants fail to recognize EF-Tu and, presumably owing to reduced defence responses, are more easily transformed by *Agrobacterium* [27••]. However, since MAPK activities in *efr1* have not been assessed, and since *Agrobacterium* contains other PAMPs in addition to EF-Tu, the importance/contribution of MAPKs in EF-Tu-triggered defence remains unclear.

Initiation of defence signalling cascades in their host can be turned into a benefit for plant pathogens: The activation of MPK3 in response to flg22 or *Agrobacterium* results in the phosphorylation and subsequent nuclear translocation of the host protein VIP1 (virE2 interacting protein 1). *Agrobacterium* has hijacked VIP1 for delivering their T-DNA into the plant nucleus, where it integrates into the host genome [50,51••]. Because VIP1 does not only serve as nuclear shuttle for the pathogenic T-DNA complex but can also induce the expression of defence

Figure 1



PAMP-induced MAPK cascades in the plant defence to bacterial and fungal pathogens. PAMP-triggered activation of MAPK cascades initiates the synthesis of pathogen-induced synthesis of PR proteins (e.g. glucanases, chitinases), cell wall depositions, stomatal closure and phytoalexin (e.g. camalexin) synthesis. Together, these modifications result in pathogen resistance. Microbial factors interfering with the signal transduction are shown in blue. Activated transcription factors (TF) are shown as semicircles. Unknown receptors and MAPK cascade components are indicated by '?'.

genes [51••], nuclear VIP1 would be counteracting *Agrobacterium* invasion. *Agrobacterium* overcomes this problem through targeting nuclear VIP1 for proteasome degradation by the *Agrobacterium* virulence factor VirF, which encodes an F-box protein [52].

Conclusions

By amplifying and transducing pathogen-derived signals perceived at membrane receptors and transducing these signals into altered gene expression, plant MAPK modules play a key role in the induction of defence mechanisms (Figure 1).

MAPKs are also prominent targets for inactivation by effector proteins. Interestingly, the mechanisms of effector-mediated interruption of MAPK signalling employed by plant and animal pathogens are similar. So far only a few components of MAPK cascades have been thoroughly studied in plants. Three reports described the systematic attempts to interconnect the MAPKK and MAPKs with their targets [53,54•,55]. The identification and *in planta* verification of additional MAPKKK-MAPKK-MAPK modules, discovery of downstream targets and the regulated processes will be a future challenge to disentangle the sophisticated network of plant MAPK signalling pathways.

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