Complexity, cross talk and integration of plant MAP kinase signalling

Claudia Jonak*, László Ökrész†, László Bögre‡ and Heribert Hirt*§

Mitogen-activated protein kinases (MAPKs) link stimuli that are activated by external sensors to cellular responses. The completed *Arabidopsis* genome sequence has revealed an extraordinary complexity in MAPK-signalling components in plants. Information obtained from *Arabidopsis* provides a framework for a unified nomenclature and for the assembly and determination of the function of MAPK-signalling pathways. Strategies and tools are evolving to connect MAPK pathways and to determine their functions. As a result, MAPK-signalling modules have emerged, one of which appears to antagonistically regulate stress- and growth-responses whereas another regulates cytokinesis.

Addresses
*Institute of Microbiology and Genetics, Vienna Biocenter, Dr. Bohrgasse 9, 1030 Vienna, Austria
†Institute of Plant Biology, Biological Research Centre of Hungarian Academy of Sciences, POB 521, H-6701 Szeged, Hungary
‡School of Biological Sciences, Royal Holloway University of London, Egham TW20 OEX, UK; §e-mail: hehi@gem.univie.ac.at
Corresponding author: Heribert Hirt

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Abbreviations

ANP1–3	*Arabidopsis* NPK1-like protein kinases 1–3
AtHK1	*Arabidopsis thaliana* HISTIDINE KINASE 1
AtMEKK1	*Arabidopsis thaliana* MAPKKK1
AtMKK1	*Arabidopsis thaliana* MAPKK1
AtMPK3	*Arabidopsis thaliana* MAPK3
CDK	cyclin-dependent kinase
CTR1	CONSTITUTIVE TRIPLE RESPONSE 1
EDR1	ENHANCED DISEASE RESISTANCE 1
flg22	flagellin22
FLS2	*FLAGELLIN SENSITIVE2*
HR	hypersensitive response
JA	jasmonic acid
MAPK	mitogen-activated protein kinase
MAPKK	MAPK kinase
MAPKKK	MAPKK kinase
MAPKKKK	MAPKKK kinase
MMK2	*Medicago* MAPK2
NACK1	NPK1-activating kinase-like protein1
NPK1	*Nicotiana* protein kinase1
Ntf6	*Nicotiana* Fus-3-like kinase6
NtMEK1	*N. tabacum* MAPKK1
PRKK	pathogen-responsive MAPKK
SA	salicylic acid
SAMK	stress-activated MAPK
SIMK	stress-induced MAPK
SIMKK	SIMK kinase
SIPK	SA-induced protein kinase
Ste20	Sterile20
WIPK	wound-induced protein kinase
ZIK	ZR1-interacting kinase

Introduction

Arabidopsis shares a large number of genes with humans and yeast, including components of conserved mitogen-activated protein kinase (MAPK) signalling pathways that are known to regulate cell growth and death, differentiation, the cell cycle and stress responses. MAPK cascades are minimally composed of three kinase modules, MAPKKK, MAPKK and MAPK, which are linked in various ways to upstream receptors and downstream targets. Receptor-mediated activation of MAPKKKs can occur through physical interaction and/or phosphorylation by the receptor itself, by intermediate bridging factors or by interlinking MAPKKKKs. MAPKKKs are serine/threonine kinases and activate MAPKKs through the phosphorylation of two serine/threonine residues in a conserved S/T-$X_{3–5}$-S/T motif. By contrast, MAPKKs are dual-specificity kinases that phosphorylate MAPKs on threonine and tyrosine residues in the T-X-Y motif. MAPKs are promiscuous serine/threonine kinases that phosphorylate a variety of substrates, including transcription factors, protein kinases and cytoskeletal proteins. The specificity of MAPK cascades functioning within the same cell is generated through the presence of docking domains that are found in various components of the MAPK modules and also through scaffold proteins [1].

During the 1.6 billion years since plants and animals diverged, the contexts in which MAPK signalling players function have diverged substantially. Owing to space limitations, not all literature on MAPKs can be discussed in this review, but reviews on the basic composition and function of MAPK pathways in animals, yeast and plants have been published [2–5]. In this update, we focus on the latest developments in the plant MAPK field, comparing different functional approaches with results from the analysis of the *Arabidopsis* genome. A specific emphasis is laid on technical and conceptual problems arising from the complexity within and crosstalk between MAPK pathways.

Components of plant MAPK cascades

Arabidopsis serves as a blueprint for assessing the complexity of plant genomes. In a recent analysis, 20 MAPKs, 10 MAPKKs and 60 MAPKKKs were identified in the *Arabidopsis* genome, and a unified nomenclature for *Arabidopsis* MAPKs and MAPKKs has been proposed [6]. It is not possible to unequivocally define homologues across species, and therefore the *Arabidopsis* nomenclature cannot be adapted to other species. However, the comparative sequence similarity of *Arabidopsis* and other plant MAPKs can be used to establish specific groups and subgroups of MAPKs, which can be used as a reference to relate plant MAPK components across species. Specific features and sequence signature motifs can be identified that reinforce the establishment of particular subgroups (Table 1). A summary of the groupings and functional characteristics of the MAPK signalling components of various species is provided in Table 2. We have established a web-based resource to provide the scientific community with updated information

Table 1

List of *Arabidopsis* MAPK signalling components.

	Number*	Kinase class	Number*	Named members	Signature motif
MAPK	23	A	3	MPK3/6/10	T(E/D)YVxTRWYRAPE(L/V)
		B	5	MPK4/5/11/12/13	
		C	4	MPK1/2/7/14	
		D	8	MPK8/9/15/16/17/18/19/20	
		MHK	3	–	
MAPKK	10	A	3	MKK1/2/6	VGTxxYMSPER
		B	1	MKK3	
		C	2	MKK4/5	
		D	4	MKK7/8/9/10	
MAPKKK	80	MEKK-like	21	MEKK1, ANP1-3, MAP3Kε1	G(T/S)Px(W/Y/F)MAPEV
		ZIK	11	ZIK1	GTPEFMAPE(L/V)Y
		Raf-like	48	EDR1, CTR1	GTxx(W/Y)MAPE
MAPKKKK	10	Ste20/PAK-like	10	–	TFVGTPxWMAPEV

*As predicted by analysis of the *Arabidopsis* genome. Known members of each class of kinase are indicated by their names and signature motifs. Direct links to *Arabidopsis* Genome Initiative (AGI) accession numbers and tools for comparative sequence analysis are available at http://www2.rhul.ac.uk/~ujba110/MAPK.htm

on the classification of novel plant MAPK signalling components (http://www2.rhul.ac.uk/~ujba110/MAPK.htm). This resource provides direct links to the *Arabidopsis* Genome Initiative (AGI) accession numbers, as well as tools for comparative sequence analysis.

The plant MAPKs are presently grouped into four subfamilies, A–D. In *Arabidopsis*, subfamilies A–C contain 12 MAPKs that have a TEY phosphorylation motif in their active site, whereas the eight MAPKs belonging to subfamily D have a TDY motif at the corresponding position. A distinguishing feature of the TDY group of MAPKs is their long carboxy-terminal extensions. Most known members of the MAPK family belong to subgroups A and B. In *Arabidopsis*, these are *Arabidopsis thaliana* MAPK3 (AtMPK3), AtMPK4 and AtMPK6; in alfalfa, stress-induced MAPK (SIMK), *Medicago* MAPK2 (MMK2), MMK3 and the stress-activated MAPK (SAMK); and in tobacco, salicylic acid (SA)-induced protein kinase (SIPK), wound-induced protein kinase (WIPK) and *Nicotiana* Fus-3-like kinase6 (Ntf6) [7–9]. A few members of subfamily D have been functionally characterized in rice [10] and alfalfa [11], but nothing is yet known about members of subfamily C. A fifth group, having three *Arabidopsis* members, contains protein kinases that have overall sequence relatedness to both MAPKs and cyclin-dependent kinases. Members of this class of kinases possess a TEY signature motif but lack the common docking domain that is required for MAPKK interaction.

Analysis of the *Arabidopsis* genome reveals four different groups of MAPKKs, with a total of just ten MAPKKs. All MAPKKs have a putative MAPK-docking domain, K/R-K/R-K/R-X_{1-6}-L-X-L/V/I, at their amino-terminus. MAPKKs of subgroups A, C and D encode relatively short proteins, whereas subgroup B members have a carboxy-terminal domain that shows homology to NTF2 (NUCLEAR TRANSPORT FACTOR 2), which mediates the nuclear import of RanGDP [6]. Functional evidence for a role of A-type MAPKKs is available for *Arabidopsis thaliana* MAPKK1 (AtMKK1) [12], alfalfa pathogen-responsive MAPKK (PRKK) [13•] and *N. tabacum* MAPKK1 (NtMEK1) [14•]. No functions are known for members of MAPKK subfamilies B and D. C-type MAPKKs include *Arabidopsis* AtMKK4 and AtMKK5 [15••,16], alfalfa SIMK kinase (SIMKK) [13•,17], and tobacco NtMEK2 [16,18•].

The MAPKKK family forms the largest group of MAPK pathway components. In *Arabidopsis*, 80 putative MAPKKKs have been found and can be subdivided into two major subtypes. One major group, whose members are most similar to animal MEKKKs and yeast MAPKKKs, is made up of 21 MEKK-like and 11 ZR1-interacting kinases (ZIKs). This group includes ANP1–3 (*Arabidopsis* NPK1-like protein kinases 1–3) and MAPKKK1 (AtMEKK1) [19] from *Arabidopsis*, *Nicotiana* protein kinase1 (NPK1) from tobacco [20•] and ZIK1 from alfalfa [8]. The other major group consists of 48 genes encoding Raf-like protein kinases including *Arabidopsis* CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) [21] and ENHANCED DISEASE RESISTANCE 1 (EDR1) [22•].

The MAPKKK–MAPKK–MAPK modules are sometimes activated by yet another protein kinase, known as MAPKKKK. In *Arabidopsis*, at least ten genes encode kinases that are related to yeast Sterile20 (Ste20)/mammalian p21-activated protein kinases (PAKs) MAPKKKKs, but none of these plant kinases has been shown to regulate a MAPK pathway.

Genomic sequences of all groups of MAPK pathway components can be found in plant species other than *Arabidopsis*, including grasses, showing that these gene families evolved before bifurcation into monocot and dicot species.

Emerging plant MAPK pathways

The phylogenetic analysis of potential MAPK cascades in *Arabidopsis* has revealed bewildering complexity. Two

Table 2

Groupings and functional characteristics of MAPKs, MAPKKs and MAPKKKs.

	Group	Biological function	References
MAPK			
AtMPK3	A3	Oxidative stress, bacterial elicitor signalling	[15••,43•]
MsSAMK	A3	Cold, drought, touch, wounding, fungal elicitor signalling	[24,52–54]
NtWIPK	A3	Hypoosmotic stress, wounding, fungal elicitor signalling, HR, viral infection	[28,36,55–57]
AtMPK6	A6	Cold, drought, high salt, touch, wounding, oxidative stress, fungal and bacterial elicitor signalling	[15••,25,26,43•,58,59]
MsSIMK	A6	Hyperosmotic stress, cold, drought, wounding, fungal elicitor signalling	[24,37]
NtSIPK	A6	Hyperosmotic and hypoosmotic stress, wounding, SA, HR, bacterial and fungal elicitor signalling, viral infection	[34–36,55–57,60–62]
AtMPK4	B4	Cold, drought, hyperosmotic, touch, wounding, pathogen resistance	[26,27••,59]
MsMMK2	B4	Fungal elicitor signalling	[24]
MsMMK3	B13	Fungal elicitor signalling, cytokinesis	[24,46]
NtNTF6	B13	Cytokinesis	[47]
MAPKK			
AtMKK1	A1	Cold, drought, hyperosmotic stress, wounding	[12]
MsPRKK	A1	Fungal elicitor signalling	[13•]
NtMEK1	A6	Cytokinesis?	[14•]
AtMKK4,5	C4	Bacterial elicitor signalling, HR	[15••,16]
MsSIMKK	C4	Hyperosmotic stress, fungal elicitor signalling	[13•,17]
NtMEK2	C4	HR	[18•]
MAPKKK			
AtMEKK1	A1	Cold, hyperosmotic stress, touch, bacterial elicitor signalling	[15••,19]
AtANP1,2,3	A3	Oxidative stress, cytokinesis, auxin signalling	[43•]
NtNPK1	A3	Cytokinesis, auxin signalling, heat, cold, hyperosmotic stress	[20•,43•,44,48••,63]
AtCTR1	B3	Ethylene signalling	[21]
AtEDR1	B3	Pathogen response	[22•]

Biochemically and/or genetically characterized MAPK components from *Arabidopsis thaliana* (At), *Medicago sativa* (Ms) and *Nicotiana tabacum* (Nt).

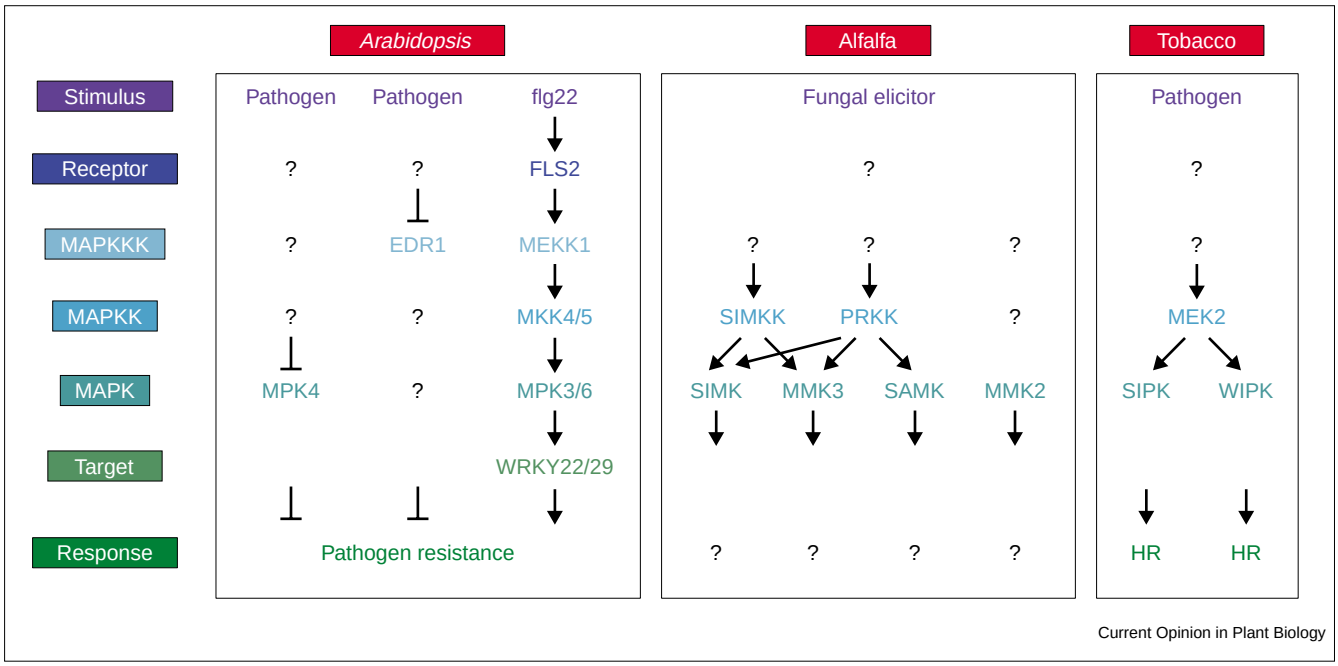
approaches have mainly been used to tackle the question of which components work in specific MAPK pathways. One is based on yeast two-hybrid and *in vitro* interaction, whereas the other uses the transient expression of various combinations of MAPK pathway components in protoplasts. Contrary to the situation in other eukaryotes, these studies show that a given plant MAPKK can interact with and activate more than one MAPK, indicating that plant MAPKKs function as divergence points in signal transduction. Direct evidence for such a mechanism comes from the analysis of SIMKK and PRKK from alfalfa, which, depending on the stimulus, activate up to three different types of MAPKs [13•]. Similarly, tobacco NtMEK2 was shown to activate both SIPK and WIPK [18•], and the *Arabidopsis* MKK4 and MKK5 can activate both MPK3 and MPK6 [15••].

The existence of a large number of putative MAPKKKs could suggest that MAPKKs function as convergence points. It should be noted, however, that almost none of the MAPKKKs have been shown to function as MAPKK activators in *sensu strictu*, leaving open the possibility that some of these kinases might not be true MAPKKKs. Several studies on the *Arabidopsis* AtMEKK1 showed that this MAPKKK can interact with and activate a total of four different MAPKKs [15••,23]. These data suggest that plant MAPKKKs might not function as convergent but rather as divergent factors in MAPK signalling pathways. As these experiments were carried out in yeast [23] or with truncated AtMEKK1 that lacks the regulatory domain [15••], however, these conclusions should be considered with care. Taking into account these limitations, the analyses of a hitherto small set of MAPK components has nonetheless provided evidence for their functional assembly into at least two modules: one functioning in stress signalling and the other in cytokinesis.

MAPKs and the pathogen response

Plants respond to pathogen attack by activating multi-step defence responses that include the rapid production of reactive oxygen species, the strengthening of cell walls and a hypersensitive response, that is, localised cell death at the sites of infection. Plant defence responses also include the synthesis of pathogen-related proteins and phytoalexins. Several MAPK cascades are associated with the induction of defence responses. In alfalfa, various fungal elicitors activate different combinations of up to four MAPKs, identified as SIMK, MMK2, MMK3 and SAMK [24]. No other external stimuli have been identified for MMK2 and MMK3. In contrast, SIMK and SAMK are activated by various abiotic stresses. Recently, the complex activation mechanism for these four kinases was further elucidated. Two alfalfa MAPKKs, SIMKK and PRKK, were found to

Figure 1



Schematic illustration of MAPK pathways for pathogen responses in plants. In *Arabidopsis*, the MAPK mutant *mpk4* and the MAPKKK mutant *edr1* show enhanced pathogen resistance, indicating that they negatively regulate pathogen response. Perception of the bacterial elicitor peptide flg22 by the LRR receptor kinase FLS2 leads to the activation of a MAPK signalling cascade, consisting of MEKK1, MKK4/5 and MPK3/6, followed by the transcriptional induction of WRKY22/29. In alfalfa, fungal elicitors activate a set of four MAPKs. SIMK and MMK3 can be activated by both SIMKK and PRKK, whereas SAMK activation is triggered by PRKK but not SIMKK. Elicitor-induced MMK2 activity is mediated by neither SIMKK nor PRKK, indicating the presence of a third, as yet unidentified, MAPKK in alfalfa pathogen signalling. In tobacco, different pathogens induce SIPK and WIPK activity. Constitutively active NtMEK2 leads to the activation of SIPK and WIPK, transcriptional induction of defence genes and HR-like cell death. Arrows indicate activation and bars indicate inhibition.

activate different sets of these MAPKs in response to the same fungal elicitor [13•]. SIMKK activates SIMK and MMK3, whereas PRKK activates SIMK, MMK3 and SAMK. This work shows that an additional, as yet unidentified, MAPKK is responsible for the elicitor-induced activation of MMK2 (Figure 1).

Various pathogenic signals activate WIPK and SIPK in tobacco [9]. High levels of WIPK and SIPK activity also result from wounding and various abiotic stresses, indicating that these MAPKs integrate different abiotic and biotic stress responses. NtMEK2 is a putative upstream activator of WIPK and SIPK in defence response signalling ([18•]; Figure 1). Overexpression of constitutively active NtMEK2 in tobacco activates a subset of defence genes and induces a hypersensitive response (HR)-like cell death, which is typical of the pathogen defence response. The HR is preceded by the activation of endogenous SIPK and WIPK. As SIPK and WIPK are induced by various stresses, it remains to be determined whether NtMEK2 acts only in defence signalling and whether different MAPKKs relay the activation of SIPK and WIPK in response to other signals.

In *Arabidopsis*, AtMPK6 is activated by the bacterial flagellin peptide (flg22) or by xylanase from the fungus *Trichoderma viride* [25]. AtMPK4 and AtMPK6 are activated by the protein elicitor harpin from *Pseudomonas syringae* [26]. Surprisingly, *Arabidopsis mpk4* mutants exhibit increased resistance to virulent pathogens [27••]. *mpk4* mutant plants have elevated SA levels and constitutive systemic acquired resistance (SAR), and they constitutively express pathogenesis-related genes that are normally induced by SA. Double-mutant studies indicated that the expression of SAR in *mpk4* is dependent upon elevated SA levels, suggesting that AtMPK4 is a negative regulator of SA-mediated defences. By contrast, the response to abiotic stresses, such as high salinity or temperature shock, is not significantly impaired in *mpk4* mutants, which also respond normally to phytohormones. However, the jasmonic acid (JA)-response genes *PLANT DEFENSIN1.2* (*PDF1.2*) and *THIONIN2.1* (*THI2.1*) are not induced in *mpk4* after treatment with methyl jasmonate (MeJA), indicating that MPK4 is required for JA-mediated gene expression.

WIPK is also involved in crosstalk between SA and JA pathways in tobacco. Silencing WIPK resulted in increased SA production and in abrogation of JA-induced gene expression upon wounding [28]. Conversely, transgenic lines with increased WIPK activity had increased JA levels, resulting in the constitutive expression of JA-responsive genes [29].

EDR1 belongs to the group of Raf-like MAPKKKs and also functions in pathogen resistance. The *edr1* mutant was isolated in a screen for mutants with enhanced resistance to virulent pathogens [22•], and encodes a truncated protein lacking the kinase domain of EDR1. *EDR1* confers resistance to the fungus *Erysiphe cichoracearum*, which causes powdery mildew. Although defences against this disease are not expressed in *edr1* mutants in the absence of a pathogen-signal, they are induced more rapidly in these mutants than in wildtype plants, indicating that EDR1 is also a negative regulator of defence responses.

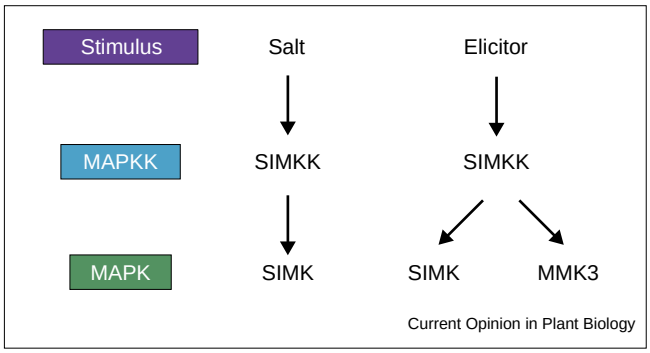
Early work on parsley cells showed that the activation of elicitor-responsive MAPK (ERMK), a homologue of AtMPK3, by a fungal elicitor results in the translocation of MAPK into the nucleus [30]. These results suggested that ERMK might phosphorylate transcription factors that are involved in the plant defence response. Recent work by Asai *et al.* [15••] substantiates this hypothesis. A genetic approach that identified the *FLAGELLIN SENSITIVE2* (*FLS2*) gene as a putative sensor of bacterial flagellin in *Arabidopsis* [31] was combined with transient expression assays of a variety of MAPKs, MAPKKs, and MAPKKKs to examine the roles of MAPK signalling components in the defence response. The results of this work place AtMPK3 and AtMPK6 downstream of the closely related MAPKKs AtMKK4 and AtMKK5, and the MAPKKK AtMEKK1 downstream of *FLS2* (Figure 1). The targets of the MAPK pathway are suggested to be two plant-specific transcription factors of the WRKY family (i.e. WRKY22 and WRKY29). Transient overexpression of truncated AtMEKK1, constitutively active AtMKK4 and AtMKK5, or WRKY29 conferred resistance of *Arabidopsis* leaves to infection by the bacterial pathogen *Pseudomonas syringae* or the fungal pathogen *Botrytis cinerea*. We still do not know how the receptor kinase activates AtMEKK1, whether AtMEKK1 interacts with and specifically activates AtMKK4 and AtMKK5, and how AtMPK3 and AtMPK6 are connected to the WRKY22 and WRKY29 transcription factors.

MAPKs in the response to osmotic stress

Hyperosmotic stress leads to changes in the volume and turgor pressure of plant cells. Cells respond to this stress with the production of stabilising osmolytes, leading to increased salt tolerance [32]. A putative receptor for osmotic stress signalling in plants has been identified as the *Arabidopsis thaliana* HISTIDINE KINASE 1 (AtHK1) [33], which has structural similarity to bacterial, yeast and plant two-component histidine kinases. The AtHK1 transcript was shown to accumulate under conditions of high and low osmolarity, and the ability of AtHK1 to transmit an osmo-signal was assayed by yeast complementation. However, it remains to be shown that AtHK1 functions in osmosensing in plants.

Several protein kinases, including AtMEKK1, are transcriptionally upregulated under high salt conditions and other abiotic stresses [19]. Although the work by Sheen and colleagues [15••] suggests that AtMEKK1 mediates flagellin signalling via the activation of AtMKK4/AtMKK5 and AtMPK3/AtMPK6, extensive functional and interaction analyses in yeast have suggested that AtMEKK1 functions upstream of AtMKK1 and AtMPK4 [23]. Recently, a role for AtMKK1 in abiotic stress signalling was confirmed. AtMKK1 is activated by wounding, cold, drought and high salt stress, and activated AtMKK1 phosphorylates AtMPK4 [12]. AtMPK4 is also involved in pathogen signalling [26,27••]. The picture is complicated by the observation that although *mpk4* mutants show altered gene expression in the defence response, no changes in gene expression were detected upon exposure to abiotic stresses such as salt stress and temperature shock.

Figure 2

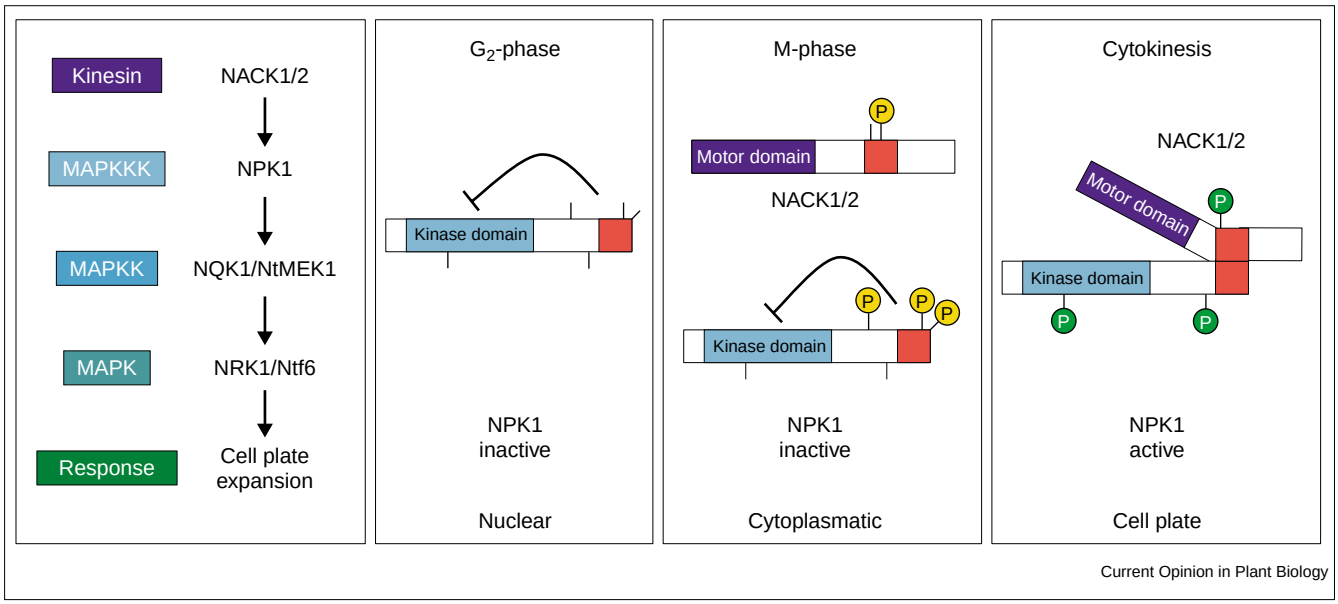


Different functions of SIMKK in high salt concentration and elicitor signalling. In response to high salt conditions, the alfalfa MAPKK SIMKK exclusively activates SIMK, whereas elicitor signalling involves the activation of SIMK and MMK3 by SIMKK. Compare with Figure 1.

The effect of osmotic stress on tobacco suspension cells was recently analysed [34–36]. In-gel kinase assays revealed the induction of multiple myelin basic protein (MBP)-phosphorylating activities. Two of these kinases could be identified, using specific antibodies, as the tobacco MAPKs SIPK and WIPK. SIPK is implicated in various stress responses and was activated by both hypoosmotic and hyperosmotic stress, with comparable kinetics. By contrast, WIPK was induced only by hypoosmotic stress. In addition, salt stress induced a novel kinase that belongs to the class of SNF1 (SUCROSE-NONFERMENTING 1) protein kinases [34].

Alfalfa cells respond to extreme hyperosmotic conditions by the induction of a 38-kDa kinase that is likely to be related to the SNF1 family, whereas moderate osmotic stress activates SIMK in these cells [37]. Yeast two-hybrid interaction screening with SIMK was used to isolate SIMKK, which is a close homologue of AtMKK4/AtMKK5 and NtMEK2 [17]. Specific activation of SIMK by SIMKK was observed upon salt stress in protoplasts, confirming that SIMKK functions as an upstream activator of SIMK in this pathway (Figure 2). The complexity of cellular

Figure 3



Cell cycle regulation of NPK1. In G_2-phase, NPK1 is inhibited through the carboxy-terminal inhibitory domain, while NACK1/2 are not present. In M-phase, NACK1/2 is expressed, but binding to NPK1 is inhibited by phosphorylation (yellow circles) of the interaction domains (red domain). During cytokinesis, NPK1 and NACK1/2 become dephosphorylated at the interaction domains and NACK1/2 binds and activates NPK1. Further activation and/or stabilisation of the interaction might occur through as yet unidentified phosphorylation sites on NPK1 and NACK1/2 (green circles). NPK1 phosphorylates and activates MAPK signalling components; plausible candidates are NQK1/NtMEK1 and NRK1/Ntf6.

signalling is shown by the fact that SIMKK not only functions in salt stress but also in elicitor signalling. Whereas SIMKK exclusively activates SIMK under salt-stress conditions, SIMK and MMK3 are activated by SIMKK in cells that are treated with elicitor. Hence, identical MAPK pathway components can function in different signalling contexts. This situation is reminiscent of the yeast MAPKKK STE11, which functions in both pheromone signalling and the osmotic stress pathway. Illegitimate cross talk of STE11 from one pathway to another is prevented by the scaffold protein STE5, which firmly tethers STE11 into a complex with the pheromone-specific components, the MAPKK STE7 and the MAPK FUS3 (Fusion-defective 3) [38]. An analogous function is overtaken by the scaffold function of the osmotic-stress-specific MAPKK PBS2 (Polymycin B Sensitive2), which forms a complex between STE11 and the MAPK HOG1 (High Osmolarity Glycerol response1)[38]. The identification of the factors that are responsible for scaffolding SIMKK in the salt and elicitor pathways should help us to decipher signal transduction pathways that share identical components.

MAPKs and hormone signalling

The isolation of the plant Raf-like MAPKKK, CTR1, as a negative regulator of ethylene signalling provided an indication of the involvement of MAPK cascades in hormone signalling [21]. *ctr1* was isolated in *Arabidopsis* as a loss-of-function mutant that exhibited constitutive expression of ethylene-inducible genes. By epistasis analysis, CTR1 was shown to act downstream of ETHYLENE-RESISTANT1 (ETR1) [39], which encodes a two-component histidine kinase receptor [40]. Both ETR1 and CTR1 are thought to act as negative regulators of ethylene signalling. The identification of two-component-like receptors as upstream components in MAPK signalling in yeast, and subsequently in the ethylene signal pathway in plants, further supports the assumption that hormone action involves MAPK-based signalling.

Conflicting results on a connection between auxin and MAPK activity levels have been previously reported (reviewed in [41]). Recently, it was shown that auxin activates a MAPK-like kinase in *Arabidopsis* roots, and that auxin- but not salt-induced MAPK-like activation was inhibited in *auxin-resistant4* (*axr4*) mutants [42]. On the other hand, there is good evidence that an H_2O_2-induced MAPK pathway blocks auxin-responsive genes while inducing genes that respond to oxidative stress [43•]. These data indicate that there is crosstalk between the oxidative-stress and auxin pathways. H_2O_2 is a signalling molecule that is implicated not only in various cellular processes, such as the response to wounding and pathogen defence, but also in the regulation of the cell cycle and in cell death. The H_2O_2-induced MAPKs were identified as AtMPK3 and AtMPK6, and only truncated *Arabidopsis* ANPs and tobacco NPK1 could induce these MAPKs. Truncated NPK1 is known to repress several auxin-inducible promoters and to result in defective embryo and seed development [44]. By contrast, other lines in which NPK1 was truncated showed no phenotypic changes in

vegetative tissues but had increased tolerance to temperature and osmotic stress [43•]. Care should be taken in interpreting the results of overexpression studies that use gain-of-function constructs or kinases that carry deletions in regulatory regions. Under these circumstances, MAPK pathway components other than the natural ones could be activated giving rise to unexpected results.

MAPKs and cytokinesis

Segregation of chromosomes during cell division is among the most spectacularly choreographed cellular events, which needs to be precisely co-ordinated both in time and space. The cyclin-dependent kinase (CDK), in complex with mitotic B-type cyclins, is a conserved master regulator of mitosis. The degradation of mitotic cyclins at the metaphase to anaphase transition marks the exit from mitosis, and thus the regulation of mitotic events is handed over to other regulators at this point. In plants, however, the localisation of CDK to cytokinetic structures points to some roles for CDK during cytokinesis [45].

On the basis of their localisation to the cell plate and their activation during cytokinesis, two related MAPKs, the alfalfa MMK3 and the tobacco Ntf6, were suggested to be involved in cytokinesis [46,47]. NtMEK1, an upstream regulator of Ntf6 was identified in a yeast two-hybrid interaction screen [14•]. Furthermore, NtMEK1 (also known as NQK1) was found to be phosphorylated by NPK1, a MAPKKK that is specifically activated during cytokinesis, localised to the cell-division plane and required for phragmoplast outgrowth to build the cell plate [20•,48••]. NPK1 has a kinase domain at its amino-terminus and a negative regulatory domain at its carboxyl terminus. Through its carboxy-terminal region, NPK1 interacts with two CENP-E-like kinesin microtubule motor proteins, NPK1-activating kinase-like protein1 (NACK1) and NACK2. Overexpression of dominant-negative mutant forms of both NACK1 and NPK1, or a lack of NACK1, results in incomplete cytokinesis owing to defects in the redistribution of phragmoplast microtubules and the lateral expansion of the cell plate. Callose deposition, and thus vesicle trafficking, appears to be unaffected in these lines. Recently, Strompen *et al.* [49] reported the isolation of the *HINKEL* gene, which is required for cytokinesis and is identical to AtNACK1.

Both NACK1 and NPK1 contain putative CDK and MAPK phosphorylation sites at their interaction surfaces. These sites overlap with a nuclear localisation signal in NPK1. Upon NACK1/NACK2 binding, NPK1 is activated, possibly by removing the carboxy-terminal inhibitory tail from its kinase domain. NACK1 is a mitosis-specific regulator of NPK1, having M-phase-specific *cis* elements within its promoter [50]. Both NACK1 and NPK1 are localised to the midplane of cell division, and localisation of NPK1 is dependent on NACK1 binding and the plus-end-directed motor activity of NACK1. On the basis of these features, the following model can be envisaged for the regulation of NPK1 during the cell cycle (Figure 3). In G_2-phase cells, NPK1 is inactive because of the activity of its inhibitory regulatory domain. Because NACK1 is not present and CDK activity is low in these cells, NPK1 is localised to the nucleus. As cells enter mitosis, CDK becomes activated and possibly phosphorylates NPK1. At this stage, NACK1 is expressed in M-phase cells, but phosphorylation of the interaction domains of NPK1 might interfere with its ability to bind NACK1, keeping NPK1 in an inactive state. As cells exit metaphase and CDK activity drops, NPK1 becomes dephosphorylated and binds NACK1, leading to the activation of NPK1 and its recruitment to phragmoplasts. As cells enter G_1 phase, both NACK1 and NPK1 disappear by unknown mechanisms.

The importance of microtubules in cytokinesis has been shown by experiments using taxol, a microtubule stabiliser that blocks the lateral expansion of phragmoplasts. The microtubule-associated protein NtMAP65-1 localises to the midplane of cell division. NtMAP65-1 contains putative MAPK and CDK phosphorylation sites, as well as a mitosis-specific destruction motif [51], and thus could be a candidate target protein for the cytokinetic MAPK pathway.

Conclusions

With the recent identification of the first MAPK signalling module for a bacterial elicitor, it appears that we have finally generated appropriate methods to assign signalling components to specific pathways. The present toolbox consists of transient expression assays using dominant-negative and active gain-of-function mutants, reporter-gene constructs, biochemical methods and the use of mutants. However, work from several groups and using various approaches indicates that extensive crosstalk among MAPK and other pathways exists, and this considerably complicates our understanding of signal transduction. Moreover, it has become clear that given MAPK components can perform very different functions in different pathways, suggesting that scaffold proteins play important roles in defining the specificity of MAPK modules. At present, we know almost nothing about plant scaffold proteins, but their identification and study will be mandatory for unravelling MAPK cascades.

Another question that comes to mind when considering the MAPK field is whether there has been a bias towards the characterisation of only a subset of MAPKs. Looking more closely into the common biochemical approaches used to characterise MAPKs, it becomes obvious that in-gel kinase assays might not work for all MAPKs, and specifically not for some of the larger TDY kinases. MAPKs that are not activated to such high levels as those related to the A- and B-type MAPKs could be overlooked. Immunokinase assays using specific antibodies against the TDY MAPKs will be necessary to characterise such MAPKs. In contrast to animal systems, no specific inhibitors are available for any plant MAPK pathway. This limiting factor might explain the current lack of known direct targets for plant MAPKs, but phosphoproteomics is

rapidly gaining momentum and should close this gap in the near future. In genetic approaches, certain loss-of-function MAPK mutants will evade detection in screens if closely related MAPK genes perform redundant functions. As MAPKs are usually activated by post-translational mechanisms, activation tagging might also be problematic. This list of challenges is not comprehensive and simply serves to illustrate that the present understanding on plant MAPK pathways is still immature. Not only more work but novel approaches may be required to finally assign specific functions to each of the many plant genes that encode MAPK pathway components.

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