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Abiotic stress series

Emerging MAP kinase pathways in plant stress signalling

Hirofumi Nakagami, Andrea Pitzschke and Heribert Hirt

Department of Genetics, Max F. Perutz Laboratories of the University of Vienna, Vienna Biocenter, Dr. Bohrgasse 9, A-1030 Vienna, Austria

Mitogen-activated protein kinase (MAPK) pathways transfer information from sensors to cellular responses in all eukaryotes. A surprisingly large number of genes encoding MAPK pathway components have been uncovered by analysing model plant genomes, suggesting that MAPK cascades are abundant players of signal transduction. Recent investigations have confirmed major roles of defined MAPK pathways in development, cell proliferation and hormone physiology, as well as in biotic and abiotic stress signalling. Latest insights and findings are discussed in the context of novel MAPK pathways in plant stress signalling.

MAPK pathways – a common theme in eukaryotic signal transduction

Humans, yeast and plants share ~60% of their genes with each other, including components of conserved protein kinase signalling pathways. In all eukaryotes, mitogen-activated protein kinase (MAPK) pathways serve as highly conserved central regulators of growth, death, differentiation, proliferation and stress responses.

A MAPK cascade minimally consists of a MAPKKK–MAPKK–MAPK module that is linked in various ways to upstream receptors and downstream targets. Receptor-mediated activation of a MAPKKK can occur through physical interaction and/or phosphorylation by either the receptor itself, intermediate bridging factors or interlinking MAPKKKKs. MAPKKKs are serine/threonine kinases that activate MAPKKs through phosphorylation on 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 cytoskeleton-associated proteins. The specificity of different MAPK cascades functioning within the same cell is generated through the presence of docking domains found in various components of MAPK modules and scaffold proteins [1].

Components of plant MAPK cascades

On the basis of the fully sequenced *Arabidopsis* genome, 20 MAPKs, 10 MAPKKs and 60 MAPKKKs were identified and a unified nomenclature for *Arabidopsis* MAPKs and MAPKKs was proposed [2]. By sequence comparison and signature motif searches, putative orthologues to most of the 20 MAPKs, 10 MAPKKs and 60 MAPKKKs can be identified in the available genomic or EST sequences of *Medicago*, tobacco and rice. However, in some cases, unequivocal definition of orthologues between different species is not possible, making a unified nomenclature for all plant MAPKs, MAPKKs and MAPKKKs impossible at present.

In all plant species, MAPKs carry either a TEY or TDY phosphorylation motif at the active site. In contrast to TEY MAPKs, all TDY MAPKs have long C-terminal extensions. To date, functional data for a TDY MAPK is only available for rice BWMK1 [3,4]. By contrast, the TEY MAPKs have been studied in many plant species, including *Arabidopsis*, *Medicago*, tobacco, tomato, parsley as well as rice (Table 1).

Analysis of the putative MAPKKs reveals conservation of the N-terminal MAPK-docking motif K/R-K/R-K/R-X_{1-6}-L-X-L/V/I, which, at least in *Medicago* SIMKK (stress-induced MAPKK), is required but not sufficient for MAPK activation [5]. Functional evidence for the role of MAPKKs is available for *Arabidopsis* MKK1, MKK2, MKK4, MKK5 and MKK6, *Medicago* PRKK and SIMKK, tobacco MEK1 and MEK2, tomato MKK2 and MKK4, parsley MKK5 and rice MEK1 (Table 1).

Although the family of MAPKKKs forms the largest and most heterogeneous group of MAPK pathway components, it has only been shown in a few cases that these kinases do function as the activator of a MAPKK. Therefore, the classification of the 60–80 *Arabidopsis* kinases as MAPKKKs must be considered with caution. Nonetheless, these kinases are all related to each other and can be divided into two large subgroups: those of the MEKK-type, for which proof of MAPKKK function has been provided in most cases, and those of the Raf-like kinases, for which evidence that they act as MAPKKKs is still lacking. The MEKK-like kinases include *Medicago* OMTK1, *Arabidopsis* ANP1, ANP2, ANP3, MEKK1 and YODA and tobacco NPK1 (Table 1). Data on the Raf-like protein kinases include those for *Arabidopsis* CTR1 and EDR1 (Table 1).

Because of space limitations, only recent literature on stress signalling MAPK pathways is discussed. Reviews on the basic composition and function of MAPK pathways in animals, yeast and plants are available [6–8].

Corresponding author: Hirt, H. (heribert.hirt@univie.ac.at).

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Table 1. A comprehensive list of references implicating MAPKs, MAPKKs or MAPKKKs in various biological processes

	Group	Biological function	Refs
MAPKKK			
AtMEKK1	A1	Cold, high salt, touch, bacterial elicitor signalling	[10,11,47]
MsOMTK1	A1	Oxidative stress, cell death	[12]
AtYODA	A2	Extra-embryonic cell fate, stomata development	[54,55]
NbMAPKKKα	A2	HR, cell death, bacterial elicitor signalling	[24]
LeMAPKKKα	A2	HR, cell death, bacterial elicitor signalling	[24,25]
AtANP1, AtANP2, AtANP3	A3	Oxidative stress, cytokinesis, auxin signalling	[56,57]
NtNPK1	A3	Heat, cold, hyperosmotic stress, cytokinesis, auxin signalling, pathogen response, drought and freezing tolerance	[20,21,23,56,58,59]
AtCTR1	B3	Ethylene signalling	[60,61]
AtEDR1	B3	Fungal pathogen response	[32,33]
LeCTR1	B3	Ethylene signalling	[62,63]
OsEDR1	B3	Defence, stress signalling and development	[64]
MAPKK			
AtMKK1	A1	Cold, drought, high salt, oxidative stress, wounding, bacterial elicitor signalling	[11,49]
AtMKK2	A1	Cold, high-salt stress	[11]
MsPRKK	A1	Fungal elicitor signalling	[9]
AtMKK6/AtANQ1	A2	Cytokinesis	[20,65]
NtMEK1/NtNQK1	A2	Cytokinesis, cell death, bacterial elicitor signalling	[19,20,24,66]
OsMEK1	A2	Cold stress	[67]
ZmMEK1	A2	Root apex proliferation	[68]
AtMKK4, AtMKK5	C1	Bacterial elicitor signalling, HR	[10,40]
MsSIMKK	C1	Heavy metal, hyperosmotic stress, fungal elicitor signalling, ethylene signalling	[5,9,43,69]
NtMEK2	C1	HR, cell death, bacterial elicitor signalling, ethylene signalling, pollen germination	[16,24,40,66,70,71]
LeMKK2	C1	Bacterial elicitor signalling	[25]
PcMKK5	C1	Fungal and bacterial elicitor signalling	[72]
LeMKK4	D1	Bacterial elicitor signalling	[25]
MAPK			
AtMPK3	A1	Osmotic, oxidative stress, bacterial elicitor signalling, ABA signalling	[10,37,41,46,56,73]
MsSAMK	A1	Heavy metal, cold, drought stress, touch, wounding, fungal elicitor signalling	[43]
NtWIPK	A1	Hypoosmotic stress, wounding, HR, cell death, fungal and bacterial elicitor signalling, viral infection	[13,14,24,66]
NbWIPK	A1	Fungal and bacterial elicitor signalling	[74]
LeMPK3	A1	UV-B, fungal and bacterial elicitor signalling, mechanical stress, wounding	[25,50,75]
PcMPK3	A1	Fungal and bacterial elicitor signalling	[72,76]
OsMAPK5/OsMAPK2/ OsMSRMK2/OsMAP1/ OsBIMK1	A1	Heavy metal, heat, cold, drought, high salt, oxidative stress, UV-C, sucrose, jasmonic acid, salicylic acid, ethylene, ABA, elicitor, pathogen resistance, abiotic stress tolerance	[27,44,67,77–79]
AtMPK6	A2	Cold, drought, high salt, osmotic, oxidative stress, touch, wounding, fungal and bacterial elicitor signalling, pathogen resistance, ethylene signalling	[10,11,28,29,31,37,45,46, 56,69,70,80]
MsSIMK	A2	Heavy metal, cold, drought, hyperosmotic stress, wounding, fungal elicitor signalling, ethylene signalling, root hair tip growth	[5,9,43,69,81]
NtSIPK	A2	Hyperosmotic and hypoosmotic stress, wounding, salicylic acid, HR, cell death, fungal and bacterial elicitor signalling, viral infection, ethylene signalling, pollen germination	[13,24,82]
NbSIPK	A2	Fungal and bacterial elicitor signalling	[74]
LeMPK1	A2	Elicitor signalling, UV-B	[50]
LeMPK2	A2	Elicitor signalling, UV-B	[25,50]
PcMPK6	A2	Heavy metal, oxidative stress, fungal and bacterial elicitor signalling	[72,76]
AtMPK4	B1	Cold, drought, hyperosmotic stress, touch, wounding, pathogen resistance	[11,28,30,45,83]
MsMMK2	B1	Heavy metal stress, fungal elicitor signalling	[43]
MsMMK3	B2	Heavy metal, oxidative stress, fungal elicitor signalling, cytokinesis, ethylene signalling, cell death	[12,43,69]
NtNTF6/NtNRK1	B2	Cytokinesis, HR, cell death, fungal and bacterial elicitor signalling	[19,20,24,66]
OsMAPK4/OsMSRMK3	C2	Heavy metal, cold, drought, salt, oxidative stress, wounding, sugar starvation, sucrose, jasmonic acid, salicylic acid, ethylene, ABA, chitosan	[84,85]
OsBWMK1	D1	Pathogen resistance, cell death, fungal elicitor signalling, oxidative stress, salicylic acid, jasmonic acid	[3,4]
OsWJUMK1	D1	Heavy metal, cold, oxidative stress	[84]

Logistics of plant MAPK pathways

Previous yeast two-hybrid and transient expression analyses of MAPK cascades in *Arabidopsis*, tobacco and *Medicago* have suggested that MAPK pathway components can function in different combinations and have distinct functions in different biological contexts. Recent genetic analyses have largely proven this unforeseen complexity to be correct. Depending on the stimulus, a given plant MAPKK can interact and activate several different MAPKs [9]. MAPKKKs can associate with different MAPKKs and function in different pathways. For example, *Arabidopsis* MEKK1 functions with MKK4 and MKK5 in pathogen defence [10], but can also activate MKK2 during abiotic stress [11]. As recently shown for *Medicago* OMTK1, some MAPKKKs also seem able to serve as scaffold proteins, assembling specific MAPK pathway components into particular modules [12].

MAPK pathways in plant pathogen response

Plants respond to pathogen attack by activating multistep defence responses, including rapid production of reactive oxygen species (ROS), strengthening of cell walls, induction of the hypersensitive response (HR) and the localized cell death at the sites of infection. Plant defence responses also include synthesis of pathogen-related proteins and phytoalexins. During the past couple of years, it has been firmly established that MAPKs play a central role in pathogen defence in *Arabidopsis*, tobacco, tomato, parsley and rice (Figure 1).

Tobacco MEK2–SIPK/WIPK pathway

Studies of the infection of tobacco leaves by TMV (tobacco mosaic virus) revealed that both SIPK and WIPK are activated in an *N* resistance gene-mediated fashion [13], preceding the HR-like cell death. Because expression and activation of WIPK was correlated with the onset of HR in response to various elicitors and TMV infection, it was assumed that WIPK might be a prime candidate for an HR inducer [14]. However, ectopic expression of SIPK was sufficient to yield active MAPK and induce HR, whereas ectopic WIPK expression yielded neither active kinase nor HR [15]. Consistent with the finding that WIPK and SIPK are substrates of MEK2, it was found that overexpression of constitutively active MEK2 activates both MAPKs followed by HR [16]. The importance of all three kinases was recently shown by VIGS (virus-induced gene silencing) of tobacco MEK2, SIPK or WIPK, resulting in strong attenuation of *N* gene-mediated resistance against TMV [17].

Dual function of the tobacco NPK1–MEK1–NTF6 pathway in pathogen defence and cytokinesis

There appears to be at least one more tobacco MAPK pathway involved in pathogen defence. VIGS of *MEK1* and its potential substrate MAPK *NTF6* attenuated *N*-mediated resistance of tobacco to TMV [18]; silencing of the potential downstream target WRKY and MYB transcription factors equally compromised *N*-mediated resistance. MEK1 and NTF6 also play an important role in cytokinesis, a specific function in the cell division cycle [19]. Overexpression of kinase-deficient mutant MEK1 resulted in multinucleate cells with incomplete cross walls [20]. A similar phenotype is obtained by overexpressing the kinase-deficient MAPKKK NPK1 [21], which is the upstream activator of MEK1 [20]. Overall, the present model envisions that the NPK1–MEK1–NTF6 module is a positive regulator of cytokinesis that is regulated by complex formation with the two kinesins NACK1 and

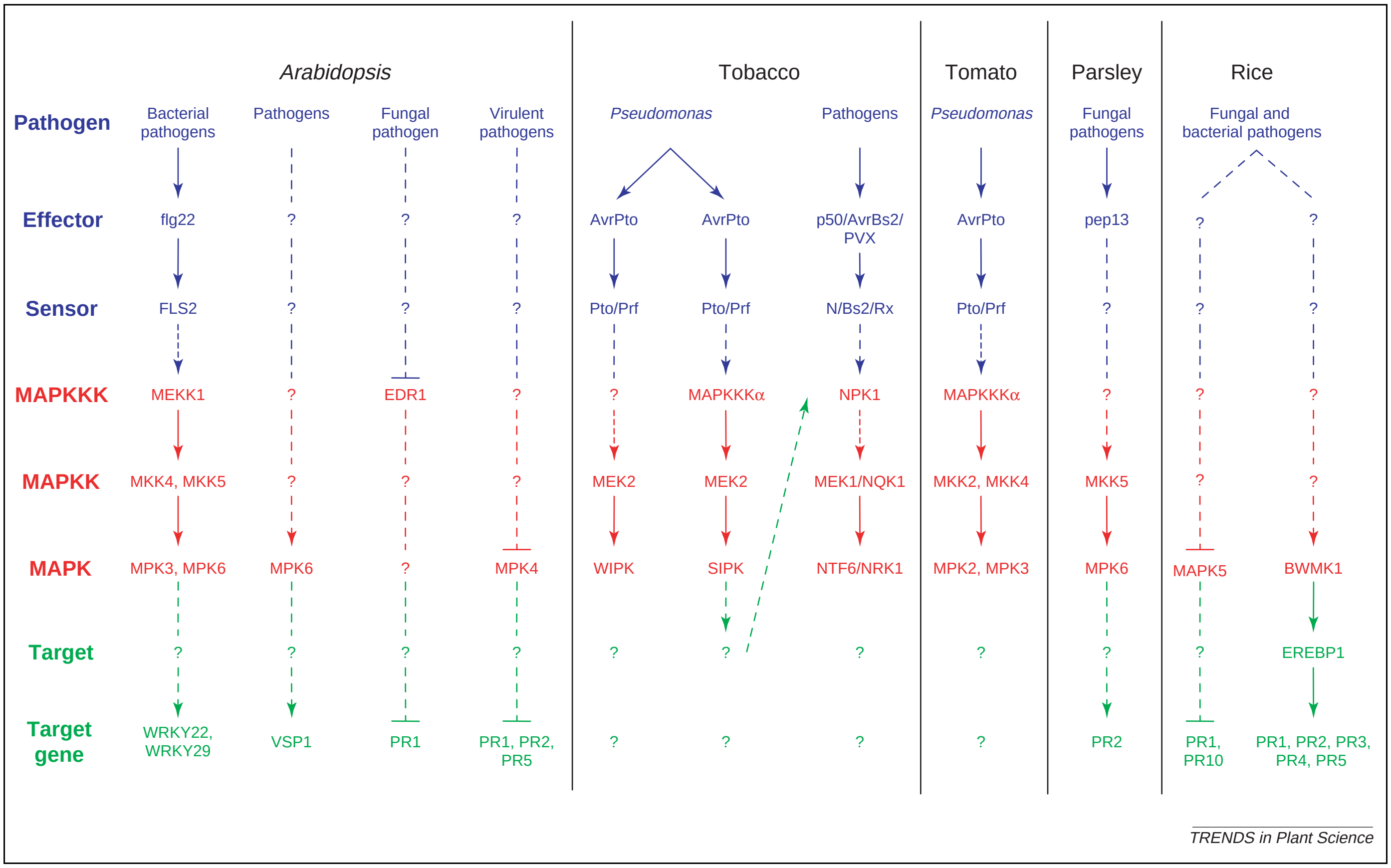

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Figure 1. Model describing the role of *Arabidopsis*, tobacco, tomato, parsley and rice MAPK pathways in pathogen defence. For explanation of gene symbols, see text. Broken arrows indicate hypothetical pathways; question marks indicate unknown factors.

NACK2 at the equatorial plane of the phragmoplast during late mitosis (reviewed in Ref. [22]). Although it seems strange that different MAPKKKs might couple MEK1 to pathogen and cell cycle stimuli, there is convincing evidence that NPK1 is involved in both processes. *NPK1*-silenced plants interfere with the functioning of several resistance genes [23]. How the same MAPK pathway can perform two different cellular functions is unclear and requires further investigations.

An even more complicated picture arises when considering recent studies on the interaction of *Pseudomonas syringae* pv. *tomato* in *N. benthamiana* and tomato. In these pathosystems, the bacterial avirulence gene product AvrPto is recognized by Pto. A novel *N. benthamiana* MAPKKK, MAPKKKα, was identified as an upstream activator of MEK2 and SIPK [24]. However, combining MEK1 with NTF6 or MEK2 with WIPK can also mediate Pto-induced cell death in *N. benthamiana*. The tomato studies confirmed the major findings in tobacco, showing that the orthologues of tobacco SIPK and WIPK, tomato MPK2 and MPK3, are activated in the AvrPto-Pto system [25]. As expected, the tomato orthologue of tobacco MEK2 was shown to function as an activator of MPK2 and MPK3. However, tomato MKK4, a member of the D group MAPKKs, for which no function is yet known in any other species, also induced cell death and, like the tomato orthologue of the *N. benthamiana* MAPKKKα, can activate MPK2 and MPK3. These studies not only indicate that multiple MAPK modules are involved in mediating AvrPto-Pto signalling but also that different combinations of the same MAPK components can serve different functions in different contexts.

Rice TEY and TDY MAPKs are involved in pathogen signalling

An increasing number of MAPK pathway components have been identified in *Oryza sativa* (reviewed in Ref. [26]). These MAPKs are either of the TEY type, belonging to the A and C groups, or of the D group, containing the TDY MAPKs. For most of the rice MAPKs, only expression data are available, indicating that almost all the genes respond to developmental and hormonal cues and/or various stresses. A more thorough functional investigation of rice MAPK5 (also called MSRMK2, MAPK2, MAP1 or BIMK1), the putative orthologue of *Arabidopsis* MPK3, revealed kinase activation by several biotic and abiotic stresses as well as by abscisic acid [27]. RNAi (RNA interference) of *MAPK5* resulted in constitutive PR gene expression and enhanced resistance to fungal (*Magnaporthe grisea*) and bacterial (*Burkholderia glumae*) infection. It is interesting that the same RNAi lines were compromised in tolerance to drought, salt and cold stress and that overexpression of *MAPK5* resulted in opposite phenotypes, as seen in the suppression lines.

To date, no functional data are available for the large group of TDY MAP kinases in dicots. Of the two TDY rice MAPKs, the rice blast and wounding-induced BWMK1 gene [4] has already been subjected to detailed functional analysis. Overexpression of *BWMK1* in tobacco resulted in constitutive PR gene expression and enhanced resistance to fungal (*Phythophthora parasitica*) and bacterial (*Pseudomonas syringae*) infection [3]. In a yeast two-hybrid screen with *BWMK1*, the *EREBP1* transcription factor was isolated. Phosphorylation of EREBP1 by BWMK1 enhanced the ability of the transcription factor to bind to GCC box elements of PR gene promoters; coexpression of BWMK1 and EREBP1 in protoplasts enhanced transcription of a GCC box-driven reporter gene. These studies indicate that pathogen defence is not only mediated by several TEY-type MAPKs, but also by at least one member of the distantly related group of TDY MAPKs.

Flagellin signalling by the *Arabidopsis* FLS2–MEKK1–MKK4/MKK5–MPK3/MPK6–WRKY22/WRKY20 pathway

In *Arabidopsis*, MPK3, MPK4 and MPK6 are all activated by bacterial and fungal PAMPs (pathogen-associated molecular patterns) [28,29]. *Arabidopsis mpk4* mutants show a dwarf phenotype, exhibit increased resistance to virulent pathogens, have elevated salicylic acid levels, show systemic acquired resistance, and constitutive expression of pathogenesis-related genes [30]. Given that no induction of the jasmonic acid-response genes *PDF1.2* and *THI2.1* is observed after treatment with methyl jasmonate, MPK4 appears to be required for jasmonic acid-mediated gene expression. By contrast, *MPK6*-silenced *Arabidopsis* plants have no obvious morphological phenotype but are compromised in resistance to avirulent *Peronospora parasitica* strains and avirulent and virulent *Pseudomonas syringae* strains [31]. With the notable exception of vegetative storage protein 1 (VSP1), most PR genes in *MPK6*-silenced plants are no different to wild-type plants.

Combining transient expression analyses with biochemical and genetic approaches has revealed that the MEKK1–MKK4/MKK5–MPK3/MPK6 module acts downstream of the flagellin receptor *FLS2* and upstream of the *WRKY22* and *WRKY29* genes [10]. Transient overexpression of the MEKK1 kinase domain, constitutively active MKK4 and MKK5 or WRKY29 rendered leaves resistant to infection by bacterial and fungal pathogens.

EDR1 (enhanced disease resistance 1) encodes a Raf-like MAPKKK that functions in pathogen resistance. *edr1* mutant plants confer resistance to the fungus *Erysiphe cichoracearum*, causing powdery mildew disease [32]. Although defence genes are not expressed in *edr1* mutant plants, they are induced more rapidly upon pathogen infection. Overexpression of wild-type *EDR1* was not feasible but kinase-deficient *EDR1* overexpression enhanced resistance to powdery mildew [33]. These data indicate that EDR1 is a negative regulator of defence, but whether EDR1 is a MAPKKK or functions in the context of a MAPK pathway is unclear.

Are MAPKs the missing link between reactive oxygen species and pathogen signalling?

MAPKs are also involved in mediating oxidative stresses (Figure 2). In tobacco, SIPK and WIPK become activated by various ROS [34,35] and overexpression or suppression of SIPK rendered plants hypersensitive to ozone treatment [36]. Ozone treatment of *Arabidopsis* activates MPK3 and MPK6 [37], the orthologues of tobacco SIPK and WIPK, respectively. Although ozone-induced activation of MPK3 and MPK6 was independent of ethylene, it was dependent on salicylic acid and resulted in nuclear translocation of the MAPKs.

Although ROS can be formed as metabolic by-products under various abiotic stress conditions, plant cells can also form ROS upon pathogen attack [38]. ROS-induced activation of MAPKs has been taken as evidence that ROS act upstream of MAPKs. However, an investigation of *Phythophthora infestans* infection of *N. benthamiana* showed that the MEK2 pathway might be part of an amplification cascade upstream of *rboh* (respiratory burst oxidase orthologue) genes, which are necessary for producing ROS in response to fungal infection [39]. In agreement with these studies, expression of constitutively active *Arabidopsis* MKK4 or MKK5, encoding the orthologues of tobacco MEK2, results in generation of hydrogen peroxide and cell death [40].

OXI1 protein kinase is an upstream mediator of ROS- and pathogen-induced activation of the *Arabidopsis* MPK3/MPK6 pathway [41]. *oxi1*-null mutants are compromised in ROS- and elicitor-induced MPK3/MPK6 activation and are hypersensitive to infection by virulent, but not avirulent strains, of the fungal pathogen *Peronospora parasitica*. Whether OXI1 is part of an amplification loop similar to that of tobacco MEK2 still needs to be tested, but it might be an interesting model to explain the genetic and biochemical data.

MAPKs in heavy metal signalling

ROS production and signalling is closely related to the topic of the response of plants to heavy metals (Figure 2). Although some of the heavy metals are required for metabolism, growth and development of plants, they are highly toxic in higher concentrations and can result in severe cellular damage. The toxicity of heavy metals is thought to result from the blocking of functional groups or the displacement of essential metal ions in biomolecules or by the autoxidation of redox-active heavy metals and the subsequent Fenton reaction producing ROS (reviewed in Ref. [42]). Recently, it was shown that heavy metals can activate MAPKs in higher plants [43,44]. Exposure of *Medicago* seedlings to excess copper or cadmium ions resulted in a complex activation pattern of four distinct MAPKs: SIMK, MMK2, MMK3 and SAMK (stress-activated MAPK) [43]. In protoplasts, the *Medicago* MAPKK SIMKK only conveyed activation of SIMK and SAMK, but not of MMK2 and MMK3. Moreover, SIMKK only mediated activation by copper but not by cadmium ions. These data show that distinct MAPK pathways are involved in signalling activated by different heavy metals.

Salt, cold, drought and wounding are mediated by overlapping sets of MAPKs

MAPKs are known to be activated by osmotic stresses in *Medicago* and tobacco (reviewed in Ref. [7]). Most progress on linking MAPKs to abiotic stress signalling has come from analysing *Arabidopsis*: MPK4 and MPK6 are activated by cold, salt, drought, wounding and touch [45]. MPK3 can also be activated by osmotic stress [46]. MEKK1 is transcriptionally induced by salt stress, drought, cold and wounding [47], but also mediates flagellin signalling through activation of MKK4 and MKK5 [10]. Functional and interaction analysis in yeast suggested that MEKK1 functions upstream of MKK1, MKK2 and MPK4 [48], and a role for the MAPK module consisting of MEKK1–MKK2–MPK4/MPK6 has now been confirmed in cold and salt stress (Figure 2). After identifying MKK2 in a yeast-based screen for activators of MPK4 and MPK6, it was found that MKK2 can become activated by cold and salt stress in transient protoplast

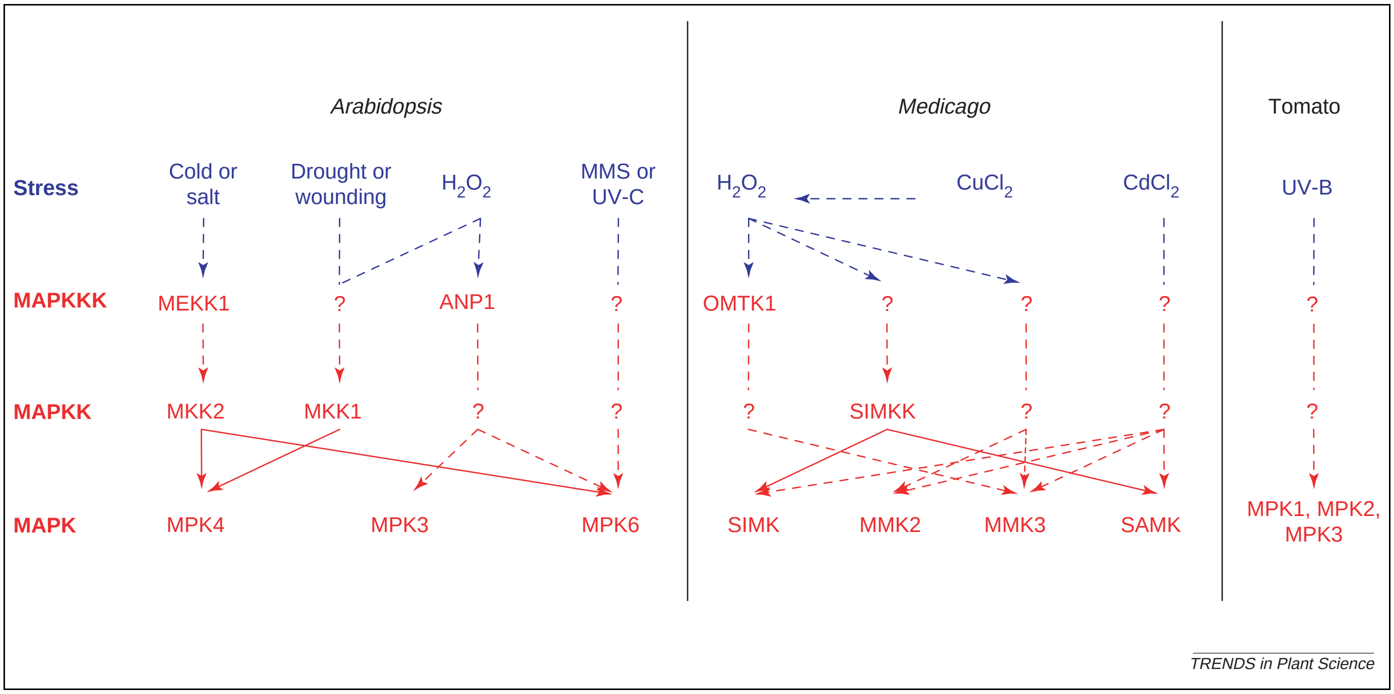

Figure 2. Model describing the role of *Arabidopsis*, *Medicago* and tomato MAPK pathways in abiotic stress responses. For explanation of gene symbols, see text. Broken arrows indicate hypothetical pathways; question marks indicate unknown cascade components. Abbreviation: MMS, methyl methanesulfonate.

assays [11]. *mkk2*-null mutant plants show no phenotype under ambient conditions but are hypersensitive to cold and salt stress. By contrast, *MKK2* overexpressor plants are more tolerant to cold and salt stress and show changes in the gene expression of 152 genes. In transient protoplast assays, MEKK1 can activate MPK4 and MPK6 in an MKK2-dependent manner. MKK1 might also be involved in abiotic stress signalling because MKK1 becomes activated by wounding, cold, drought and salt stress and can phosphorylate MPK4 [11,49].

MAPKs are downstream of the wounding, UV-B and brassinosteroid receptor

Tomato MPK1, MPK2 and MPK3 are orthologues of *Arabidopsis* MPK3 and MPK6; they are activated by wounding, systemin, various elicitors and UV-B [50]. The wounding signalling peptide systemin binds SR160, a leucine-rich repeat membrane-spanning protein kinase that is identical to the brassinosteroid receptor BRI1 [51]. SR160 also affects UV-B signalling, suggesting that systemin, brassinosteroid and UV-B might be sensed by the same receptor [52]. Whether MAPKs are downstream components of all three signalling pathways is currently unclear, as is the question of how a cell can differentiate between systemin, brassinosteroids and UV-B.

Dual specificity phosphatase MKP1 targets MPK6 and is a negative regulator of genotoxic stress

Isolation of an *Arabidopsis* mutant with increased sensitivity to the chemical mutagen methyl methanesulfonate and UV-C resulted in the identification of the *MKP1* gene, encoding a dual-specific phosphatase that affects genotoxic stress [53]. Surprisingly, although *mkp1* mutants showed hypersensitivity to genotoxic stress they were more resistant to salt stress. Subsequent analysis revealed that MKP1 preferentially interacts with MPK6 and to a lesser extent with MPK3 and MPK4 [53]. Although these results suggest that MKP1 controls the genotoxic and salt stress-inducible MPK6 pathway, it is not clear whether MPK6 is of central importance for the adaptation of *Arabidopsis* to these stresses and if MPK6 is the only target of the MKP1 phosphatase.

Conclusions

The first complete MAPK modules have been identified for signalling biotic and abiotic stresses as well as for cytokinesis. These studies provided compelling evidence for the functioning of a partially overlapping set of MAPKs in different pathways, indicating that knowledge on the presence or absence of other signalling components will be indispensable. Therefore, a future important task will be to monitor the expression of all available MAPK components in a developmental-, tissue- and signal-dependent context. However, the same modules can serve different functions in different cellular contexts, as shown for the dual function of NPK1–MEK2–NTF6 in pathogen defence and cytokinesis pathways. Integration of diverse signal transduction pathways has become a prominent feature that equally holds true for MAPK cascades, as seen with the systemin, UV-B and brassinosteroid pathways potentially targeting the same MAPKs in tomato. Investigations of signal transduction have uncovered unexpected levels of cross talk between signalling cascades. It comes as no surprise that MAPK pathways are found centre stage, affecting diverse physiological processes. This suggests that MAPK cascades are tightly interlinked with other signalling pathways. The different phenotypic effects caused by overexpression of NPK1 might be explained by cross talk to various cellular pathways. Whereas some NPK1 overexpressor plants showed an embryo lethal phenotype related to auxin signalling, others were tolerant to various abiotic stresses. The opposite phenotypes of mutants in the dual specificity phosphatase MKP1 on chemical mutagens and salt stress could be taken as another example for cross talk of a MAPK with other pathways.

The advent of VIGS in tobacco and tomato and the availability of insertion mutants for almost any gene in *Arabidopsis* have made it feasible to undertake high-throughput analysis of signalling pathways by genetic means. However, depending on the genetic background, any genetic analysis might result in cellular reprogramming and subsequent misleading interpretations. Therefore, unbiased approaches analysing the global set of cellular pathways will be necessary to improve our understanding of the underlying molecular signalling events. Full genome transcript profiling has become a standard analysis for investigating organisms and mutants at a global scale. Novel methods for performing large-scale proteome profiling, including the various types of post-translational modifications, should be of tremendous value for complementing our understanding of the structure and functioning of signalling pathways in a cellular context.

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