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Mitogen-activated protein kinase cascades in plants: a new nomenclature

MAPK Group (Kazuya Ichimura *et al.*)

Mitogen-activated protein kinase (MAPK) cascades are universal signal transduction modules in eukaryotes, including yeasts, animals and plants. These protein phosphorylation cascades link extracellular stimuli to a wide range of cellular responses. In plants, MAPK cascades are involved in responses to various biotic and abiotic stresses, hormones, cell division and developmental processes. Completion of the *Arabidopsis* genome-sequencing project has revealed the existence of 20 MAPKs, 10 MAPK kinases and 60 MAPK kinase kinases. Here, we propose a simplified nomenclature for *Arabidopsis* MAPKs and MAPK kinases that might also serve as a basis for standard annotation of these gene families in all plants.

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Mitogen-activated protein kinase (MAPK; see Glossary) cascades are universal modules of signal transduction in eukaryotes. These protein phosphorylation cascades mediate the intracellular transmission and amplification of extracellular stimuli, resulting in the induction of appropriate biochemical and physiological cellular responses [1–4]. MAPKs form the terminal components of the prototypical sequential cascades, and are activated by MAPK kinases (MAPKKs or MEKs) via dual phosphorylation of conserved threonine and tyrosine residues in the motif TxY located in the activation loop (T-loop) between kinase subdomains VII and VIII. MAPKKs are themselves activated by MAPKK kinases (MAPKKKs or MEKKs) through phosphorylation of conserved serine and/or threonine residues in their T-loop [1–4].

In plants, MAPK cascades are associated with various physiological, developmental and hormonal responses. Molecular and biochemical studies using specific antibodies to particular MAPKs have revealed that MAPK activation correlates with stimulatory treatments such as pathogen infection, wounding, low temperature, drought, hyper- and hypo-osmolarity, high salinity, touch, and reactive oxygen species [5–8]. Genetic studies of the *Arabidopsis* mutants *constitutive triple response 1* (*ctr1*) and *enhanced disease resistance 1* (*edr1*), which exhibit altered responses to ethylene and pathogens, respectively, show that their wild-type alleles encode MAPKKKs related to the RAF protein kinase [9,10]. Reverse genetic analysis of the *Arabidopsis mpk4* knockout mutant revealed its importance in regulating systemic acquired resistance, including the ability to accumulate salicylic acid [11]. A gain-of-function study

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Glossary

ANP
Arabidopsis NPK1 homolog
AtMRK1
Arabidopsis thaliana MLK/Raf-related protein kinase 1
BWMK1
Blast- and wound-induced MAP kinase 1
Bck1
Bypass of C kinase 1
CD domain
Common docking domain
JNK
c-Jun amino(NH_2)-terminal kinase
LRR
Leucine-rich repeat
MAP3K
Mitogen-activated protein kinase kinase kinase
MAPK
Mitogen-activated protein kinase
MAPKK
Mitogen-activated protein kinase kinase
MAPKKK
Mitogen-activated protein kinase kinase kinase
MEK
MAPK/ERK kinase; another name for a MAPKK
MEKK
MAPK/ERK kinase kinase; another name for a MAPKKK
MHK
Mak-homologous kinase
MKK
Another name for a MAPKK
MMK
Medicago MAPK
MPK
Mitogen-activated protein kinase
NB-ARC
Nucleotide-binding adaptor shared by APAF-1, certain *R*-gene products and CED-4
NPK1
Nucleus- and phragmoplast-localized protein kinase (renamed from *Nicotiana* protein kinase 1)
RAF
Transforming gene
Ran
ras-related nuclear protein
SAMK
Stress-activated MAPK
SIMK
Salt-stress-inducible MAPK
SIMKK
SIMK kinase
SIPK
Salicylic-acid-induced protein kinase
Ste11
Sterile 11
TDY
Putative activation motif in MAPK gene TDY1
TIR domain
Toll- and interleukin-1-receptor domain
WIPK
Wound-induced protein kinase
WRKY
Zinc-finger-type transcription factor
WRKY domain
60-amino-acid motif that binds to the W-box domain

showed that ectopic expression of a constitutively active mutant form of tobacco (*Nicotiana tabacum*) *NtMEK2* induced the activation of downstream MAPK, the expression of defense genes and hypersensitive-response-like cell death [12]. Recently, an *Arabidopsis* MAPK cascade (MEKK1–MKK4-5–MPK6) and WRKY transcription factors were shown to be involved in innate immunity and function downstream of the flagellin receptor FLS2 [13].

In this article, we describe the complete sets of *Arabidopsis* MAPKs and MAPKKs, and a proposed set of MAPKKKs, and analyze them by phylogenetic methods, together with other plant MAPKs, MAPKKs and MAPKKKs (for supplementary information see Online Tables 1–6; http://archive.bmn.com/supp/plants/plants0707a.pdf). Our analysis allows us to derive a systematic nomenclature for *Arabidopsis* MAPKs (for which we have adopted the gene name *MPK*) and MAPKKs (for which we have adopted the gene name *MKK*). In this nomenclature, the *Arabidopsis* MPKs and MKKs are classified on the basis of structural features and identified by simple numbering. The architecture of this system should allow it to be extended to MAPKs in other plant species, as demonstrated by integrating the predicted structures of all known plant MPK, MKK and putative MAPKKK proteins, and comparing their domains and relationships. We also include a short summary of what is known about the functions of plant MPKs, MKKs and MAPKKKs.

We are not proposing a definitive nomenclature of plant MAPKKKs at this time because it has yet to be shown that the RAF-related MAPKKKs (EDR1 and CTR1) actually function as MAPKKKs. We therefore recommend that members of this class retain their original names until the putative RAF-like MAPKKKs have been investigated biochemically. Within the proposed plant MPK and MKK nomenclature, we respect the established rules for plant gene naming and numbering, both for consistency and to avoid implying possible functions for specific MPKs and MKKs. In keeping with the TAIR suggestions for naming *Arabidopsis* genes (http://www.arabidopsis.org/info/guidelines.html), we have also not incorporated an 'At' prefix into the formal gene name in this species. Although this is appropriate within *Arabidopsis*, we also recognize the informational value of a taxonomic prefix for situations in which homologous genes from different plant species are under discussion. In the interests of clarity, it is sometimes desirable to use an informal prefix nomenclature that allows the reader to discern the taxonomic origin of each homolog.

Completion of the *Arabidopsis* genome has made it possible to define for the first time the full complement of MAPK family members in a single plant species. Although other species will have variations on this pattern, we feel that the timely introduction of systematic naming of all the *Arabidopsis* MPKs, MKKs and MAPKKKs should provide an important baseline. Applied consistently, it should help to guide the encyclopedic annotation of the other protein kinases involved in plant MAPK cascades as these are identified through future genome sequencing and gene discovery efforts.

MAPKs

In the completed *Arabidopsis* genome sequence [14], we identified 20 genes encoding possible MPKs (Online Table 1; http://archive.bmn.com/supp/plants/plants0707a.pdf). These MPKs can be divided into at least four groups (A–D) (Fig. 1). Sequence

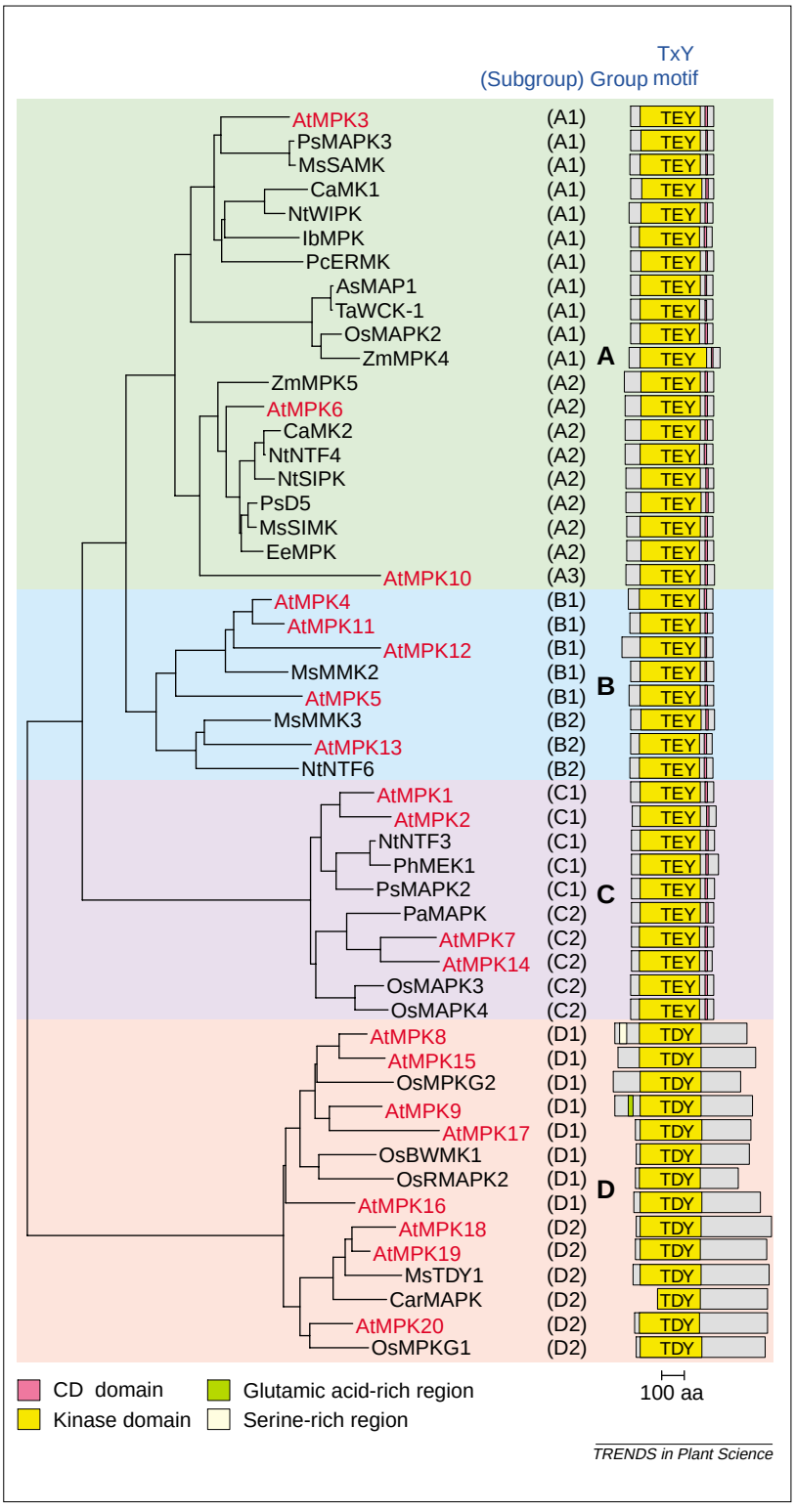


Fig. 1. Phylogenetic tree and domain structure of plant mitogen-activated protein kinases (MPKs). *Arabidopsis* MPKs are shown in red letters. The neighbor-joining phylogenetic tree (shown as a dendrogram) was created using ClustalW [57] on the DNA Data Bank of Japan and DendroMaker. The entire length of each MPK was used for the alignment. The organization of the functional domains and motifs, including the phosphorylation motif (TxY) of each MPK, is shown in cartoon format on the right. Scanning of the protein sequences for the presence of known motifs and domains was performed at the website PlantsP (http://plantsp.sdsc.edu/) [58]. Scale bar = 100 amino acids. To identify the species of origin for each MPK, a species acronym is included before the protein name: As, *Avena sativa*; At, *Arabidopsis thaliana*; Ca, *Capsicum annuum*; Car, *Cicer arietinum*; Ee, *Euphorbia esula*; Ib, *Ipomoea batatas*; Ms, *Medicago sativa*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*; Pa, *Prunus armeniaca*; Pc, *Petroselinum crispum*; Ph, *Petunia×hybrida*; Ps, *Pisum sativum*; Ta, *Triticum aestivum*; Zm, *Zea mays*.

comparison of the conserved amino acid motif TxY, which is phosphorylated by MAPKKs, clearly classified *Arabidopsis* MPKs into two subtypes: those containing the amino acid motif TEY (TEY subtype) at the phosphorylation site and those with the amino acid motif TDY (TDY subtype). The TEY subtype can be classified into three Groups, A, B and C, whereas the TDY subtype forms a more distant Group D. Major clades within each subgroup have been assigned arbitrary numbers (Fig. 1).

Group A MPKs have been most frequently found to be involved in environmental and hormonal responses. MPK6 and its apparent orthologs in other species are activated by many environmental stresses [15–19]. Tobacco SIPK, initially identified as a salicylic-acid-induced protein kinase, has since been shown to be activated during both biotic and abiotic stress responses [6]. Alfalfa SIMK is also involved in stress responses [20]. Expression of the *MPK3* gene is readily induced by environmental stress [21], and the MPK3 protein is also activated by oxidative stress [15]. Tobacco WIPK shares high sequence similarity with MPK3 and is involved in wound-signal transduction [22]. The closely related alfalfa SAMK is also involved in biotic- and abiotic-stress responses [23].

Group B MPKs have been less well studied but appear to be involved in both environmental stress responses and cell division. For example, disruption of the *MPK4* gene by transposon insertion created a constitutive systemic-acquired-resistance phenotype [11]. In the wild-type background, biochemical analysis using a MPK4-specific antibody revealed both biotic- and abiotic-stress-induced activation of MPK4 [17,18]. By contrast, alfalfa MMK3 and tobacco Ntf6, which (with MPK13) form a distinct cluster (B2) within Group B, are activated in a cell-cycle-dependent manner and specifically localized in the phragmoplast during telophase [24,25]. Information on the Group C MPKs is limited, although microarray analysis detected circadian-rhythm-regulated expression of *MPK7* [26].

Group D MPKs, which include eight members of the *Arabidopsis* MPKs, are notable for the TDY motif in their T-loop and their extended C-terminal region relative to Groups A, B and C. Group D MPKs also lack the C-terminal CD DOMAIN, which is consistently found in members of the other MPK groups. MPK8, MPK9 and MPK15 contain short extensions (~60–80 amino acids) in their N-termini, and MPK8 and MPK9 possess a serine-rich and a glutamic-acid-rich region, respectively, in their N-terminal regions. Two reported Group D genes, rice *BWMK1* and alfalfa *TDY1*, are induced by blast fungus and wounding [27,28], respectively. It is worth noting that no plant MAPK homolog is known to possess the TGY motif that is found in the budding yeast Hog1p and the mammalian p38 MAPKs, or the TPY motif of mammalian JNK MAPKs.

MPKs belonging to Groups A and B possess an evolutionarily conserved CD domain [29] in their

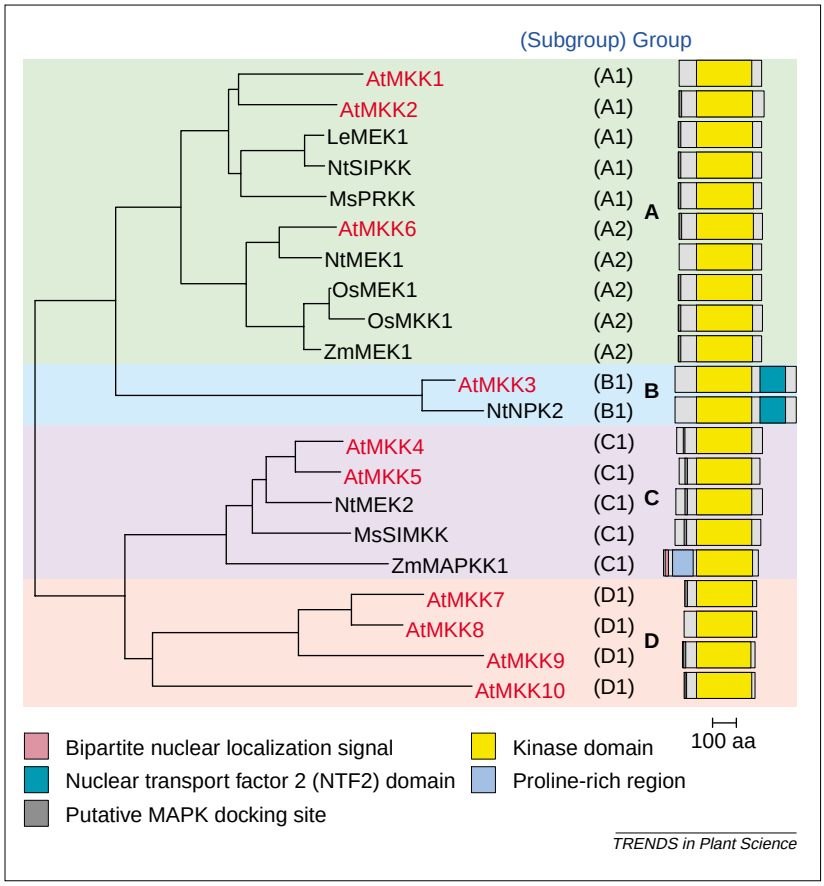


Fig. 2. Phylogenetic tree and domain structure of plant mitogen-activated protein kinase kinases (MKKs). *Arabidopsis* MKKs are shown in bold red letters. The neighbor-joining phylogenetic tree (shown as a dendrogram) was created using ClustalW [57] on the DNA Data Bank of Japan and DendroMaker. The organization of the functional domains and motifs is shown in cartoon format on the right. Scanning of the protein sequences for the presence of known motifs and domains was performed at the website PlantsP (http://plantsp.sdsc.edu/) [58]. Scale bar = 100 amino acids. To identify the species of origin for each MKK, a species acronym is included before the protein name: At, *Arabidopsis thaliana*; Le, *Lycopersicon esculentum*; Ms, *Medicago sativa*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*; Zm, *Zea mays*.

C-terminal extension. The CD domain functions as a docking site for MAPKKs, phosphatases and protein substrates, and contains an amino acid sequence [LH][LHY]Dxx[DE]xx[DE]EPxC (where x represents any amino acid) that includes two adjacent acidic residues (D and E) that are crucial for interaction with a cluster of basic amino acids (K and R) that is observed in MAPKKs [29]. Similarly, the bulky hydrophobic residues (L, H and Y) in the MAPK CD domain bind to their counterpart residues (LxLxL) in the MAPKK docking site. The CD domain in Group C MPKs appears to be modified, and neither version of the domain is found in the Group D sequences (Fig. 1).

We do not classify *Arabidopsis* Mak-homologous kinase (MHK) [30] and its homologs as MPKs even though MHKs do have the TEY motif in their T-loop and show overall sequence similarity to MPKs. This is because: (1) MHK shows similar sequence relatedness to MPKs and CDC2-like kinases; (2) the biochemical characteristics of MHKs are largely unknown; and (3) MHK sequences lack the CD domain observed in most of the MPKs.

MAPKKs

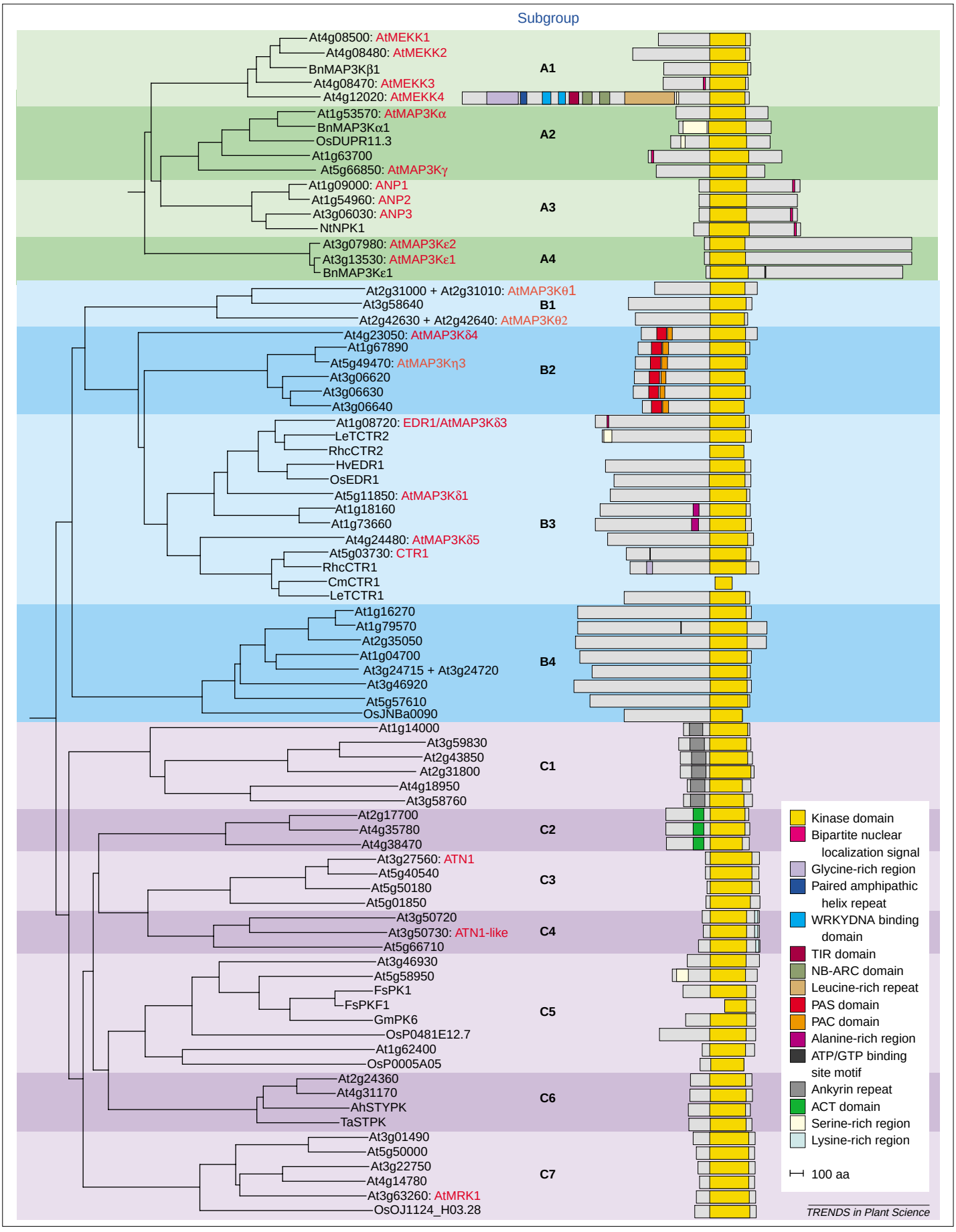
There are ten putative MKKs encoded in the *Arabidopsis* genome (Online Table 3; http://www.archive.bmn.com/supp/plants/plants0707a.pdf), which is only half as many as there are MPKs. This suggests that MKKs are likely to activate multiple MPKs, and that cross-talk between various signal-transduction pathways might be concentrated at this level in plant MAPK cascades. To date, 21 plant MAPKKs have been identified.

The sequence of the phosphorylation site of plant MKKs is different from that in the mammalian kinases. Plant MKKs have the consensus sequence S/TxxxxxS/T, whereas mammalian enzymes have S/TxxxS/T. This consensus sequence is not found in *Arabidopsis* MKK10 because of the deletion of three amino acid residues in this region. Sequence alignment placed the plant MKKs into four groups (A–D) (Fig. 2). Clades within each subgroup are numbered sequentially. The *Arabidopsis* C- and D-group MKK genes do not have introns. The N-terminal extension of plant MKKs shows a putative MAPK docking site [K/R][K/R][K/R]x(1–5)[L/I]x[L/I] similar to that found in animal MAPKKs [31] (Fig. 2). The putative MAPK docking site is characterized by a cluster of basic residues (R and K) N-terminal to hydrophobic residues (L and I).

The Group A MKKs *Arabidopsis* MKK1 (renamed from MEK1) and MKK2 are thought to be upstream factors for MPK4, based on *in vitro* kinase assays, yeast two-hybrid analysis and complementation of yeast MAPK cascade mutants [32–35]. Analysis of MKK1 using a specific antibody revealed that multiple abiotic stresses activated MKK1 [35]. Alfalfa PRKK (pathogen-responsive MAPKK) is also classified in Group A and transmits elicitor signaling to downstream MAPKs [36]. *Arabidopsis* MKK6 is similar to NtMEK1, which has been shown to be involved in cell division and in activation of the tobacco MPK, Ntf6 [37].

Arabidopsis MKK3 and tobacco NPK2 in Group B, have an unusual structural feature consisting of a nuclear transport factor 2 (NTF2) domain in the extended C-terminus region. NTF2 is a small protein that mediates the nuclear import of RAN–GDP and

Fig. 3. (right) Phylogenetic tree and domain structure of putative plant mitogen-activated protein kinase kinase kinases (MAPKKKs). The kinase catalytic domains of MEKK-like and RAF-related kinases were used for alignment. *Arabidopsis* MAPKKKs are listed using their AGI (*Arabidopsis* Genome Initiative) names, and the protein names of those *Arabidopsis* MAPKKKs that have already been identified are shown in red. The neighbor-joining phylogenetic tree (shown as a dendrogram) was created using ClustalW [57] on the DNA Data Bank of Japan and DendroMaker. The organization of the functional domains and motifs is shown in cartoon format on the right. Scanning of the protein sequences for the presence of known motifs and domains was performed at the website PlantsP (http://plantsp.sdsc.edu/) [58]. Scale bar = 100 amino acids. To identify the species of origin for each MAPKKK, a species acronym is included before the protein name: Ah, *Arachis hypogaea*; At, *Arabidopsis thaliana*; Bn, *Brassica napus*; Cm, *Cucumis melo*; Fs, *Fagus sylvatica*; Gm, *Glycine max*; Hv, *Hordeum vulgare*; Le, *Lycopersicon esculentum*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*; Rhc, *Rosa hybrid cultivar*; Ta, *Triticum aestivum*.


Subgroup
At4g08500: AtMEKK1
At4g08480: AtMEKK2
BnMAP3Kβ1
At4g08470: AtMEKK3
At4g12020: AtMEKK4
A1
At1g53570: AtMAP3Kα
BnMAP3Kα1
OsDUPR11.3
At1g63700
At5g66850: AtMAP3Kγ
A2
At1g09000: ANP1
At1g54960: ANP2
At3g06030: ANP3
NtNPK1
A3
At3g07980: AtMAP3Kε2
At3g13530: AtMAP3Kε1
BnMAP3Kε1
A4
At2g31000 + At2g31010: AtMAP3Kθ1
At3g58640
At2g42630 + At2g42640: AtMAP3Kθ2
B1
At4g23050: AtMAP3Kδ4
At1g67890
At5g49470: AtMAP3Kη3
At3g06620
At3g06630
At3g06640
B2
At1g08720: EDR1/AtMAP3Kδ3
LeTCTR2
RhcCTR2
HvEDR1
OsEDR1
At5g11850: AtMAP3Kδ1
At1g18160
At1g73660
At4g24480: AtMAP3Kδ5
At5g03730: CTR1
RhcCTR1
CmCTR1
LeTCTR1
B3
At1g16270
At1g79570
At2g35050
At1g04700
At3g24715 + At3g24720
At3g46920
At5g57610
OsJNBa0090
B4
At1g14000
At3g59830
At2g43850
At2g31800
At4g18950
At3g58760
C1
At2g17700
At4g35780
At4g38470
C2
At3g27560: ATN1
At5g40540
At5g50180
At5g01850
C3
At3g50720
At3g50730: ATN1-like
At5g66710
C4
At3g46930
At5g58950
FsPK1
FsPKF1
GmPK6
OsP0481E12.7
At1g62400
OsP0005A05
C5
At2g24360
At4g31170
AhSTYPK
TaSTPK
C6
At3g01490
At5g50000
At3g22750
At4g14780
At3g63260: AtMRK1
OsOJ1124_H03.28
C7
Kinase domain
Bipartite nuclear localization signal
Glycine-rich region
Paired amphipathic helix repeat
WRKYDNA binding domain
TIR domain
NB-ARC domain
Leucine-rich repeat
PAS domain
PAC domain
Alanine-rich region
ATP/GTP binding site motif
Ankyrin repeat
ACT domain
Serine-rich region
Lysine-rich region
100 aa
TRENDS in Plant Science

binds to both Ran–GDP and FxFG-repeat-containing nucleoporins [38]. Characterized MKKs in Group C include stress-responsive upstream factors of Group A MPKs. Transient expression of alfalfa SIMKK and SIMK in parsley protoplasts results in the activation of SIMK [39]. Unlike PRKK, SIMKK conducts both salt- and elicitor-induced signals with different substrate specificities [36]. Dexamethasone-induced transient overproduction of a constitutively active form of tobacco NtMEK2 in tobacco leaves caused not only activation of SIPK and WIPK but also hypersensitive cell death [12]. Moreover, stable transformation with *Arabidopsis* MKK4 and MKK5, putative orthologs of tobacco NtMEK2, showed similar effects [40]. There is no information on the function and production of Group D MKKs.

MAPKKKs

Compared with MPKs and MKKs, the MAPKKK family has many more members and greater variety in primary structures and domain composition (Fig. 3). Relationship analysis based on the amino acid sequences of the protein kinase catalytic domain shows that *Arabidopsis* MAPKKKs fall into two main classes: MEKKs such as MEKK1/STE11/BCK1 and RAF-like (Fig. 3). Group A comprises MAPKKKs whose kinase domains have significant similarity to typical MAPKKKs, such as MEKK/STE11/BCK1. Group A can be further divided into five subgroups. Subgroup A1 comprises four protein kinases (AtMEKK1–4). *AtMEKK1* expression is enhanced by drought, high salinity and touch [21]. Functional analyses using yeast two-hybrid and complementation of yeast mutants suggested a role for AtMEKK1 upstream of MPK4, MKK1 and MKK2 [33,34]. AtMEKK2–AtMEKK4 were isolated as partial cDNA clones by low-stringency hybridization using AtMEKK1 as a probe (T. Mizoguchi *et al*., unpublished).

AtMEKK1–AtMEKK4 and BnMEKK1 (from *Brassica napus*) possess common motifs in their N-terminal regions, which vary in organization [41]. However, AtMEKK4 has a unique structure, with several functional domains in its N-terminus [glycine-rich region, paired-amphipathic-helix repeat, WRKY DOMAIN, TIR DOMAIN, NB-ARC domain, LEUCINE-RICH REPEAT (LRR) and protein kinase domain]. The WRKY domain is likely to confer direct DNA-binding capacity. WRKY proteins are plant-specific zinc-finger transcription factors that are transcriptionally activated during some plant defense responses [42]. In addition, the extended AtMEKK4 N-terminal region contains sequences similar to the class-I TIR–NB–LRR resistance-gene structure [43], which might suggest a further connection to plant defense-response signaling. The unusual structure of the *AtMEKK4* gene might have arisen through recombination between a disease resistance gene and a *MAPKKK* gene of the MEKK type because several class-I TIR–NB–LRR resistance genes are located in the neighboring chromosomal region near *AtMEKK4*.

Subgroup A3 comprises ANP1, ANP2 and ANP3, whose cDNAs were isolated using tobacco *NPK1* as a probe [44]. ANP1–ANP3 have a putative regulatory region in their C-terminus. Tobacco NPK1, an apparent tobacco ortholog of ANP1–ANP3, is involved in cytokinesis [45]. NPK1 interacts and localizes with its activators NACK1 and NACK2, kinesin-like proteins, during M phase, which is required for the intracellular events that lead to cytokinesis [46]. Moreover, a reverse-genetic approach using multiple-knockout mutants of the *ANP* genes revealed their function as a positive regulator for cytokinesis and a possible negative regulator for stress responses [47]. In addition, ANPs and NPK1 function in oxidative-stress-response signaling and as negative regulators of the auxin-response pathway [15,48].

Subgroup A4 comprises AtMAP3Kε1 and AtMAP3Kε2, which are also involved in cell division [49]. They are functional homologs of fission yeast Cdc7, which is involved in cell division control. However, Cdc7 is not a MAPKKK, which suggests that AtMAP3Kε1 and AtMAP3Kε2 also might not function as MAPKKKs. Functions for the MAPKKKs of subgroup A2 remain to be determined.

AtMAPKKKs classified into Groups B and C are related to the RAF kinases, whose sequences differ from those of MEKK/Ste11/Bck1 [50]. All Group B, RAF-related AtMAPKKKs have extended N-terminal domains. Among them, CTR1 [9] and EDR1 [10] are involved in ethylene and disease resistance signaling, respectively. Interestingly, the AtMAPKKKs in RAF-related subgroup B2 contain Per, Arnt and Sim (PAS) domains and PAS-associated C-terminal (PAC) domains in their N-termini; the PAS domain functions as a signal sensory domain in many signaling pathways [51]. Two MAPKKKs of subgroup B3, At1 g18160 and At1 g73660, also have central alanine-rich regions.

Group C members of the AtMAPKKKs are related to RAF protein kinases and to *Dictyostelium discoideum* developmentally regulated protein-tyrosine kinase 1 (DPYK1) [52]. Only two *Arabidopsis* protein kinases, ATN1 and ATMRK1, have been described in this subgroup [53,54], most of which are known only from genomic sequence analysis. The biological functions of Group C RAF-related MAPKKKs thus remain largely unknown. Six C1 members have ankyrin repeats in their N-terminal region, a motif that is known to play a role in protein–protein interaction [55]. Three subgroup-C2 RAF-related sequences have an aspartokinase, chorismate mutase and TyrA (ACT) domain, which is known to play a role in the regulation of a wide range of metabolic enzymes by responding to amino acid concentration [56]. Three RAF-related genes from subgroup C4, as well as At5 g58950, contain a lysine-rich region in their C-terminus and a serine-rich region in their N-terminus.

Conclusion

Plant MAPK cascades are thought to play an important role in biotic- and abiotic-stress responses,

hormone responses, cell division, and development. In the *Arabidopsis* genome, genes for 20 MAPKs, 10 MAPKKs, 12 MEKK-like MAPKKKs and 48 RAF-related MAPKKKs have been discovered. The 'pre-genomics' nature of MAPK discovery in plants has led to a nonsystematic and confusing nomenclature. The adoption of a rigorous phylogeny and naming system, as presented here, will help to rationalize the functional analysis of these genes and proteins in *Arabidopsis*, where the full array is known. However, this proposed nomenclature should also provide a basis for systematic naming of the elements of plant MAPK cascades in other plant species for which genome sequencing projects are in progress. At the same time, it must be emphasized that sequence-based phylogenetic clustering of orthologs cannot reliably predict *in vivo* functions. These must be established experimentally, on a case-by-case basis.

Both biochemical and genetic evidence indicate that MPKs, MKKs and MAPKKKs in plants include complex multigene families, which points to the possibility of extensive functional redundancy. This question is being explored from various directions, including the development of T-DNA and transposon insertion lines for specific genes, systematic reverse-genetics analysis using post-transcriptional gene silencing, creation and analysis of multiple mutants derived from appropriate crosses, and the use of transient expression systems such as protoplasts and agroinfiltration [12,13,15,36,39,40,48]. Combining these approaches with profiling technologies such as microarray and proteomics analysis should allow the community to make rapid progress in defining the individual and collective functions of these important plant signaling components.

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