Mitogen-Activated Protein (MAP) Kinase Pathways in Plants: Versatile Signaling Tools

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Mitogen-activated protein kinases (MAPKs) are important signaling tools in all eukaryotes, and function in mediating an enormous variety of external signals to appropriate cellular responses. MAPK pathways have been studied extensively in yeast and mammalian cells, and a large body of knowledge on their functioning has accumulated, which is summarized briefly. Plant MAPK pathways have attracted increasing interest, resulting in the isolation of a large number of different components of MAPK cascades. Studies on the functions of these components have revealed that MAPKs play important roles in the response to a broad variety of stresses, as well as in the signaling of most plant hormones and in developmental processes. Finally, the involvement of various plant phosphatases in the inactivation of MAPKs is discussed.

KEY WORDS: Signal transduction, MAP kinase, Protein kinase, Phosphorylation, Stress, Hormones, Cell cycle. © 2001 Academic Press.

I. Introduction

Like all living organisms, plants must be able to sense many external stimuli and signal them to cellular targets to stimulate appropriate

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responses. Mitogen-activated protein kinases (MAPKs[2]) are important components for some of these signaling pathways. MAPKs are serine/threonine protein kinases that can be found in all eukaryotes and that perform their function as part of protein kinase cascades. Besides MAPKs, these cascades are composed of MAPK kinases (MKKs) and MAPK kinase kinases (MKKKs).

MAPKs were first discovered by their ability to phosphorylate microtubule-associated protein 2 (MAP2) and therefore were named MAP2 kinases (Ray and Sturgill, 1987). MAP2 kinase turned out to be identical to a 42-kDa protein that previously had been identified to become tyrosine-phosphorylated upon the mitogenic stimulation of cells (Cooper *et al.,* 1982; Rossomando *et al.,* 1989). MAP2 kinase therefore was renamed mitogen-activated protein kinase (Rossomando *et al.,* 1989). The cloned gene had high homology with the previously isolated yeast kinases FUS3 and KSS1 (Elion *et al.,* 1990; Courchesne *et al.,* 1989), and because this family of kinases was activated not only by mitogens but also by other stimuli, it was named ERK1 for extracellular-signal-regulated kinase 1 (Boulton *et al.,* 1990). MAPK and ERK often are used interchangeably, but it was suggested that ERK should refer to a particular group of isoforms within the MAPK family (Seger and Krebs, 1995).

Soon after the identification of MAPKs, a MAPK activator was identified (Ahn *et al.,* 1991) that was able to phosphorylate MAPKs (Seger *et al.,* 1992). This activator was cloned in 1992 by Crews and co-workers (Crews *et al.,* 1992), and was named MEK (for MAPK/ERK kinase). The MEKs also turned out to be activated by phosphorylation (Gomez and Cohen, 1991; Ahn *et al.,* 1993). The proto-oncogene Raf-1 was the first MEK activator identified (Kyriakis *et al.,* 1992), but other MEK activators, like MEKK1 and c-Mos, were isolated soon after (Lange-Carter *et al.,* 1993; Posada *et al.,* 1993). The high degree of homology of MEK and MEKK1 to yeast protein kinases upstream of FUS3 and KSS1 was the first sign of an evolutionarily conserved structure for MAPK cascades.

[2] Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; ABA, abscisic acid; CDPK, calcium-dependent protein kinase; CKI, cytokinin-insensitive; CTR, constitutive triple response; EIN, ethylene-insensitive; ERK, extracellular-signal-regulated kinase; EST, expressed sequence tag; ETR, ethylene-resistant; GA, gibberellin; hcd, hypersensitive cell death; HOG1, high-osmolarity glycerol 1; HR, hypersensitive response; JA, jasmonic acid; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; MeJA, methyl jasmonate; MEK, MAPK/ERK kinase; MEKK, MEK kinase; MKK, MAPK kinase; MKKK, MKK kinase; NPK, *Nicotiana tabacum* protein kinase; NtF, *Nicotiana tabacum* FUS3-like kinase; PiE, *Phytophthora infestans* elicitor; PKC, protein kinase C; PLA, phospholipase A; PP2C, protein phosphatase 2C; PR, pathogen response; PTP, protein tyrosine phosphatase; SA, salicylic acid; SAMK, stress-activated MAPK; SAPK, stress-activated protein kinase; SIPK, SA-induced protein kinase; SMK1, sporulation MAP kinase 1; TMV, tobacco mosaic virus; TvX, xylanase from *Trichoderma viride;* WIPK, wound-induced protein kinase.

Over the past few years, several plant MAPK pathway components have been isolated, and this review will focus on what is known about the role of these components in signal transduction in plants.

II. Structure of Mitogen-Activated Protein Kinase (MAPK) Cascades

A. MAPKs

MAPKs belong to the superfamily of protein kinases and are classified into the CMGC group, which further consists of cyclin-dependent kinases (Cdks), glycogen synthase kinase-3 isoforms, and the Cdk-like kinases (Hanks and Hunter, 1995). All MAPKs exhibit 11 subdomains that are characteristic for all serine/threonine protein kinases (Hanks *et al.,* 1988). The different MAPKs can be divided into three major groups: the ERKs (extracellular regulated kinases), the SAPKs/JNKs (stress-activated protein kinases/c-Jun N-terminal kinases), and the p38 kinases. Crystallographic analysis of members of all three groups, ERK2, p38, and JNK3, revealed a highly similar 3D architecture (Zhang *et al.,* 1994; Wilson *et al.,* 1996; Wang *et al.,* 1997; Xie *et al.,* 1998), having a two-lobed structure, where the active site is found at the domain interface (Goldsmith and Cobb, 1994). The N-terminal domains are mainly composed of β-strands, whereas the C-terminal domains are predominantly α-helical. This overall topology is similar to that of cAMP-dependent protein kinase and CDK2 (Taylor and Radzio-Andzelm, 1994).

One of the most important and least stable secondary structure elements of MAPKs is the activation loop that forms the mouth of the active site (Zhang *et al.,* 1995) and contains the MAPK-specific TXY (threonine–X–tyrosine) dual-phosphorylation motif (Payne *et al.,* 1991; Gartner *et al.,* 1992). Phosphorylation of both threonine and tyrosine in this motif is required for full activation of the MAPKs. In the inactive form, some of the catalytic residues are misaligned and the substrate-binding pocket of the kinase is blocked. Both kinase-inhibiting features are corrected by conformational changes upon MAPK activation, as determined by comparing the crystal structures of inactive and active, dual-phosphorylated ERK2 (Canagarajah *et al.,* 1997).

Distinct MAPKs have specific lengths of the activation loop and sequences of the dual-phosphorylation motif. ERK, JNK, and p38 kinases generally have activation loop lengths of 25, 21, or 19 residues and TEY, TPY, or TGY dual-phosphorylation motifs, respectively. By using site-directed mutagenesis, it was found that the particular amino acid residues of

the dual-phosphorylation motif and the activation loop influence substrate specificity, but are not crucial for the specificity of activation by upstream signals, whereas the length of the activation loop plays a role in controlling autophosphorylation (Robinson *et al.,* 1996; Jiang *et al.,* 1997).

Several groups have tried to elucidate the requirements of MAPKs for specific recognition by MKKs. In chimeras constructed from p38 and ERK2, an amino-terminal region of 40 residues was identified to be involved in specifying the response of the kinase to different external signals, whereas the carboxyl-terminal half of the molecule specified substrate recognition (Brunet and Pouysségur, 1996). Other work suggests that multiple MAPK regions in the amino- and carboxyl-terminal domains are responsible for the specific interaction with MKKs (Wilsbacher *et al.,* 1999).

Downstream of the MAPK cascade, the signal is translated into different cellular responses. MAPKs may be translocated to the nucleus to phosphorylate, and thereby activate, specific transcription factors or may stay in the cytoplasm to phosphorylate cytoskeleton-associated proteins or certain enzymes such as protein kinases, phosphatases, and phospholipases. Phosphorylation of these substrates occurs only at serine or threonine residues that are followed immediately by a proline (Gonzalez *et al.,* 1991). This substrate specificity is regulated by the so-called P+1 loop in the substrate-binding pocket of MAPKs. In ERK2, specific binding of substrate proline by this loop is only possible after activation of the kinase (Canagarajah *et al.,* 1997).

B. MKKs

MKKs are dual-specificity protein kinases that activate MAPKs by phosphorylation of both the threonine and tyrosine residues of the TXY motif of MAPKs. MKKs are activated themselves by phosphorylation of two conserved serine or threonine residues between kinase subdomains VII and VIII (Alessi *et al.,* 1994; Zheng and Guan, 1994). In most MKKs, these conserved amino acids have the motif $^{S}/_{T}XXX^{S}/_{T}$.

Besides phosphorylation-mediated activation of MKKs, it also has been demonstrated that phosphorylation on other residues can negatively regulate MKK activity (Brunet *et al.,* 1994; Rossomando *et al.,* 1994). This phosphorylation can be mediated by MAPKs (Wu *et al.,* 1993; Brunet *et al.,* 1994), although phosphorylation of MKKs by MAPKs also is thought to facilitate the binding of MKK to Raf (Catling *et al.,* 1995). MKK function can be regulated not only at the posttranslational level by phosphorylation but also posttranscriptionally by differential splicing. There are indications that different splicing forms have different cellular localizations (English *et al.,* 1995).

MKKs have restricted substrate specificity in that they have no other known substrates beyond MAPKs (Seger *et al.,* 1992). Furthermore, MKKs normally function in only one or two distinct MAPK cascades (Robinson and Cobb, 1997), and therefore MKKs are thought to be convergence points of MAPK cascades by integrating different input signals into a given pathway. As mentioned previously, the substrate specificity of MKKs is thought to be determined by multiple MAPK domains, and MKKs are known to recognize the tertiary structure of their substrates, effectively restricting the interaction of different MKKs and MAPKs (Seger *et al.,* 1992). In MKKs, functioning in cell proliferation and differentiation, a conserved N-terminal sequence can be found that is suggested to function as a MAPK docking site (Bardwell and Thorner, 1996). This region could determine the binding specificity between these MKKs and MAPKs. It is not known whether a site with similar function exists in other MKKs.

The lack of cross talk between different MAPK cascades at the MKK level implies that the activation of MAPKs by MKKs primarily has a role in signal amplification (Brown *et al.,* 1997). However, because MKKs and MAPKs are present in roughly equal concentrations (Ferrell, 1996), this hypothesis seems unlikely, and MKKs could function in kinetic regulation (Huang and Ferrell, 1996). Because MKKs normally are present in considerable excess of MKKKs, amplification is more likely to occur at this step of the cascade (Ferrell, 1996).

C. MKKKs

MKKKs can be classified into four major families: MEKK/STE11, Raf, MLK, and Mos kinases (Widmann *et al.,* 1999). All yeast MKKKs belong to the MEKK/STE11 family, whereas MKKKs of higher eukaryotes can be found in all four groups.

In contrast to the MAPKs and MKKs, the structure of the different MKKKs is rather diverse, and different regulatory motifs can be found in MKKKs. Among them are Pleckstrin homology (PH) domains, proline-rich sequences involved in SH3 binding, zinc finger motifs, leucine zippers, binding sites for G-proteins, and several tyrosine and serine/threonine phosphorylation sites (Garrington and Johnson, 1999). In accordance with their diverse structures, MKKKs can be activated by different mechanisms, such as phosphorylation through MKKK kinases and PKCs, by interaction with G-proteins of the Ras and Rho families, or by direct activation through two-component receptor systems (Whitmarsh and Davis, 1996; Fanger *et al.,* 1997; Wurgler-Murphy and Saito, 1997; Rommel and Hafen, 1998). Thus, the heterogeneity in the structure and the diversity of regulatory domains of the different MKKKs confer on MAPK cascades the flexibility

to respond to a wide range of stimuli. Furthermore, whereas MKKs have very restricted substrate specificity, functioning mainly in a single MAPK cascade, MKKKs can feed into multiple MAPK pathways (Fanger *et al.,* 1997; Gustin *et al.,* 1998), and there are examples of the involvement of MKKKs in MAPK-independent pathways (Lee *et al.,* 1997a).

The best-studied MKKK is Raf, and it is a good example of the complexity of MKKK activation. Raf activation is initiated by Ras-GTP-mediated translocation to the plasma membrane (Vojtek *et al.,* 1993; Rommel and Hafen, 1998), whereby Raf binds to activated Ras with two amino-terminal domains (Brtva *et al.,* 1995). The exact mechanism of Raf activation is not known, but it is thought that binding of Raf to activated Ras and possibly other unknown membrane components will relieve the repression of Raf activity by its amino-terminal noncatalytic region (Cutler *et al.,* 1998). Repression of MKKK activity by their kinase-unrelated domains also is observed for other MKKKs like the yeast STE11 kinase (Cairns *et al.,* 1992).

Activated Ras is not sufficient for full Raf activation, and other components contribute to Raf activation, including 14–3–3 proteins, phospholipids, serine/threonine kinases like PKC, and tyrosine kinases like Src (Morrison and Cutler, 1997).

The amino terminus of Raf seems to be important for its activation, and many binding events with both upstream and downstream kinases as well as proteins outside the cascade and also tyrosine and serine phosphorylation of its carboxyl-terminal region are important for Raf activation (Chow *et al.,* 1995; Mason *et al.,* 1999). Furthermore, autophosphorylation is thought to play a role in the complex mechanism of Raf regulation (Morrison *et al.,* 1993).

MAPK cascade components are linked not only in a linear manner in an activation cascade. MAPKs and MKKs can also phosphorylate Raf or upstream activators of Raf as part of a negative feedback loop (Ueki *et al.,* 1994; Waters *et al.,* 1996; Holt *et al.,* 1996), and MAPKs can mediate activation of Raf via a positive feedback mechanism (Zimmermann *et al.,* 1996).

Much less is known about the activation mechanisms of other MKKKs. MEKK1 can be activated by Ras and also by Ras-independent pathways involving MKKK kinases and other small G-proteins (Fanger *et al.,* 1997; Widmann *et al.,* 1999). It is also thought that autophosphorylation of its activation loop can activate MEKK1 (Deak and Templeton, 1997). In contrast, the Mos MKKK is thought to be regulated mainly by its synthesis and degradation, whereby phosphorylation of Mos is probably important for its stability (Nishizawa *et al.,* 1992).

The physiological significance of the modular arrangement of MAPK cascades is not entirely understood, but it will enable amplification of a certain input signal and branching or cross talk between signaling pathways.

Furthermore, together with the possibility for positive feedback loops between MAPKs and MKKs or MKKKs, it makes the MAPK cascade suitable for regulating developmental decisions by converting graded inputs into an all-or-none switch (Ferrell and Machleder, 1998). Switchlike responses are thought to be enhanced by the dual-phosphorylation activating event of MAPKs and the translocation of MAPK cascade components (Ferrell and Bhatt, 1997; Ferrell, 1998).

III. MAPK Pathways in Eukaryotes

A. MAPK Cascades in Yeast

The yeast genome encodes at six MAPKs (Hunter and Plowman, 1997). Only five of them have been assigned to a specific pathway (Fig. 1)

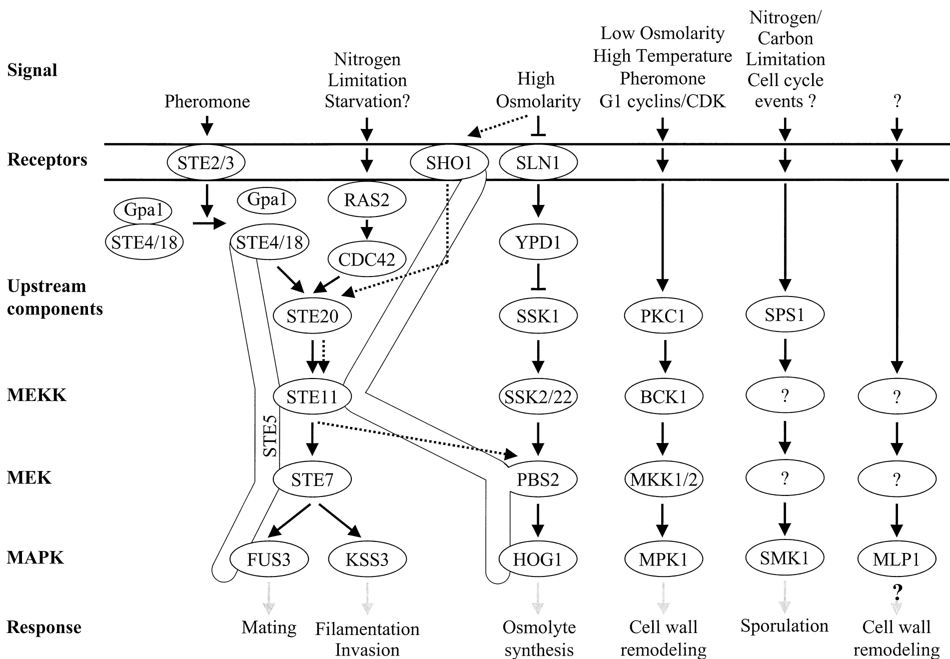


FIG. 1 MAPK cascades in *S. cerevisiae*. Question marks indicate uncertainties or unknown components. Black arrows indicate direct interaction/activation, whereas gray arrows indicate an indirect connection. The dotted arrows show the SHO1-mediated hyper-osmosensing pathway that is thought to be complexed by PBS2.

(Herskowitz, 1995; Gustin *et al.,* 1998). From the upstream MKKs and MKKKs, four members of each class of kinases can be found in yeast. The first yeast MAPKs isolated were FUS3 (for cell fusion defective 3) and KSS1 (for kinase suppression of SST2 1), and initially they were thought to have a redundant function in the pheromone response pathway. New data, however, suggest that FUS3 has a direct function in the mating pathway and that KSS1 functions in the signaling pathway leading to pseudohyphal and invasive growth. STE7 is the MKK that activates both MAPKs, and STE7 is activated by STE11.

The high-osmolarity glycerol (HOG1) pathway has a role in signaling hyperosmotic stress. Activation of this pathway upon hyperosmotic stress leads to increased production of the osmotic stabilizer glycerol, and HOG1-deficient yeast mutants have reduced salt tolerance. The upstream MKK of HOG1 is PBS2. Two pathways have been discovered that feed into the HOG1 pathway. One pathway includes the MKKKs SSK2 and SSK22. The other pathway includes the osmosensor SHO1, which activates the MKKK STE11 that in turn activates PBS2 (Ruis and Schüller, 1995).

MPK1 (for MAP kinase 1), also known as SLT2 (for suppression of low temperature 2), has a function in regulating cell wall integrity. MPK1 is activated in response to high temperature, hypo-osmolar stress, and mating pheromone, in a cell cycle-dependent manner. The MPK1 pathway further consists of the MKKs, MKK1 and MKK2, and the MKKK, BCK1. Input into the pathway occurs via protein kinase C (PKC1). Cells lacking MPK1 or one of the other members of the pathway are unable to grow at elevated temperatures and under low osmotic conditions.

The SMK1 (for sporulation MAP kinase 1) pathway is activated upon the growth of yeast on a nitrogen-deficient medium that lacks a fermentable carbon source. Under these conditions, yeast starts to form spores. As part of this process, the SMK1 pathway is responsible for the assembly of spore walls. At the moment, no MKK or MKKK has been identified for this pathway, and it remains unclear whether SMK1 is part of a classical MAPK module. However, the protein kinase SPS1, which is similar to MKKK kinases, has a role as an upstream activator of the SMK1 pathway.

The putative protein kinase MLP1 (for MPK1-like protein kinase 1) was discovered by the yeast genome project and clusters together with the MAPKs. Because of this fact, MLP1 is believed to be a MAPK, although it has a KXY motif instead of the TXY at its activation site (Hunter and Plowman, 1997). No clear function is known for this kinase at the moment, but there are some indications that MLP1 has a function partially complementary to MPK1 (Watanabe *et al.,* 1997).

Besides the three core elements of the MAPK cascade, it has been shown that so-called scaffold proteins also play an important role in MAPK pathway activation and regulation. At the moment, two scaffold proteins

are known in yeast. STE5 functions in the pheromone pathway and is known to bind STE11, STE7, and FUS3 (Printen and Sprague, 1994), as well as the upstream G-protein STE4 (Gustin *et al.,* 1998). Besides STE5, PBS2 also functions as a scaffold protein and, via its N-terminal SH3-binding domains, binds to both STE11 and HOG1 to form a signaling complex (Posas and Saito, 1997). Scaffold proteins are thought to be important for preventing cross talk between different cascades, allowing single kinases to function in more than one MAPK module without affecting the specificity of their responses.

B. MAPK Cascades in Animals

To date, thirteen different MAPKs have been identified in mammalian cells: six different ERKs, three JNKs, and four p38 kinases (Widmann *et al.,* 1999; Abe *et al.,* 1999). It should be mentioned that many MAPKs exist in several isoforms and splicing variants. The three JNK genes are known to code for at least ten different isoforms (Gupta *et al.,* 1996). The relevance of this complexity is poorly understood. In addition, the human genome codes for at least seven MKKs and fourteen MKKKs, of which different isoforms and splicing variants also exist (Tournier *et al.,* 1999; Widmann *et al.,* 1999). Figure 2 gives a summary of the mammalian MAPK pathways.

The six members of the ERK family can be divided into four subfamilies, of which ERK1 and -2 have been studied extensively. ERK1 and -2 have a role in cell proliferation, differentiation, cell cycle control, and cell survival. They can be activated by the MKKs MEK1 and -2, which in turn are activated most often by MKKKs of the Raf family. Members of almost all other MKKK subfamilies are reported to be able to activate MEK1 and -2. Upon activation, cytoplasmic ERK1 and -2 both translocate to the nucleus (Lenormand *et al.,* 1993), and this translocation is important for their function (Brunet *et al.,* 1999). The mechanism of ERK translocation is not well-understood, but it is thought that MEK1 keeps inactive ERK1 and -2 in the cytoplasm (Fukuda *et al.,* 1997). Furthermore, it is suggested that homodimerization of ERK1 and -2 could also play a role in their nuclear translocation (Khokhlatchev *et al.,* 1998).

ERK3 and -4 form the second ERK subfamily, but almost nothing is known about the function of these kinases. ERK3 is a constitutively nuclear kinase that can be activated by PKC kinases and has an SEG motif instead of the common TXY motif in the activation loop (Cheng *et al.,* 1996). ERK4 has been shown to be activated by growth factors (Widmann *et al.,* 1999). The third and fourth subfamilies consist of only ERK5 and ERK7, respectively. Both kinases have long distinct carboxyl-terminal extensions. ERK5 is able to induce c-Jun expression upon serum treatment and is

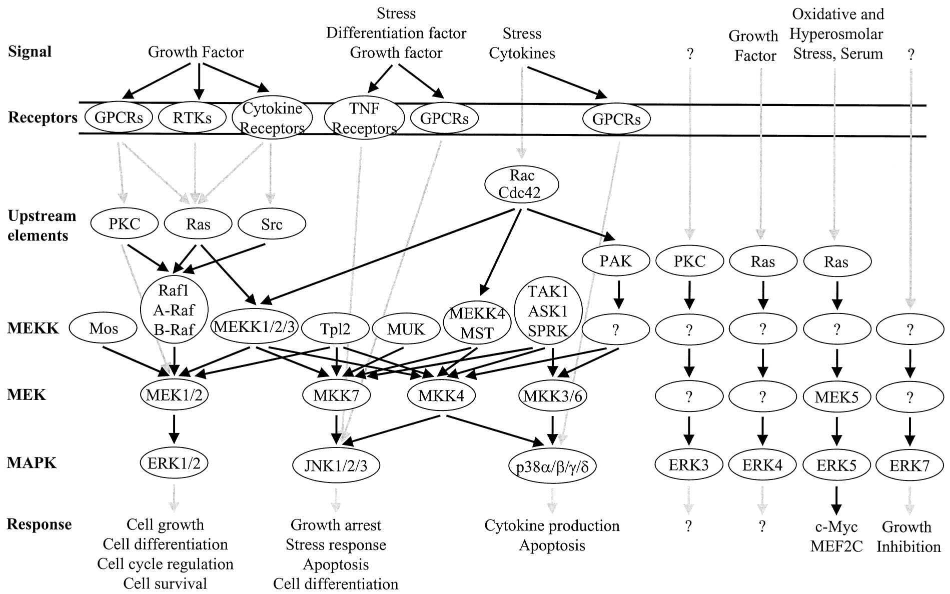


FIG. 2 Overview of mammalian MAPK pathways. Question marks indicate unknown components, black arrows indicate direct activation, and gray arrows indicate indirect connections. This scheme only shows major players up- and downstream of the MAPK cascades.

activated by oxidative and hyperosmolar stress. Cytoplasmic ERK5 is translocated to the nucleus upon activation, and its C terminus seems to inhibit its autophosphorylation activity (Zhou *et al.,* 1995). ERK7 can function as a negative regulator of cell growth, is a constitutively active nuclear kinase, and requires its carboxyl terminus for these functions (Abe *et al.,* 1999).

The SAPK/JNK group of MAPKs can be activated by stresses, such as heat shock, hyperosmolar stress, and ultraviolet irradiation. Furthermore, this group of kinases has a role in the cellular response to different extracellular ligands, like differentiation and growth factors. The SAPK/JNKs can be activated by the MKKs, MKK4 and MKK7, and activation of MKK4/7 occurs by MKKKs from almost all MKKK subtypes, except for members of the Raf subfamily. To date, all known substrates of the JNK pathway are transcription factors (Widmann *et al.,* 1999).

MAPKs of the p38 family are known to be activated by cytokines and certain cellular stresses, such as ultraviolet irradiation, osmotic shock, and heat shock. p38 MAPKs can be activated by MKK3 and MKK6, and also by MKK4. TAK1, ASK1, and SPRK can act as MKKKs in these pathways. Substrates for p38 MAPKs are MAPK-activated protein kinases 2 and 3

(MAPKAP2/3) and several transcription factors. Besides activation through cytokines, p38 also seems to be important for cytokine production (Widmann *et al.,* 1999).

The role of scaffold proteins in mammalian MAPK pathways is less clear, but potential mammalian MAPK–scaffold complexes have been described. The JNK-interacting protein 1 (JIP1) is known to interact with multiple components of the JNK-signaling pathway and is thought to function in a manner similar to STE5. Furthermore, MEKK1 might function as a scaffold protein in a PBS2-like way, because it is able to bind both MKK4 and JNK (Xu and Cobb, 1997; Whitmarsh and Davis, 1998). MP1 seems to function as a third class of scaffold proteins. It is able to bind MKK1 and ERK1 and facilitates the activation of ERK1 by MKK1 (Schaeffer *et al.,* 1998).

Despite an obvious lack of cross talk between yeast MAPK pathways, single receptors can activate multiple MAPK cascades in mammalian cells, and one substrate can be regulated by different MAPK pathways. Some ligands seem to activate one MAPK pathway while inhibiting another. Furthermore, other signaling pathways are present in cells and have the ability to interact (cross talk) with each other and with the MAPK cascades. This complex interplay between different signal transduction pathways gives mammalian cells the capacity to respond to extracellular stimuli in a sophisticated manner.

IV. MAPK Cascades in Plants: Structure and Phylogenetic Classification

A. MAPKs

To date, 28 full-length MAPK cDNAs have been isolated from a variety of plant species (Table I). A total of seven cDNAs were published from *Arabidopsis.* Among the other MAPKs are five from tobacco, five from alfalfa, two from maize, and single genes from apricot, oat, pea, parsley, petunia, sweet potato, and wheat. Besides the cDNAs, additional sequences of MAPK homologues can be found in the databases. Besides the seven *Arabidopsis* cDNAs in the database, six genomic and seven EST sequences encoding additional MAPK homologues can be found. Thus, the *Arabidopsis* MAPK family consists of at least 20 members. On the basis of an additional preliminary search for MAPK genes using several methods, it is postulated that the *Arabidopsis* genome may code for more than 30 different MAPK genes (Mizoguchi *et al.,* 1999).

The predicted amino acid sequences show the highest homology in the 11 kinase domains. The sequences outside the kinase domains often are

TABLE I
Summary of the Isolated Plant MAPK Sequences

Name	Species	Accession number	Number of amino acids	MW (kDa)	p*I* (pH units)	Subfamily	Reference
ATMPK1	*Arabidopsis thaliana*	D14713	370	42.7	6.8	PERKα5	Miz *et al.*, 1994
ATMPK2	*Arabidopsis thaliana*	D14714	376	43.1	6.1	PERKα5	Mizoguchi *et al.*, 1994
ATMPK3	*Arabidopsis thaliana*	D21839	370	42.7	5.5	PERKα2	Mizoguchi *et al.*, 1993
ATMPK4	*Arabidopsis thaliana*	D21840	376	42.9	5.7	PERKα3	Mizoguchi *et al.*, 1993
ATMPK5	*Arabidopsis thaliana*	D21841	376	43.1	5.4	PERKα3	Mizoguchi *et al.*, 1993
ATMPK6	*Arabidopsis thaliana*	D21842	395	45.1	5.2	PERKα1	Mizoguchi *et al.*, 1993
ATMPK7	*Arabidopsis thaliana*	D21843	368	42.4	6.8	PERKα5	Mizoguchi *et al.*, 1993
ATMPK8	*Arabidopsis thaliana*	Unpublished				PERKβ	Mizoguchi *et al.*, 1997
ATMPK9	*Arabidopsis thaliana*	Unpublished				PERKβ	Mizoguchi *et al.*, 1997
NtF3	*Nicotiana tabacum*	X69971	372	42.8	6.2	PERKα5	Wilson *et al.*, 1993
NtF4	*Nicotiana tabacum*	X83880	393	45.1	5.5	PERKα1	Wilson *et al.*, 1995
NtF6	*Nicotiana tabacum*	X83879	371	42.7	5.0	PERKα4	Wilson *et al.*, 1995
SIPK	*Nicotiana tabacum*	U94192	393	45.2	5.4	PERKα1	Zhang and Klessig, 1997
WIPK	*Nicotiana tabacum*	D61377	375	42.9	5.1	PERKα2	Seo *et al.*, 1995
MMK1	Medicago sativa	X66469	387	44.4	5.4	PERKα1	Jonak *et al.*, 1993; Duerr *et al.*, 1993
MMK2	*Medicago sativa*	X82268	371	42.3	6.1	PERKα3	Jonak *et al.*, 1995
MMK3	*Medicago sativa*	AJ224336	374	43.0	4.8	PERKα4	Bögre *et al.*, 1998
MMK4	*Medicago sativa*	X82270	371	43.0	5.6	PERKα2	Jonak *et al.*, 1996
MsTDY1	*Medicago sativa*	AF129087	608	68.9	9.8	PERKβ	Schoenbeck *et al.*, 1997
AsPK9	*Avena sativa*	S56638	369	42.9	5.4	?	Huttly and Phillips, 1995
lbMAPK	*Ipomoea batatas*	AF149424	365	41.7	5.5	PERKα2	Unpublished
ERMK	*Petroselinum crispum*	Y12785	371	42.8	5.7	PERKα2	Ligterink *et al.*, 1997
PhERK1	*Petunia x hybrida*	X83440	384	44.4	6.8	PERKα5	Decroocq-Ferrant *et al.*, 1995
PsD5	*Pisum sativum*	X70703	394	45.1	5.3	PERKα1	Stafstrom *et al.*, 1993
PaMAPK	*Prunus armeniaca*	AF134730	368	42.4	8.2	PERKα5	Unpublished
WCK1	*Triticum aestivum*	AF079318	369	42.8	5.3	?	Unpublished
ZmMPK4	*Zea mays*	AB016801	406	46.7	5.7	?	Unpublished
ZmMPK5	*Zea mays*	AB016802	399	44.9	5.2	PERKα1	Unpublished

more conserved in specific MAPKs from different species than in MAPKs of different subfamilies of the same species. This makes it likely that similar MAPKs from different species also perform similar functions, and evidence supporting this assumption exists for at least some MAPK groups, as will be discussed in Section V.

When compared with mammalian MAPKs, all plant MAPKs have highest homolgy to the ERK subfamily. Analysis of the plant MAPKs indicates that most of them cluster together in one group (PERKα). Only three plant MAPKs fall into a different group, denoted PERKβ. According to sequence similarity, at least five different subfamilies can be established within PERKα, named PERKα1–5 (Fig. 3 and Table I). The different PERK groups have distinct lengths of their activation loops. The activation loops are 25, 22, and 21 amino acids long for the PERKβ, PERKα5, and PERKα1–4 groups, respectively.

Almost all plant MAPKs isolated have the TEY motif as the dual-phosphorylation site. The only exceptions are AtMPK8 and -9 and the alfalfa MAPK TDY1, which have a TDY motif (Mizoguchi *et al.,* 1997; Schoenbeck *et al.,* 1997). So far, a TDY dual-phosphorylation motif has been found only in some MAPKs from protozoa (Kültz, 1998). Interestingly, these three plant MAPKs also have a long C-terminal extension (Mizoguchi *et al.,* 1999; Schoenbeck *et al.,* 1997). Analysis of genomic sequences and ESTs coding for MAPKs has shown that additional MAPKs for this new group exist (Ligterink, 1999).

B. MKKs

Despite the rapidly accumulating information about plant MAPKs, little is known about their upstream activators. At the moment, eight MKK cDNA clones have been isolated from plants. There are five MKKs from *Arabidopsis* and one each from tobacco, tomato, and maize (Table II). In contrast to the MAPKs and MKKKs, no genomic sequences for additional MKKs can be found in the databases.

The first plant MKK was reported in 1995 and was named NPK2 (for *Nicotiana tabacum* protein kinase 2). On the basis of hybridization data, sequences with strong similarity to NPK2 are also expected in the genomes of tomato, potato, Atropa belladonna, sweet potato, and *Arabidopsis* (Shibata *et al.,* 1995). In maize, at least two more MKKs highly related to ZmMEK1 are expected (Hardin and Wolniak, 1998), and rapeseed possesses at least three AtMAP2Kα homologues (Hamal *et al.,* 1999).

Similar to most MAPK cDNAs isolated, all isolated plant MKK cDNAs belong to one group when compared to MKK sequences from other species. Interestingly, the PMKK group can be clustered only with a *Dictyostelium*

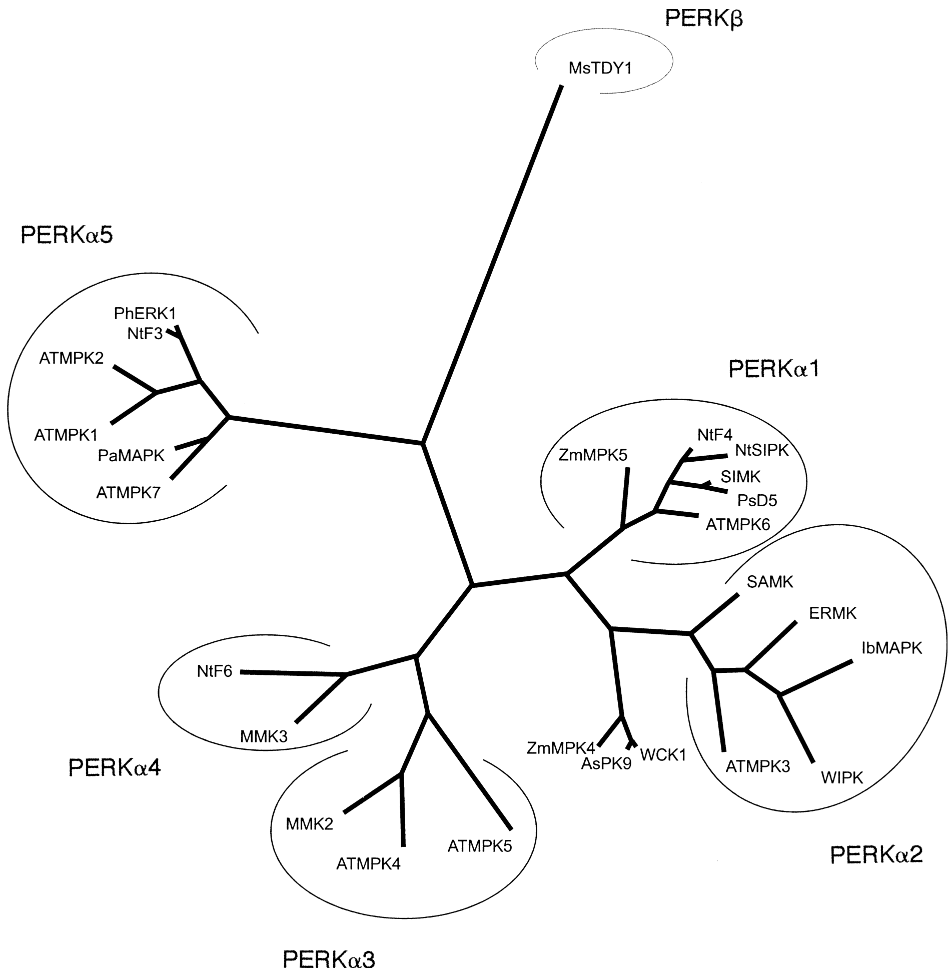


FIG. 3 Phylogenetic tree based on the amino acid sequences of plant MAPKs (Table I). This tree was reconstructed using the neighbor-joining method implemented in version 4.0b1 of PAUP* written by David L. Swofford. The six different subfamilies are denoted as PERKα1–5 and PERKβ.

MKK and not with animal MKKs. The PMKK group itself can be divided into three subfamilies (Table II, Fig. 4). Because no functional data exist for plant MKKs, it is not yet clear whether members within one subfamily have similar functions, but the high homology in the kinase-unrelated regions within each subfamily suggests some functional similarities. Homology to yeast and animal MKKs could be detected only in the conserved kinase domains, but not in the kinase-unrelated regions.

TABLE II

Summary of the Isolated Plant MKK Sequences

Name	Species	Accession number	Activation sites	Number of amino acids	MW (kDa)	p*I* (pH units)	Subfamily	Reference
AtMEK1	*Arabidopsis thaliana*	AF000977	TSSLANS	354	39.2	7.5	PMKK1	Morris *et al.*, 1997
AtMKK2/ AtMAP2Kβ	*Arabidopsis thaliana*	AB015313 AJ006871	TAGLANT	363	39.9	6.0	PMKK1	Ichimura *et al.*, 1998b; Hamal *et al.*, 1999
AtMKK3	*Arabidopsis thaliana*	AB015314	SMAMCAT	520	57.5	5.3	PMKK3	Ichimura *et al.*, 1998a
AtMKK4	*Arabidopsis thaliana*	AB015315	TMDPCNS	348	40.1	9.7	PMKK2	Ichimura *et al.*, 1998a
AtMKK5/ AtMAP2Kα	*Arabidopsis thaliana*	AB015316 Y07694	TMDPCNS	348	38.3	8.8	PMKK2	Ichimura *et al.*, 1998a; Jouannic *et al.*, 1996
NPK2	*Nicotiana tabecum*	D31964	SIAMCAT	518	57.5	5.3	PMKK3	Shibata *et al.*, 1995
LeMEK1	*Lycopersicon esculentum*	AJ000728	ISGLANT	357	39.7	5.5	PMKK1	Hackett *et al.*, 1998
ZmMEK1	*Zea mays*	U83625	SIGQRDT	355	39.9	5.4	PMKK1	Hardin and Wolniak, 1998

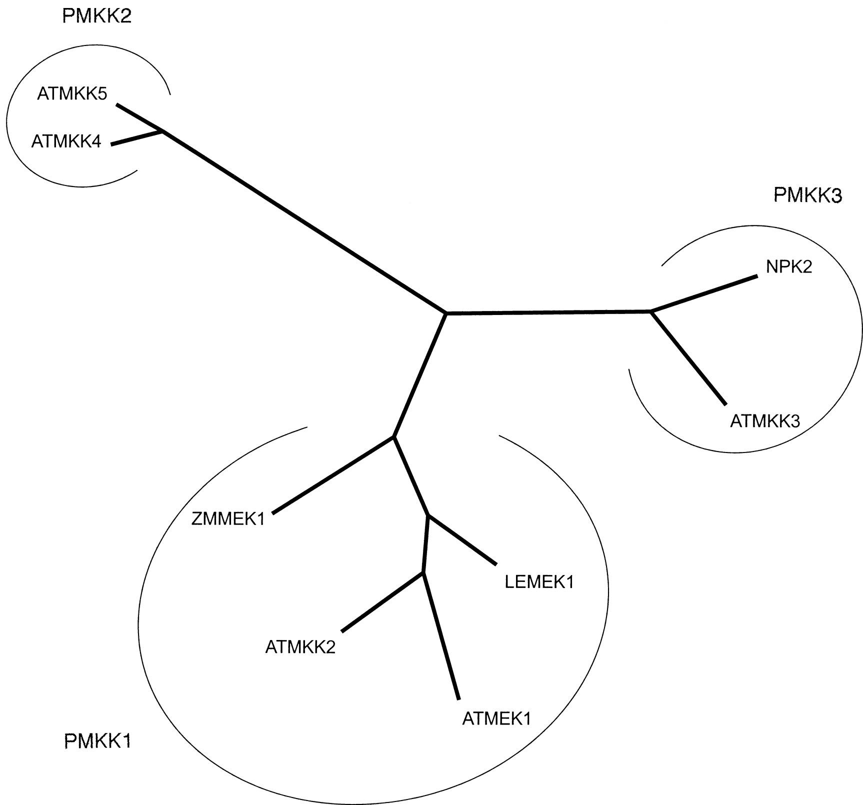


FIG. 4 Phylogenetic tree based on the amino acid sequences of plant MKKs (Table II). This tree was reconstructed using the neighbor-joining method implemented in version 4.0b1 of PAUP* written by David L. Swofford. The three plant MKK subfamilies are denoted as PMKK1–3.

Whereas most yeast and animal MKKs contain an $SXXX^{S}/_{T}$ motif as the phosphorylation site in the activation loop, all plant MKKs have an $^{S}/_{T}XXXXX^{S}/_{T}$ motif. Such a motif so far has been found only in MKKs from *Candida albicans* and *Dictyostelium discoideum.* However, it should be noted that there is no experimental evidence that the serine/threonine residues in this motif become phosphorylated in plant MKKs.

Some characteristic features for the different PMKK groups can be found. The PMKK1 group and the *Dictyostelium* DdMEK1 share a conserved 15-amino-acid motif in their amino-terminal kinase-unrelated domain, and it is to be expected that this motif has some specific, as yet unknown function. Members of the PMKK2 group possess two proline-rich sequences in the

amino- and carboxyl-terminal parts (Hamal *et al.,* 1999), which have been shown to be involved in the efficient activation of MAPKs in animal systems (Dang *et al.,* 1998) and contain putative SH3-binding motifs (PXXP; Cohen *et al.,* 1995). PMKK3 members are different from other plant MKK sequences in that they have long noncatalytic carboxyl-terminal regions.

C. MKKKs

Seventeen plant cDNAs with homology to MKKKs have been cloned to data (Table III). There is no direct evidence that these plant proteins also function as MKKKs in plants, but their homology to yeast and mammalian MKKKs and the ability of AtARAKIN and NPK1 to complement yeast *ste11* and *bck1* mutants, respectively, strongly suggests that some plant MKKKs also function as activators of MKKs *in vivo* (Covic and Lew, 1996; Banno *et al.,* 1993a). The isolated plant MKKKs seem to cluster mainly with the MEKK/STE11 and the Raf groups, named PMEKK and PRaf, respectively (Jouannic *et al.,* 1999b). The PMEKKs can be subdivided into three subfamilies and the PRaf group into two (Table III, Fig. 5a).

In the databases, at least 19 additional genomic sequences for MKKK homologues can be found, and several ESTs coding for putative MKKKs have been identified. There are also cDNAs that share weak homology with both Raf and mixed lineage kinases (MLK), making them candidates for an additional putative MKKK group in plants (Feng *et al.,* 1993; Tregear *et al.,* 1996; Ichimura *et al.,* 1997). Because MKKK sequences are very diverse, additional plant MKKK groups may still be discovered in the future. The considerable variability that can be observed in protein organization and noncatalytic sequences of the plant MKKKs suggests that, like in other systems, plant MKKKs have a wide variety of substrate specificities and regulatory mechanisms.

Whereas the PMEKK members do not have a general structure, both plant and animal proteins of the Raf family share the same structural organization, with a carboxyl-terminal catalytic domain and a long amino-terminal noncatalytic extension (Fig. 5b). Furthermore, weak homology can be observed in the noncatalytic domains of mammalian Raf and PRaf members, like serine- and cysteine-rich regions.

The largest group of plant MKKKs is formed by NPK1 and its homologues. Three *Arabidopsis* NPK1 homologues have been isolated (ANP1–3 for *Arabidopsis* NPK1-related protein kinase), but Southern blot analysis has shown that homologous genes are also present in maize and rice (Banno *et al.,* 1993a). *ANP1* transcripts exist in a long (ANP1L) and a short (ANP1S) version, which are probably the result of differential splicing. The activity of ANP1S is higher than that of ANP1L, and the differential splicing could be involved in the regulation of ANP1 activity (Nishihama *et al.,* 1997).

TABLE III

Summary of the Isolated Plant MKKK Sequences

Name	Species	Accession number	Number of amino acids	MW (kDa)	p*I* (pH units)	Group/subfamily	Reference
AtMEKK1	*Arabidopsis thaliana*	D50468	608	66.0	5.4	1/PMEKK2	Mizoguchi *et al.*, 1996
AtMAP3Kα	*Arabidopsis thaliana*	AJ010090	582	63.6	9.7	1/PMEKK3	Jouannic *et al.*, 1999
AtMAP3Kβ3	*Arabidopsis thaliana*	AJ010092	572	63.7	5.5	1/PMEKK2	Jouannic *et al.*, 1999
AtMAP3Kγ	*Arabidopsis thaliana*	Y14316				1/PMEKK3	Jouannic *et al.*, 1999
AtMAP3Kδ1	*Arabidopsis thaliana*	Y14199				2/PRaf1	Jouannic *et al.*, 1999
ANP1L	*Arabidopsis thaliana*	AB000796	661	72.8	5.2	1/PMEKK1	Nishihama *et al.*, 1997
ANP1S	*Arabidopsis thaliana*	AB000797	376	41.4	7.3	1/PMEKK1	Nishihama *et al.*, 1997
ANP2	*Arabidopsis thaliana*	AB000798	642	70.8	5.3	1/PMEKK1	Nishihama *et al.*, 1997
ANP3	*Arabidopsis thaliana*	AB000799	651	71.7	8.1	1/PMEKK1	Nishihama *et al.*, 1997
AtARAKIN	*Arabidopsis thaliana*	L43125				1/PMEKK2	Covic and Lew, 1996
CTR1	*Arabidopsis thaliana*	L08789	821	90.3	5.4	2/PRaf2	Kieber *et al.*, 1993
BnMAP3Kα1	*Brassica napus*	AJ010091	591	64.5	9.3	1/PMEKK3	Jouannic *et al.*, 1999
BnMAP3Kβ1	*Brassica napus*	AJ010093	575	62.6	5.9	1/PMEKK2	Jouannic *et al.*, 1999
TCTR1	*Lycopersicon esculentum*	AF110518	829	91.9	6.4	2/PRaf2	Unpublished
TCTR2	*Lycopersicon esculentum*	AJ005077	982	107.3	6.0	2/PRaf1	Lin *et al.*, 1998
Put MEKK	*Lycopersicon esculentum*	Y13273	829	91.9	6.0	2/PRaf2	Wang and Li 1997
NPK1	*Nicotiana tabacum*	D26601	690	76.2	7.9	1/PMEKK1	Banno *et al.*, 1993a

A similar regulatory mechanism has been demonstrated for mammalian MEKK1, whose activity is altered by proteolytic cleavage (Cardone *et al.,* 1997).

D. MAPK Cascades

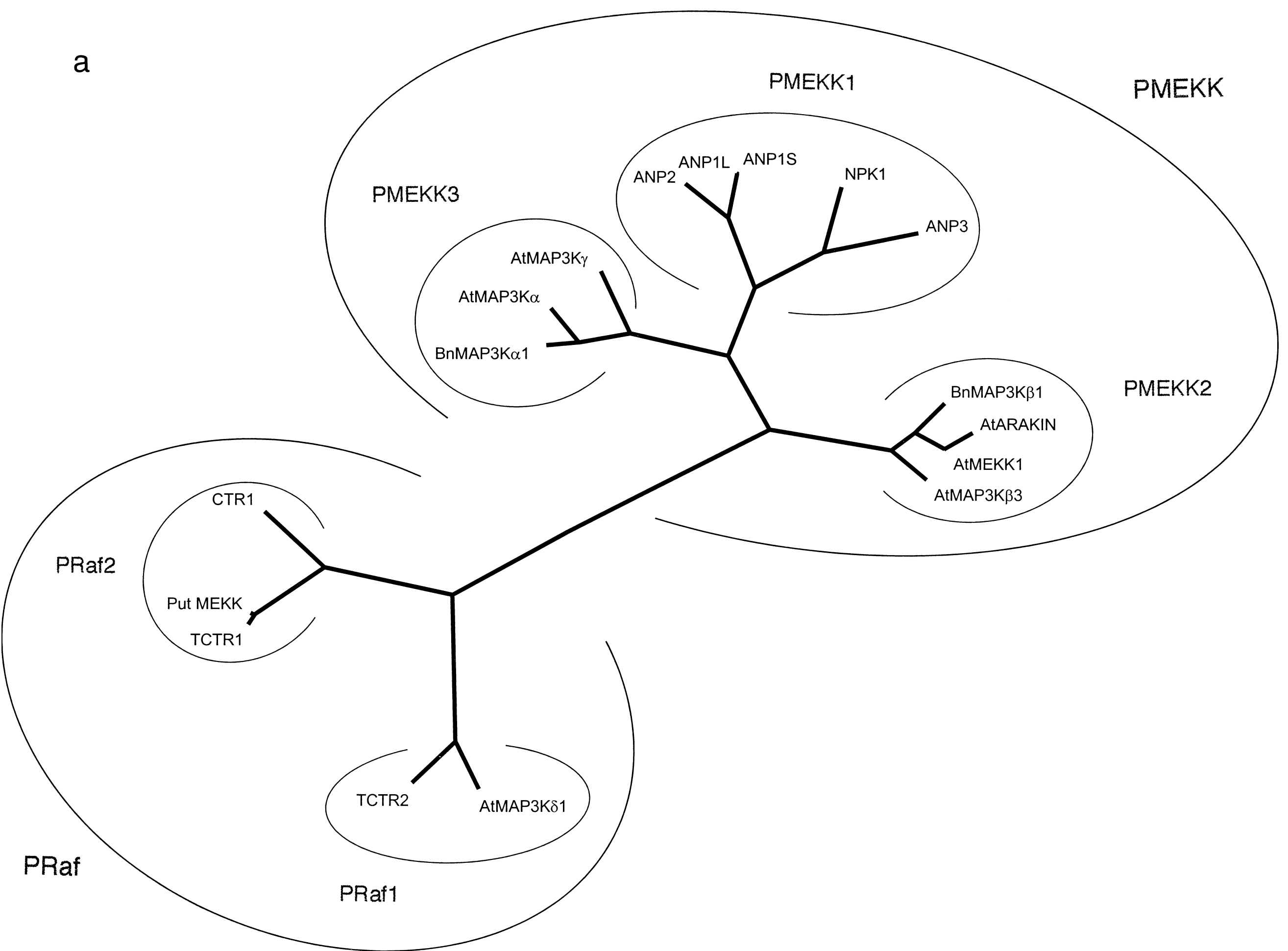
Various plant MAPKs, MKKs, and MKKKs have been identified, indicating that MAPK cascades like those known for yeast and animals could also exist in plants. However, no MAPK cascade has been established conclusively in plants. A possible MAPK cascade has been identified for *Arabidopsis* by two-hybrid assays and yeast complementation experiments.

By yeast two-hybrid assay, specific interaction between AtMPK4 and the MKKs AtMKK2 and AtMEK1, as well as the interaction of these MKKs with AtMEKK1, has been shown (Mizoguchi *et al.,* 1998; Ichimura *et al.,* 1998b). AtMPK4 is not able to complement the yeast *mpk1* mutant, but complementation could be observed when AtMPK4 was expressed together with AtMEK1 (Mizoguchi *et al.,* 1998). Similarly, AtMKK2 was able to partially complement the yeast *pbs2* mutant when expressed together with AtMEKK1, and expression of AtMKK2 and AtMEK1 with an N-terminal-truncated form of AtMEKK1 resulted in complementation in both cases. These results indicate that deletion of the N-terminus enhances the kinase activity of AtMEKK1 and that AtMEKK1 is able to activate AtMKK2 and AtMEK1 in yeast (Mizoguchi *et al.,* 1998; Ichimura *et al.,* 1998b). Therefore, AtMEKK1, AtMEK1, AtMKK2, and AtMPK4 could constitute a MAPK cascade, but there is no proof that these components also interact *in planta.* In contrast to the interaction data, expression data correlate AtMEKK1 with AtMPK3 (Mizoguchi *et al.,* 1996). This could indicate either that there is no functional meaning for their correlated expression or that AtMEKK1 activates the ATMPK3 pathway via a different, as yet unknown MKK.

Surprisingly, AtMEKK1 interacts not only with both MKKs but also with AtMPK4 (Ichimura *et al.,* 1998b). This could suggest a role for AtMEKK1 as a scaffold protein, as has been postulated for the rat MEKK1 (Whitmarsh and Davis, 1998).

E. Upstream of the MAPK Cascade

In yeast and mammals, several kinases have been isolated that act upstream of MKKKs. STE20 was the first MKKK kinase isolated, and a large number of STE20-related kinases have been identified in various organisms. In general, there are two MKKK kinase subfamilies. The STE20/PAK subfam-


a
PMEKK
PMEKK1
PMEKK2
PMEKK3
ANP3
NPK1
ANP1S
ANP1L
ANP2
BnMAP3Kβ1
AtARAKIN
AtMEKK1
AtMAP3Kβ3
AtMAP3Kγ
AtMAP3Kα
BnMAP3Kα1
AtMAP3Kδ1
TCTR2
PRaf1
CTR1
Put MEKK
TCTR1
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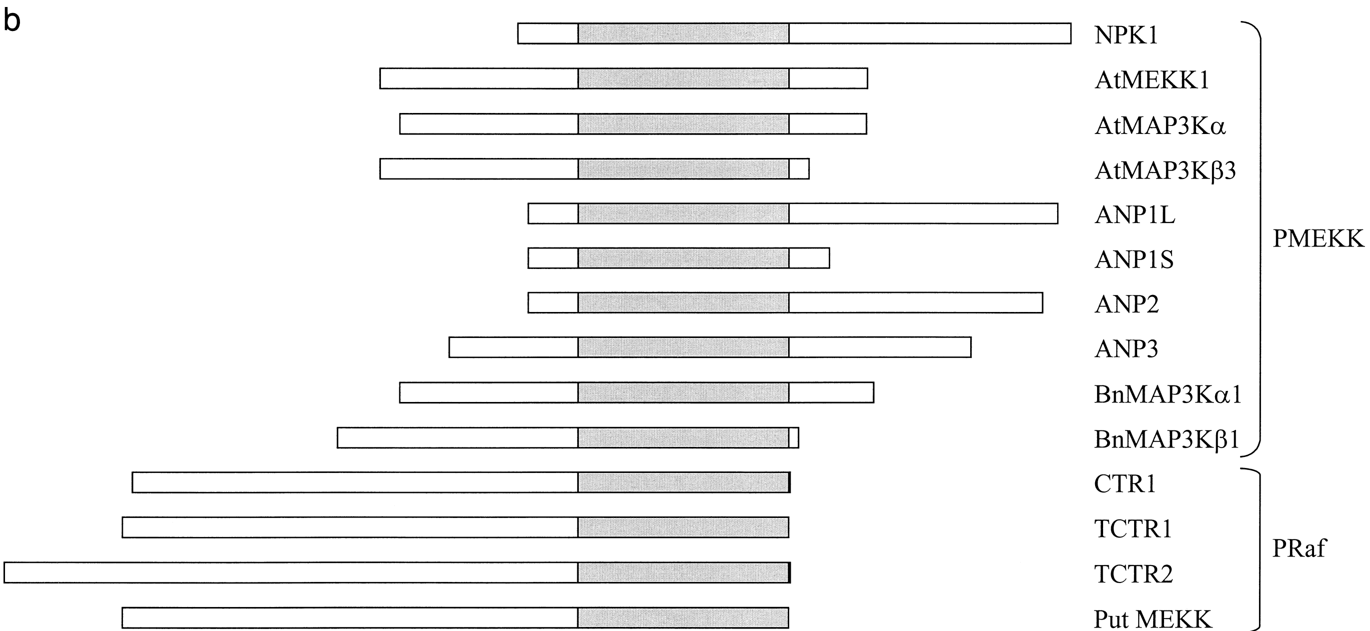

FIG. 5 (a) Phylogenetic tree based on the amino acid sequences of plant MKKKs (Table III). This tree was reconstructed using the neighbor-joining method implemented in version 4.0b1 of PAUP* written by David L. Swofford. The two plant MKKK subgroups are denoted as PMEKK and PRaf and the six different subfamilies as PMEKK1–4 and PRaf1–2. (b) Overview of the primary structures of plant MKKKs. Catalytic domains are indicated as gray boxes.

ily possesses a C-terminal catalytic domain and an N-terminal domain with putative G-protein-binding motifs. The second MKKK kinase subfamily is the GCK/SPS1 subfamily, which possesses an N-terminal catalytic domain and a long C-terminal kinase-unrelated region and is mostly activated by stress (Fanger *et al.,* 1997). Two putative MKKK kinases isolated from rapeseed, BnMAP4Kα1 and -2 (Leprince *et al.,* 1999), and the *Arabidopsis* SIK1 (for stress-induced kinase 1) (Accession number U96613) could be grouped into the GCK/SPS1 subfamily. However, several genomic sequences for plant MKKK kinases belonging to the STE20/PAK subfamily can also be found in the databases.

Other possible MKKK activators are PKC-like kinases. NPK1 probably can be activated by the yeast PKC1 (Banno *et al.,* 1993b), and this could indicate that a similar activation mechanism occurs in plants. PKC-like activities have been reported to be involved in elicitor-induced defense responses in plants (Xing *et al.,* 1996; Subramaniam *et al.,* 1997), but no plant PKC genes have been isolated to date.

Small G-proteins have an important role in the activation of mammalian MKKKs, and several small G-proteins have been isolated from plants (Bischoff *et al.,* 1999). To date, however, there is no direct evidence whether they play a role in activating plant MAPK cascades.

V. Functions of MAPK Pathways in Plants

A. Role of MAPK Pathways in Stress Signaling

Due to their sessile habit, plants are exposed to a wide variety of environmental stresses, and they have developed a broad range of responses to resist these stresses. A function for MAPK pathways has been implicated in the signal transduction for a broad variety of stress responses.

1. Mechanical Stress

Plants are exposed to different forms of mechanical stress like touch, wind, and rain. These stresses are known to induce several physiological responses in plants, the majority of which result in differential growth, reduction of growth rates, and thickening of the cell wall. The developmental changes in response to mechanostimulation collectively are named thigmomorphogenesis (Trewavas and Knight, 1994). Most of the responses serve to enhance resistance to subsequent mechanical stress.

In tobacco seedlings, touch induces an immediate increase of cytoplasmic calcium (Knight *et al.,* 1991), and several calmodulin and calmodulin-related

genes have been shown to be activated transcriptionally upon touch in *Arabidopsis* (Braam and Davis, 1990). Changes in cytoplasmic calcium and protein phosphorylation are among the earliest responses of plants to mechanical stress, and several reports indicate that MAPKs are responsible for phosphorylating at least some of the proteins. Mechanical stimulation of *Arabidopsis* leaves results in transcript accumulation of both MAPK and MKKK genes (*AtMPK3* and *AtMEKK1,* respectively) (Mizoguchi *et al.,* 1996). The transcriptional response is fast and transient, with transcript levels starting to accumulate at 5–10 min after touching and reaching a maximum at around 20 min. These results suggest a role for a MAPK pathway in signaling mechanical stimuli. Evidence for such a role was provided by experiments with alfalfa leaves. Touching of alfalfa leaves activated an alfalfa MAPK, named SAMK for stress-activated MAPK (previously called MMK4) (Bögre *et al.,* 1996). SAMK becomes activated within 1 min after mechanical stimulation, and activity disappears after 10 min. A constant SAMK activity could be seen in suspension-cultured alfalfa cells. This is probably due to constant mechanical stimulation of the cells in suspension. The SAMK activity was abolished after the cells were allowed to rest for 1 hr. SAMK activity was restored again when the cells were shaken for only 2 s (Bögre *et al.,* 1996). The fast activation of SAMK suggests that this MAPK pathway may be one of the cell's immediate responses to mechanical stimulation. Because SAMK and AtMPK3 are highly homologous, it is likely that AtMPK3 also may be activated at the posttranslational level and has a similar function in mechanical signaling. These results show that, like in yeast and animal systems, MAPKs are involved in mechanosignaling in plants, but their specific roles remain to be elucidated.

2. Wound Signaling

Among the most severe environmental stresses with which plants have to cope is wounding. Wounding can be the result of physical injury, herbivore, or pathogen attack and induces a wide range of responses, primarily encompassing the activation of genes involved in healing and defense. Wound healing is associated with the onset of genes involved in cell cycle and cell differentiation, whereas defense is mediated by the expression of genes encoding pathogen-response (PR) proteins and proteinase inhibitors (PIN). Proteinase inhibitors are proposed to protect plants against pests and herbivores by decreasing the digestibility and nutritional quality of leaf proteins (Bowles, 1993).

Whereas some wound-induced genes only are expressed locally at the site of attack, others, such as *PR* and *PIN* genes, are expressed systemically throughout the plant and protect it from attack at distant sites. The products of these genes are collectively named SWRPs for systemic wound-response

proteins (Bergey *et al.,* 1996). There are strong indications that the plant peptide systemin is a mediator of the systemic wound signal in tomato (Pearce *et al.,* 1991; Ryan and Pearce, 1998). However, other signals, including jasmonates (Seo *et al.,* 1997), and changes in hydraulic pressure (Malone, 1992) or electrical potential (Wildon *et al.,* 1992) are discussed as candidate systemic wound signals. The jasmonates jasmonic acid (JA) and its methyl ester (MeJA) have been implicated as important mediators of the wound signal, both at local and systemic sites (Seo *et al.,* 1997).

Compared to the systemic response to wounding, little is known about the primary sensing and signaling. Protein kinases have been implicated to have a role in primary wound signaling. The first suggestion for a role of protein kinases in wound signaling came from the observation that polygalacturonide, a local mediator of the wound response, induced phosphorylation of plasma membrane proteins of tomato (Farmer *et al.,* 1989). Since then, several reports have implicated MAPKs in signaling the wound response. In tobacco, a kinase activity of 46 kDa was detected after cutting of leaves (Usami *et al.,* 1995). This kinase activity appeared as early as 1 min after wounding, reached its maximum at 2–5 min, and decreased to basal levels within 30 min. The active form of the 46-kDa kinase was phosphorylated on both serine and/or threonine and tyrosine residue(s), and dephosphorylation inactivated the kinase. These phosphorylation properties, together with the size of approximately 46 kDa and the ability to use myelin basic potein (MBP) as an artificial substrate, strongly suggested that this kinase was a MAPK. A similar wound-inducible kinase activity was detected in a broad range of plant species, including both monocots and dicots (Usami *et al.,* 1995).

It was shown that, in tobacco leaves, transcripts of a MAPK gene accumulate within 1 min after wounding (Seo *et al.,* 1995). The corresponding MAPK was termed WIPK for wound-induced protein kinase. Accumulation of *WIPK* transcripts was not limited to the wounded leaves, but was also observed in adjacent and distant unwounded leaves at approximately the same level. These results showed that the *WIPK* gene is expressed systemically upon wounding, as it is known for many different wound-inducible genes. The fast kinetics of *WIPK* transcript accumulation suggested that WIPK could be one of the earliest responses to the wound stress.

A study showed that *WIPK* transcript accumulation upon wounding is accompanied by an increase in WIPK activity (Seo *et al.,* 1999). By using a WIPK-specific antibody, it was shown that WIPK is activated within 3 min after wounding and that activity returns to basal levels after 30 min. WIPK activation also occurred in adjacent unwounded leaves with similar kinetics, although this activation was less pronounced. By using the same antibody, it could be shown that WIPK protein levels do not change upon wounding (Seo *et al.,* 1999).

The alfalfa SAMK, which is very similar to the tobacco WIPK, also becomes activated upon wounding (Bögre *et al.,* 1997). Activation of SAMK can already be detected 1 min after wounding of alfalfa leaves and has kinetics similar to that of WIPK activation. It was shown that activation of SAMK is a posttranslational process, because α-amanitin and cycloheximide do not block the activation. However, these drugs did block SAMK inactivation, indicating that *de novo* transcription and translation of protein factors are required for inactivation of SAMK. An alfalfa PP2C-like phosphatase was isolated that was shown to be this or another of these factors (Meskiene *et al.,* 1998a), as discussed in Section VI.

Similar to WIPK in tobacco, *SAMK* mRNA levels increase upon wounding, although transcripts of the *SAMK* gene accumulate with different kinetics, starting 20 min after wounding. The accumulation of *SAMK* transcripts at a later point in time than SAMK activation suggests that the *SAMK* gene may be a direct target of the SAMK pathway. At the moment, the reason why transcript accumulation is not accompanied by an increase in SAMK protein levels is unclear, but could be explained by a more rapid turnover rate of SAMK protein after activation. No evidence is available yet for such a model, and further experiments are required to clarify this point.

Besides wound-induced transcriptional and posttranslational activation of WIPK, another tobacco MAPK has been shown to be activated posttranslationally by wounding. This 48-kDa MAPK, termed SIPK for salicylic acid-induced protein kinase, was shown to be activated by wounding with kinetics similar to that of WIPK (Zhang and Klessig, 1998a). Unlike WIPK, *SIPK* transcripts did not accumulate upon wounding.

There is also evidence of a role for MAPKs in the wound response of tomato. Wounding of tomato leaves was shown to activate an MBP kinase of 48 kDa that was also found to be activated at lower levels in adjacent unwounded leaves (Stratmann and Ryan, 1997). This kinase was phosphorylated on tyrosine residues with similar kinetics as the activity increased upon wounding, strongly suggesting it to be a MAPK. Activation of this kinase also was observed when the leaves were exposed to known secondary messengers of the wound signal, like systemin, polygalacturonic acid, and chitosan (Stratmann and Ryan, 1997). It was shown that activation of the kinase and other defense responses by systemin could be suppressed by simultaneous addition of the N-terminal part of systemin, supporting the idea that the N-terminus of systemin is involved in receptor binding, whereby the C-terminal part plays a role in the subsequent activation of the MAPK pathway (Meindl *et al.,* 1998). In alfalfa, it was shown that other proposed mediators of the wound signal, like MeJA and electrical signals, were not able to activate SAMK (Bögre *et al.,* 1997).

Activation data have provided information about the involvement of MAPK pathways in the wound response and have helped researchers study

some of the upstream components of this pathway. To elucidate the specific role of MAPKs and possible downstream targets, overexpression of sense-oriented WIPK cDNA was used in tobacco. In some transformants that were selected for high transgene expression, endogenous *WIPK* gene expression was silenced (Seo *et al.,* 1995). These transgenic plants showed a constitutive low level of MBP kinase activity, and wounding could not increase this activity. Wounding of leaves of the transgenic plants did not result in accumulation of the wound- and JA-inducible *PI-2* and basic *PR-1* genes. Moreover, no increase in JA and MeJA levels could be observed. In contrast, transgenic plants showed increased levels of salicylic acid and transcripts for acidic PR-1 and PR-2 proteins, responses that normally occur only after pathogen attack, but not wounding. A similar phenotype was found in transgenic plants overexpressing a small GTP-binding *rab*-related protein, and these authors showed that MeJA and SA accumulations antagonize each other (Sano *et al.,* 1994, 1996). Thus, the different expression patterns in transgenic WIPK plants can be explained by suppressed JA and MeJA synthesis and thereby release of the otherwise repressed SA synthesis.

Extending these studies, Seo and colleagues (1999) also searched the WIPK-overproducing transformants for constitutive accumulation of *PI-2* gene transcripts. These transgenic plants consistently showed higher transgene *WIPK* transcript and WIPK activity levels. JA and MeJA amounts of the unwounded transgenic plants were 3- to 4-fold higher than in the wild-type plants. These results clearly demonstrated a role for WIPK in the production of jasmonates and their subsequent activation of wound-induced genes.

The mechanism by which WIPK induces the accumulation of JA and MeJA is not known, but it has been proposed that this could occur by a mechanism similar to that of prostaglandin production in mammals (Bergey *et al.,* 1996). Prostaglandins are signaling molecules in the inflammatory stress response in mammals, and the chemical structure of prostaglandins is similar to that of JA. Inflammatory stress in mammals is signaled by the activation of MAPKs that phosphorylate and thereby activate cytoplasmic phospholipase A_2 ($cPLA_2$) (Lin *et al.,* 1993). $cPLA_2$ cleaves phospholipids to release arachidonic acid, which is then further converted to prostaglandins. By analogy to mammalian prostaglandin synthesis, one of the substrates of WIPK could be a $cPLA_2$-like enzyme. Although no plant PLAs have yet been isolated, increases in PLA activity have been observed after wounding and elicitor treatment (Lee *et al.,* 1997b; Chandra *et al.,* 1996).

A role for MAPKs acting upstream of the octadecanoid pathway also agrees with the results of Stratmann and Ryan (1997). Wound-induced activation of the MAPK-like kinase in tomato was still possible in *def1,* a wound-signaling-deficient tomato mutant. *def1* has a mutation in the

octadecanoid pathway and is effectively blocked in the wound-induced synthesis of JA (Howe *et al.,* 1996). Because products of the octadecanoid pathway like JA and PDA were able to induce several wound-expressed genes, but not the kinase (Stratmann and Ryan, 1997), the MAPK is upstream of the octadecanoid pathway. The inability of JA to activate the wound-inducible SAMK in alfalfa supports these results (Bögre *et al.,* 1997). Overall, these data show that the MAPK cascade functions to activate the octadecanoid pathway.

Besides MAPKs, the *Arabidopsis* MKK AtMEK1 and its tomato homologue LeMEK1 have also been suggested to play a role in wound response (Morris *et al.,* 1997; Hackett *et al.,* 1998). Transcript levels of *AtMEK1* increased remarkably after wounding of *Arabidopsis* leaves. Transcriptional activation was observed only in wounded leaves, starting to accumulate 6 hr after wounding, which is markedly slower than the transcriptional activation of wound-induced MAPKs. Although no protein or activation data are available for AtMEK1, the local and late transcriptional activation of *AtMEK1* makes it unlikely that AtMEK1 is the MKK upstream of the wound-induced kinases. It might be possible that AtMEK1 has no direct role in wound signaling, but instead functions in the reentry of cells into the cell cycle. Such a role would fit with AtMEK1's expression in meristematic regions (Morris *et al.,* 1997).

In general, it can be concluded that MAPK pathways play an important role in the wound response of plants, and a preliminary model for its action can be made in which a MAPK pathway is activated by wounding via secondary messengers of the wound response, like systemin. Subsequently, the MAPK induces the production of JA and MeJA, which will activate a broad set of defense responses (Fig. 6).

3. Abiotic Stress Signaling

Plants have to cope with extreme changes in the abiotic environmental conditions. These abiotic stresses include high and low temperature, drought, and osmotic stress. The possible role of MAPK pathways in translating these stresses into cellular responses will be discussed next.

a. Drought Stress *AtMEKK1* and *AtMPK3* are activated transcriptionally not only by mechanical stimulation but also by dehydration (Mizoguchi *et al.,* 1996). The mRNA of the *AtPK19* gene, a ribosomal S6 kinase homologue, accumulated simultaneously under the same stress conditions (Mizoguchi *et al.,* 1996). Ribosomal S6 kinases have been shown to be phosphorylated and activated by MAPKs in several mammalian systems (Sturgill *et al.,* 1988; Gregory *et al.,* 1989; L'Allemain *et al.,* 1991; Sadoshima and Izumo, 1993), and these results could indicate the existence of a similar pathway

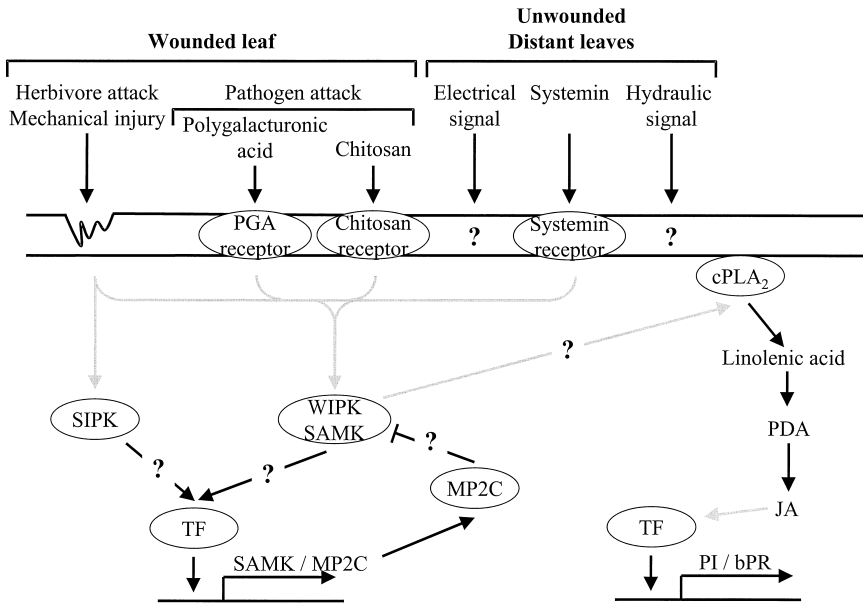

FIG. 6 Schematic illustration of the wound-response pathways in which MAPKs are involved. Question marks indicate uncertain connections or unknown components. Black arrows indicate direct interaction/activation, whereas gray arrows indicate indirect connections.

in *Arabidopsis,* whereby AtPK19 could be phosphorylated and thereby activated by AtMPK3.

In accordance with a role for AtMPK3 during dehydration, the closely related alfalfa homologue *SAMK* is also transcriptionally upregulated upon drought stress (Jonak *et al.,* 1996). Besides transcriptional induction of the gene, SAMK is activated at the posttranslational level. However, in contrast to mRNA and SAMK activity levels, no changes in SAMK protein levels were observed upon dehydration (Jonak *et al.,* 1996).

b. Low-Temperature Stress Low-temperature stress has been shown to have a strong effect on the phosphorylation status of several plant proteins. An important role of protein kinases in mediating low-temperature responses is emphasized by the finding that kinase inhibitors are able to markedly inhibit cold-induced freezing tolerance (Monroy *et al.,* 1993). The finding that protein phosphatase 2A (PP2A) activity is downregulated upon cold stress (Monroy *et al.,* 1998) underscores the importance of protein kinases in cold signaling.

AtMEKK1, AtMPK3, AtPK19, and SAMK react to cold stress in the same way as to dehydration, and all show transcriptional upregulation (Mizoguchi *et al.,* 1996; Jonak *et al.,* 1996). Also the *Arabidopsis* MKKK, *AtMAP3Kβ3,* showed cold-induced transcript accumulation (Jouannic *et al.,* 1999a). In contrast, the transcript levels of the maize MKK, *ZmMEK1,* were reduced substantially after cold treatment of roots (Hardin and Wolniak, 1998).

c. High-Temperature Stress It has been shown that heat stress is able to inactivate a 50-kDa protein kinase in tomato-suspension-cultured cells (Heider *et al.,* 1998). The ability of this kinase to phosphorylate MBP, together with its molecular mass, could be an indication that this kinase is a member of the MAPK family. Because transcriptional activation of heat shock genes by the human transcription factor Hsf1 is repressed by phosphorylation of Hsf1 by ERK1 (Chu *et al.,* 1996), the 50-kDa protein kinase could play a similar role in silencing heat-inducible genes in unstressed cells.

d. Osmotic Stress In the halotolerant green alga *Dunaliella tertiolecta,* protein kinases have an important role in conferring resistance to changes in external osmolarity. Hypo-osmotic stress induced a kinase of 40 kDa that could phosphorylate MBP and histone. In the same system, hyperosmotic stress induced, among other things, two kinases of 40 and 45 kDa, respectively (Yuasa and Muto, 1996). The 40-kDa kinase could use MBP, histone, and casein as a substrate, whereas the 45-kDa kinase could only phosphorylate MBP. The activity of the kinases could be shown to be dependent on their phosphorylation state. All three kinases were active in the absence of Ca^{2+}, proving that they are not CDPKs. The kinases could be MAPKs, although MAPKs generally are not able to use casein as a substrate. The use of histone as a substrate by plant MAPKs has been reported (Wilson *et al.,* 1995; Zhang and Klessig, 1997), suggesting that hypo-osmotic stress activates a 40-kDa MAPK and that hyperosmotic stress activates a 45-kDa MAPK. However, an argument against the possibility that the 40- and 45-kDa protein kinases are MAPKs was the inability to detect tyrosine phosphorylation of the proteins with an antiphosphotyrosine antibody (Yuasa and Muto, 1996), leaving open the possibility that the kinases are not MAPKs.

D. tertiolecta cells change volume if exposed to changes in external osmolarity, but the original volume is recovered within 30 min after treatment. Volume recovery was blocked by the kinase inhibitor K-252a, which also inhibited the hypo- and hyperosmotic stress-induced protein kinase activities, suggesting that these kinases are involved in mediating the osmotic tolerance of *D. tertiolecta* (Yuasa and Muto, 1996).

There are also indications of a role for MAPKs in the signaling of osmotic stress in higher plants. Hyperosmotic stress was able to activate MAPK-like kinases in tobacco (Usami *et al.,* 1997; Tena and Renaudin, 1998). Exposure of tobacco-suspension-cultured cells to hypo-osmotic stress was shown to activate three MBP kinases with sizes 50, 70, and 80 kDa (Takahashi *et al.,* 1997). The 50-kDa protein kinase has all the properties of a MAPK. It uses MBP as a substrate and becomes tyrosine-phosphorylated upon activation. Protein kinase and phosphatase inhibitor studies suggested that activation and inactivation of the kinases are regulated by phosphorylation and dephosphorylation events, respectively (Takahashi *et al.,* 1997, 1998). It was shown that an increase in cytosolic Ca^{2+} is a prerequisite for the activation of the kinases and that the G-protein activator mastoporan is able to activate the same three kinases (Takahashi *et al.,* 1998). These results suggest a role for G-proteins, Ca^{2+} influx, and protein kinases in mediating hypo-osmotic stress in tobacco.

In yeast, hyperosmotic stress is sensed by so-called osmosensors. One hyperosmotic sensor in yeast is a two-component regulatory system, a well-known system of signal transduction pathways in prokaryotes. Two-component systems normally consist of a histidine kinase, which functions as a sensory kinase and a response-regulator protein. After autophosphorylation on a histidine residue, the sensor kinase transfers the phosphate group to a response-regulator protein, thereby activating the latter (Wurgler-Murphy and Saito, 1997; Chang and Stewart, 1998). In the yeast hyperosmotic signaling pathway, the two-component regulatory system consists of a phosphorelay between three proteins. The yeast osmosensor SLN1 is a fused two-component system that autophosphorylates on histidine in the N-terminal sensor domain and then transfers the phosphate group to an aspartate residue in the C-terminal-located response-regulator domain. The phosphate is then transferred further to YPD1, which functions as a second histidine phosphorelay intermediate between SLN1 and the response regulator SSK1. SSK1 finally feeds into the HOG1 MAPK pathway by interaction with the MKKKs SSK2 and SSK22 (Posas *et al.,* 1996). A similar osmosensing system could also exist in plants, because the *Arabidopsis* SLN1 homologue AtHK1 can function as an osmosensor in yeast and can complement SLN1-deficient yeast cells (Shinozaki and Yamaguchi-Shinozaki, 1997).

In yeast the hyperosmotic stress response is mediated through the HOG1 MAPK pathway. HOG1-deficient yeast cells are unable to survive under salt stress, demonstrating that the HOG1 pathway is essential for stress adaptation under hyperosmotic conditions. The pea PsD5 MAPK was able to complement a HOG1-deficient yeast mutant (Pöpping *et al.,* 1996). In accordance with these results, it was assumed that PsD5 may have a role in hyperosmotic stress signaling in pea. There is, however, no evidence for

such a role, and studies on the alfalfa MAPK MMK2 showed that care must be taken in using functional yeast complementation data to assign functions to plant MAPKs. The alfalfa MMK2 MAPK is specifically able to complement the yeast MPK1 kinase, which has a function in hypo-osmotic and heat-stress signaling (Jonak *et al.,* 1995). Furthermore, MMK2, but not three other alfalfa MAPKs, was able to phosphorylate a 39-kDa protein that is part of or tightly associated with the cytoskeleton of carrot cells (Jonak *et al.,* 1995). Cytoskeletal-associated proteins have been shown to be the targets of several MAPKs (Ray and Sturgill, 1987), and several lines of evidence suggest a role for cytoskeletal components in the activation of the MPK1 pathway (Kamada *et al.,* 1995). These results could suggest a similar role of MMK2 in the hypo-osmotic and heat-stress response in alfalfa, but no evidence could be found for this assumption (Jonak *et al.,* 1996).

Interestingly, some stress-activated MAPKs like AtMPK3 and SAMK seem to be involved in mediating multiple abiotic stresses. Both genes become transcriptionally activated upon touch, drought, and cold stress, and *AtMPK3* also shows transcriptional upregulation upon salt stress (Mizoguchi *et al.,* 1996). Although these stresses appear very different at first sight, drought, cold, and high-salt stress all result in dehydration. Therefore, the AtMEKK1/AtMPK3 and SAMK pathways could function in signaling general dehydration.

4. Pathogen Response

Plants are threatened by a wide variety of pathogens and have developed many ways to protect themselves against them. Plants are protected by chemical and physical barriers and also have defenses that are induced upon pathogen attack, like reinforcement of cell walls, production of phytoalexins, and transcriptional activation of defense genes (Hammond-Kosack and Jones, 1996; Yang *et al.,* 1997; Somssich and Hahlbrock, 1998). Induction of defense responses is mostly a result of the interaction between pathogen-produced signals (elicitors) and plant receptors. Elicitors can be involved in the induction of race-specific incompatible (gene-for-gene interactions), species-specific compatible, or nonhost incompatible responses (Ebel and Mithöfer, 1998). An important feature of plant defense responses is the hypersensitive response (HR), which can be recognized by localized host cell death at the infection sites (Dangl *et al.,* 1996; He, 1996). HR is often associated with the onset of a broad-spectrum, long-lasting systemic resistance to subsequent infections, called systemic acquired resistance (SAR) (Ryals *et al.,* 1996).

Extensive research has been performed to elucidate the components of the signaling pathways that lead to these defense responses, and G-proteins,

ion flux, calcium homeostasis, and the formation of reactive oxygen species are thought to play imporant roles (Blumwald *et al.,* 1998; Yang *et al.,* 1997).

A number of reports have demonstrated an essential role for phosphorylation and dephosphorylation events in the activation of defense genes and the hypersensitive response. Elicitor treatment of plant cells resulted in changes in phosphoprotein profiles (Dietrich *et al.,* 1990; Felix *et al.,* 1991; Grab *et al.,* 1989), and inhibition of certain defense responses were observed by applying inhibitors of both protein kinases (Felix *et al.,* 1991; Grosskopf *et al.,* 1990; Schwacke and Hager, 1992; Viard *et al.,* 1994) and protein phosphatases (Dunigan and Madlener, 1995; Conrath *et al.,* 1997). In some cases, phosphatase inhibitors were also able to activate defense responses on their own (Chandra and Low, 1995; Felix *et al.,* 1994; Levine *et al.,* 1994; MacKintosh *et al.,* 1994). Furthermore, several groups have reported the elicitor-induced phosphorylation and thereby activation of transcription factors (Dröge-Laser *et al.,* 1997; Stange *et al.,* 1997; Subramaniam *et al.,* 1997).

The first indication of a possible function of MAPKs in the pathogen response of plants was provided by Suzuki and Shinshi (1995). Treatment of tobacco-suspension-cultured cells with a fungal elicitor, derived from the cell walls of *Phytophthora infestans* (PiE), resulted in fast and transient activation of a 47-kDa MAPK-like kinase, and staurosporine was able to block both elicitor-induced MAPK activity (Suzuki and Shinshi, 1995) and defense gene accumulation (Suzuki *et al.,* 1995).

Direct proof of the involvement of MAPKs in pathogen response came from studies of parsley-suspension-cultured cells treated with the elicitor Pep13. Most responses of parsley cells to the fungal pathogen *Phytophthora sojae* are mimicked by this elicitor, which is a 13 amino acid long oligopeptide fragment derived from a 42-kDa extracellular glycoprotein from *P. sojae*. Treatment of parsley-suspension-cultured cells with Pep13 results in binding of Pep13 to a specific plasma membrane receptor and subsequent activation of plant defense responses, such as phytoalexin synthesis and activation of defense genes (Nürnberger *et al.,* 1994). Ion flux and the oxidative burst were shown to be involved in the signal transduction pathway leading from the Pep13 receptor to the defense responses (Jabs *et al.,* 1997). A MAPK was found to become activated at both the posttranslational as well as the transcriptional level after elicitor treatment of parsley cells, and it could be shown that this activation was the result of the binding of Pep13 to its specific receptor (Ligterink *et al.,* 1997). Moreover, inhibitor studies could place the MAPK downstream of elicitor-induced ion channel activation and upstream of or parallel to the oxidative burst. Similar to many mammalian and yeast MAPKs (Chen *et al.,* 1992; Sanghera *et al.,* 1992; Ferrigno *et al.,* 1998), elicitor-induced activation of the parsley MAPK resulted in translocation to the nucleus. MAPKs phosphorylate transcrip-

tion factors in the nucleus and thereby regulate gene transcription (Treisman, 1996). The parsley MAPK may play a similar role and could be the link between cytosolic signal transduction and nuclear activation of defense genes.

The tobacco SIPK was isolated because of its transient activation upon treatment of tobacco-suspension-cultured cells with salicylic acid (SA) (Zhang and Klessig, 1997). Salicylic acid is known to play an important role in signaling pathogens and is able to activate several plant defense responses (Dürner *et al.,* 1997). SIPK also is activated by treatment of the cells with different elicitors, like parasiticein, cryptogein, and a cell-wall-derived carbohydrate elicitor from the pathogenic fungus *Phytophthora parasitica,* but in these cases its activation is more prolonged (Zhang *et al.,* 1998). Besides activating SIPK, parasiticein and cryptogein also are able to activate WIPK and a 40-kDa MBP kinase (Zhang and Klessig, 1998b; Zhang *et al.,* 1998). WIPK, but not the 40-kDa kinase, was activated by the fungal cell wall elicitor, but activation occurred to a much lesser extent (Zhang *et al.,* 1998). Cryptogein had already been shown to induce protein phosphorylation in tobacco cells (Viard *et al.,* 1994), and Lebrun-Garcia *et al.,* (1998) showed the activation of two MAPKs with molecular masses of 50 and 46 kDa, which are likely to correspond to SIPK and WIPK, respectively.

Besides activation by fungal elicitor and SA, SIPK also becomes activated upon treatment of tobacco plants with tobacco mosaic virus (TMV) in an *N*-gene-dependent manner (Zhang and Klessig, 1998b). Besides SIPK, WIPK is activated by this treatment with similar kinetics. Similar to the wounding response of WIPK (Seo *et al.,* 1995), TMV treatment resulted in elevated *WIPK* transcript levels. In contrast to the wound system, WIPK protein levels increased upon TMV treatment and transcript accumulation preceded the increase in WIPK protein amounts, which in turn preceded the activation of WIPK (Zhang and Klessig, 1998b). These results suggest that activation of WIPK by TMV requires not only posttranslational phosphorylation but also *de novo* transcription and translation. WIPK activation is independent of SA, because TMV-induced WIPK activation was not altered in a transgenic tobacco line expressing the *NahG* gene, in which SA-mediated defense responses are blocked (Gaffney *et al.,* 1993; Zhang and Klessig, 1998b).

Experiments with a tobacco cell line expressing the tomato Cf-9 resistance gene elicited with the corresponding avr protein Avr9 from the fungal pathogen *Cladosporium fulvum* suggest that MAPKs also play an important role in signaling gene-for-gene interaction-dependent defense responses (Romeis *et al.,* 1999). In this system, both SIPK and WIPK become activated upon Avr9 treatment in a Cf-9-dependent manner. *WIPK* also is upregu-

lated transcriptionally, but unlike the TMV-induced WIPK activation, no accumulation of WIPK protein is observed (Romeis *et al.,* 1999).

Not only are fungal elicitors or viruses able to activate MAPKs, but harpins from the bacteria *Erwinia amylovora* and *Pseudomonas syringae* pv. *syringae* also were able to activate a MAPK-like kinase in tobacco leaves (Ádám *et al.,* 1997). Data suggest that this kinase is identical to SIPK (Hoyos *et al.,* 1998).

Besides pathogen-induced MAPK activation in tobacco and parsley, a MAPK-like activity was detected in soybean cells treated with a β-glucan elicitor isolated from the pathogenic fungus *Phytophthora sojae* (Ebel and Mithöfer, 1998).

Elicitors and pathogens that are able to activate WIPK, such as cryptogein and parasiticein (Zhang *et al.,* 1998), the harpin-producing bacterial pathogen *Pseudomonas syringae* pv. *syringae,* and TMV (Zhang and Klessig, 1998b) all induce HR-like host hypersensitive cell death (hcd). This could indicate a role for MAPKs in hcd, similar to mammalian cells, where the SAPK/JNK and p38 subfamilies of MAPKs play an important role in stress-induced apoptosis (Ichijo *et al.,* 1997; Schwenger *et al.,* 1997; Verheij *et al.,* 1996). Because kinase inhibitors are able to block hcd, protein kinases are definitely involved in the signaling pathway leading to hcd (Levine *et al.,* 1994; He *et al.,* 1994). Suzuki and co-workers (1999) gave additional correlative data for a role for MAPKs in hcd. Treatment of tobacco-suspension-cultured cells with the fungal elicitor xylanase from *Trichoderma viride* (TvX) was able to activate both hcd and a 47-kDa MAPK (Suzuki *et al.,* 1999). The kinase was activated by TVX to the same level as after treatment with the *P. infestans* elicitor PiE (Suzuki and Shinshi, 1995), but with more prolonged kinetics. Interestingly, only TvX is able to induce hypersensitive cell death (Yano *et al.,* 1998). Similar to the role of JNK in apoptosis of mammalian cells (Chen *et al.,* 1996), sustained activation of the 47-kDa MAPK may trigger hypersensitive cell death in plants. In accordance with such a role, it was shown that the phosphatase inhibitor calyculin A can both induce prolonged activation of the 47-kDa MAPK and hypersensitive cell death. Moreover, staurosporine can selectively block PiE-induced activation of the kinase and the induction of defense gene accumulation by both PiE and TvX elicitor without affecting TvX-induced MAPK activation and hcd (Suzuki and Shinshi, 1995; Suzuki *et al.,* 1995, 1999).

In general, MAPK activation has been shown for a large number of plant–pathogen interactions, during both race-specific and nonhost resistance responses. However, little is known about the other components of the elicitor-induced MAPK pathways. Several groups have tried to address this question by the use of different inhibitors and activators of known components of the signaling pathways in plant–pathogen interactions. From these results, it can be concluded that MAPK cascades in most cases func-

tion downstream of a Ca^{2+} influx. It is known that Ca^{2+} plays an important role in many signaling pathways (Bush, 1995), and an important role for Ca^{2+} in the pathogen response of plants has been proposed by different groups (Dietrich *et al.,* 1990; Viard *et al.,* 1994; Nürnberger *et al.,* 1994; Zimmerman *et al.,* 1997). Although an influx of extracellular Ca^{2+} appears to be necessary, it is not sufficient for elicitor-induced MAPK activation in several systems (Suzuki and Shinshi, 1995; Ádám *et al.,* 1997; Lebrun-Garcia *et al.,* 1998; Romeis *et al.,* 1999). Two reports showed that MAPKs act upstream or independent of the oxidative burst (Ligterink *et al.,* 1997; Lebrun-Garcia *et al.,* 1998), and Romeis and co-workers (1999) demonstrated that in their system activation of both WIPK and SIPK occurs independently of the oxidative burst, because both processes can be blocked separately. Treatment of cells with a PLA inhibitor resulted in strongly reduced Avr9-induced MAPK activation (Romeis *et al.,* 1999), suggesting that phospholipases may also be involved in MAPK activation. These results are summarized in Fig. 7, but it should be noted that the signaling pathways activated by the different elicitors are not necessarily composed of the same components.

B. Role of MAPK Pathways in Plant Hormone Signaling

The focus of investigations concerning MAPK pathways in plants has been on stress responses. It is known that MAPK pathways also play an important role in stress signaling in yeast and mammals, but mammalian MAPK pathways certainly play an equally important role in the signaling of hormones. In plants, five ''classical'' hormones are known. The plant hormones cytokinin, gibberellin, and auxin are known to have a global effect in promoting and ethylene and abscisic acid in inhibiting growth and cell division (Kende and Zeevaart, 1997). Little is known about the role of MAPK pathways in signaling plant hormones, but there is accumulating information that suggests at least some roles for MAPKs in the signaling pathways of all of these hormones (Fig. 8).

Besides the five classical plant hormones, other plant hormones have been discovered (Creelman and Mullet, 1997), including brassinosteroids and compounds that play an important role in the defense response in plants, like SA, JA, and systemin (Dürner *et al.,* 1997; Wasternack and Parthier, 1997; Ryan and Pearce, 1998). The possible role of MAPKs in the signaling pathway of these hormones has already been discussed in Section V.A.

1. Abscisic Acid

Abscisic acid (ABA) plays a role in seed maturation and germination, stomatal regulation, and many abiotic stress responses. Evidence exists

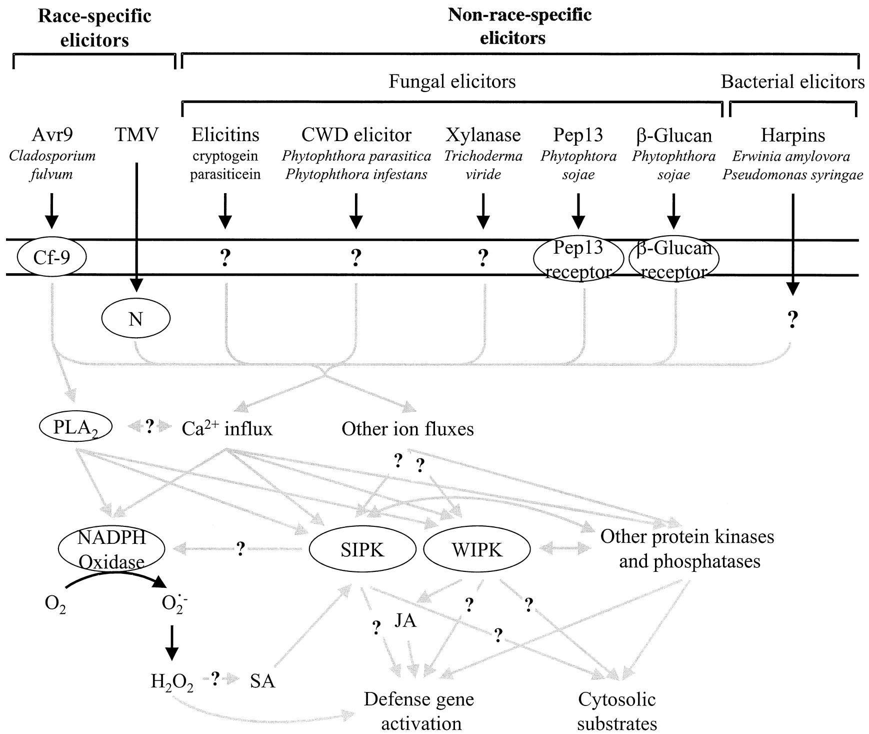

FIG. 7 Schematic illustration of the major components involved in pathogen-induced MAPK activation. Question marks show uncertain connections or unknown components. Black arrows indicate direct interaction/activation, whereas gray arrows indicate indirect connections. See text for details.

of a role for ABA in response to dehydration, salt, osmotic, and low-temperature stresses (Leung and Giraudat, 1998). The important role for ABA in stress adaptation is emphasized by its ability to induce certain stress-induced genes in the absence of stress (Shinozaki and Yamaguchi-Shinozaki, 1996).

To make use of protein kinase and protein phosphatase inhibitors, a role for phosphorylation in ABA-mediated signaling has been shown in several systems (Chapman *et al.,* 1975; Hey *et al.,* 1997). Several ABA-responsive kinases (Anderberg and Walker-Simmons, 1992; Gómez-Cadenas *et al.,* 1999; Hwang and Goodman, 1995) and phosphatases (Leung *et al.,* 1997) have also been cloned. Important information about ABA signaling mechanisms has come from the analysis of ABA-insensitive *Arabidopsis* mutants (Bonetta and McCourt, 1998). Among these are the well-studied *abi1* and *abi2* mutants, which are affected in ABA-mediated effects on germination, stomatal closure, and root growth, implying ABI1 and -2 in the early steps

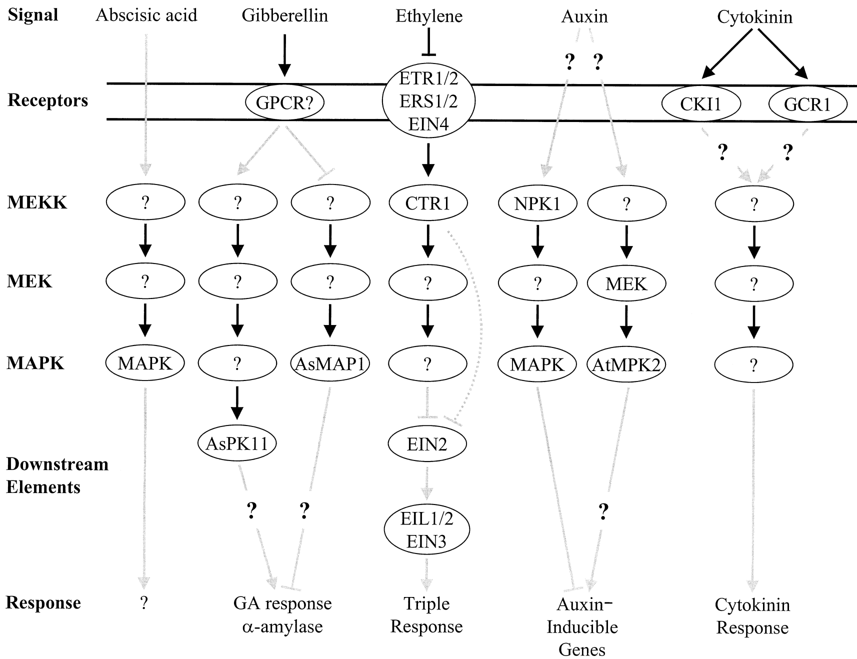

FIG. 8 Schematic illustration of the (possible) role of MAPK cascades in plant hormone signaling. Question marks show uncertain connections or unknown components. Black arrows indicate direct interaction/activation, whereas gray arrows indicate indirect connections. See text for details.

of ABA signaling. ABI1 and -2 turned out to be protein phosphatases of type 2C (PP2Cs) (Leung *et al.,* 1997). Components of MAPK pathways have been shown to be possible substrates for PP2Cs in plants and other organisms (Meskiene *et al.,* 1998a; Hanada *et al.,* 1998), but there are no indications of such a role for ABI1 or -2. ABA signaling involves a complex interplay between various kinases and phosphatases, and this complexity is illustrated by the fact that both kinase and phosphatase inhibitors block ABA-mediated gene expression of the ABA-responsive dehydrin gene in pea (Hey *et al.,* 1997).

Although no ABA-responsive MAPKs have been cloned so far, there are indications of a role for MAPKs in ABA signaling. Aleurone protoplasts are a model system for the analysis of ABA signaling. In this system, ABA inhibits the production of hydrolitic enzymes and apoptosis (Grill and Himmelbach, 1998; Wang *et al.,* 1996). Analysis of MAPK activity in barley aleurone protoplasts identified a MAPK-like protein that cross-reacted with an ERK1 antibody and was activated rapidly and transiently after ABA

treatment (Knetsch *et al.,* 1996). ABA treatment in the presence of the protein tyrosine phosphatase inhibitor phenylarsine oxide (PAO) resulted in the complete block of both MAPK activity (Knetsch *et al.,* 1996) and ABA-induced gene expression (Heimovaara-Dijkstra *et al.,* 1996). These results suggest a model in which a tyrosine phosphatase activates a MAPK pathway, which in turn activates the transcription of several ABA-response genes. Although MAPK pathways usually are activated by protein kinases, there are examples of phosphatase regulation of MAPK pathways in *Xenopus,* mammals, and yeast (Tang *et al.,* 1995; Sun and Tonks, 1994; Nguyen and Shiozaki, 1999).

Another system often used to study ABA signaling is guard cells. Guard cells respond to ABA with osmotic-controlled shrinking, resulting in stomatal closure (Grill and Himmelbach, 1998). In guard cells of fava bean, a calcium-independent protein kinase of 48 kDa was activated upon ABA treatment and named ABR kinase for ABA-responsive protein kinase (Mori and Muto, 1997). This kinase preferentially used MBP as a substrate and was poorly phosphorylating histone or casein. Considering its size and substrate specificity, this kinase could be a MAPK. However, the kinase could not be immunoprecipitated by an anti-phosphotyrosine antibody (Mori and Muto, 1997). Li and Assmann (1996) detected a kinase of similar molecular mass, which was named AAPK for ABA-activated rotein kinase, that could efficiently use histone as a substrate. These results indicate a clear role for serine/threonine protein kinases in ABA signaling in guard cells, but whether these kinases are MAPKs remains to be resolved.

The facts that many abiotic stresses can induce MAPKs (see Section V.A.3) and that ABA was able to activate a MAPK-like kinase might suggest that MAPK activation by all abiotic stresses is mediated by ABA. However, it was clearly shown that the alfalfa SAMK is not activated by ABA (Jonak *et al.,* 1996), making this mechanism unlikely to be generally valid. Compared to ABA accumulation, MAPKs are activated much earlier by abiotic stresses. Therefore, MAPKs might be involved in activating ABA synthesis. On the other hand, it is equally likely that the stress-activated MAPKs act on ABA-independent pathways. Future investigations are required to differentiate between these possibilities and may be helped by a genetic analysis of MAPK mutants.

2. Gibberellin

Gibberellins (GA) have an important role in the regulation of plant growth. They affect germination, leaf expansion, shoot elongation, flowering, fruit development, and mobilization of seed reserves (Hooley, 1994). Some components of the signaling pathways that GA uses to perform its functions have been identified, and many more have been suggested to play a role.

Among these are heterotrimeric G-proteins, calmodulins, CDPKs, and transcription factors (Bethke and Jones, 1998).

Huttly and Phillips (1995) isolated several kinase genes from oat aleurone cells. Among these, two turned out to be responsive to GA. The first one turned out to code for an MAPK named AsMAP1. Aleurone cells incubated in the absence of GA had much higher *AsMAP1* transcript levels, as had cells incubated in the presence of GA (Huttly and Phillips, 1995). Aleurone cells are known to synthesize and secrete hydrolitic enzymes into the endosperm and start the breakdown of stored aleurone reserves in response to gibberellin. AsMAP1 could have a role as negative regulator of GA-induced events, preventing premature degradation of the grain resources. The finding that treatment of aleurone cells with the phosphatase inhibitor okadaic acid leads to insensitivity to GA (Kuo *et al.,* 1996) supports such a model, because okadaic acid has been shown to be able to block MAPK pathway inactivation (Haystead *et al.,* 1994; Chajry *et al.,* 1996).

The other gene, *AsPK11,* has some homology to mammalian ribosomal S6 kinases, and its transcripts are strongly induced by GA. As discussed previously, ribosomal S6 kinases are known MAPK substrates in mammals, and a similar pathway could exist in plants. Because G-proteins are known to activate yeast and mammalian MAPK cascades (Herskowitz, 1995; Gutkind, 1998), the involvement of heterotrimeric G-proteins in gibberellin-induced gene expression could be a further indication of a role for MAPKs in this process (Jones *et al.,* 1998), but more work is required to clarify the role of MAPK pathways in GA signaling.

3. Ethylene

The plant hormone ethylene plays a role in many signaling pathways in plants. Besides roles in plant growth and development, like fruit ripening, senescence of leaves and flowers, and sex determination, it also has a function in the induction of certain defense responses, such as after flooding, wounding, and pathogen attack (Johnson and Ecker, 1998).

Raz and Fluhr (1993) were the first to show a role for protein phosphorylation in ethylene signaling. Ethylene treatment of tobacco leaves induced rapid protein phosphorylation, and the kinase inhibitor K252a could block both protein phosphorylation and ethylene-induced defense responses. Besides these reports, most of our knowledge about ethylene signaling has come from analysis of mutants with an altered ethylene response (Solano and Ecker, 1998; Fluhr, 1998).

Dark-grown dicot seedlings show shortening and radial swelling of the hypocotyl, inhibition of root elongation, and exaggerated apical curvature when exposed to ethylene. These responses collectively are called the "triple response" and can easily be used to screen for mutants with an altered

ethylene response. The mutants that are insensitive to ethylene are termed *etr* for ethylene-resistant (ETR1 and -2) or *ein* for ethylene-insensitive (EIN1–7). Another class of mutants shows a constitutive triple response in the absence of ethylene. This class can be divided into two subgroups. One group of mutants overproduces ethylene, whereas the other group does not produce more ethylene that normal and therefore must be affected in the perception or transduction of ethylene. Several mutants of the second group fall into a single complementation group termed *ctr1* for constitutive triple response (Kieber *et al.,* 1993).

Of the genes responsible for the ethylene-insensitive mutants, *ETR1,* and *ETR2,* and *EIN4* turned out to code for proteins with homology to two-component histidine receptor kinases and are thought to be ethylene receptors. On the basis of their homolgy, several additional members of the ethylene receptor family have been isolated, among which are ERS1 and -2 for ethylene response sensor. Two-component receptors consist of a sensor protein with a histidine kinase domain and a response-regulator protein (Wurgler-Murphy and Saito, 1997; Chang and Stewart, 1998). In ETR1, ETR2, and EIN4, these two components are present in the same protein. ERS1 and -2 lack the response regulator. Of the ethylene receptor family, ETR1 was the first member to be cloned, and most information is available for this receptor. Besides the fact that *etr1* mutants bind significantly less ethylene than wild-type plants, it was shown that ETR1 has histidine kinase activity *in vitro* (Gamble *et al.,* 1998) and is able to bind ethylene with its N-terminus when expressed in yeast (Schaller and Bleecker, 1995).

The *CTR1* gene codes for a protein with strong homology to the Raf family of protein kinases. *ctr1* mutants are recessive loss-of-function or reduction-of-function mutations (Kieber *et al.,* 1993), and CTR1 therefore must act as a negative regulator of ethylene signaling. By epistasis analysis, it was shown that CTR1 is downstream of the isolated ethylene receptors (Hua and Meyerowitz, 1998). Furthermore, it was shown that CTR1 can interact with ETR1 and ERS1 *in vitro* (Clark *et al.,* 1998). From these results, it is speculated that the ethylene receptors bind to and subsequently activate CTR1 by as yet unknown mechanisms. However, additional factors could be involved in CTR1 activation. A 14–3–3 protein is a candidate to be one of these factors, because certain isoforms were found to interact with both CTR1 and ETR1 in a two-hybrid assay (Solano and Ecker, 1998). CTR1 homologues are expected to be present in a range of other plant species (Kieber *et al.,* 1993), and it is clear that, at least in tomato, a similar ethylene response pathway exists. The tomato ERS1 homologue is encoded by the *Never-ripe* gene (Wilkinson *et al.,* 1995), and two CTR1 homologues from tomato have been isolated (TCTR1 and -2) (Johnson and Ecker, 1998;

Lin *et al.,* 1998). TCTR1, which is 95% identical to CTR1, is upregulated transcriptionally upon ethylene treatment (Johnson and Ecker, 1998).

Besides the ethylene receptor family, all other ethylene-insensitive mutants act downstream of CTR1. EIN2, -5, -6, and -7 are thought to have a role in mediating the ethylene signal from the putative MAPK cascade to the nucleus. EIN3 is a protein with sequence features similar to transcription factors and possesses a functional nuclear localization signal (Chao *et al.,* 1997). Furthermore, EIN3 showed transactivation activity in yeast. Besides EIN3, two other proteins with similar characteristics have been cloned that were named EIL1 and -2 (for EIN3-like). It has been proposed that these proteins represent a novel class of transcriptional regulators involved in the regulation of ethylene response genes (Chao *et al.,* 1997).

At the moment, the only pathway known to contain both a two-component phosphorelay system and a complete MAPK cascade is the hyper-osmosensing pathway of yeast (Posas *et al.,* 1996). Interestingly, SLN1, the receptor kinase of this pathway, is inactivated upon sensing high osmolarity, similar to the proposed inactivation of the ethylene receptors upon ethylene binding (Hua and Meyerowitz, 1998). It will be interesting to determine whether the similarity of these two pathways can be extended and whether a complete MAPK cascade exists for ethylene signaling. To date, no MKKs or MAPKs have been identified that could be downstream targets of CTR1. However, ethylene treatment of tobacco leaves resulted in fast activation of a 50-kDa MBP kinase (Sessa *et al.,* 1996). This kinase could be a candidate for a MAPK downstream of CTR1. It was suggested, however, that this kinase activity could come from the ethylene-responsive PK12 kinase. PK12 is a dual-specificity kinase of the LAMMER family and was shown to be activated by ethylene with kinetics similar to that of the 50-kDa MBP kinase (Sessa *et al.,* 1996). It should be noted that there are also cases in mammals where MKKKs activate pathways not involving MAPKs (Lee *et al.,* 1997a), and this could be the case for the CTR1 pathway.

4. Auxin

Many important aspects of plant growth and development are regulated by auxin. Among these are apical dominance, tropic responses, lateral root formation, root hair formation, and vascular tissue differentiation (Abel and Theologis, 1996; Hobbie *et al.,* 1994). Little is known about the signaling pathway(s) that mediates the multiple auxin effects.

Cumulative evidence suggests a role for protein kinases and/or phosphatases in auxin signaling. In 1978, Murray and Key reported for the first time an effect of auxin on protein phosphorylation. Treatment of soybean with auxin led to enhanced phosphorylation of nuclear proteins. Other indications of a role for kinases or phosphatases in auxin signaling came

from experiments with pea epicotyl segments and bean plants, which also showed auxin-induced changes in protein phosphorylation (Reddy *et al.,* 1987; Poovaiah *et al.,* 1988).

The first indication that a MAPK could be one of the kinases involved in auxin signaling came from experiments where it was shown that treatment of auxin-starved tobacco BY-2 cells with the synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), resulted in rapid and transient activation of an MBP protein kinase of 46 kDa (Mizoguchi *et al.,* 1994). Moreover, the activity of a protein kinase, which was able to phosphorylate recombinant AtMPK2 protein, also increased rapidly after auxin treatment. These results suggest that MKKs and MAPKs could have a role in auxin-mediated cell-cycle reentry. However, Tena and Renaudin (1998) could not reproduce the protein kinase activation upon auxin treatment. Only treatment of cells with very high and very toxic 2,4-D concentrations resulted in activation of a 47-kDa MBP kinase, but the same activation was also observed after treatment with the inactive auxin analogue 2,3-D. It was also shown that the activated MBP kinase is most probably a MAPK, because it could be detected by MAPK-specific antibodies. Activation of this MAPK was shown to be correlated with and probably triggered by cytosolic acidification (Tena and Renaudin, 1998). The activation of a MAPK pathway by such a mechanism is likely to be of physiological relevance, because elicitor treatment of cells was shown to induce cytosolic acidification (Mathieu *et al.,* 1996).

MAPKs may also be involved in negatively regulating auxin responses, because expression of the tobacco MKKK, NPK1, in maize cells results in inhibition of auxin-induced gene expression (Kovtun *et al.,* 1998). Blocking of auxin activation of the GH3 and ER7 auxin-responsive promoters by NPK1 was shown to be dependent on NPK1 kinase activity. Furthermore, there was a correlation between the kinase activities of the different NPK1 mutants and a MAPK activity in the maize cells. Deactivation of this MAPK by a MAPK-specific phosphatase (mouse MPK1) completely eliminated the NPK1 effect (Kovtun *et al.,* 1998). These results indicate the NPK1 is able to activate an endogenous MAPK cascade in maize, which in turn is responsible for inhibiting auxin-induced gene expression. Interestingly, *NPK1* was also reported to be activated transcriptionally upon auxin treatment of tobacco roots (Nakashima *et al.,* 1998), and therefore it could be postulated that NPK1 serves a role in a negative feedback mechanism for auxin-induced gene expression.

5. Cytokinin

Cytokinins are suggested to have a role in a wide variety of developmental processes like photomorphogenesis, chloroplast biogenesis and maintenance, and senescence. In combination with auxin, it also regulates several

aspects of growth and development (Hobbie *et al.,* 1994). Little is known about the mechanisms of cytokinin action and the signaling pathways that lead to these responses. However, the isolation of mutants for cytokinin requirement has given some information about its signal transduction pathway (Kakimoto, 1998).

At the moment there is no indication of a role for a MAPK pathway in cytokinin signaling. However, a possible upstream component of such a pathway has been isolated. Two *Arabidopsis* mutants were isolated that exhibit a constitutive cytokinin response in the absence of cytokinin (Kakimoto, 1996). One of the genes responsible for such a phenotype turned out to be a homologue of the ethylene receptor ETR1, consisting of a histidine kinase domain, a receiver domain, and a putative input domain. It turned out that overexpression of this gene, named *CKI1* for cytokinin-insensitive 1, was responsible for the constitutive cytokinin response phenotype (Kakimoto, 1996), suggesting that CKI1 could function as a cytokinin receptor.

Several cytokinin-induced genes encode proteins with similarity to the response regulators of two-component systems and may function together with CKI1. The same proteins also have regions that could function as transcriptional activators (Kakimoto, 1998). Thus, cytokinin signaling could function similar to the *Escherichia coli* EnvZ–OmpR system, where EnvZ is the sensory kinase that activates the response-regulator OmpR, which functions directly as a transcription factor (Chang and Stewart, 1998). However it is also feasible that CKI1 could be the start of a signaling pathway, leading into a MAPK cascade, as is proposed for the ethylene pathway (Fluhr, 1998) and as is known for the yeast osmosensing pathway (Posas *et al.,* 1996).

Besides the CKI1 signaling pathway, an additional cytokinin pathway has been proposed. A putative *Arabidopsis* seven-transmembrane receptor was isolated (GCR1), and antisense GCR1 plants show a reduced cytokinin response (Plakidou-Dymock *et al.,* 1998). This makes GCR1 another candidate for a cytokinin receptor. At the moment nothing is known about possible downstream components of the GCR1 pathway, but yeast and animal signaling pathways are known where seven-transmembrane receptors feed into MAPK cascades via heterotrimeric G-proteins (Herskowitz, 1995; Gutkind, 1998).

C. Role of MAPK Pathways in Plant Development

1. Cell Cycle

Mammalian and yeast MAPKs also function in regulating the cell division cycle. In fact, the name "mitogen-activated protein kinase" refers to their

ability to function in transducing signals that induce the proliferation of cell-cycle-arrested mammalian cells, but MAPKs also are involved in other cell-cycle events. In *Xenopus,* a MAPK becomes activated upon the entry of meiosis (Gotoh *et al.,* 1991) and is required for the spindle assembly checkpoint (Takenaka *et al.,* 1997). ERK1 and ERK2 play important roles in cell-cycle reentry (Pagès *et al.,* 1993; Brunet *et al.,* 1999), and FUS3 and KSS1 from *Saccharomyces cerevisae* and SPK1 from *Schizosaccharomyces pombe* are yeast MAPKs involved in cell-cycle regulation (Gustin *et al.,* 1998).

The first evidence of a role for protein phosphorylation in the regulation of the plant cell cycle was the finding that kinase inhibitors can block the entry into mitosis and the disassembly of the preprophase band (Katsuta and Shibaoka, 1992). Furthermore, protein kinase inhibitor treatment prolongs metaphase (Wolniak and Larsen, 1995).

Several studies have looked for plant MAPKs with a function in the cell cycle or cell-cycle reentry. The cDNA for the pea MAPK PsD5 was isolated from a growing bud library, and therefore it was hoped to have some role in cell-cycle control (Stafstrom *et al.,* 1993). It could be shown that *PsD5* transcript accumulation was correlated with cell proliferation, but not with a particular phase of the cell cycle (Devitt and Stafstrom, 1995).

A tobacco homologue of PsD5 could also have a function in controlling the cell cycle. Tobacco-suspension-cultured cells can be arrested in G_1 by phosphate starvation. After readdition of phosphate, the cells reenter the cell cycle, and a MAPK is activated fast and transiently during this process (Wilson *et al.,* 1998). Activation occurred prior to the entry of cells into the S-phase and therefore could have a function in the reactivation of the cell cycle. There is, however, no direct proof of such a role for this MAPK. Additional data that suggest a role for PsD5 homologues in cell-cycle control came from the finding that the alfalfa PsD5 homologue, the stress-induced MAPK (SIMK, previously named MsK7), is expressed in a cell-cycle-dependent manner (Jonak *et al.,* 1993). *SIMK* transcripts could be detected at low levels in G_1, but transcript levels increased during the S- and G_2-phases. A clone identical to SIMK was isolated from the infectable zone of *Rhizobium*-inoculated alfalfa roots, but there are no indications of a specific role for SIMK in mitogenic induction in symbiotic root nodules (Duerr *et al.,* 1993). Besides these MAPKs, two isolated MKKK kinases, BnMAP4Kα1 and -2, also show cell-cycle-dependent expression, with the highest expression in G_2 (Leprince *et al.,* 1999).

Receptor-like kinases (RLKs) from plants are structurally similar to mammalian receptor protein kinases (RPKs) and are thought to play an important role in plant development (Becraft, 1998; Lease *et al.,* 1998). The best-characterized RLK is CLAVATA1 (CLV1), which has been proposed to maintain the proper balance between proliferation and differentiation

of meristematic cells (Clark *et al.,* 1997). It was found that, upon activation, CLV1 forms a protein complex together with a PP2C-like phosphatase (KAPP), a Rho-GTPase-related protein, and other unknown factors (Trotochaud *et al.,* 1999). The fact that mammalian RPKs often feed into MAPK pathways and the apparent involvement of small G-proteins in CLV1 signaling might suggest a role for MAPKs in the CLAVATA pathway.

The best indication of the involvement of plant MAPKs in the cell cycle has been derived from studies on the alfalfa MMK3 MAPK and its tobacco homologue, *Nicotina tabacum* FUS3-like kinase 6 (NtF6) (Bögre *et al.,* 1999; Calderini *et al.,* 1998). It was shown that the activation levels for these MAPKs fluctuate in a cell-cycle-dependent manner. The kinases were active exclusively in ana- and telophase. Furthermore, destabilizing microtubules blocked their activation in anaphase. During mitosis they are localized at the phragmoplast in late anaphase and stay localized to the cell plate during telophase. These results strongly suggest that MMK3 and NtF6 have a function in cytokinesis, but their targets so far are unknown. The kinases could function in phragmoplast construction or in regulating vesicle transport to the cell plate. Both the localization (Shapiro *et al.,* 1998; Zecevic *et al.,* 1998) and the activation (Tamemote *et al.,* 1992) of MMK3 and NtF6 during mitosis in plants are similar to those of ERK1/2 in mammals, and these results suggest a similar role for these MAPKs in mitosis. Data on the mRNA and protein localization of NtF6 homologues in pepper and onion support a role for NtF6 in mitosis. Both protein and mRNA levels were significantly higher in proliferating cells than in quiescent cells (Préstamo *et al.,* 1999).

The tobacco MKKK, NPK1, was shown to have the highest expression levels in suspension-cultured cells during the logarithmic growth phase (Banno *et al.,* 1993a) and in meristematic and developing tissues (Nakashima *et al.,* 1998). Furthermore, it could be shown that *NPK1* transcripts accumulate after the induction of cell proliferation (Nakashima *et al.,* 1998). In addition, the *Arabidopsis* NPK1 homologues (*ANP1–3*) showed higher transcript levels in proliferating tissues (Nishihama *et al.,* 1997). During the cell cycle, NPK1 protein and mRNA are present from the S- to M-phases, with slightly higher levels in the M-phase (Machida *et al.,* 1998).

Two kinesin-like proteins were isolated that can interact with NPK1 and also are able to activate NPK1 in a yeast complementation assay; therefore, they were named NACK1 and -2 for NPK1-activating kinesin-like proteins (Machida *et al.,* 1998). Interestingly, ERK1 and -2 are also complexed with a microtubule motor protein, CENP-E (Zecevic *et al.,* 1998). Similar to CENP-E, NACK1 accumulates only in the M-phase and is localized to the center of the phragmoplast together with NPK1. NACK1 not only is a possible activator of NPK1 but also may be its substrate, implying some

kind of feedback mechanism. A MKK belonging to the NACK1–NPK1 pathway has also been identified (Machida *et al.,* 1998), but the identity of the MAPK that functions in this pathway is not yet known. Because MMK3 and NtF6 have a localization similar to that of NACK1 in mitosis, these kinases could be good candidates to belong to the same pathway.

2. Pollen Development

Changes in protein phosphorylation during pollen development have been reported by several groups (Clarke *et al.,* 1998; Hiscock *et al.,* 1995; Op den Camp and Kuhlemeier 1998). A role for protein kinases in the onset of embryogenesis after starvation of immature tobacco pollen has been proposed (Garrido *et al.,* 1993). Although these kinases have not been identified, MAPKs could be candidates. MAPKs have been shown to play an important role in the reactivation of the cell cycle in quiescent cells in other systems and plant homologues of upstream components of mammalian MAPK pathways, like small G-proteins, and a receptor-like kinase has been shown to be involved in pollen development (Lin and Yang, 1997; Lee *et al.,* 1996).

Direct evidence of a function for MAPKs in pollen development was provided by the observations that transcripts of the tobacco MAPK *NtF4* could be found solely from the mid-bicellular to the mature stage of pollen development and that during germination of pollen there is a strong increase in NtF4 kinase activity (Wilson *et al.,* 1997). The activation of the kinase could be correlated with the rehydration of the pollen and was not dependent on pollen germination (Wilson *et al.,* 1997). NtF4 activation occurs before the pollen tube emerges and therefore is likely to play a role in the early events of pollen activation. Because one of the earliest events that follows pollen hydration is rapid rearrangement of the cytoskeleton, it is possible that NtF4 has a function in this process. Such a role for MAPKs has been shown in mammalian systems (Reszka *et al.,* 1995; Morishima-Kawashima and Kosik, 1996). It should be mentioned that it is not completely clear whether NtF4 activation has a specific function in pollen germination or whether it is only a stress response to the swelling of pollen upon hydration. However, activation of NtF4 as a response to hypo-osmotic stress would not necessarily rule out a function in pollen germination.

D. Role of MAPK Pathways in Sugar Signaling

In plants, sugars not only have metabolic functions but also are important signals. It is known that sugars are able to regulate the expression of genes encoding sink-specific enzymes, such as extracellular invertase, and specific

pathogen- and stress-response genes, such as proteinase inhibitor genes and the gene for phenylalanine ammonia lyase (*PAL*). On the other hand, genes involved in photosynthesis are repressed by sugars (Koch, 1996).

Treatment of *Chenopodium rubrum* suspension-cultured cells with glucose results in the accumulation of several transcripts encoding enzymes, like PAL and extracellular invertase (CIN1). Furthermore, high-steady-state mRNA levels of the ribulose biphosphate carboxylase (*RbcS*) gene were strongly reduced upon glucose treatment (Ehness *et al.,* 1997). Besides transcriptional effects, glucose treatment of the cell culture resulted in the activation of a 44- and 46-kDa MBP kinase (Ehness *et al.,* 1997). The two kinases could be MAPKs, and because the same kinase activity and transcriptional changes also seem to be triggered by different elicitors, it is tempting to speculate that they could be the homologues of WIPK and SIPK, the two elicitor-induced MAPKs identified in tobacco (Zhang and Klessig, 1998b; Zhang *et al.,* 1998).

VI. MAPK Pathway Inactivation

As important as the activation of MAPK pathways is their inactivation. Inactivation of MAPKs is mediated by threonine and/or tyrosine dephosphorylation of the TXY motif. Several yeast and mammalian phosphatases have been cloned and characterized that are able to inactivate MAPK pathways. They can be divided into at least three groups: (i) the dual-specificity phosphatases that can dephosphorylate the MAPKs on both tyrosine and threonine residues (DsPTPases) (Sun *et al.,* 1993; Doi *et al.,* 1994); (ii) the tyrosine phosphatases that can solely dephosphorylate tyrosine residues (PTPase) (Wurgler-Murphy *et al.,* 1997; Van Vactor *et al.,* 1998); and (iii) the serine/threonine phosphatases (PPases) like PP2A- and PP2C-type phosphatases (Alessi *et al.,* 1995; Millward *et al.,* 1999; Shinozaki and Russell, 1995; Takekawa *et al.,* 1998).

A broad range of plant protein phosphatases has been isolated, but little is known about their interaction with MAPK pathways. Most information about the function of phosphatases in the deactivation of plant MAPK pathways has come from inhibitor studies. Both serine/threonine and tyrosine phosphatases were shown to inactivate plant MAPKs *in vitro* (Usami *et al.,* 1995; Ádám *et al.,* 1997; Zhang and Klessig, 1997, 1998b). Furthermore, it could be shown that activation of MAPKs can be sustained (Suzuki and Shinshi, 1995) or even induced solely by the use of phosphatase inhibitors (Suzuki *et al.,* 1999), indicating a role for phosphatases in downregulating basal MAPK activity. Several groups also showed a requirement of *de novo*

protein sythesis for MAPK inactivation (Suzuki and Shinshi, 1995; Bögre *et al.,* 1997; Ádám *et al.,* 1997).

Several plant protein phosphatases have been isolated that could be involved in the inactivation of MAPK pathways. Among them are members of all three MAPK phosphatase classes. The first report of a possible MAPK phosphatase in plants came from Haring and co-workers (1995). They isolated a dual-specificity phosphatase from *Chlamydomonas eugametos* referred to as VH–PTP13, which had homology to members of the vaccinia virus H1 (VH)-like PTPs. VH-like PTPs can dephosphorylate and thereby inactivate MAPKs efficiently. A role for VH–PTP13 as a MAPK phosphatase was suggested by its ability to inactivate the alfalfa MAPKs SIMK and MMK2 *in vitro* (Haring *et al.,* 1995). Furthermore, VH–PTP13 has relative high homology to the human CL-100 MAPK phosphatase and a similar expression pattern, being restricted to the beginning of the G_1-phase (Keyse and Emslie, 1992).

A role in inactivating plant MAPK pathways has also been proposed for the *Arabidopsis* dual-specificity protein phosphatase AtDsPTP1 (Gupta *et al.,* 1998), which also seems to be a member of the VH-like PTPase subfamily. AtDsPTP1 was shown to be an active phosphotyrosine and phosphoserine/threonine phosphatase and was able to dephosphorylate and inactivate the *Arabidopsis* AtMPK4 *in vitro*. AtDsPTP1 was demonstrated to be expressed constitutively in various tissues under various conditions, leaving open the possibility that AtDsPTP1 may be regulated exclusively at the posttranslational level as the tyrosine phosphatases PTP2 and -3 from yeast (Wurgler-Murphy *et al.,* 1997). So far, such a mechanism has not been seen for other VH–PTPs.

The *Arabidopsis* protein tyrosine phosphatase AtPTP1 has high homology to cytoplasmic mammalian PTPases (Xu *et al.,* 1998) and is able to dephosphorylate and thereby deactivate a MAPK *in vitro* (Luan, 1998). Expression of AtPTP1 was strongly induced by high-salt conditions, and its transcript levels declined under low-temperature conditions. Pea and soybean homologues of AtPTP1 also have been isolated (Fordham-Skelton *et al.,* 1999). There are, however, no indications that these PTPs are MAPK phosphatases, and it should be noted that protein tyrosine phosphorylation events are implicated in many signaling cascades other than MAPK pathways and that there are indications that protein tyrosine phosphorylation is a relatively frequent event in plants (Barizza *et al.,* 1999; Trojanek *et al.,* 1996).

Tyrosine phosphorylation in correlation with MAPK activation has been shown for MsERK1 (Duerr *et al.,* 1993), NtF3, -4, and -6 (Wilson *et al.,* 1995, 1997), SIPK (Zhang and Klessig, 1997, 1998a), and MAPK-like activities in ABA signaling (Knetsch *et al.,* 1996), elicitor response (Suzuki and Shinshi, 1995; Suzuki *et al.,* 1999; Ádám *et al.,* 1997), and wounding (Usami *et al.,*

1995; Stratmann and Ryan, 1997). Surprisingly, Romeis *et al.* (1999) reported transient activation of WIPK and SIPK upon Avr9 treatment of Cf9 tobacco cells, whereas tyrosine phosphorylation of these MAPKs was sustained. This would suggest an important role for threonine dephosphorylation in MAPK inactivation in this system.

The only putative serine/threonine MAPK phosphatase isolated to date is the alfalfa protein phosphatase 2C, MP2C (Meskiene *et al.,* 1998a). MP2C was isolated by a functional yeast screen, where it could intervene in the pheromone-induced MAPK pathway, and it was able to inactivate the wound-induced SAMK pathway *in vitro*. The *MP2C* gene was activated transcriptionally upon wounding of alfalfa leaves, and the kinetics of this transcriptional activation correlated very well with the inactivation of SAMK.

It was shown that the inactivation mechanism is induced after the activation of SAMK, preventing reactivation of the SAMK pathway for a certain time (Bögre *et al.,* 1997). This refractory period extends over a time period of approximately the same length as the presence of *MP2C* transcripts (Meskiene *et al.,* 1998a). These results suggest that MP2C is one of the factors that is able to reset the SAMK pathway to make it accessible for subsequent sensing of changes in the environment. Furthermore, it is possible that the expression of the *MP2C* gene is regulated by the SAMK pathway itself and that it is part of a negative feedback loop (Meskiene *et al.,* 1998b), as has been shown for other systems (Sun *et al.,* 1993; Doi *et al.,* 1994; Brondello *et al.,* 1997).

VII. Concluding Remarks and Perspectives

Over the past few years, many components of plant MAPK cascades have been isolated and information on their function is accumulating rapidly. It is clear that some MAPKs can be activated by a whole range of stimuli and that some environmental conditions can activate more than one MAPK. This leaves open the question of how one MAPK can give rise to different responses. From our knowledge of MAPK pathways in mammals and yeast, activation of one MAPK can lead to different cellular responses, and different levels and kinetics of activation may determine the outcome of the signal transduction process. Such a mechanism has been shown for both the human ERK1 MAPK cascade and the yeast SMK1 pathway. ERK1 can activate both cell proliferation and differentiation in the same cell type (Traverse *et al.,* 1992; Cowley *et al.,* 1994), depending on the amplitude and kinetics of ERK1 activation (Marshall, 1995). SMK1 has an important role in spore morphogenesis, and distinct steps of spore morphogenesis

were shown to be directly correlated with the magnitude of SMK1 activity. Increasing SMK1 activity levels allowed more and later spore morphogenesis events (Wagner *et al.,* 1999). Differences in plant MAPK activation kinetics by different stimuli have been observed, and further research will need to clarify the importance of these observations.

Furthermore, specificity can be achieved by the physical separation of signaling pathways. This can happen through specific cellular localization of components of each pathway or through differential expression of certain signal pathway components and substrates in specific cells, but the most important way to generate specificity probably occurs with the help of scaffold proteins that tether specific signaling components into a complex. Therefore, the isolation of plant scaffold proteins will be important to understand the exact regulation of plant MAPK pathways.

Despite the increasing amount of data on the functions of certain components of MAPK cascades, little is known on the interaction between different components and or on their upstream activators and downstream targets. To date, not a single MAPK substrate has been identified. Several plant transcription factors have been isolated (Liu *et al.,* 1999) and the effect of phosphorylation on the function of some of these is known, but none could be assigned to be a MAPK substrate unequivocally. Little is known about the possible upstream activators of plant MAPK cascades. There are, however, some indications that the upstream activators will be at least as diverse as in yeast and animals. Finally, despite the isolation of multiple MAPKs, MKKs, and MKKKs, placement of these components into distinct signaling pathways remains to be established. The use of both biochemical and genetic approaches should reveal the role and function of different MAPK pathways, finally unravelling the complexity of signal transduction networks that link appropriate responses to distinct extracellular signals.

Acknowledgments

This work was supported by the Training and Mobility of Researchers program of the European Union and by grants P14114-GEN and P13535-GEN from the Austrian Science Foundation.

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