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A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*

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ABSTRACT **We describe here the cloning and characterization of a cDNA encoding a protein kinase that has high sequence homology to members of the mitogen-activated protein kinase (MAPK) kinase kinase (MAPKKK or MEKK) family; this cDNA is named cATMEKK1 (*Arabidopsis thaliana* MAP kinase or ERK kinase kinase 1). The catalytic domain of the putative ATMEKK1 protein shows ≈40% identity with the amino acid sequences of the catalytic domains of MAPKKKs (such as Byr2 from *Schizosaccharomyces pombe*, Ste11 from *Saccharomyces cerevisiae*, Bck1 from *S. cerevisiae*, MEKK from mouse, and NPK1 from tobacco). In yeast cells that overexpress ATMEKK1, the protein kinase replaces Ste11 in responding to mating pheromone. In this study, the expression of three protein kinases was examined by Northern blot analyses: ATMEKK1 (structurally related to MAPKKK), ATMPK3 (structurally related to MAPK), and ATPK19 (structurally related to ribosomal S6 kinase). The mRNA levels of these three protein kinases increased markedly and simultaneously in response to touch, cold, and salinity stress. These results suggest that MAP kinase cascades, which are thought to respond to a variety of extracellular signals, are regulated not only at the posttranslational level but also at the transcriptional level in plants and that MAP kinase cascades in plants may function in transducing signals in the presence of environmental stress.**

Recently, the phosphorylation cascades including mitogen-activated protein kinases (MAPKs), MAPK kinases (MAPKKs), and MAPKK kinases (MAPKKKs) have been reported to function in various signal transduction pathways of organisms from yeasts to vertebrates (1–4). MAPKs are activated by the phosphorylation of threonine and tyrosine residues by upstream kinases, MAPKKs (2, 3), that are themselves activated by the phosphorylation of serine and threonine residues by their upstream kinases, MAPKKKs (3). Several subgroups of MAPKs have been reported to exist in mammalian and yeast cells. In mammalian cells, ERK (also known as MAPK) (5–7), JNK (also known as SAPK) (8, 9), and p38 MAPK (10) have been reported. The ERKs are activated by various growth or differentiating factors and function in the M-phase-specific phosphorylation cascades (1–3). The JNK1 and p38 MAPKs are activated in response to environmental stress in mammalian cells. In *Saccharomyces cerevisiae* at least five different MAPK signaling pathways composed of MAPKKK, MAPKK, and MAPK homologs have been described: the mating-pheromone response pathway (Ste11, Ste7, and Fus3/Kss1), the protein kinase C-dependent signal pathway (Bck1, Mkk1/Mkk2, and Mpk1), the osmoregulatory pathway (Pbs2 and Hog1), the pseudohyphal differentiation pathway (Ste11 and Ste7), and the spore wall assembly pathway (Smk1) (4).

A number of genes for MAPKs have been reported in higher plants (11). We have demonstrated that MAPKs constitute a gene family of at least nine members (ATMPK1 through ATMPK9) in *Arabidopsis thaliana* and that plant MAPK homologs can be classified into four subgroups based on their sequence analyses (refs. 12 and 13; unpublished data). NPK1 (14) and NPK2 (15), which are structurally related to MAPKKK and MAPKK, respectively, have been isolated from tobacco. We have also isolated from *Arabidopsis* two closely related cDNAs, cATPK6 and cATPK19 (16), which are related to p70 and p90 S6 kinases (17–19). The mammalian MAPKs have been reported to phosphorylate and activate p90 S6 kinase. The *CTR1* gene, encoding a Raf homolog that functions as an activator of MAPKK, has a key role in the ethylene signal transduction pathway (20). However, the functions of these plant protein kinases, except for those of CTR1, are still unknown. It is important to determine which protein kinases constitute phosphorylation cascades, and what signals activate MAPK cascades in plants.

We began by isolating nine cDNAs for MAPKs (refs. 12 and 13; unpublished data) and two cDNAs for S6 kinases (16) in *Arabidopsis*. Here we report the isolation and characterization of a cDNA (cATMEKK1)¶ which has an extensive homology with mammalian MAPKKK (MEKK; ref. 21) and yeast MAPKKKs (Byr2; ref. 22, Ste11; ref. 23, Bck1; ref. 24). We then examined the expression levels of plant protein kinases which are structurally related to the protein kinases of MAPK cascades in animals and yeasts. The transcript levels of three protein kinases, ATMEKK1 and ATMPK3 (12) and ATPK19 (16) increased markedly and simultaneously when plants were treated with touch stimuli, low temperature, and salinity stress. We discuss the possible roles of these three protein kinases in signal transduction pathways under environmental stresses such as mechanical stimuli, low temperature, and osmotic stress.

MATERIALS AND METHODS

Plant Materials and Treatments. *A. thaliana* (Columbia ecotype) was used in this study. Growth and stress conditions were the same as those previously reported (16, 25). Mechanical stimulation (touch) was conducted as reported (26). Briefly, the rosette leaves were gently moved back and forth 20

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Abbreviations: MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. D50468).

times by a gloved hand. In all cases, the plants were subjected to the stress treatments for various times and then frozen in liquid nitrogen for further analyses.

cDNA Cloning and Sequencing. All of the cDNA clones were generated by plaque hybridization (27) of two *Arabidopsis* cDNA libraries, one prepared from rosette plants grown for 4 weeks and the other prepared from rosette plants dehydrated for 10 hr (28). A total of 8.0×10^5 plaques were screened under low-stringency hybridization conditions (13, 16) with the insert DNA (≈700 bp) from the cDNA clone 23A12T7 (GenBank T04696) as a probe. This cDNA clone had been obtained as an expressed sequence tag clone from the *Arabidopsis* Biological Research Center (Ohio State University, Columbus), and partially sequenced by T. Newman (Michigan State University, East Lansing). The clone 23A12T7 contains a partial sequence for a MAPKKK; positive clones were plaque-purified and classified into four distinct groups based on restriction endonuclease mapping and partial sequencing analyses.

The longest inserted DNA fragments of the cATMEKK clones were subcloned into the plasmid vector pBluescript II SK(−) (Stratagene) and named pATMEKK1-4. A model 373A DNA sequencer (Applied Biosystems) was used for DNA sequencing. Nucleotide and amino acid sequences were analyzed with the GENETYX (Software Development, Tokyo) and GENEWORKS (IntelliGenetics) software systems.

DNA and RNA Blot Hybridization Analyses. Genomic Southern hybridization and Northern hybridization were performed as described (12, 13, 16). A nylon membrane blotted with size-separated total RNA (40 μg) was stained with methylene blue (28). The inserted DNA fragments of cATMEKK1, cATMPK1 (13), cATMPK3 (12), cATPK19 (16), and cdc2a (29) cDNAs and the partial cDNA corresponding to the 3′ noncoding region of the ATCAL5 cDNA (45) from *Arabidopsis* were used as probes. ATCAL5 encodes calmodulin (26, 45) and its mRNA level increases in response to a water spray. The hybridization signals corresponding to the ATMEKK1, ATMPK1, ATMPK3, ATPK19, ATCAL5, and cdc2a mRNAs were quantified with a BioImage analyzer (FUJIX model BAS2000, Fuji, Tokyo).

Yeast Strains, Growth Conditions, and Transformation. The *S. cerevisiae* strains used were SY1984 (*MATα ste11Δ::ura3 FUS1::HIS3 leu2 ura3 trp1 his3Δ200::ura3 pep4Δ::ura3 can1*) (30) and SY1493 (*MATα ste7Δ::URA3 FUS1::HIS3 leu2 ura3 trp1 his3Δ200::ura3 pep4Δ::ura3 can1*) (30). Yeast cultures were grown in YEP (1% Bacto yeast extract/2% Bacto Peptone) supplemented with 2% glucose. SD medium [0.7% yeast nitrogen base without amino acids (Difco)/2% glucose], supplemented with the appropriate nutrients, was employed for the selection of cells with plasmids. Yeast cells were transformed by the lithium acetate method. General genetic manipulations were carried out as described (31).

Construction of Plasmids for Complementation Analysis. Polymerase chain reaction (PCR) amplification was used to generate fragments of the cATMEKK1 (encoding aa 1–608). The plasmid YEpGAP112-ATMEKK1 was constructed by inserting the DNA fragment that contained cATMEKK1 into the *Bam*HI site of the yeast expression vector YEpGAP112 (14), a YEp-based YRP1 plasmid containing the *TDH3* promoter.

RESULTS

Cloning and Sequence Analysis of cDNAs for Protein Kinases Related to MAPKKKs. Thirty-nine positive *Arabidopsis* cDNA clones were obtained by screening 8.0×10^5 plaques with an expressed sequence tag cDNA, 23A12T7, as a probe. Partial sequence analysis revealed that the cloned DNA inserts had at least four distinct but closely related sequences. We subcloned and sequenced the largest inserts of four cDNAs and determined the nucleotide sequence of one cDNA clone (cATMEKK1) that was 2386 bp long. There is an in-frame stop codon (TAA) 27 bp upstream of the putative initiation codon at nt 136. The cDNA encodes a protein of 608 aa with a molecular mass of 66 kDa (Fig. 1).

Primary Structure of the Putative ATMEKK1 Protein. As shown in Fig. 2*A*, the putative ATMEKK1 protein shows extensive homology to NPK1 (46% identity) from tobacco (14), Byr2 (40% identity) from *Schizosaccharomyces pombe* (22), Bck1 (42% identity) from *S. cerevisiae* (24), Ste11 (42%

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GTCTTCTTCACTCCCGCGAGTTTTTTTTCTCGGGAAAATTTTCCAATTCCAAGCTCCATTGAAGCCAAGTAAGAGGTGCGATTCTCTTCAATTTTGACTT   100
GGAACTTGTAAATAGCGTATGGATTTGAGCTGGTTATGGACAGAATTCTAGCTCGTATGAAGAAATCAACTGGACGAAGAGGAGGAGATAAGAATATTAC   200
                                  M  D  R  I  L  A  R  M  K  K  S  T  G  R  R  G  G  D  K  N  I  T
TCCGGTACGGCGGTTAGAGCGTCGCGATGCGGCCAGGAAATATCAATTACGCCGCAGCTTCATGTTCTAGTTCGTCAGCTGAAGATCTTTCCGTTTCGACT   300
 P  V  R  R  L  E  R  R  D  A  A  R  N  I  N  Y  A  A  A  S  C  S  S  S  S  A  E  D  L  S  V  S  T
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S  S  L  M  T  R  S  L  E  F  P  E  P  T  S  F  R  I  G  G  G  V  G  E  M  D  R  I  Y  R  S  L  G  V
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  S  G  P  D  D  L  A  I  S  F  D  A  W  E  A  C  K  K  R  S  S  S  D  V  V  N  R  F  K  S  F  D  L
TGATAAGGTTCGTGATCAGGATTTGAGTGAAGAAGGTCCTAGTGGTGTTGTTGTTGGTTCTGATTCAATGAATCATAAGGTTCAGGGTCAGGATTTGAGT   600
  D  K  V  R  D  Q  D  L  S  E  E  G  P  S  G  V  V  V  G  S  D  S  M  N  H  K  V  Q  G  Q  D  L  S
GAAGCAGGTCCTAGTGGTGGAATTGTTACTGAGTTGAGTGAGATTGGGAATTTAATCACTCCTGTGGATAGGTTAGTAGCTGATGGTGTGGTAGAGAATA   700
E  A  G  P  S  G  G  I  V  T  E  L  S  E  I  G  N  L  I  T  P  V  D  R  L  V  A  D  G  V  V  E  N  R
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  R  V  M  E  R  T  P  T  I  V  K  S  K  G  Y  L  V  P  N  N  V  V  A  V  G  V  G  V  G  G  G  I  K
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S  E  T  V  K  R  P  S  S  S  S  S  S  S  S  E  D  G  C  D  E  E  E  G  K  E  E  E  A  E  A  E  E  M  G
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CTACCAAAGATACCAGCTTCGGGACTCTGTAGTCTCCTTGTACACTAGACAGATTCTTGACGGTCTGAAATATCTCCACGATAAAGGTTTTATTCACAGG   1500
  Y  Q  R  Y  Q  L  R  D  S  V  V  S  L  Y  T  R  Q  I  L  D  G  L  K  Y  I  H  D  K  G  F  I  H  R
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D  I  K  C  A  N  I  L  V  D  A  N  G  A  V  K  L  A  D  F  G  L  A  K  V  S  K  F  N  D  I  K  S  C
GCAAGGGAACTCCATTTTGGATGGCTCCAGAGGTTATTAACCGAAAGGATAGTGATGGCTATGGAAGTCCAGCTGATATATGGAGCCTCGGGTGCACTGT   1700
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L  S  L  D  A  R  L  F  I  L  K  C  L  K  V  N  P  E  E  R  P  T  A  A  E  L  L  N  H  P  F  V  R  R
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  P  L  P  S  V  G  S  G  G  S  G  A  S  P  L  L  R  R  *
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CAAACTTACTTAGTTTCCTGTGTCATTCGTTGGTTATTTGTTGCAAGTTATGTGTTGGGCTTGATTTTAAAACTGAAGAGCCTTATTTTTCTAGGAATTA   2200
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AATTATAGTTGGTTCTTATAGTTCCTGGCTTGTTGTAACTGTGTACACACATCGATGGGTTTAAGTTCTATTTGTCTCATATTTTA                  2386
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FIG. 1. Nucleotide and deduced amino acid sequences of the cATMEKK1 cDNA. The DNA sequence includes the putative coding region and the 5′ and 3′ noncoding regions. The amino acid sequence of the putative coding region is shown beneath the DNA sequence. The amino acid sequence corresponding to the putative catalytic domain is underlined.

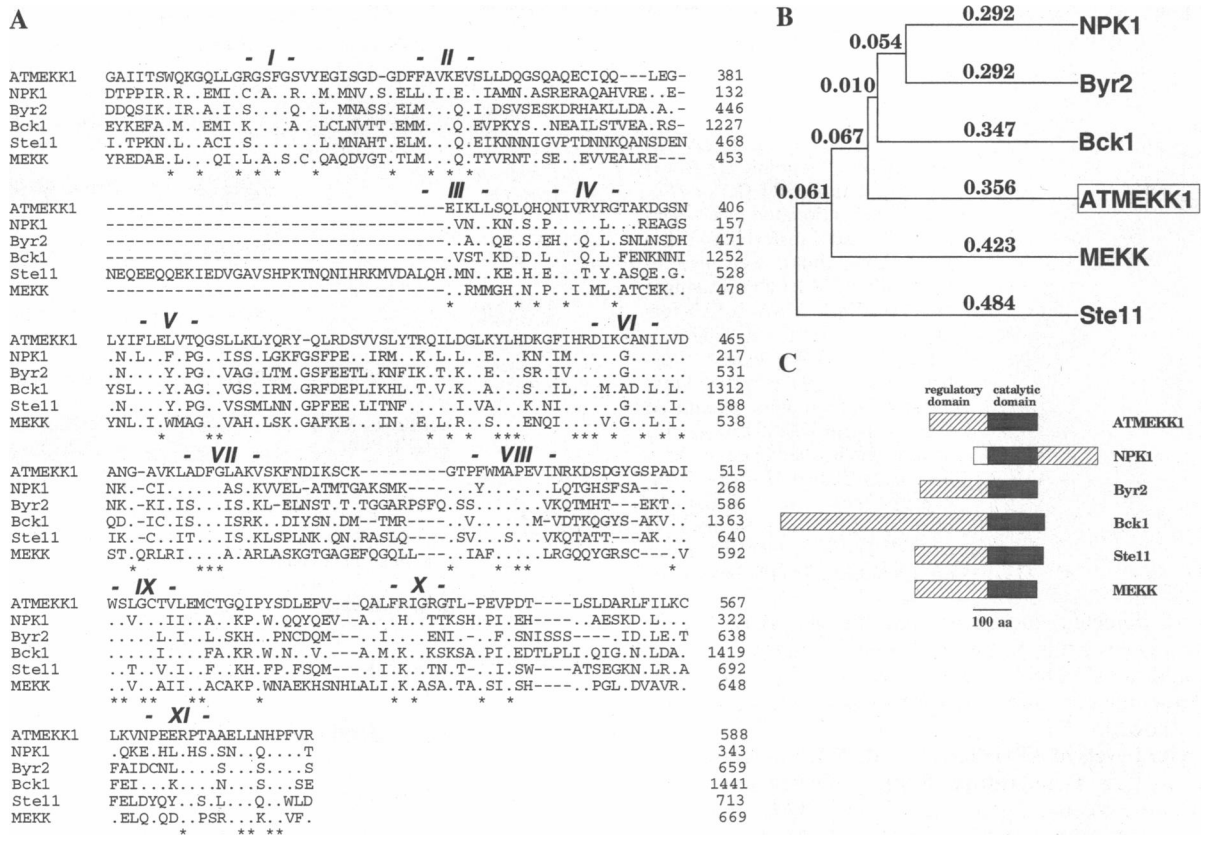

FIG. 2. (*A*) Comparison of the deduced amino acid sequences of putative catalytic domains of *Arabidopsis* ATMEKK1, tobacco NPK1 (14), *Schizosaccharomyces pombe* Byr2 (22), *S. cerevisiae* Bck1 (24), *S. cerevisiae* Ste11 (23), and mouse MEKK (21). Dots represent identical amino acid residues and dashes indicate gaps introduced to maximize alignment. Stars indicate the amino acid residues conserved among these six protein kinases. Roman numerals indicate the 11 major conserved subdomains of protein kinases (32). (*B*) A phylogenetic tree showing evolutionary relationship among ATMEKK1, NPK1, Byr2, Bck1, Ste11, and MEKK proteins was constructed from the matrix of sequence similarities calculated with the UPGMA program (33). Numbers above the horizontal lines indicate the evolutionary distance between one protein and another. (*C*) Predicted structures of ATMEKK1, NPK1, Byr2, Bck1, Ste11, and MEKK proteins. Solid boxes, putative catalytic domains; hatched boxes, the noncatalytic domains, which are thought to be regulatory domains.

identity) from *S. cerevisiae* (23), and MEKK (36% identity) from mouse (21). The ATMEKK1 protein contains all of the conserved residues and the 11 subdomains that are typical of protein kinases (32). A phylogenetic tree indicating the evolutionary distances among the ATMEKK1, Ste11, Byr2, Bck1, NPK1, and MEKK proteins is shown in Fig. 2*B*.

The putative ATMEKK1 protein has a noncatalytic flanking region in the N-terminal half (Fig. 2*C*). The Byr2, Bck1, Ste11, and MEKK proteins also have kinase-unrelated regions in their N-terminal halves; these regions are thought to regulate protein kinase activity, although the C-terminal half of the NPK1 protein has a kinase-unrelated region (14, 21–24). In contrast to the homologies among the catalytic domains of these protein kinases, the kinase-unrelated region of ATMEKK1 has no significant homology with those of NPK1, Byr2, Bck1, Ste11, and MEKK.

Functional Analysis of the ATMEKK1 Gene with Mutants of *S. cerevisiae*. The overexpression of cATMEKK1 with a multicopy plasmid suppressed the *ste11*Δ mutation (MEKK-deficient) of *S. cerevisiae*; this suppression was monitored by the complementation of the histidine-requiring genotype with the *FUS1*::*HIS3* reporter gene (30, 34) (Fig. 3*A*). However, the *ste7*Δ mutation (MEK-deficient) (30) could not be suppressed by cATMEKK1 (Fig. 3*B*). The Ste11 protein is shown to phosphorylate and activate the Ste7 protein directly in the mating-pheromone response pathway (4). These results indicate that the *STE11* deficiency is overcome by cATMEKK1. Overexpression of cATMEKK1 weakly complemented the *bck1*Δ mutation (data not shown).

Southern Blot Analysis of the ATMEKK1 Gene. *Arabidopsis* nuclear DNA was digested with *Eco*RI or *Hin*dIII, blotted onto nylon membranes, and hybridized under both high- and low-stringency conditions with the cATMEKK1 insert as a probe (Fig. 4). Under high-stringency conditions, the cATMEKK1 insert hybridized with one sharp band and several faint bands. However, under low-stringency conditions, several additional

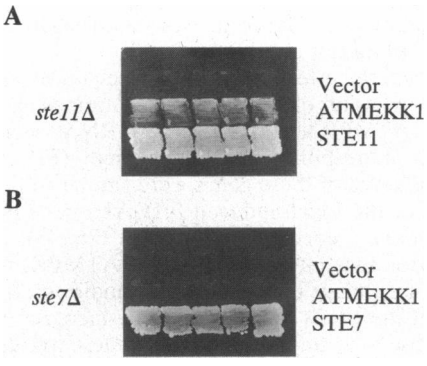

FIG. 3. Effects of expression of cATMEKK1 in yeast mutants. The yeast mutants are indicated at left. The photographs show the growth of cells. Each patch represents an independent transformant. The plasmids expressed in the cells are indicated at right. (*A*) Suppression of *ste11*Δ (SY1984) by overexpression of cATMEKK1. (*B*) Effects of expression of cATMEKK1 in the yeast *ste7*Δ (SY1493). Yeast strains were grown for 4 days at 30°C on plates with synthetic complete lacking histidine.

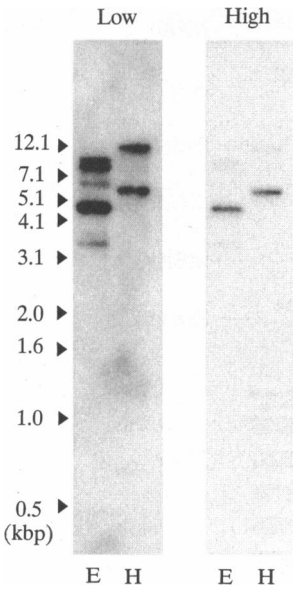

FIG. 4. Genomic DNA gel blot analysis. Genomic DNA was digested with *Eco*RI (E) or *Hin*dIII (H), fractionated in 1% agarose gels, and transferred to nylon membranes. Filters were hybridized with a ^{32}P-labeled fragment of ATMEKK1 cDNA in 50% formamide/5× standard saline citrate (SSC)/25 mM sodium phosphate buffer, pH 6.5/10× Denhardt's solution with denatured salmon sperm DNA (250 μg/ml) at 42°C and washed in either 0.5× SSC/0.5% SDS at 37°C (low stringency) (*Left*) or 0.1× SSC/0.1% SDS at 65°C (high stringency) (*Right*). The sizes of DNA markers are indicated in kilobase pairs.

bands were detected, indicating that the ATMEKK genes constitute a gene family on the *Arabidopsis* genome. Isolation of three additional cDNAs (cATMEKK2, cATMEKK3, and cATMEKK4) related to cATMEKK1 supports this conclusion (data not shown).

Transcript Levels of ATMEKK1, ATMPK3, and ATPK19 in Response to Low Temperature, Salinity, Dehydration, and Touch. To analyze the expression of the ATMEKK1 gene under stress conditions, we carried out Northern blot analysis and found that the mRNA level of ATMEKK1 increased within 1 hr when plants were under cold or high-salinity stress conditions (Fig. 5*A*). Interestingly, the mRNA levels of two protein kinases, ATMPK3 and ATPK19, increased simultaneously in response to these stresses. ATMPK3 is structurally related to MAPKs (12), while ATPK19 is structurally related to ribosomal S6 kinases ($p70^{S6K}$ and $pp90^{rsk}$) (16). The levels of the ATMEKK1, ATMPK3, and ATPK19 mRNAs did not change when plants were transferred from GM agar plates to water as controls. By contrast, the mRNA level of cdc2a (29) was not affected by these treatments. The mRNA level of ATMPK1 (13) was not affected by water and low-temperature treatments but was slightly affected by high-salinity stress. Then we examined the levels of the ATMEKK1, ATMPK3, ATPK19 mRNAs within 1 hr after a variety of stresses, including dehydration stress. The levels of the ATMEKK1, ATMPK3, and ATPK19 mRNAs in response to low temperature, high-salinity, and dehydration stresses began to increase within 5 min (data not shown).

We examined the effect of touch or mechanical stimuli (26, 35) on the expression of the three protein kinase genes. The ATMEKK1, ATMPK3, and ATPK19 mRNAs increased simultaneously in response to touch stimuli (Fig. 5*B*). The expression patterns of these genes were similar to the expression pattern of the touch-induced ATCAL5 gene (see *Materials and Methods*), encoding calmodulin (26, 45), used as a positive control. By contrast, the levels of ATMPK1 and cdc2a mRNAs did not change under the same conditions. It has been reported that the cdc2a mRNA markedly increases within 30 min in response to wounding stress (36). These results indicate that the accumulation of the transcripts for ATMEKK1, ATMPK3, and ATPK19 is not due to wounding stress but due to touch.

DISCUSSION

We have cloned a cDNA which encodes a MAPKKK in *Arabidopsis*. The putative ATMEKK1 protein has extensive

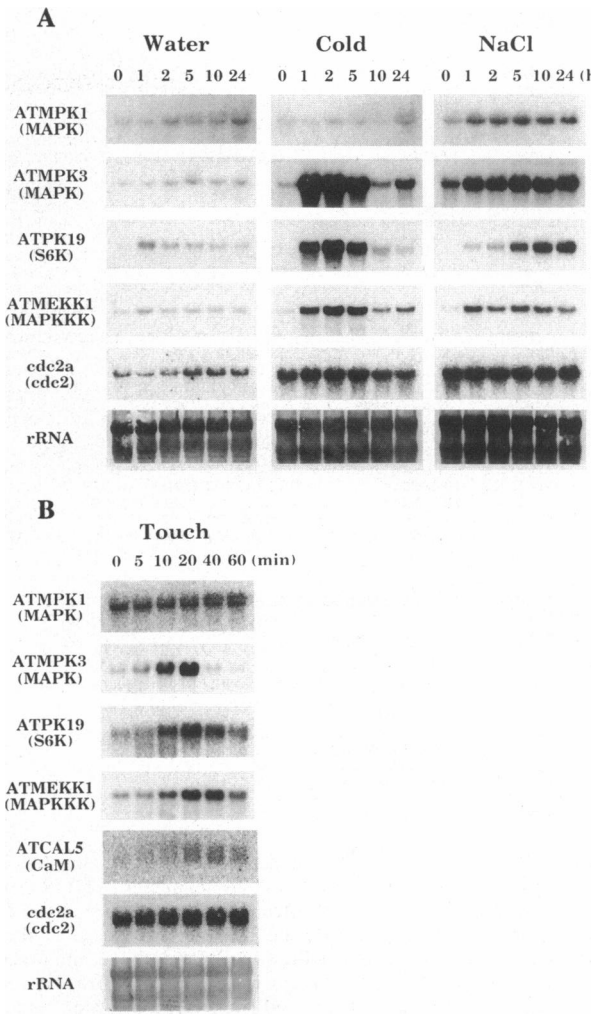

FIG. 5. Similar expression profiles of three genes encoding protein kinases that function in a MAPK cascade—ATMEKK1, ATMPK3, and ATPK19—in response to a variety of environmental stresses. The mRNA levels were examined by Northern blot analysis with ATMEKK1, ATMPK1, ATMPK3, ATPK19, ATCAL5 (encoding calmodulin, CaM), and cdc2a cDNAs as probes. The number above each lane indicates the time (in hours or minutes) between the initiation of treatment and the isolation of RNA. For the rRNA panel, a nylon membrane blotted with size-separated total RNA (40 μg) was stained with methylene blue to show that similar amounts of RNA were loaded per lane. The two prominent bands are rRNAs. (*A*) Expression of genes for ATMEKK1, ATMPK3, and ATPK19 in response to low temperature (4°C) and high salinity (250 mM NaCl) treatments. Water, control. (*B*) Expression of genes for ATMEKK1, ATMPK3, and ATPK19 in response to touch stimuli.

homology in the catalytic domain with other MAPKKKs and has a unique noncatalytic flanking region in the N-terminal region (Figs. 1 and 2). Overexpression of cATMEKK1 overcame a *ste11*Δ mutation (Fig. 3*A*) and weakly restored the functionality of a *bck1*Δ mutant (data not shown). We also examined the effect of cATMEKK1 overexpression in a mutant lacking the *STE7* gene, whose product is known to act downstream of Ste11 (Fig. 3*B*). Overexpression of cATMEKK1 could not suppress the *ste7*Δ mutation. Furthermore, weak mating responses were observed in the *ste11*Δ mutant when cATMEKK1 was overexpressed in the presence of $Ste7^{P368}$ (data not shown). $Ste7^{P368}$ is a gain-of-function mutant of Ste7 (34) and has an increased kinase activity to induce mating responses without pheromone stimulation, although this kinase activity is still dependent on the presence of Ste11 (34). An activated form of mammalian Raf (RafΔN) was

shown to compensate for the suppressed Ste11 activity in this system (34). These results indicate that ATMEKK1 can function as a MAPKKK in *S. cerevisiae*, at least in the Ste11-mediated signaling system.

Northern blot analysis showed that the ATMEKK1 mRNA accumulated in response to environmental stresses, such as low temperature, high salinity, and dehydration (Fig. 5*A*). Moreover, the mRNA of the ATMPK3 gene, which encodes a MAPK homolog, and the mRNA of the ATPK19 gene, which encodes an S6 kinase homolog, also accumulated simultaneously under the same stress conditions. These results suggest that these three protein kinases in a MAPK cascade may function in the signal transduction pathway under osmotic stress conditions and that the MAPK cascade may be regulated not only at the protein-modification level but also at the transcriptional level. In yeast, one of the MAPK cascades, the *PBS2/HOG1* pathway, functions under osmotic stress response (37) and is linked to an upstream two-component osmosis-sensing system, named *sln1/ssk1* (38). Recently, a similar signal transduction pathway has been reported to function in the ethylene response of higher plants. The *ETR1* gene encodes the two-component histidine kinase (39) and the *CTR1* gene encodes the Raf homolog (20). Two MAPK homologs, JNK1 and p38, were demonstrated to be activated in response to osmotic shock in animal systems and to complement a *hog1* mutation in yeast (8, 37, 40). These results suggest that the MAPK cascade generally functions in response to osmotic stress in yeast, animal, and plant systems.

The genes for ATMEKK1, ATMPK3, and ATPK19 also respond to touch or mechanical pressure at the mRNA level (Fig. 5*B*). Three of the touch-inducible genes (Tch1, Tch2, and Tch3) encode calmodulin or calmodulin-related proteins (26). It has been reported that touch and cold shock cause an immediate increase of cytoplasmic calcium ion in tobacco seedlings (41). These results suggest that touch-inducible genes for calmodulin, MAPKKK, MAPK, and S6 kinase may function in calcium-signaling pathways in response to touch and low temperature.

Under stress conditions, the elevated levels of the mRNAs encoding the protein kinases ATMEKK1, ATMPK3, and ATPK19 may increase their protein levels, which is likely to amplify the signal transduction efficiency of the cascade. In higher plants, the mRNAs of various genes involved in signal transduction pathways accumulate in response to environmental stimuli or stress. We have also demonstrated that the mRNAs of a transcription factor MYB homolog (42), one phospholipase C (43), and two calcium-dependent protein kinases (44) accumulate under osmotic stress conditions. Based on these observations, we can speculate that factors involved in signal transduction pathways are controlled at not only posttranslational but also transcriptional levels in higher plants.

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