Two genes that encode ribosomal-protein S6 kinase homologs are induced by cold or salinity stress in *Arabidopsis thaliana*

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Abstract **We have isolated two closely related cDNA clones (cATPK19 and cATPK6) with homology to protein-serine/threonine kinases from *Arabidopsis thaliana* using the polymerase chain reaction (PCR). The deduced amino acid sequences of the ATPK19 and ATPK6 contain all 11 conserved regions of the catalytic domain of protein kinases, and have homology to p70 ribosomal S6 kinases (52%). ATPK19 and ATPK6 have putative PEST regions in their N- and C-terminal regions, and these regions also contain putative phosphorylation sites that are recognized by casein kinases or proline-directed protein kinases such as cdc2, MAP kinase, and p54 MAP-2 kinase (SAPK). The transcription levels of the *ATPK19* and *ATPK6* genes rapidly and markedly increased when plants were subjected to cold or high-salt stresses. These observations suggest that ATPK19 and ATPK6 may function in the adaptation of plant cells to cold or high-salt conditions, providing an understanding of the role of protein phosphorylation in plant responses to environmental stresses.**

Key words: Arabidopsis thaliana; Protein kinase; Ribosomal S6 kinase

1. Introduction

Protein kinases play important roles in the transduction of environmental and developmental signals in animals and yeasts [1]. The molecular cloning of genes that encode animal and yeast protein kinases, and the knockout/disruption of these genes in the organisms themselves, have provided much information about the functions of protein kinases in vivo. Recent studies using molecular cloning and biochemical techniques have shown that there are also a variety of protein kinases in plants [2,3]. The roles of protein kinases in a variety of signal transduction pathways remain to be elucidated in plants, although some of their functions have been studied using mutant analyses [4–6].

The availability of amino acid sequences for a number of protein kinases from various organisms [1] provides a new method for the isolation and characterization of additional protein kinases from plants. Using the polymerase chain reaction (PCR) method, we have isolated several distinct DNA fragments containing partial sequences of protein kinases from a small crucifer plant, *Arabidopsis thaliana* [7,8]. We found that one of these amplified DNA sequences has a high degree of homology with ribosomal protein S6 kinases in animals [9–13].

Two types of ribosomal S6 kinases have been reported, p90 ribosomal S6 kinase (now referred to as $pp90^{rsk}$ or RSK) [10,11], and p70 ribosomal S6 kinases ($p70^{S6K}$) in animals [12,13]. Both kinases are activated by serine/threonine phosphorylation [9], and MAP kinase was shown to phosphorylate and partially reactivate dephosphorylated $pp90^{rsk}$ [14]. However, MAP kinase and $p70^{S6K}$ were shown to function on different signalling pathways, and the direct upstream kinases of $p70^{S6K}$ have not been identified [15]. The phosphorylation of the 40 S ribosomal protein S6 by $p70^{S6K}$ activates certain ribosomes, which preferentially translate messenger RNAs containing a polypyrimidine tract at their 5′ end. This type of RNA encodes proteins for progression through the G_1 phase of the cell cycle [16].

In the present study, we report the characterization and expression of two cDNA clones of *A. thaliana*, cATPK19 and cATPK6, which encode for proteins most closely related to ribosomal S6 kinases, $p70^{S6K}$ [12,13] and $pp90^{rsk}$ [10,11]. We also demonstrate that the mRNA levels of *ATPK19* and *ATPK6* increase in response to low temperatures and salinity. Finally, we discuss the possible roles of these two protein kinases in the signal transduction pathways in plants under these stress conditions.

2. Materials and methods

2.1. Polymerase chain reaction

The template DNA was obtained from an amplified cDNA library, prepared from 3-week-old *A. thaliana* rosettes by phenol extraction and CsCl gradient centrifugation. The DNA inserts were amplified by PCR using two oligonucleotide primers corresponding to the boundary sequences of the arms of λgt11 (5′-GGTGGC-GACGACTCCTGGAG-CCCG-3′ and 5′-TTGACACCAGACCAACTGGT-AATG-3′). The following oligonucleotide sequences were used as mixed primers C (PK0IC1, PK0IC2 and PKIXC) for each reaction.

PK0IC1 5′-(T/C)TIGGIAA(G/A)GGITCITT(T/C)GGIAA(G/A)-GT-3′
PK0IC2 5′-(T/C)TIGGIAA(G/A)GGIAG(T/C)TT(T/C)GGIAA-(G/A)GT-3′
PKIXC 5′-A(G/A)IA(G/A)IACICC(G/A)(A/T)AIGCCCACCA-(G/A)TC-3′

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The nucleotide sequences reported in this paper have been submitted to the DNA Data Bank of Japan with accession numbers D42056 (ATPK6) and D42061 (ATPK19).

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All the primers were phosphorylated with T4 polynucleotide kinase under standard reaction conditions [17]. PCR was performed using a GeneAmp kit according to the manufacture's protocol (Perkin Elmer Cetus, Norwalk, CT, USA). The procedure was executed over 35 cycles, consisting of denaturation at 93°C for 1 min, annearing at 37°C for 2 min, and polymerization at 72°C for 3 min. The amplified DNA fragments, which ranged from 400 to 600 base pairs (bp) in length, were cloned into the pBluescript vector, pSKII$^-$, (Stratagene, La Jolla, CA, USA), digested with *Sma*I and dephosphorylated.

2.2. Cloning and sequencing of two full-length cDNAs, cATPK19 and cATPK6, from an A. thaliana *cDNA library*

The *A. thaliana* cDNA library (described above) was screened by plaque hybridization using procedure of Maniatis et al. [17]. A PCR product, C11, which contained the conserved sequence of protein kinase, was used as a probe. This probe was labeled with ^{32}P using a random primer kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacture's instruction. Positive clones were plaque-purified and the DNA was isolated by phenol extraction and CsCl gradient centrifugation [17].

A 2.0 kbp *Bam*HI fragment containing the ATPK19 cDNA and a 1.8 kbp *Eco*RI fragment containing the ATPK6 cDNA were each subcloned into the pBluescript vector, pSKII$^-$, (Stratagene, La Jolla, CA, USA). A DNA Sequencer Model 373A (ABI, San Jose, CA, USA) was used for DNA sequencing. Nucleotide and amino acid sequences were analyzed using the GENETYX software system (Software Development Co., Tokyo, Japan).

2.3. Plant growth and stress treatments

A. thaliana (Columbia ecotype) plants were grown on GM agar plates under a continuous illumination of 2500 lux at 22°C for 4 to 5 weeks, and subsequently used in stress treatment experiments prior to bolting as reported previously [18]. Plants subjected to treatment with abscisic acid (ABA), 2,4-D (2,4-D) and salt stress (NaCl) were grown hydroponically under dim light in solutions of 100 μM ABA, 100 μM 2,4-D, and 250 mM NaCl, respectively. Heat treatment (Heat) was performed under continuous light by exposing plants grown at 22°C to a temperature of 40°C. Plants subjected to treatments with water (Water) were grown hydroponically under dim light in 22°C water, while plants subjected to treatment with cold stress (Cold) were grown under similar conditions at 4°C. In all cases, the plants were subjected to the stress treatments for various time periods and frozen in liquid nitrogen for later analysis.

2.4. DNA and RNA blot hybridization analyses

Genomic Southern hybridization was performed as described previously [8,17]. Total RNA was isolated according to the method of Nagy et al. [19]. Northern hybridization was carried out as described previously [8,17]. The hybridization signals corresponding to the *ATPK19* or *ATPK6* mRNAs were quantified with a Bio Image Analyzer (FUJIX BAS2000, Fuji Film Co., Ltd., Tokyo, Japan).

3. Results

3.1. Isolation of a PCR-amplified DNA fragment containing a conserved sequence of protein kinase from A. thaliana

The remarkable conservation of protein kinase genes among a wide variety of organisms [1] allowed us to use PCR to isolate homologous sequences from *A. thaliana*. In this work two DNA sequences encoding conserved regions I and IX were used to perform PCR [1]. Degenerate oligonucleotide primers corresponding to two conserved amino acid sequences, LGKGSFGKV and DWWAF/YGVLL, were used for PCR amplification of an *A. thaliana* cDNA template prepared from 3-week-old rosette plants.

We obtained PCR-amplified fragments of about 540 bp in length. The 540 bp PCR products were cloned into a pBluescript II vector, pSKII$^-$, at the *Sma*I site and sequenced. One of the PCR-products has conserved sequences of protein kinases. The deduced amino acid sequence of the 540 bp insert is closely related to those of the ribosomal protein S6 kinases [9–13].

3.2. Cloning and sequence analysis of two cDNAs that encode ribosomal protein S6 kinase homologs

The 540 bp PCR-amplified fragment was used as a probe in screening a cDNA library to clone its corresponding cDNAs. Eight positive clones were obtained out of 6×10^5 plaques. Partial sequence analysis revealed that the cloned DNA inserts have two distinct but closely related sequences. We subcloned and sequenced the largest inserts for each of these cDNAs and named them cATPK19 and cATPK6. cATPK19 contains a 1413 bp open reading frame, encoding a polypeptide of 471 amino acids having a predicted molecular weight of 53,111. cATPK6 contains a 1395 bp open reading frame, encoding a polypeptide of 465 amino acids having a predicted molecular weight of 52,587. cATPK19 and cATPK6 are 82.0% identical at the nucleotide sequence level.

The deduced amino acid sequences of the putative ATPK19 and ATPK6 proteins are highly homologous to the conserved catalytic subdomains of serine/threonine-specific protein kinases (Fig. 1A). Conserved amino acids characteristic of each of the 11 catalytic subdomains found in almost all serine/threonine-specific protein kinases are present [1]. ATPK19 and ATPK6 are closely related to each other and 85% identical at the amino acid level. A search of the sequence data bases (GenBank, EMBL, and Swiss-Prot, October 1994) using GCG version 7.0 software showed that the ATPK19 and ATPK6 sequences within the putative catalytic domains are most closely related to human p70^{S6K} [12] (51.7–52.1% identity), followed by *Xenopus* pp90^{rsk} [10] (49.0–49.4% identity), human protein kinase A [20] (41.5–41.1% identity), and human protein kinase C [21] (40.2–41.4% identity). Comparisons of the deduced amino acid sequences of ATPK19 and ATPK6 with these protein kinases showed a high degree of sequence identity

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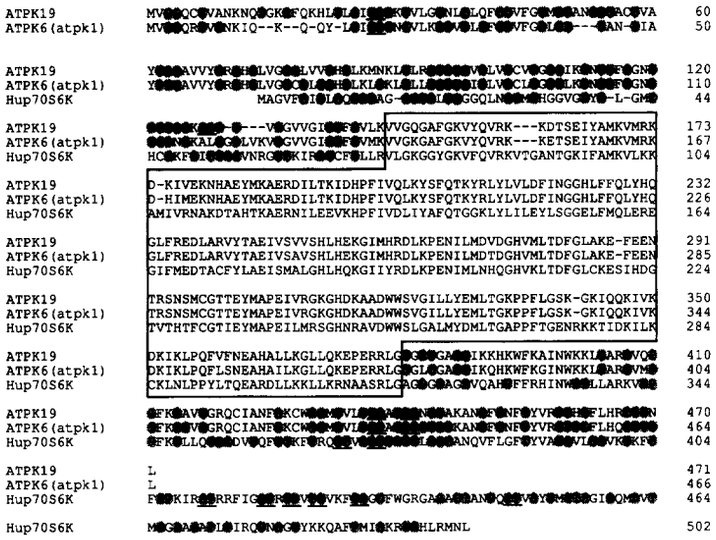
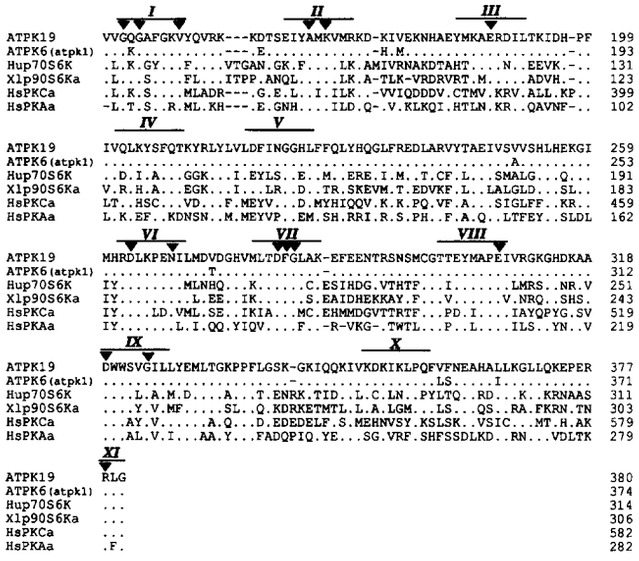
Fig. 1. (A) Comparison of the deduced amino acid sequences of putative catalytic domains of ATPK19, ATPK6, human p70 ribosomal S6 kinase (Hup70S6K) [12], *Xenopus* p90 S6 kinase II α (XIp90S6Ka) [10], human PKCα (HsPKCa) [21], and human PKAα (HsPKAa) [20]. Dots represent identical amino acid residues with ATPK19 and dashes indicate gaps introduced to maximize alignment. Closed triangles indicate the conserved amino acid residues among protein kinases. Roman numerals indicate the eleven major conserved subdomains of protein kinases identified previously [1]. When this work had been completed, a cDNA clone, atpk1 (accession number L29030), encoding apparently the same protein kinase as ATPK6 was isolated from *A. thaliana* by Zhang et al. [29]. (B) The putative PEST sequences in the N- and C-terminal regions of the ATPK19, ATPK6, and human p70 ribosomal S6 kinase (Hup70S6K) [12]. Proline (P), glutamic acid (E), serine (S), threonine (T), and aspartic acid (D) residues are underlined. The consensus phosphorylation sites for casein kinase II (S/TX$_2$E/D) and proline-directed protein kinases such as cdc2 (XS/TPX), MAP kinase (PX$_{1\text{ or }2}$S/TP) are boxed. (C) A phylogenetic tree showing evolutional relationship among ATPK19, ATPK6, ribosomal S6 kinase (RSK) family, protein kinase C (PKC) family, and protein kinase A (PKA) family was constructed from the matrix of sequence similarities calculated with the UPGMA program. Numbers above the horizontal lines indicate the evolutional distance between one protein and another. Species abbreviations: Hs and Hu, *Homo sapiens*; Rn, *Rattus norvegicus*; Gg, *Gallus gallus*; Mm, *Mus musculus*; Xl, *Xenopus laevis*; Dm, *Drosophila melanogaster*; Sc, *Saccharomyces cerevisiae*. All sequences used for these analyses are from the SwissProt and GenBank data bases.

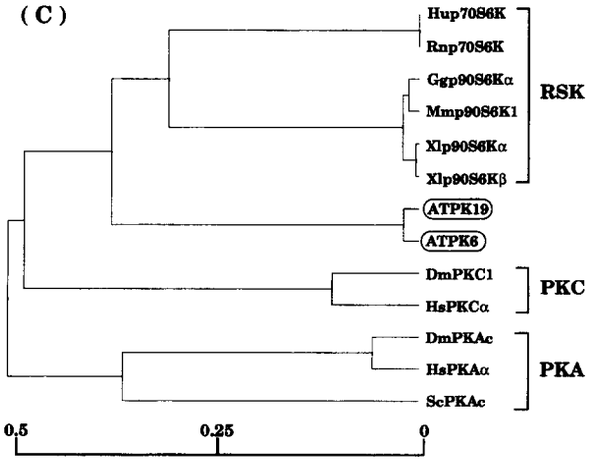
(A)



(B)



(C)


Hup70S6K
Rnp70S6K
Ggp90S6Kα
Mmp90S6K1
Xlp90S6Kα
Xlp90S6Kβ
RSK
ATPK19
ATPK6
DmPKC1
HsPKCα
PKC
DmPKAc
HsPKAα
ScPKAc
PKA
0.5
0.25
0

and similarity within 11 conserved subdomains of the catalytic domain (Fig. 1A). To analyze the relation of ATPK19 and ATPK6 to other protein kinases, an unrooted phylogenetic tree was constructed (Fig. 1B). Based on this analysis, ATPK19 and ATPK6 are most closely related to the RSK family, and are less closely related to the PKC or the PKA families.

3.3. *Southern blotting analysis of the ATPK19 and ATPK6 genes*

We estimated the number of genes related to *ATPK19* and *ATPK6* in the *A. thaliana* genome by Southern blot analyses (Fig. 2). *A. thaliana* nuclear DNA was digested with *Bam*HI, *Eco*RI and *Hin*dIII, blotted onto nylon membranes, and hybridized under both high and low stringency conditions using the ATPK19 and ATPK6 cDNAs as probes. Under high stringency hybridization conditions, ATPK19 hybridized with one strong and one weak *Bam*HI, four *Eco*RI, and three strong and two weak *Hin*dIII fragments. ATPK6 hybridized with one strong and one weak *Bam*HI, one strong and one weak *Eco*RI, and two strong and three weak *Hin*dIII fragments under the same conditions. Under low stringency hybridization conditions, no signals other than cross-hybridizing bands corresponding to *ATPK19* or *ATPK6* genes were detected. This result indicates that there are no other genes closely related to *ATPK19* and *ATPK6* in the *Arabidopsis* genome.

3.4. *Northern analysis of the induction of the ATPK19 and ATPK6 genes under stress conditions*

Recently, the transcription levels of protein kinases [22–24], transcription factors [25,26], G proteins [27], and calmodulins [28] in plants have been reported to be affected by environmental stresses (e.g. light, temperature, mechanical stress, and dessication). Therefore, we analyzed the accumulation of the

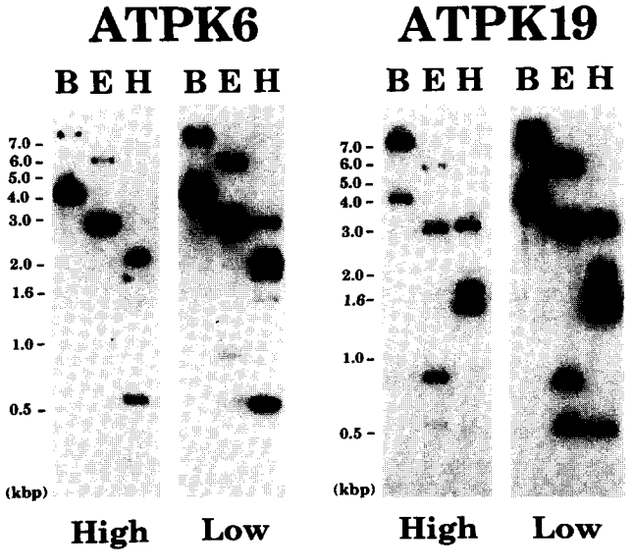


Fig. 2. Genomic DNA gel blot analysis. Genomic DNA was digested with *Bam*HI (B), *Eco*RI (E) and *Hin*dIII (H), fractionated on 1% agarose gels and transferred to nylon membranes. Filters were hybridized with ^{32}P-labeled fragment of cATPK19 or cATPK6 cDNAs at 42°C and washed in either 0.5× SSC/0.5% SDS at 37°C (low stringency) or 0.1× SSC/0.1% SDS at 65°C (high stringency). High and Low represent high- and low-stringency hybridization conditions, respectively. The sizes of DNA markers are indicated in kbp.

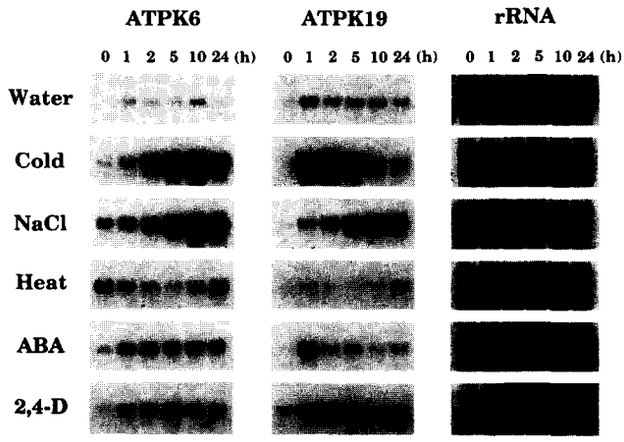


Fig. 3. Effects of a variety of stresses on the expression of the *ATPK19* and *ATPK6* genes. Each lane was loaded with 40 μg of total RNA prepared from unbolted *Arabidopsis* plants that had been transferred from agar plates to 22°C water and grown at 22°C (Water), transferred to 4°C water and grown at 4°C (Cold), transferred from agar plates for hydroponic growth in 250 mM NaCl (NaCl), transferred to and grown at 40°C (Heat), transferred from agar plates for hydroponic growth in 100 μM ABA (ABA) or 100 μM 2,4-D (2,4-D), as described in section 2. RNA was subjected to electrophoresis on 1% agarose gels that contained formaldehyde. A nylon membrane blotted with RNA were hybridized with ^{32}P-labeled cATPK19 or cATPK6 cDNAs. The number above each lane indicates the number of hours after the initiation of treatment prior to isolation of RNA. A nylon membrane blotted with RNA was stained with Methylene blue to show that similar amounts of RNA were loaded per lane. The two prominent bands are rRNAs. The ratio of hybridization signals for the *ATPK19* and *ATPK6* mRNAs were measured by a Bio Image Analyzer (FUJIX BAS2000, Fuji Film Co., Tokyo, Japan).

ATPK19 and *ATPK6* mRNAs in *A. thaliana* in response to a variety of stress conditions for various exposure times of up to 24 h (Fig. 3). The levels of the *ATPK19* and *ATPK6* mRNAs were low in our controls, fully hydrated plants grown on GM agar plates. However, when the plants were transferred from GM agar plates to water at 4°C, the *ATPK19* mRNA began to accumulate in response to the low temperature within 1 h, peaked (8.8-fold) after 2 h, and then fell gradually at least for the first 24 h of the treatment. The level of the *ATPK6* mRNA also began to increase within 2 h in response to the same treatment and peaked (6.8-fold) after 10 h. Under high-salinity conditions, the *ATPK19* mRNA began to accumulate within 1 h, peaked (6.3-fold) after 10 h, and remained at an elevated level for at least 24 h. The level of the *ATPK6* mRNA also increased within 2 h, peaked (4.8-fold) after 10 h, and remained at an elevated level for at least 24 h. When plants were transferred from GM agar to water at room temperature as a control, the levels of the *ATPK19* and *ATPK6* mRNAs slightly increased within 1 h and then decreased immediately. Although the *ATPK19* mRNA increased in response to the application of exogenous 2,4-D and the *ATPK6* mRNA increased in response to ABA or 2,4-D, the levels of the accumulated mRNAs of *ATPK19* and *ATPK6* were relatively low compared with those observed under cold or high-salinity stress conditions.

4. Discussion

In the present study, we demonstrated that transcription levels of the *ATPK19* and *ATPK6* genes for putative ribosomal

S6 kinase markedly increased in response to cold stress (Fig. 3). The level of the *ATPK19* mRNA began to increase and peaked earlier than that of *ATPK6*. The transcription levels of *ATPK19* and *ATPK6* also increased in response to high-salinity stress (Fig. 3). Their mRNAs accumulated transiently by dehydration (Mizoguchi, unpublished observation), while their gene expression was scarcely affected by the application of exogenous ABA. We have shown that the dehydration-responsive *rd29A* gene is induced under low-temperature and high-salinity conditions but is not responsive to exogenous ABA [18]. A cis acting element named DRE (Dehydration Responsive Element, TACCGACAT) is involved in its stress-responsive expression [18]. The induction pattern of *ATPK19* and *ATPK6* is similar to that of *rd29A*, which suggests that DRE may function in the induction of *ATPK19* and *ATPK6*. Recently, we found that the transcription levels for two putative protein kinases involved in MAP kinase cascade, MAP kinase (ATMPK3) and MAP kinase kinase kinase (ATMEKK1) in *A. thaliana*, increased in response to cold or high salinity stresses within 1 h (Mizoguchi et al., submitted for publication) but do not respond to exogenous ABA. *ATPK19* showed a similar pattern of expression to *ATPK3* and *ATMEKK1*, and encodes ribosomal protein S6 kinase, which is one of the target proteins of MAP kinase in animals. These results suggest that three genes for protein kinases involved in the MAP kinase cascade are responsive to low temperature and osmotic stress at the transcription level, and that the MAP kinase cascade may function in the signal transduction pathway under these stress conditions. Many plant genes involved in signal transduction pathways have been shown to be induced by a variety of stimuli. These upregulated factors probably function in the amplification of signals under a variety of environmental or developmental stimuli in plants.

We found that ATPK19 and ATPK6 from *A. thaliana* are closely related to animal $p70^{S6K}$ and $pp90^{rsk}$ (Fig. 1). In animals, $p70^{S6K}$ phosphorylates 40S ribosomal protein S6 and activates protein synthesis [12,13,16]. Recently, Zhang et al. has demonstrated that the Atpk1 protein, which is apparently identical with the ATPK6 protein, can phosphorylate two plant ribosomal proteins [29,30]. We speculate that ATPK19 and ATPK6 may play important roles in increasing the capacity of protein synthesis through the phosphorylation of plant ribosomal proteins when plants are exposed to cold or high-salinity stress conditions. The production of a nucleolin-like protein NSR1, which is associated with pre-rRNA processing and ribosome assembly in yeast, increases upon cold shock of yeast cells, which suggests that yeasts adapt to cold temperatures by increasing their capacity for protein synthesis [31]. Therefore, increasing the capacity of protein synthesis may be one of the general strategies of cells to adapt to environmental stimuli or stresses [31]. Another possibility is that ATPK19 and ATPK6 may phosphorylate and regulate the proteins which function mainly under low temperature or high-salinity stress conditions. These two protein kinases may have important roles in the adaptive response of plant cells to environmental stress.

ATPK19 and ATPK6 have putative regulatory domains in their N- and C-terminal regions (Fig. 1A), and contain the putative PEST sequences in these same regions [32,33]. Proteins that contain PEST sequences are thought to be rapidly degraded. Several consensus phosphorylation sites for casein kinase II ($S/TX_2E/D$), and proline-directed protein kinases such as cdc2 (XS/TPX), and MAP kinase ($PX_{1\text{ or }2}S/TP$), were found in the putative PEST regions of ATPK19 and ATPK6 [34]. The $p70^{S6K}$ protein also has the PEST sequence in its N- and C-terminal regions. The C-terminal PEST sequence, which is thought to function as an auto-inhibitory domain [35], has been demonstrated to be phosphorylated but not activated by three proline-directed protein kinases (cdc2, MAP kinase, and p54 MAP-2 kinase) [35]. In addition to the actions of these known kinases, phosphorylation by other as-yet unidentified protein kinases may be necessary to activate $p70^{S6K}$ [35]. We found that the transcription levels of ATPK19 as well as two other genes involved in the MAP kinase cascade, MAP kinase (ATMPK3) and MAP kinase kinase kinase (ATMEKK1), increased in response to cold or high salinity stresses within 1 h (Mizoguchi et al., submitted for publication). It is possible for us to speculate that MAP kinase may phosphorylate and regulate the function of the ATPK19 protein in *Arabidopsis*.

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References

[1] Hanks, S.K. and Quinn, A.M. (1991) Methods Enzymol. 200, 38–61.
[2] Roberts, D.M. and Harmon, A.C. (1992) Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 43, 375–414.
[3] Jonak, C., Heberle-Bors, E. and Hirt, H. (1994) Plant Mol. Biol. 24, 407–416.
[4] Kieber, J.J., Rothenberg, M., Roman, G., Feldmann, K.A. and Ecker, J.R. (1993) Cell 72, 427–441.
[5] Martin, G.B., Brommonschenkel, S.H., Chunwongse, J., Fray, A., Ganal, M.W., Spivey, R., Wu, T., Earle, E.D. and Tanksley, S.D. (1993) Science 262, 1432–1436.
[6] Roe, J.L., Rivin, C.J., Sessions, R.A., Feldmann, K.A. and Zambryski, P.C. (1993) Cell 75, 939–950.
[7] Mizoguchi, T., Yamaguchi-Shinozaki, K., Hayashida, N., Kamada, H. and Shinozaki, K. (1993) Plant Mol. Biol. 5, 279–289.
[8] Mizoguchi, T., Gotoh, Y., Nishida, E., Yamaguchi-Shinozaki, K., Hayashida, N., Iwasaki, T., Kamada, H. and Shinozaki, K. (1994) Plant J. 5, 111–122.
[9] Downward, J. (1994) Nature 371, 378–379.
[10] Jones, S.W., Erikson, E., Blenis, J., Maller, J.L. and Erikson, R.L. (1988) Proc. Acad. Sci. USA 85, 3377–3381.
[11] Alcorta, D.A., Crews, C.M., Sweet, L.J., Bankston, L., Jones, S.W. and Erikson, R.L. (1989) Mol. Cell. Biol. 9, 3850–3859.
[12] Grove, J., Banerjee, P., Balasubramanyam, A., Coffer, P., Price, P.J., Avruch, J. and Woodgett, J.R. (1991) Mol. Cell. Biol. 11, 5541–5550.
[13] Kozma, S.C., Ferrari, S., Bassand, P., Siegmann, M., Totty, N. and Thomas, G. (1990) Proc. Natl. Acad. Sci. USA 87, 7365–7369.
[14] Sturgill, T.W., Ray, L.B., Erikson, E. and Maller, J.L. (1988) Nature 334, 715–718.
[15] Ballou, L.M., Luther, H. and Thomas, G. (1991) Nature 349, 348–350.
[16] Jefferies, H.B.J., Reinhard, C., Kozma, S.C. and Thomas, G. (1994) Proc. Natl. Acad. Sci. USA 91, 4441–4445.
[17] Maniatis, T., Fitsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[18] Yamaguchi-Shinozaki, K. and Shinozaki, K. (1994) Plant Cell. 6, 251–264.
[19] Nagy, F., Kay, S.A. and Chua, N.-H. (1988) in: Plant Molecular Biology Manual B4 (Gelvin, S.V. and Schilperoort, R.A., Eds.) pp. 1–29, Kluwer Academic Publishers, Dordrecht.

[20] Maldonado, F. and Hanks, S.K. (1988) Nucleic Acids Res. 16, 8189–8190.
[21] Finkenzeller, G., Marme, D. and Hug, H. (1990) Nucleic Acids Res. 18, 2183.
[22] Urao, T., Katagiri, T., Mizoguchi, T., Yamaguchi-Shinozaki, K., Hayashida, N. and Shinozaki, K. (1994) Mol. Gen. Genet. 244, 331–340.
[23] Lin, X., Feng, X. and Watson, J.C. (1991) Proc. Natl. Acad. Sci. USA 88, 6951–6955.
[24] Anderberg, R.J. and Walker-Simmons, M.K. (1992) Proc. Natl. Acad. Sci. USA 89, 10183–10187.
[25] Aguan, K., Sugawara, K., Suzuku, N. and Kusano, T. (1993) Mol. Gen. Genet. 240, 1–8.
[26] Urao, T., Yamaguchi-Shinozaki, K., Urao, S. and Shinozaki, K. (1993) Plant. Cell. 5, 1529–1539.
[27] Yoshida, K., Nagano, Y., Murai, N. and Sasaki, Y. (1993) Proc. Natl. Acad. Sci. USA 90, 6636–6640.
[28] Braam, J. and Davis, R.W. (1990) Cell 60, 357–364.
[29] Zhang, S.-H., Lawton, M.A., Hunter, T. and Lamb, C.J. (1994) J. Biol. Chem. 269, 17586–17592.
[30] Zhang, S.-H., Broome, M.A., Lawton, M.A., Hunter, T. and Lamb, C.J. (1994) J. Biol. Chem. 269, 17593–17599.
[31] Kondo, K. and Inoue, M. (1992) J. Biol. Chem. 267, 16252–16258.
[32] Rogers, S., Wells, R. and Rechsteiner, M. (1986) Science 234, 364–368.
[33] Rechsteiner, M., Rogers, S. and Rote, K. (1987) Trends Biochem. Sci. 12, 390–394.
[34] Pearson, R.B. and Kemp, B.E. (1991) Methods Enzymol. 200, 62–81.
[35] Mukhopadhyay, N.K., Price, D.J., Kyriakis, J.M., Pelech, S., Sanghera, J. and Avruch, J. (1992) J. Biol. Chem. 267, 3325–3335.