

Identification of a Receptor-Like Protein Kinase Gene Rapidly Induced by Abscisic Acid, Dehydration, High Salt, and Cold Treatments in *Arabidopsis thaliana*¹

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A cDNA clone for a receptor-like protein kinase gene (*RPK1*) was isolated from *Arabidopsis thaliana*. The clone is 1952 bp long with 1623 bp of an open reading frame encoding a peptide of 540 amino acids. The deduced peptide (*RPK1*) contains four distinctive domains characteristic of receptor kinases: (a) a putative amino-terminal signal sequence domain; (b) a domain with five extracellular leucine-rich repeat sequences; (c) a membrane-spanning domain; and (d) a cytoplasmic protein kinase domain that contains all of the 11 subdomains conserved among protein kinases. The *RPK1* gene is expressed in flowers, stems, leaves, and roots. Expression of the *RPK1* gene is induced within 1 h after treatment with abscisic acid (ABA). The gene is also rapidly induced by several environmental stresses such as dehydration, high salt, and low temperature, suggesting that the gene is involved in a general stress response. The dehydration-induced expression is not impaired in *aba-1*, *abi1-1*, *abi2-1*, and *abi3-1* mutants, suggesting that the dehydration-induced expression of the *RPK1* gene is ABA-independent. A possible role of this gene in the signal transduction pathway of ABA and the environmental stresses is discussed.

Plants encounter a variety of external and internal environmental changes. Among the external environmental factors critical for survival of plants are water, temperature, light, and other organisms. Internal environmental factors include plant hormones such as ABA, auxins, cytokinins, ethylene, GA₃, jasmonic acid, and brassinosteroids. The ability of plant cells to respond and adapt to these environmental factors is crucial in choosing a proper developmental strategy and for surviving outdoors.

It is becoming evident that protein kinases are important components in the signal transduction pathways of various environmental signals in plants, as they are in yeasts and animals (Ma, 1993; Stone and Walker, 1995). In animal systems many of the extracellular signals are first perceived by membrane-associated receptor kinases (Lemmon and Schlessinger, 1994). These receptor kinases are typically composed of an extracellular ligand-binding domain,

a transmembrane domain, and a cytosolic kinase domain. Binding of an extracellular ligand(s) to the extracellular domain activates the cytoplasmic kinase domain, thus transducing an extracellular signal(s) into intracellular targets. In higher plants protein kinases with structures similar to those of animal receptor protein kinases have been discovered recently (reviewed by Walker, 1994; Stone and Walker, 1995). These plant receptor protein kinases may be classified into several groups according to the characteristics of their extracellular domains; one of these groups contains LRRs in the extracellular domain. Since the discovery of the *TMK1* gene, which encodes an LRR containing receptor-like kinases in *Arabidopsis thaliana* (Chang et al., 1992), several other receptor-like kinases of this type have been identified. The plant protein kinases in this group with a known function include the proteins encoded by the *Arabidopsis ER* (Torii et al., 1996), the rice *Xa21* (Song et al., 1995), and the petunia *PRK1* (Mu et al., 1994; Lee et al., 1996) genes. Thus, it became clear that the receptor-like protein kinases containing LRRs are critical components in the signal transduction pathways of developmental and environmental signals.

A major environmental stress that plants have to cope with is the change in osmotic potential. The knowledge about the molecular mechanism that operates in the signal transduction pathway of the cellular response to osmotic stress is still minimal, although the adaptive physiological and molecular responses to osmotic stress have been studied intensively in plants (Mansfield, 1988; Zeevaart and Creelman, 1988; Skriver and Mundy, 1990; Trewas and Jones, 1991; Bray, 1994; Davies et al., 1994). However, increasing evidence suggests that protein phosphorylation may be involved in the pathway. Some polypeptides induced by ABA and osmotic stress, such as maize RAB-17 (Plana et al., 1991) and tomato dehydrin (Godoy et al., 1994), are accumulated in a phosphorylated form. Specific protein kinases that can phosphorylate these proteins *in vitro* have been identified. It has been shown that some dehydration responses are impaired in the mutants of the *Arabidopsis abi1* gene (reviewed by Finkelstein and Zeevaart, 1994; Giraudat et al., 1994) that encodes a type 2C

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Abbreviations: LRR, Leu-rich repeat; RACE, rapid amplification of cDNA ends; RPK1, receptor-like protein kinase 1.

phosphatase (Leung et al., 1994; Meyer et al., 1994). Furthermore, expression of several plant protein kinase genes has been shown to be induced by dehydration. These protein kinases include two *Arabidopsis* calcium-dependent protein kinase genes (Urao et al., 1994; Holappa and Walker-Simmons, 1995; Hwang and Goodman, 1995; Mizoguchi et al., 1996). Whereas the structures of the protein kinases encoded by these genes imply that they are intracellular proteins, there has been no report on a receptor-like protein kinase that is induced by osmotic stress.

As a part of our efforts to understand both the structure and function of protein kinases in plants (Park et al., 1993, 1995), we have attempted to isolate the cDNA clones for receptor-like protein kinases. Here we report an *Arabidopsis* cDNA clone encoding a new receptor-like protein kinase, which we named RPK1. We further show that expression of the gene is rapidly induced by ABA, dehydration, high salt, and cold treatments. The possible involvement of this protein kinase in a signal transduction pathway of osmotic stress and ABA is discussed.

MATERIALS AND METHODS

Seeds of wild-type *Arabidopsis thaliana* (ecotypes Col-O and La-O) were obtained from the *Arabidopsis* Biological Resource Center (The Ohio State University, Columbus). The seeds of the mutants were obtained from the Nottingham *Arabidopsis* Stock Center (The University of Nottingham, University Park, Nottingham, UK). The mutants used are *aba-1* (Koorneef et al., 1982), *abi1-1*, *abi2-1*, and *abi3-1* (Koorneef et al., 1984). Seeds were sown on a compound soil mixture of vermiculite:peat moss:perlite (1:1:1, v/v) and treated at 4°C for 3 d to promote even germination. Plants were grown in a growth chamber (Vision Scientific, Seoul, Korea) with a 16-h light/8-h dark cycle at 23°C.

BA, ACC, and ABA as mixed isomers were purchased from Sigma. GA₃ and IAA were purchased from GIBCO-BRL.

Plants at 17 d after germination were used for various treatments. BA (100 μM), GA₃ (100 μM), IAA (100 μM), and ABA (100 μM; Straub et al., 1994) treatments were done by spraying the hormone solutions onto whole plants. For each treatment, 1 L of the respective solution was used on 120 plants at once. The ACC treatment was done by floating detached third or fourth foliar leaves (Oh et al., 1996) on the ACC solution (100 μM). The salt treatment was done by spraying 250 mM NaCl solution (Holappa and Walker-Simmons, 1995) onto whole plants. In this experiment 1 L of each solution was applied to 120 plants. For the dehydration treatment, the third or fourth foliar leaves were detached and placed on 3MM filter papers (Whatman) set in 9-cm Petri dishes at 23°C. For rehydration treatment, dehydrated leaves were floated on water as described previously (Oh et al., 1996). For cold treatment, the plants were transferred to a homemade 4°C chamber equipped with timer-controlled fluorescent lighting.

PCR

Two oligonucleotides were designed to match the sequence motifs Ile/Met-His-Arg-Asp-Leu-Lys or Ile-His-Arg/Ser-Asp-Ile-Lys. The oligonucleotides synthesized were 5'-C GAA TCG ATI CAT CGI GAT CTI AA(A/G)-3' (primer 1) and 5'-A TCT CGA GTI CA(C/T) (A/C)GI GA(C/T) ATC AA(A/G)-3' (primer 2), respectively. *Clai* and *XhoI* restriction sites were included at the 5' ends of primers 1 and 2, respectively, for the purpose of cloning. Another oligonucleotide was synthesized in the antisense direction for the peptide motif Asp-Val-Tyr-Ser-Phe-Gly. The sequence of the synthesized oligonucleotide was 5'-AGC GGA TCC (G/A)AA I(G/C)(A/T) (A/G)TA IAC (A/G)TC-3' (primer 3). To reduce the degeneracy of the oligonucleotides, the codon preference in *A. thaliana* was considered and the nucleoside analog inosine was used where needed.

The first-strand cDNA was prepared from total cellular RNA of *Arabidopsis* leaf tissues, using a cDNA synthesis kit (Pharmacia). PCR was performed by employing two pairs of oligonucleotides, primer 1/primer 3 and primer 2/primer 3. The PCR mixture contained 10 ng of the first-strand cDNA and 5 pmol of each oligonucleotide. PCR amplification was carried out for 20 cycles of 1 min of denaturation at 94°C, 2 min of annealing at 45°C, and 2 min of extension at 72°C. The products of PCR amplification were examined by 1.2% agarose gel electrophoresis, and then were cloned into pBS KS⁺.

Screening of cDNA Library

Approximately 100,000 plaques of the *Arabidopsis* leaf cDNA library (Park et al., 1993) constructed in the λgt11 vector were screened by filter-hybridization, as described previously (Park et al., 1993). The filters were washed in 1× SSC, 1% SDS at room temperature for 10 min and twice at 35°C for 10 min. The insert DNA fragments of the isolated cDNA clones were subcloned into the *EcoRI* site of pBS KS⁺ for further analysis.

RACE

The RACE approach was employed to clone the 5' ends of the cDNA clone. For use as primers, two oligonucleotides corresponding to the 5' end region of the pRPK1-1 cDNA clone (oligomer 1, 5'-GATGAGCTGTCGGAAAC-CCAGCTAG-3'; oligomer 2, 5'-CTCCATTTGGATCCA-AGAACTACTC-3') were synthesized in the antisense direction. The sequences of the oligonucleotides correspond to the nucleotide positions 296 to 318 and 262 to 287, respectively, in Figure 1. Oligomer 2 contains the *BamHI* site at its 5' end for the purpose of cloning. The first-strand cDNA was prepared using poly(A)⁺ RNA. Synthesis of the second-strand cDNA and adaptor ligation were carried out as recommended by the manufacturer (Marathon cDNA amplification kit, Clontech, Palo Alto, CA). The first-round PCR was conducted with oligomer 1 and the AP1 primer (5'-CCATCCTAATACGACTCACTATAGGGC-3') supplied by the manufacturer. The first-round PCR was conducted for 20 cycles with a regime of 94°C for 30 s, 60°C for 30 s,

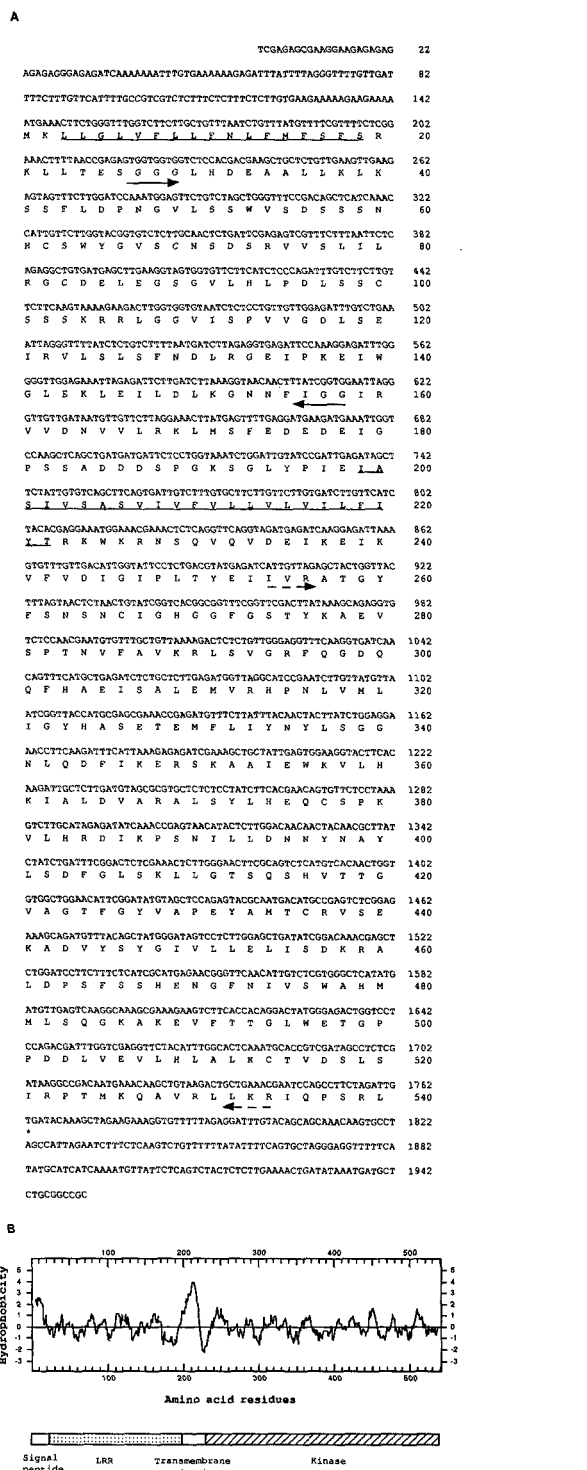


Figure 1. The sequence and the domain structure of RPK1. A, Nucleotide sequence of the cDNA and the deduced peptide sequence. Amino acid residues are shown in the single-letter codes. The two hydrophobic motifs, the amino-terminal signal peptide, and the membrane-spanning region are underlined. The LRR domain and the kinase domain are marked with solid and broken arrows, respectively. The asterisk indicates the termination codon. B, Hydropathy plot and domain structure of RPK1. The hydropathy was deduced by the method of Kyte and Doolittle (1982) with a window of nine amino acid residues.

and 68°C for 2 min. The first-round PCR product was diluted and used as a PCR template for the second-round PCR. Oligomer 2 and the AP2 primer (5'-ACTCACTATAGGGCTCGAGCGGC-3') supplied by the manufacturer were used in the second-round PCR. The PCR mixture was heated at 94°C for 2 min and then subjected to 30 cycles of PCR with a regime of 94°C for 30 s and 68°C for 2 min. The amplified DNA fragments were size-fractionated in a 1.2% agarose gel and subcloned into pBS KS⁺ for further characterization.

Screening of Genomic Library

Approximately 200,000 plaques of the genomic library of *Arabidopsis* ecotype La-O were screened. The genomic library was kindly provided by D. Voytas (Iowa State University, Ames). The probe used for this screening was the pRPK1-2 clone isolated in the RACE approach described above and contained the 5' side of the *RPK1* gene. The screening procedure was the same as described above for cDNA library screening, except that the final washing was done at 42°C.

DNA and RNA Gel-Blot Analysis

For DNA-blot analysis, 2 µg of *EcoRI*- or *HindIII*-digested genomic DNA was size-fractionated in a 0.8% agarose gel and transferred onto a nylon membrane (ICN). For RNA gel-blot analysis, 10 µg of total cellular RNA was denatured, size-fractionated in 1.2% formaldehyde-agarose gels, and transferred onto nylon membranes (ICN). The filter hybridization reaction was performed as described previously (Park et al., 1994), except that the washing of the membranes was done at 55°C. The probe was the pRPK1-2 clone.

DNA Sequencing and Analysis

An ordered set of deletion clones was generated with the Erase-a-Base system (Promega). The deletion clones were subjected to double-stranded DNA sequencing using the Sequenase kit (United States Biochemical). Some parts of the sequences were determined by an automated sequencer (model 381A, ABI Applied Biosystems, Inc., Foster, CA) using the cycle-sequencing protocol and the fluorescently labeled primer, according to the procedure provided by the manufacturer (ABI). Analyses of amino acid sequence data were carried out using the GeneWorks program (IntelliGenetics, Inc., Mountain View, CA). The deduced amino acid sequence was compared against the National Center for Biotechnology Information nonredundant protein database with the BLAST search program (Altschul et al., 1990).

RESULTS

Isolation of a cDNA Clone for a Receptor-Like Protein Kinase

To isolate a cDNA clone for a receptor-like protein kinase, we first generated a cDNA probe corresponding to a plant receptor-like protein kinase gene by PCR amplifica-

tion. To design oligonucleotides for PCR amplification, we compared the protein sequences of a few receptor-like protein kinases that had been previously identified in plants. One notable consensus motif among the receptor-like protein kinases was found in the kinase subdomain IX. Many of the putative receptor kinases, including the Arabidopsis TMK1 (Chang et al., 1992), maize ZmPK1 (Walker and Zhang, 1990), RLK1 (Walker, 1993), and an S-locus receptor kinase of *Brassica oleracea* (Stein et al., 1991), contained the sequence motif Asp-Val-Phe/Tyr-Ser-Phe/Tyr-Gly. One of the oligonucleotides, therefore, was synthesized to match this peptide sequence motif. We could not find another motif notably conserved among the plant receptor-like protein kinases. We chose the protein kinase subdomain Vib, which is relatively well conserved among protein kinases (Hanks and Quinn, 1991), to design the other oligonucleotides for PCR amplification. The consensus motifs we derived were Ile/Met-His-Arg-Asp-Leu-Lys and Ile-His-Arg/Ser-Asp-Ile-Lys. By cloning of the PCR-amplified product and subsequent sequencing, we were able to identify one cDNA fragment that encodes the protein kinase subdomains Vib, VII, VIII, and IX. The clone was obtained from the primer pairs 2 and 3 (see "Materials and Methods").

The PCR clone was then used to screen a leaf cDNA library. By partial sequencing of the screened cDNA clones, we identified a cDNA clone that contains LRR sequences at its amino terminus; the clone was named pRPK1-1. Sequencing of the pRPK1-1 clone revealed that it contained an open reading frame for a receptor-like protein kinase (see Fig. 1). However, the putative open reading frame extended beyond the 5'-most ATG codon without interruption by the stop codons. Thus, the open reading frame appeared to extend beyond the 5' end of the cDNA clone. To obtain more complete sequence information about the coding region, we employed the RACE approach. The clone obtained from RACE was named pRPK1-2. The nucleotide sequence obtained by combining the sequences of the pRPK1-1 and pRPK1-2 cDNA clones is shown in Figure 1.

The 5' side of the sequence contained in the pRPK1-2 clone generated by RACE may not have faithfully represented the real mRNA sequence due to the low fidelity of thermostable DNA polymerases. To confirm the sequence of this region, we isolated a genomic clone using the pRPK1-2 clone as a probe. Sequencing of the genomic fragment corresponding to the pRPK1-2 clone revealed that the cDNA and the genomic DNA sequences matched completely (data not shown).

Characteristics of the RPK1 Sequence

The complete cDNA sequence contains 142 bp of a 5' untranslated region, 1623 bp of an open reading frame, and 187 bp of a 3' untranslated region. The open reading frame encodes a 540 amino acid peptide with a calculated molecular mass of 59,703 D. The protein for this open reading frame was designated RPK1. The open reading frame we assigned is likely the complete open reading frame. The 5' untranslated region contains two stop codons in the same

reading frame. No other ATG codons are present beyond the first ATG codon of the assigned open reading frame. The size of the RPK1 transcript, as estimated from RNA gel-blot analysis, is close to the size of the cDNA clone. The nucleotide sequence context surrounding the putative initiation codon is consistent with the consensus sequence surrounding the translational initiation codon in plants (Joshi, 1987).

The deduced amino acid sequence of RPK1 is shown in Figure 1. The amino acid sequence has four distinctive domains (Fig. 1B): an amino-terminal hydrophobic domain, an LRR sequence domain, a second hydrophobic domain in the middle part, and a protein kinase domain at the carboxy terminus.

The carboxy-terminal kinase domain (amino acids 251–540; Fig. 2A) carries all of the 11 subdomains conserved among known protein kinases (Hanks and Quinn, 1991). Notable among them are the GXGXXG motif in subdomain I and the Lys residue in subdomain II, which is necessary for phosphotransfer reaction. Although we have not shown that RPK1 is a protein kinase by biochemical means, the presence of all of the subdomains conserved among known protein kinases strongly suggests that RPK1 is very likely a protein kinase. The amino acids in subdomains VI and VIII are characteristically conserved for either Ser/Thr kinases or Tyr kinases. The amino acid sequences of RPK1 in subdomain VI (DIKPSN) and subdomain VIII (GTFGYVAPE) are closer to the consensus sequences of Ser/Thr kinases (DLKXXN and GT/SXXY/FXAPE, respectively) than those of Tyr kinases. This structural feature, therefore, suggests that RPK1 may be a Ser/Thr protein kinase. However, the identity of RPK1 as a Ser/Thr protein kinase needs to be confirmed. Comparison of the kinase domain of RPK1 with the sequences in the databases revealed that RPK1 is related to other plant receptor-like protein kinases (Fig. 2A), including Arabidopsis ER (40% identity, 50% similarity) and TMK1 (38% identity, 45% similarity).

The hydropathy plot analysis revealed that the protein contains two highly hydrophobic regions (Fig. 1). The hydrophobic domain near the amino terminus (amino acids 2–19) is indicative of a signal peptide associated with translocation of peptides into the ER (von Heijne, 1990). The second hydrophobic domain (amino acids 199–222), which is immediately followed by several positively charged amino acids (amino acids 223–227), is indicative of a membrane-spanning segment. The charged residues are likely to function as a stop transfer sequence in inhibiting further translocation of the carboxy side of the protein (Weinstein et al., 1982).

The domain between the amino-terminal and the second hydrophobic regions contains five peptide motifs related to the LRR sequences (Figs. 1 and 2B). A database search with this sequence region revealed that repeats three, four, and five are related to repeats two, three, and four, respectively, of the Arabidopsis ER. The LRR repeat region also shows similarity to the LRR sequences of other LRR-containing plant proteins, including Arabidopsis RLK5 (Walker et al., 1993) and tomato Cf-2.1 (Dixon et al., 1996) and Cf9 (Jones et al., 1994).

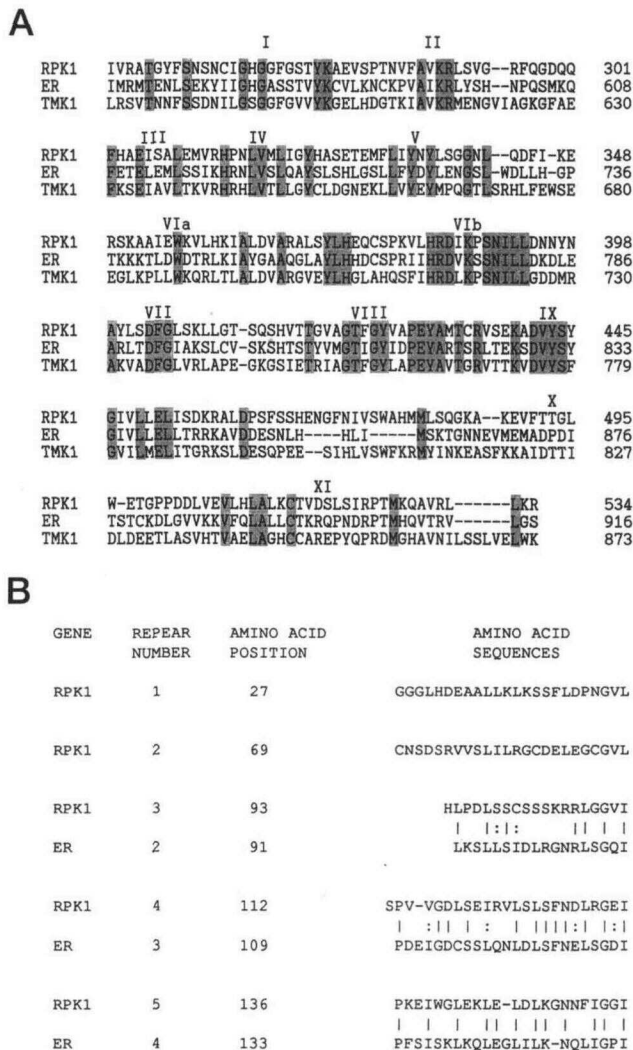


Figure 2. Comparison of the RPK1 sequence with related sequences. A, The kinase domain of RPK1 and its similarity to Arabidopsis ER (Torii et al., 1996) and TMK1 (Chang et al., 1992). The sequence alignment was conducted with the GeneWorks program (IntelliGenetics). Amino acid residues identical in all sequences are indicated by gray boxes. The conserved subdomains of the protein kinase family (Hanks and Quinn, 1991) are indicated by the Roman numerals. B, The LRR motif of RPK1 and its similarity to the LRR sequence of Arabidopsis ER. Identical and similar amino acid residues are marked by vertical lines and dots, respectively. Gaps are marked with dashes and were introduced to maximize the alignment.

DNA Gel-Blot Analysis

The complexity of the RPK1 gene in the genome of *A. thaliana* was examined by DNA gel-blot analysis. By probing with the pRPK1-2 clone, which contains the 5' end 276 bp of the cDNA sequence presented in Figure 1, we detected single bands in *EcoRI*- or *HindIII*-digested genomic DNA (Fig. 3). Thus, the RPK1 gene is most likely a single-copy gene in the haploid genome of Arabidopsis. However, the probe used here corresponds to the 5'-most sequences of the RPK1 transcript and contains only a short stretch of the protein-coding sequence. We cannot rule out the pos-

sibility that related genes with sequence similarity in the protein coding region exist in Arabidopsis. This result, however, shows that the transcript detected with this probe in the following RNA gel-blot analyses is specific to the RPK1 gene.

Expression of the RPK1 Gene in Various Organs of *A. thaliana*

To get an insight into the regulation of the RPK1 gene, we first examined its expression pattern in various organs by RNA gel-blot analysis. As shown in Figure 3, the pRPK1-2 probe detected a single band of transcript approximately 2.5 kb in size in all of the organs examined.

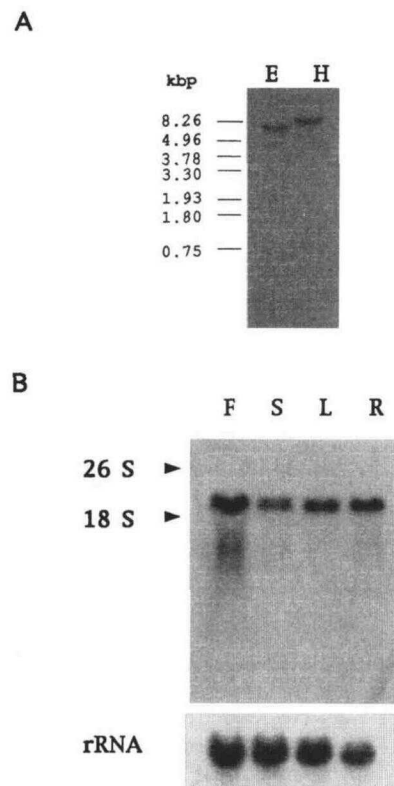


Figure 3. Genomic DNA-blot analysis of the RPK1 gene (A) and expression pattern of the RPK1 gene in various organs of *A. thaliana* (B). For genomic DNA-blot analysis, total cellular DNA of Arabidopsis (ecotype Col-O) digested with *EcoRI* (E) or *HindIII* (H) was separated on a 0.8% agarose gel. The positions of the size markers are indicated. The size markers used here were generated by combining the fragments of the pBM150 vector digested with *EcoRI/XhoI*, *EcoRI/PstI*, and *EcoRI* alone. For RNA gel-blot analysis, 10 μ g of total cellular RNA prepared from flower (F), stem (S), leaf (L), or root (R) was size-fractionated in a 1.2% formaldehyde-agarose gel. The sample of the floral organ was a collection of whole flowers and included the sepal, petal, stamen, and pistil. The probe was the pRPK1-2 cDNA clone, which contains the 5' end 276 bp of the sequence shown in Figure 1. The same RNA blot was rehybridized with an 18S rDNA probe as a control after removing the pRPK1-2 probe. The positions of the 26S and 18S ribosomal RNAs are indicated.

ABA Induction of the *RPK1* Gene Expression

Plant hormones play major roles in various cellular responses, including regulation of many plant genes. Thus, we examined *RPK1* gene expression in response to treatments with various plant hormones. We first examined the hormonal response of *RPK1* gene expression at a single time (3 h) after treatment with the five plant hormones IAA, BA, ACC, GA₃, and ABA. We found that expression of the *RPK1* gene is increased upon ABA treatment, whereas treatment with the other four hormones did not result in a significant change (data not shown, see Fig. 4 for effect of ethylene and GA₃ treatment). This result does not mean that the *RPK1* gene is not regulated by the other four hormones, since expression of the *RPK1* gene was examined only at a single time. It is still possible that the gene is regulated by the other four hormones, but we simply did not detect the regulation under our experimental conditions. However, the data suggested that the *RPK1* gene is induced by ABA. We then proceeded with a detailed kinetic analysis of the effect of ABA on expression of the

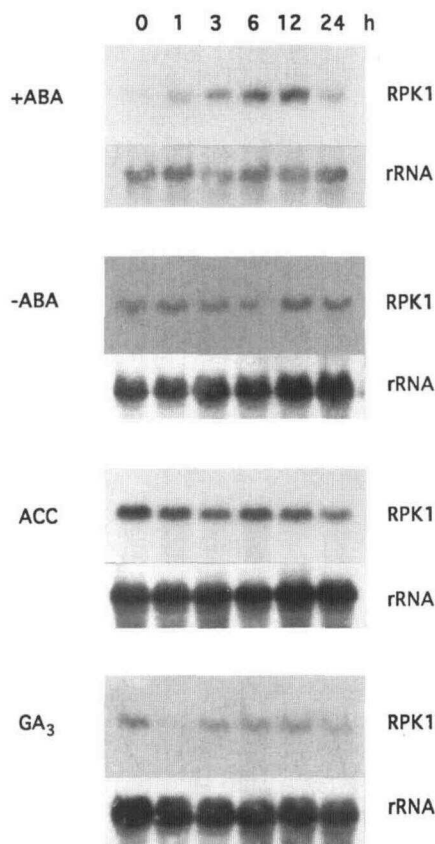


Figure 4. ABA induction of *RPK1* gene expression. Ten micrograms of total cellular RNA isolated from the wild-type (Col-O) foliar leaves at the indicated times (h) after treatment with ABA (+ABA), a buffer solution (-ABA), ACC, or GA₃ was used in each lane. The probe was the pRPK1-2 cDNA clone containing the 5' end 276 bp of the sequence shown in Figure 1. The -ABA, ACC, and GA₃ gels were obtained by exposing the film several times longer than the +ABA gel. The same blots were reprobated with 18S rDNA after removing the pRPK1-2 probe.

RPK1 gene. In this experiment we performed RNA gel-blot analyses using total cellular RNA of foliar leaves harvested from 0 to 24 h after the ABA treatment.

The data in Figure 4 show that expression of the *RPK1* gene is markedly induced within 1 h of ABA application. The transcript level reached a maximum at 12 h and then declined after 24 h. This increased expression was not due to the effect of spraying the buffer solution or to circadian regulation, since expression of the *RPK1* gene was not altered in control plants during the same time course. In contrast to ABA, ACC or GA₃ treatments did not result in induction of the gene (Fig. 4). It is interesting that GA₃ caused a transient reduction in the level of the *RPK1* transcript at 1 h after the treatment. The data suggest that the *RPK1* gene is induced by ABA in a rather specific manner.

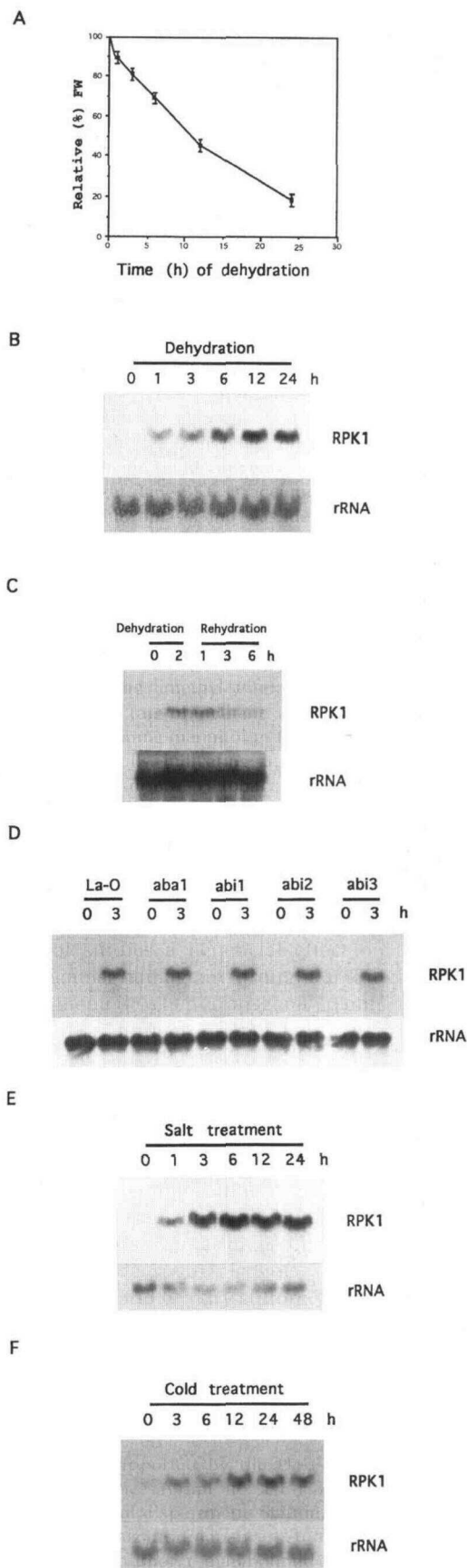
Induction of *RPK1* Gene Expression by Dehydration, High Salt, and Cold Treatments

ABA has been implicated to function in plant responses to many environmental stresses, including dehydration, high salinity, and low temperature (Mansfield, 1988; Zeevaart and Creelman, 1988; Skriver and Mundy, 1990; Trewavas and Jones, 1991; Davies et al., 1994; Giraudat et al., 1994; Straub et al., 1994). It is now well established that an increase of ABA concentration is one important physiological response to the environmental stresses. Furthermore, many of the ABA-induced genes are also induced by the environmental stresses (reviewed by Finkelstein and Zeevaart, 1994; Giraudat et al., 1994).

Since expression of the *RPK1* gene showed a rapid increase after exogenous ABA treatment, we sought to determine if expression of the gene is also regulated in response to other environmental stresses. We first examined the dehydration response of *RPK1* gene expression. The data in Figure 5, A and B, show that expression of the *RPK1* gene is markedly induced within 1 h of the dehydration treatment. The mRNA level gradually increased until 6 h of the gradual loss of water content and remained at a similar level thereafter. Furthermore, transfer of dehydrated leaves to water caused a decrease in the steady-state amount of *RPK1* mRNA induced by dehydration (Fig. 5C), confirming that the *RPK1* gene is dehydration-regulated.

The result above showed that *RPK1* gene expression is induced by dehydration and exogenous ABA. Since dehydration-induced expression of many of the ABA- and dehydration-induced genes is dependent on ABA (reviewed by Finkelstein and Zeevaart, 1994; Giraudat et al., 1994), we asked whether the dehydration induction of the *RPK1* gene is also dependent on ABA. For this purpose, we examined dehydration-responsiveness of the *RPK1* gene in the ABA-deficient (*aba-1*) or ABA-insensitive mutants (*abi1-1*, *abi2-1*, and *abi3-1*). As shown in Figure 5D, the levels of *RPK1* mRNA induced by dehydration in all of the mutants were almost the same as that in wild type. This result showed that *RPK1* gene expression was not impaired in these mutant plants.

We also examined the effect of high salt and cold treatments on *RPK1* gene expression. The salt treatment, which was given by spraying 250 mM NaCl solution on the plants,



also resulted in a marked increase of the transcript level of the *RPK1* gene within 1 h of the treatment (Fig. 5E). Cold treatment at 4°C, as shown in Figure 5F, also resulted in the induction of the *RPK1* gene expression.

DISCUSSION

We have identified a cDNA clone for a receptor-like protein kinase gene of *A. thaliana*. The structural features of a typical signal peptide sequence, the membrane-spanning hydrophobic segment, and the highly conserved kinase domain strongly suggest that the encoded protein functions as a receptor protein kinase.

We have shown that the gene is rapidly induced by ABA, which is involved in a variety of stress responses. We have further shown that the gene is induced by several environmental stresses, including dehydration, high salt, and low temperature. Considering *RPK1* to be a receptor protein kinase, this observation suggests that *RPK1* may function in the transmission of ABA and various environmental stress signals into intracellular reactions. In addition, regulation of the gene by a variety of environmental stresses and ABA may indicate that the gene is involved in a general stress response, as has been suggested for a wheat protein kinase, PKABA1 (Holappa and Walker-Simmons, 1995). Whereas only a part of the molecular mechanism of

Figure 5. Induction of *RPK1* gene expression by dehydration, high salt, and cold treatments. **A**, The degree of dehydration of the detached leaves used for the RNA gel-blot analysis. Third or fourth foliar leaves of plants at 17 d after germination were used. Detached leaves were placed on 3MM filter papers set in 9-cm Petri dishes at 23°C for the indicated time periods. The degree of dehydration was measured by comparing the fresh weight (FW) of the leaves before and after the dehydration treatment. The assay was performed in triplicate. Six leaves were used for each time point in each assay. Shown are the average values. The vertical bars indicate SD values. **B**, The expression pattern of the *RPK1* gene during dehydration treatment. Ten micrograms of total cellular RNA isolated from the leaves subjected to dehydration treatment for the indicated time periods was loaded in each lane. **C**, The expression pattern of the *RPK1* gene upon rehydration. Ten micrograms of total cellular RNA isolated from the leaves before (0) and after 2 h (2) of dehydration treatment, or from the leaves rehydrated for the indicated time periods after 2 h of dehydration was loaded in each lane. **D**, Dehydration response of *RPK1* gene expression in ABA-deficient or ABA-insensitive mutants. Ten micrograms of total cellular RNA isolated from the leaves of the wild-type (La-O), *aba-1* (*aba1*), *abi1-1* (*abi1*), *abi2-1* (*abi2*), and *abi3-1* (*abi3*) before (0) or after 3 h (3) of dehydration treatment was loaded in each lane. **E**, Induction of *RPK1* gene expression by salt treatment. Ten micrograms of total cellular RNA isolated from the foliar leaves at the indicated times (h) after treatment with 250 mM NaCl was loaded in each lane. **F**, Induction of *RPK1* gene expression by cold treatment. Ten micrograms of total cellular RNA isolated from the foliar leaves incubated for the indicated times (h) at 4°C was loaded in each lane. The probe was the pRPK1-2 cDNA clone containing the 5' end 276 bp of the sequence shown in Figure 1. The same blots were reprobed with 18S rDNA after removing the pRPK1-2 probe also resulted in a marked increase of the transcript level of the *RPK1* gene within 1 h of the treatment (Fig. 5E). Cold treatment at 4°C, as shown in Figure 5F, also resulted in the induction of *RPK1* gene expression.

the signal transduction pathway of these environmental stresses has been revealed in plants, the discovery of a receptor-like protein kinase induced by various environmental stresses, as described here, may provide some significant insight into the mechanism.

Although the *RPK1* gene appears to be involved in a general stress response, one possible common physiological and physical environment that plant cells experience as a result of dehydration, high salt, and low temperature is osmotic stress or water stress. Dehydration causes osmotic stress by a direct loss of water. High salt causes osmotic stress by reducing water potential. Cold stress is also known to cause osmotic stress by reducing the water supply from the roots to green tissues (Yamaguchi-Shinozaki and Shinozaki, 1993). ABA is also known to be involved in the osmotic stress response that is caused by several environmental stressors. Thus, it is possible that induction of the *RPK1* gene is due to a common osmotic stress and that the gene is involved in an osmotic stress response rather than in a general stress response. However, it is true that the gene is involved in a dehydration response, whether the involvement is through a dehydration-specific signal, a common osmotic-stress signal, or a general-stress-response signal. We have demonstrated in this experiment that the gene is regulated by water availability; it is induced when dehydrated and repressed when rehydrated.

Plant responses to dehydration, including gene expression, appear to involve both ABA-dependent and ABA-independent pathways (Guerrero et al., 1990; Yamaguchi-Shinozaki et al., 1992; Yamaguchi-Shinozaki and Shinozaki, 1993; Giraudat et al., 1994; Urao et al., 1994; Gosti et al., 1995). In this report we have shown that dehydration-induced expression of the *RPK1* gene is not impaired in the ABA-deficient and ABA-insensitive mutations. This result suggests that dehydration induction of the *RPK1* gene is ABA independent, although the gene is induced by exogenous ABA treatment. This suggestion is further supported by the induction kinetics of the *RPK1* gene. The *RPK1* gene was highly induced within 1 h of dehydration treatment. In *Arabidopsis* the level of endogenous ABA is detectable 2 h after dehydration (Yamaguchi-Shinozaki and Shinozaki, 1993). Thus, the early induction of the *RPK1* gene upon dehydration may be due to an ABA-independent response and not to the increased level of endogenous ABA. This ABA-independent regulation is similar to the case of the *rd29* and *rab17* genes of *Arabidopsis*. These two genes are responsive to both ABA and dehydration, but the dehydration-induced expression of the genes appears to be independent of ABA (Yamaguchi-Shinozaki and Shinozaki, 1993; Vilardell et al., 1994). The signal transduction pathways for the cellular response to dehydration involve protein phosphorylation.

It has been suggested that the calcium-dependent protein kinases of *Arabidopsis* are involved in an ABA-independent pathway (Urao et al., 1994). ABA-independent induction of the *RPK1* gene by dehydration suggests that the receptor-like protein kinase encoded by the *RPK1* gene may also be involved in an ABA-independent dehydration pathway. However, the expression patterns we examined in the ABA-

deficient and ABA-insensitive mutations may need further consideration. It may still be possible that *RPK1* gene expression is dependent on ABA. There may be an additional ABA signal transduction pathway that does not involve the *abi1*, *abi2*, or *abi3* genes (Finkelstein and Zeevaert, 1994). In addition, induction of the *RPK1* gene may require only a small amount of endogenous ABA, and the residual amount of ABA produced in the *aba-1* mutation may still be enough to induce *RPK1* gene expression. Our discovery of the *RPK1* gene adds supporting evidence that the signal transduction pathway of the plant responses to dehydration involves protein phosphorylation that is mediated by a receptor protein kinase.

RPK1 has the structural features of a transmembrane protein kinase, with an intracellular kinase domain and an extracellular ligand-binding domain. These structural features are highly reminiscent of animal receptor kinases, such as the large class of Tyr receptor kinases and a few Ser/Thr receptor kinases that are involved in transmembrane signal transduction (Lemmon and Schlessinger, 1994). The structure thus suggests that an ABA-dependent osmotic response in plants may involve a transmembrane signaling mechanism, and that *RPK1* may function in transmitting an extracellular signal into intracellular target molecules.

The presence of the LRR sequences in the extracellular domain of *RPK1* is intriguing. The LRR sequence is known to be involved in strong protein-protein interactions (Kobe et al., 1995). In animals LRR-containing transmembrane receptor proteins, such as the lutropin-choriogonadotropin receptor (McFarland et al., 1989) and follicle-stimulating receptors (Heckert et al., 1992), are involved in the transmembrane transduction of peptide hormone signals into intracellular targets. In plants a peptide encoded by the tomato Cf-9 contains the extracellular LRR sequence, which is likely to be an extracellular binding domain for the Avr9 peptide (Jones et al., 1994). Whereas there is no report on a peptide signal that is involved in an ABA-dependent osmotic response, the presence of the LRR sequences in *RPK1* suggests that such an extracellular peptidic ligand may exist. Alternatively, a protein-protein interaction including oligomerization of *RPK1* on the outside of the plasma membrane may occur, as in the case of some animal receptor kinases. However, the possibility that the extracellular LRR sequence may be involved in binding a small nonpeptidic chemical ligand(s) cannot be excluded.

It should be noted that the gene is induced in leaves that are detached and subjected to dehydration. Many of the physiological and developmental responses of plant shoots are regulated by a signal(s) moving from dehydrated roots (Davies et al., 1994). Expression of the *RPK1* gene in detached leaves suggests that the dehydration response involving *RPK1* does not depend solely on a signal(s) that is generated in roots.

In animals the action of the receptor Tyr kinase is often associated with protein Tyr phosphatase in controlling downstream target molecules (Sun and Tonks, 1994). Recently, *RLK5*, a receptor protein kinase of *Arabidopsis*, was

found to be associated with a type 2C phosphatase, and this association was dependent on phosphorylation of RLK5 (Stone et al., 1994). In analogy to RLK5 and animal receptor kinases, it is not unreasonable to imagine that RPK1 may be associated with a protein phosphatase. Identifying an extracellular ligand(s) and an intracellular target molecule(s) interacting with RPK1 along with the functional study of RPK1 using a biochemical or a transgenic approach, should shed some light on the signal transduction pathway of osmotic stress and/or ABA in plants.

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