Stress-responsive expression of genes for two-component response regulator-like proteins in *Arabidopsis thaliana*

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Abstract Four cDNAs that encode two-component response regulator-like proteins were cloned from *Arabidopsis thaliana*. Putative proteins (ATRR1–4) contain a receiver domain with a conserved aspartate residue – a possible phosphorylation site – at the N-terminal half. ATRR2 lacks the C-terminal half; the others contain a C-terminal domain abundant in acidic amino acids or proline residues. ATRR1 and ATRR2 are expressed more in roots than in other tissues and are induced by low temperature, dehydration and high salinity. Levels of ATRR3 and ATRR4 were not affected by stress treatments. These results suggest that ATRRs play distinct physiological roles in *Arabidopsis*, and that some are involved in stress responses.

Key words: *Arabidopsis thaliana*; Stress response

1. Introduction

The two-component system is a simple and elaborate signaling module that transduces extracellular signals to cytoplasm through phosphotransfer between two components [1–8]. Typically, the two-component system is composed of two types of proteins, a sensory histidine kinase and a response regulator, and 40 different kinase–regulator pairs operate in *Escherichia coli* alone. A typical histidine kinase contains an N-terminal input domain and a C-terminal transmitter domain with an invariant histidine residue. A typical response regulator contains an N-terminal receiver domain with an invariant aspartate residue and a C-terminal output domain. The input domain of the sensory histidine kinase detects environmental parameters and promotes autophosphorylation of a histidine residue within its transmitter domain, followed by transfer of the phosphoryl group to an aspartate residue within the receiver domain of the cognate response regulator. The phosphorylation state of the response regulators alters the activity of the output domain to control the transcription of signal-responsive genes.

Recently, a number of histidine kinases have been cloned and have been shown to be involved in osmosensing [9–13], development [14,15], and plant hormone responses [16–19] in eukaryotes. It is thus apparent that the two-component system is not confined to prokaryotes. As the information on histidine kinases accumulates, it is now of interest to identify additional regulators that form a particular two-component signaling module with these histidine kinases. However, there are only three reports on the isolation and characterization of genes for response regulators in eukaryotes [10,20,21]. In this report, we describe molecular cloning of four cDNAs that encode two-component response regulator-like proteins from *Arabidopsis thaliana*, and the differential expression of these genes in various tissues and under environmental stresses.

2. Materials and methods

2.1. Plant materials

A. thaliana (Columbia ecotype) was grown on GM agar plates under continuous illumination of approximately 2000 lux at 22°C for 3–4 weeks and was used for the construction of a cDNA library and in stress treatments. Plants used for the analysis of tissue-specific expression were grown in 15-cm pots filled with a 1:1 mixture of perlite and vermiculite and were watered with 0.1% Hyponex (Hyponex, Tokyo, Japan).

2.2. Stress treatments

For the growth regulator and salt treatments, plants were transferred to and grown hydroponically in a solution of either 100 μM ABA or 250 mM NaCl, under dim light. For the temperature treatments, plants were grown under continuous light at temperatures of 40°C or 4°C. For the dehydration treatment, plants were removed from the agar and desiccated in plastic dishes at 22°C and 60% humidity under dim light. In each case, plants were subjected to the treatments for 2 h, and then were frozen in liquid nitrogen and stored at −80°C.

2.3. cDNA library screening, cloning and sequencing

A cDNA library was constructed in λgt11 (Amersham International, UK) with poly(A)$^+$ RNA prepared from *Arabidopsis* rosette plants. Screening was performed by the plaque hybridization method under low-stringency condition as described by Mizoguchi et al. [22]. The cDNA inserts were isolated from the positive phage clones and were subcloned into pBluescript II SK$^-$ (Stratagene, La Jolla, CA, USA). The sequences were determined by the dye-primer cycle sequencing method (Applied Biosystems, San Jose, CA, USA). Gene Works software (IntelliGenetics, Mountain View, CA, USA) was used for the analysis of the DNA and amino acid sequences.

2.4. Northern blot analysis

Total RNA was prepared by the method described by Ausubel [23]. 30 μg of total RNA was fractionated on a 1% agarose gel containing formaldehyde and blotted onto a nitrocellulose filter [24]. The filter was hybridized with ^{32}P-labeled full-length cDNA inserts in 50% formamide, 5×SSC, 25 mM sodium phosphate buffer (pH 6.5), 10 Denhardt's solution, and 250 μg/ml of denatured salmon sperm DNA, at 42°C. The filter was washed twice with 0.1×SSC, 0.1% SDS at 60°C for 15 min and autoradiographed.

3. Results and discussion

To isolate cDNAs that encode *response regulators*, we first searched the *Arabidopsis* expression sequence tag (EST) database against amino acid sequences of conserved two-component receiver domains as queries, and found that several EST

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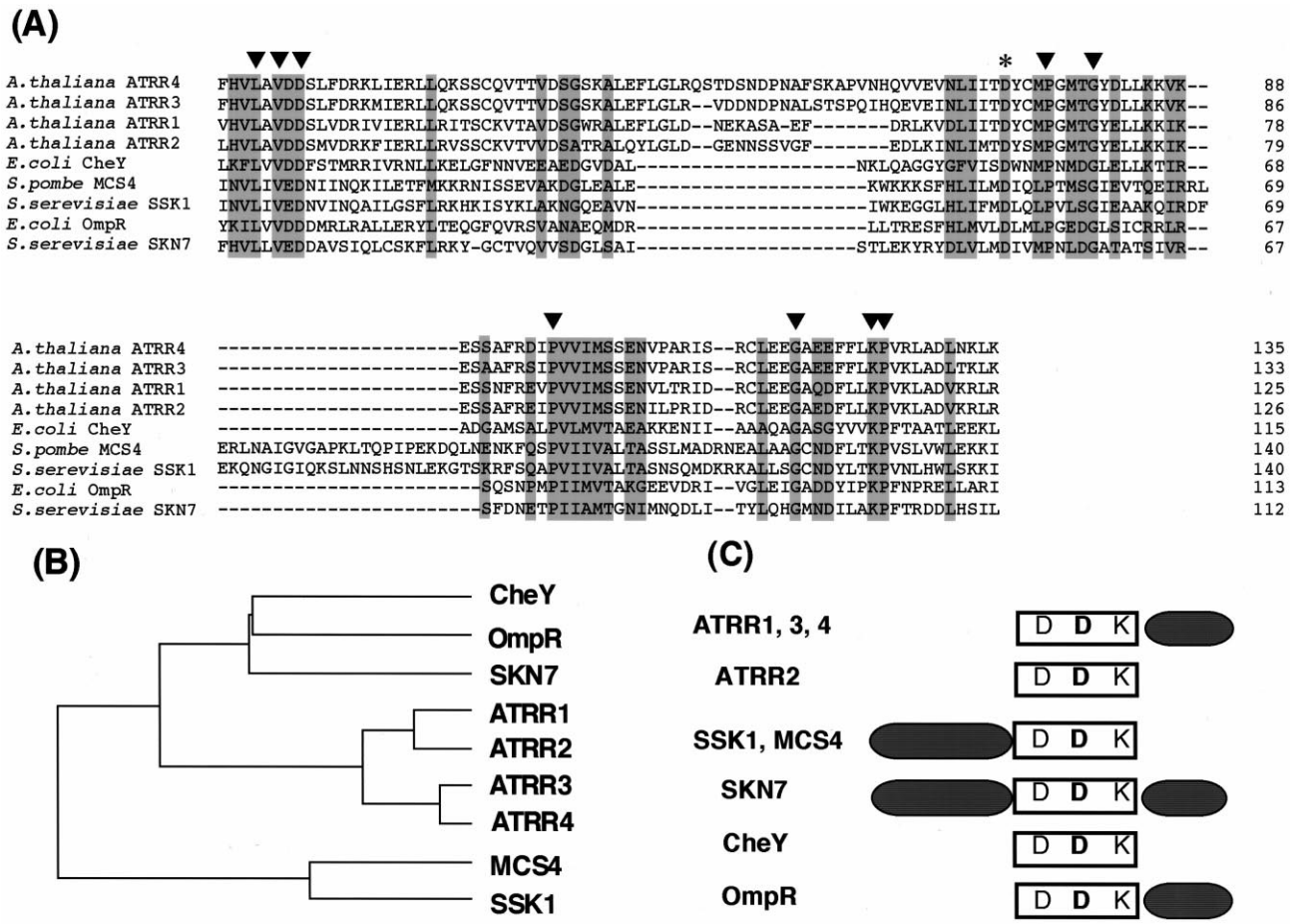

Fig. 1. A: Alignment of amino acid sequences of ATRRs and the known two-component response regulators, *S. cerevisiae* SSK1 [10], *S. cerevisiae* SKN7 [20], *S. pombe* MCS4 [21], *E. coli* CheY [26], and *E. coli* OmpR [27]. Triangles represent completely conserved amino acid residues, shading represents mostly conserved residues, and dashes indicate gaps introduced to maximize alignment. An asterisk indicates potential phosphorylation sites. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the following accession numbers: AB010915 (ATRR1), AB010916 (ATRR2), AB010917 (ATRR3), and AB010918 (ATRR4). B: Evolutional relationship among the two-component response regulators. The phylogenetic tree was constructed from the matrix of sequence similarities calculated with the UPGMA program [28]. C: Schematic representation of ATRRs. An open box represents the receiver domains. Potential phosphorylation sites are indicated with bold letters. Highly conserved amino acids in the two-component response regulators are also represented.

clones have sequence homology with bacterial response regulators. We then screened an *Arabidopsis* cDNA library using EST clone GAGa67 (GenBank accession number Z18216) as a probe under low-stringency hybridization conditions. Twenty-six positive clones were isolated and their cDNA inserts were subcloned. Partial sequence analysis revealed that these cDNAs belong to four distinct groups based on their sequence identities. The four with the longest inserts were selected and their nucleotide sequences were determined.

Fig. 1A shows a sequence comparison of the receiver domains of the putative proteins (ATRR1–4; *Arabidopsis thaliana* response regulator) with five known two-component response regulators. A phylogenetic tree (Fig. 1B) indicates the evolutional relationship among the regulators. ATRR3 and ATRR4 are closely related (70% identity within the receiver domain). As shown in Fig. 1C, all ATRRs contain a putative receiver domain with a conserved aspartate residue, which is a possible phosphorylation site, on the N-terminal half. Several amino acids that are located in an acidic pocket within the receiver domain – Asp near the N-terminus and Lys near the C-terminus – are also conserved in all ATRRs (Fig. 1C). Only ATRR2 lacks the C-terminal half, like CheY, which is involved in chemotaxis in *E. coli*, whereas the other ATRRs contain the C-terminal domain, which has an unknown function. Although there are no marked similarities beyond the receiver domain to each other, the C-terminal halves of ATRRs 1, 3 and 4 have an abundance of acidic amino acids in common. Most bacterial response regulators have a DNA binding domain and act as transcription factors [1–8]. However, no significant homology to any sequences that encode DNA binding domains in the database was found in the ATRRs. This suggests that ATRRs function as signal mediators but not as transcription factors.

To examine whether other genes related to ATRRs exist in *Arabidopsis*, we carried out genomic Southern analysis. Several hybridized bands with ATRR probes were detected under low-stringency conditions, suggesting that each ATRR constitutes a small multigene family on the *Arabidopsis* genome (data not shown). This is consistent with our observation that a number of response regulator genes exist in the *Arabidopsis* EST database. We investigated the gene expression of ATRRs in flowers, stems, roots, developing seeds, and leaves

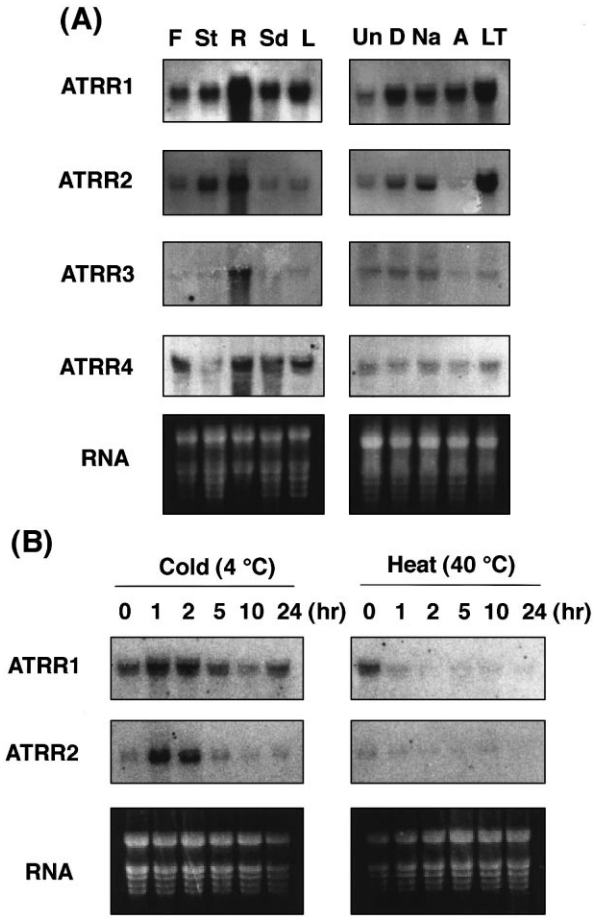

Fig. 2. A: Expression of the ATRR genes in various tissues and under various stress conditions. B: Induction kinetics of the ATRR1 and ATRR2 genes by low- and high-temperature treatments. Each lane was loaded with 30 μg of total RNA prepared from flowers (F), stems (St), roots (R), developing seeds (Sd), leaves (L), and rosette plants including roots that had been subjected to treatments for 2 h with dehydration (D), 250 mM NaCl (Na), and 100 μM ABA (A), and at 4°C (LT), or untreated (Un). The blots were probed with each ^{32}P-labeled full-length cDNA clone. Ethidium bromide-stained RNAs before blotting are also shown as controls.

by Northern blot analysis. The ATRR1 and ATRR2 mRNAs were more abundant in roots than in other tissues (Fig. 2A). The ATRR3 mRNA appeared to be root-specific, suggesting a functional importance of ATRR3 in root tissue (Fig. 2A). The ATRR4 mRNA was very low or absent in stem tissue (Fig. 2A). We then examined the induction of the ATRR genes in rosette plants including roots by a variety of stress treatments lasting 2 h. The ATRR1 and ATRR2 mRNAs accumulated significantly in the low-temperature treatment, and also accumulated in the dehydration and salt treatments (Fig. 2A). The ATRR1 gene was induced by exogenous ABA treatment (Fig. 2A). By contrast, the levels of ATRR3 and ATRR4 mRNA were not affected by any stress treatments (Fig. 2A). We further analyzed the induction of the ATRR1 and ATRR2 genes over 24 h by 2 h low- or high-temperature treatment. The ATRR1 and ATRR2 mRNAs began to accumulate within 1 h after exposure to low temperature, and then returned to their basal levels by 5 h (Fig. 2B). By contrast, both mRNAs disappeared following high-temperature treatment (Fig. 2B). Thus, the gene expression of ATRRs is differentially regulated in different tissues and in response to different environmental stresses. These results suggest that some of two-component response regulators may play an important role in roots and others in response to environmental stresses.

It is interesting to note that the ATHK1 gene, an *Arabidopsis* gene for a hybrid-type histidine kinase that has recently been cloned in our group [25], is abundantly expressed in roots and is induced by high osmolarity and low temperature, which is similar to the expression of the ATRR1 and ATRR2 genes. In *Saccharomyces cerevisiae*, the osmosensing two-component system is composed of three proteins, a sensory histidine kinase SLN1, an intermediary molecule YPD1, and a cognate response regulator SSK1 [12]. YPD1 contains a histidine-phosphotransfer domain and functions in multistep phosphorelay reaction. In *Arabidopsis*, we have shown that the dehydration-inducible ATHK1 functions as an osmosensor in yeast [25]. Further experiments are now in progress to determine which ATRR molecules participate in water-stress signaling in *Arabidopsis*.

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