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Plant Gene Register

Cloning and Sequencing of a Novel Serine/Threonine Protein Kinase in *Arabidopsis thaliana*[1]

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Since the first report of cloning of plant protein kinases (Lawton et al., 1989), the numbers of identified kinases and signal pathways regulated by protein phosphorylation have increased markedly. Activation of protein kinases and the subsequent phosphorylation of their target proteins are thought to be common strategies in the transduction of environmental and developmental signals among yeasts, animals, and plants. In animals and yeasts, protein kinases are classified into several groups based on their structure, substrate specificity, and regulatory ligands (Hanks and Quinn, 1991). In plants, the number of groups of identified protein kinases is more limited than in animals and yeasts, and the counterparts of the cAMP-dependent protein kinases, cGMP-dependent protein kinases, protein kinase C, and tyrosine kinases have yet to be reported. One aim of the present study was to identify the types of protein kinases and their function in plants.

Since protein kinases in eukaryotic cells have highly conserved regions (Hanks and Quinn, 1991), the PCR can be used with degenerate oligonucleotides corresponding to those conserved regions to isolate cDNAs encoding putative protein kinases. Using this strategy, we isolated PCR-amplified DNA fragments containing partial sequences of protein kinases from *Arabidopsis thaliana*. Included among these were DNA fragments encoding conserved regions for casein kinase II, mitogen-activated protein kinase, and calcium-dependent protein kinase. We then isolated and characterized the cDNAs corresponding to the PCR fragments (Mizoguchi et al., 1993, 1994; Urao et al., 1994) and screened a cDNA library pre-

Table I. *Characteristics of cDNA clone cATPK10 encoding a Ser/Thr-protein kinase in A. thaliana*

Organism:
Arabidopsis thaliana L. (Columbia ecotype).
Function:
Ser/Thr-protein kinase.
Sources:
cDNA library in λgt11 constructed from poly(A)$^+$ RNA of rosette plants grown under normal conditions.
Isolation:
A DNA fragment homologous to sequence encoding protein kinase was amplified from cDNA by the PCR method using degenerate oligonucleotide primers corresponding to amino acid sequences of subdomain I and IX conserved in protein kinases. To isolate the corresponding full-length cDNA, a cDNA library was screened with the PCR-amplified DNA fragment as a probe.
Sequence:
Deletion subcloning and complete dideoxy sequencing of both strands.
Identification:
Similarity of the deduced amino acid sequence to previously reported sequences.
Features of the cDNA:
2112 bp in length containing a 445-bp 5′ untranslated region, a 1266-bp open reading frame, and a 401-bp 3′ untranslated region.
Features of the Deduced Protein:
The deduced protein has 421 amino acids with a predicted molecular mass of 47.089 kD. Eleven catalytic subdomains for protein kinases and an ATP-binding site were found. The deduced protein exhibits 49, 45, 38, 42, and 35% identity to SNF1, RKIN1, CaMII-β, NIM1, and KIN1, respectively.
Expression:
The *ATPK10* mRNA was detected in flowers, leaves, roots, and stems, although the expression level was quite low in stems. The mRNA was almost undetected in developing seeds or siliques by northern analysis.
Antibody:
Not available.
Subcellular Location:
Not determined.

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pared from *A. thaliana* rosette plants grown under normal conditions with one of the PCR-amplified fragments that contained a partial sequence of a novel protein kinase. We isolated a corresponding cDNA clone, cATPK10, that encodes a novel Ser/Thr-type protein kinase (Table I).

The insert DNA of cATPK10 contains an open reading frame of 1266 bp. The putative ATPK10 protein has a length of 421 amino acids and a predicted molecular mass of 47.089 kD. ATPK10 contains all the conserved amino acid residues in protein kinases and the 11 subdomains that are typical of the catalytic subunit of protein kinases (Hanks and Quinn, 1991). ATPK10 shows highest degree of homology (38–49%) to SNF1, RKIN1, CaMII-β, NIM1, and KIN1 in the catalytic domains (Celenza and Carlson, 1986; Bennett and Kennedy, 1987; Russell and Nurse, 1987; Levin and Bishop, 1990; Alderson et al., 1991). These kinases are reported to map quite close to each other within the phylogenetic tree of protein kinases (Hanks and Quinn, 1991). The predicted ATPK10 protein has a noncatalytic domain in the C-terminal half. The SNF1, RKIN1, CaMII-β, NIM1, and KIN1 proteins also have large noncatalytic domains in their C-terminal halves, which are thought to regulate their kinase activity. In contrast to the high homologies in the catalytic domains of these protein kinases, the noncatalytic domain of ATPK10 exhibits no apparent homology to those of SNF1, RKIN1, cAMII-β, NIM1, or KIN1.

We examined the relative levels of the *ATPK10* transcript in a variety of organs of *A. thaliana*. A single hybridizing band of 2.2 kb was detected in flowers, leaves, roots, and stems, although the expression level was quite low in stems. The *ATPK10* mRNA was found at such low levels as to be nearly undetectable in developing seeds or siliques by northern analysis.

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