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Plant Gene Register

An *Arabidopsis thaliana* cDNA Encoding Ca^{2+}-Dependent Protein Kinase[1]

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Cytoplasmic Ca^{2+} plays important roles as a second messenger in plants as well as in animals and yeast. Changes in cytoplasmic Ca^{2+} concentration occur in response to various stimuli or stresses, such as mechanical stress, light, drought, or pathogen attack (Trewavas and Gilroy, 1991). The plant hormone ABA, which causes stomatal closure during drought conditions, also causes increases in cytoplasmic Ca^{2+}. The increase in free Ca^{2+} in the cytoplasm may trigger a signal for stomatal closure. We attempted to isolate cDNAs encoding Ca^{2+}-related proteins to understand the role of cytoplasmic Ca^{2+} in various signal transduction pathways of plants.

CDPKs or their genes have been found in various plants (Putnam-Evans et al., 1990; Harper et al., 1991, 1993; Suen and Choi, 1991; Kawasaki et al., 1993). The CDPK proteins have two domains; one is a catalytic domain that contains conserved amino acid residues found in calmodulin-dependent protein kinases and the other is a Ca^{2+}-binding domain that contains four typical E-F hand structures found in calmodulins (Roberts and Harmon, 1992). To isolate cDNA clones encoding CDPKs, we used PCR with oligonucleotide primers corresponding to the conserved regions on cDNAs that had been prepared from dehydrated *Arabidopsis thaliana* plants. Several PCR-amplified fragments of DNA that contain partial sequences of CDPKs were isolated. We screened a cDNA library prepared from 1-h-dehydrated plants with the PCR fragments and isolated three cDNA clones that encode CDPKs, cATCDPK1, 2, and 3 (Table I).

Northern blot analyses indicated that the mRNAs corresponding to *ATCDPK1* and *ATCDPK2* genes are rapidly induced by drought and high salt stresses but not by low-temperature or heat stresses (Urao et al., 1994). By contrast,

Table I. *Characteristics of cDNA clone ATCDPK3, encoding a calcium-dependent protein kinase in A. thaliana*

Organism:
Arabidopsis thaliana L. (Columbia ecotype).
Function:
CDPK.
Sources:
cDNA library in λgt11 constructed from poly(A)$^+$ RNA of 1-h-dehydrated rosette plants.
Isolation:
A DNA fragment homologous to sequence encoding CDPK was amplified from cDNA using degenerated oligonucleotide primers corresponding to amino acid sequences of subdomain VIb and the third E-F hand by a PCR method. To isolate the corresponding full-length cDNA, a cDNA library was screened with the PCR-amplified DNA fragment as a probe.
Sequencing:
Deletion subcloning and complete dideoxy sequencing of both strands.
Identification:
Similarity of the deduced amino acid sequence to previously reported sequences.
Features of cDNA:
1876 bp in length containing a 107-bp 3′ untranslated region, a 1449-bp open reading frame, and a 320-bp 3′ untranslated region. No typical poly(A) signal was found in the 3′ untranslated region.
Features of Deduced Protein:
The deduced protein has 483 amino acids with a predicted molecular mass of 54,279 D. Eleven catalytic subdomains for protein kinases, an ATP-binding site, an autoinhibitory domain, and four E-F hand structures were found. The deduced protein exhibits 66% identity with soybean CDPK.
Expression:
The mRNA was detectable in unstressed plants. The accumulation of the mRNA was not stimulated by drought, osmotic, cold, heat stresses, or treatment with exogenous ABA.
Antibody:
Not available.
Subcellular Location:
Not determined.

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Abbreviation: CDPK, Ca^{2+}-dependent, calmodulin-independent protein kinase.

the *ATCDPK3* gene did not respond to such environmental stresses. The cDNA (cATCDPK3) contains an open reading frame of 1449 bp encoding 483 amino acids with a predicted molecular mass of 54,279 D. The putative protein (ATCDPK3) exhibits significant similarity to a soybean CDPK (66%) and *A. thaliana* CDPKs ATCDPK2 (65%), ATCDPK1 (53%), and AK1 (56%). Genomic Southern blot analysis suggests the existence of a few additional genes that are related to *ATCDPK3* in the *A. thaliana* genome.

ATCDPK3 contains a catalytic domain that is typical of Ser/Thr protein kinases and a regulatory domain that is homologous to the Ca^{2+}-binding sites of calmodulin (Roberts and Harmon, 1992). ATCDPK3 contains all of the conserved amino acid residues in protein kinases and the 11 subdomains that are typical of the catalytic subunit of protein kinases. The catalytic domain most closely resembles the family of calmodulin-dependent protein kinases, which coincides with those of other CDPKs. A possible ATP-binding site was found in the N-terminal region of ATCDPK3. ATCDPK3 also contains a putative autoinhibitory domain composed of basic amino acids that probably inactivates kinase activity in Ca^{2+}-free conditions. The C-terminal domain of ATCDPK3, as well as the known CDPKs, resembles calmodulin and has four E-F hand structures. The Ca^{2+}-binding proteins, including calmodulin and calpain, have four E-F hand, Ca^{2+}-binding loops that have been shown to function as a Ca^{2+} sensor. Each of the four Ca^{2+}-binding sites is flanked by polypeptides that form α helices. The presence of Ca^{2+}-binding sites indicates that Ca^{2+} directly binds to ATCDPK3 protein and regulates its kinase activity.

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