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Plant Gene Register

Arabidopsis thaliana cDNA Encoding a Novel Member of the EF-Hand Superfamily of Calcium-Binding Proteins[1]

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Calcium plays a vital role in any living cell because of its structural as well as regulatory functions. In the plant cell, calcium acts as a second messenger and may be released from internal stores (McAinsh et al., 1990; Knight et al., 1992; Roberts and Harmon, 1992) or enter the cell from the apoplast (Schroeder and Hagiwara, 1989) in response to various stimuli, such as elicitation during pathogen attack (Stab and Ebel, 1989), the phytohormone ABA (McAinsh et al., 1990), or mechanical stress (Knight et al., 1992). How the intracellular calcium signal serves to regulate plant cell function is still largely unknown, but it is clear that immediate targets of calcium action are calcium-modulated proteins (for a review, see Roberts and Harmon, 1992).

A member of an *Arabidopsis thaliana* cDNA library that overrepresents plasma membrane-associated proteins (Bartling et al., 1992) was found to encode a novel EF-hand protein clearly different from the common calmodulins. Southern analysis of the cDNA indicates a single gene locus in *A. thaliana* (Table I). The 892-bp cDNA (PM129) contains one continuous open reading frame corresponding to a protein of 215 amino acids with a predicted molecular mass of 23,278 D. The protein sequence shares 34.8% and 34.0% amino acid identity with calmodulins from *A. thaliana* (Perera and Zielinski, 1992), wheat (Toda et al., 1985), and spinach (Lukas et al., 1984). The protein encoded by PM129 is thus clearly related to, but certainly not a member of, the calmodulin family, which is highly conserved among plants and even between plants and animals. The deduced amino acid sequence of PM129 displays four typical helix-turn-helix domains, or EF-hands (Nakayama et al., 1992), which contain all of the elements required for functional calcium-binding sites. PM129 is a member of a novel class of EF-hand proteins having no known counterpart in the animal kingdom.

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The EMBL accession number for the sequence reported in this article is X68054.

Table I. *Characteristics of the cDNA PM129 from A. thaliana*

Organism:
Arabidopsis thaliana (L.) Heynh., ecotype Landsberg erecta.
Location on Chromosome:
Unknown, one single-gene locus in Southern analysis.
Gene Product:
Calmodulin-like EF-hand protein.
Clone Type:
cDNA, full length.
Techniques:
Antibody screening; plasmid sequencing; Southern and northern analysis.
Method of Identification:
Sequence comparison of the deduced amino acid sequence with an *A. thaliana* calmodulin gene ACaM-3 (34.8% homology) (Perera and Zielinski, 1992), wheat calmodulin (34.0% homology) (Toda et al., 1985), and spinach calmodulin (34.0% homology) (Lukas et al., 1984).
Expression Characteristics:
Single transcript of 0.95- to 1-kb size; transcript termini not determined.
Regulation:
Unknown.
Features of cDNA Structure:
The ATG at bp position 54 is found in seven independently screened cDNAs as the first translational start codon of the open reading frame; typical stop codon at bp position 699.
Codon Usage:
No significant differences in codon usage (G + C) content: 44.6%.
Structural Features of Protein:
Open reading frame 215 amino acids; M_r = 23.278; four EF-hand putative Ca^{2+}-binding loops with helix-turn-helix secondary structure prediction (amino acid positions 69 to 97, 106 to 134, 144 to 172, 181 to 209).
Antibodies:
None available.
Subcellular Location:
Not tested, probably plasma membrane.

LITERATURE CITED

Bartling D, Seedorf M, Mithöfer A, Weiler EW (1992) Cloning and expression of an *Arabidopsis* nitrilase which can convert indole-3-acetonitrile to the plant hormone, indole-3-acetic acid. Eur J Biochem **205:** 417–424

Knight MR, Smith SM, Trewavas AJ (1992) Wind-induced plant motion immediately increases cytosolic calcium. Proc Natl Acad Sci USA **89:** 4967–4971

Lukas TJ, Iverson DB, Schleicher M, Watterson DM (1984) Structural characterization of a higher plant calmodulin: *Spinacia oleracea*. Plant Physiol **75:** 788–795

McAinsh MR, Brownlee C, Heterington AM (1990) Abscisic acid-

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induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure. Nature **343:** 186–188

Nakayama S, Moncrief ND, Kretsinger RH (1992) Evolution of EF-hand calcium modulated proteins. II. Domains of several subfamilies have diverse evolutionary histories. J Mol Evol **34:** 416–448

Perera IY, Zielinski RE (1992) Structure and expression of the *Arabidopsis* CaM-3 calmodulin gene. Plant Mol Biol **19:** 649–664

Roberts DM, Harmon AC (1992) Calcium-modulated proteins: targets of intracellular calcium signals in higher plants. Annu Rev Plant Physiol Plant Mol Biol **43:** 375–414

Schroeder JI, Hagiwara S (1989) Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba* guard cells. Nature **338:** 427–430

Stab MR, Ebel J (1989) Effects of Ca^{2+} on phytoalexin induction by fungal elicitor in soybean cells. Arch Biochem Biophys **257:** 416–423

Toda H, Yazawa M, Sakiyama F, Yagi K (1985) Amino acid sequence of calmodulin from wheat germ. J Biochem **98:** 833–842