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Plant Gene Register

Isolation and Sequence Comparison of a Maize Calmodulin cDNA[1]

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CaM is a key regulatory protein involved in the Ca^{2+} signal transduction pathway of all organisms investigated to date. It participates in transducing signals of external stimuli to intracellular targets and thereby controls the activity of a large number of enzymes or other regulatory proteins in response to changes of intracellular Ca^{2+} concentrations. CaM contains four EF-hand structures, each of which can bind one Ca^{2+} ion. Such binding triggers a conformational change in the protein, which then facilitates interactions of CaM with its target proteins. The conformational change is one of the initial intracellular events in the signal transduction pathway using Ca^{2+} as second messenger.

A notable feature of the CaM protein is the high conservation of its primary structure even between different kingdoms. This may be interpreted as the result of a strong selective pressure to maintain its amino acid sequence in the course of evolution to enable interactions with CaM-dependent proteins common to all eukaryotes and their ancestors.

To extend the knowledge of plant CaMs further (for review, see Roberts and Harmon, 1992; Poovaiah and Reddy, 1993) and to augment extensive physiological studies undertaken previously in the maize (*Zea mays* L.) system (Dieter and Marme, 1986), we isolated a cDNA encoding CaM from a maize cDNA library by a homology screening approach. Positive clones were isolated from a λ-Uni-ZAP XR cDNA library commercially prepared (Stratagene) from the leaves and sheaths of a 5-week-old maize plant. Phagemid inserts of plaque-purified clones were excised using the in vivo excision protocol supplied by the manufacturer. Since the cDNA library used was cloned unidirectionally, cDNAs were first sequenced on one strand only by a dideoxy method and using an automated EMBL-Sequencer prototype of ALF (Pharmacia).

The partial sequence of one clone, designated ZMCALM1, showed extensive homology to nucleic acid sequences of CaMs from various species. This clone was therefore completely sequenced from both sides and by use of an internal primer (Igloi and Schiefermayr, 1993).

The complete nucleotide sequence of the ZMCALM1 cDNA is 743 bp in length and contains an open reading frame of 447 nucleotides coding for 149 amino acids (Table I). The predicted mass of the corresponding protein is 16,812 D, the isoelectric point is 3.81, and the protein is rich in acidic residues (25% Asp or Glu). The open reading frame shows very high homology to all CaMs and some CaM-like proteins from plants and high homology to CaM protein sequences from other organisms.

To analyze the deduced amino acid sequence of ZMCALM1 further we aligned all 15 available full-length plant CaM protein and deduced amino acid sequences together with the deduced amino acid sequence of ZMCALM1 and set up a consensus sequence for these plant CaMs. All

Table I. *Characteristics of the ZMCALM1 cDNA from maize*

Organism:
Zea mays [L.] cv B73, inbred line.
Location on Chromosome:
Unknown.
Function:
Encodes a CaM protein of maize.
Techniques:
λ-Uni-ZAP XR cDNA library screening, in vivo excision of pBluescript SK phagemid inserts, dideoxy sequencing.
Method of Identification:
Similarity of deduced amino acid sequence to CaM proteins from rice, *Arabidopsis*, barley, and other plants as well as to those of other organisms. Identity (99%) with a plant CaM consensus sequence derived from all full-length plant CaM protein or deduced amino acid sequences.
Feature of cDNA Structure:
Full-length cDNA of 743 bp designated ZMCALM1. Translational start site at nucleotide 14 and stop site at nucleotide 461. Poly(A)$^+$ tail of 21 nucleotides at 3′ end.
G + C Content:
52.7% G + C; 59.2% G + C in coding region only.
Structure Feature of Deduced Amino Acid Sequence:
The coding region codes for a polypeptide of 149 amino acids with a calculated molecular mass of 16,812 D and a predicted isoelectric point of 3.81. Twenty-five percent of amino acids are Asp or Glu. A Phe appearing at a position next to the second Ca^{2+}-binding site in all other full-length plant CaM sequences is replaced by a Leu in maize.
Expression Characteristics and Subcellular Location:
Not determined.
Antibody:
Not available.

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Abbreviation: CaM, calmodulin.

regions in the maize sequence comprising the four Ca^{2+} binding sites as well as the other regions are nearly identical to the consensus sequence. Only one mismatch appears next to the second Ca^{2+} binding site, where a Phe appearing at this position in all other sequences is replaced by a Leu in maize. The possible relevance of this substitution is currently unknown.

In summary we have isolated a CaM-encoding cDNA from maize and compared the encoded protein sequence with other plant CaM sequences by use of a consensus sequence that was set up for the available full-length plant CaMs. The isolated CaM clone will enable us to investigate further the possible occurrence of isoforms of CaM and CaM gene structure in the maize system as well as to undertake experiments on CaM physiology using this CaM coding region.

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