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Plant Gene Register

The cDNA Sequence Encoding an Annexin from *Medicago sativa*[1]

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Annexins are a family of at least 13 calcium-binding proteins in higher eukaryotes. They share the common features of binding phospholipids in a Ca^{2+}-dependent manner and contain a 4- or 8-fold repeated sequence of about 70 amino acid residues termed the annexin repeat. An N-terminal domain is of greater variability and may confer specific functions for each type. At least some of the annexins seem to be differentially expressed with respect to cell proliferation (Schlaepfer and Haigler, 1990). However, the biological function of the annexins is still not clearly defined. Proposed roles include exocytosis and membrane trafficking (Creutz, 1992; Gruenberg and Emans, 1993), inhibition of phospholipase A_2 (Haigler et al., 1987), and mitogenic signaling (Haigler et al., 1987).

Until now, plant annexins have been characterized biochemically and by partial protein sequences (Boustead et al., 1989; Smallwood et al., 1990; Blackbourn et al., 1992; Andrawis et al., 1993). In cotton, an annexin-containing protein fraction was found to inhibit callose synthase in vitro (Andrawis et al., 1993). In addition, a 95-bp PCR fragment was isolated from tomato that was used as a probe in northern analysis to detect varying transcript levels in different plant organs (Smallwood et al., 1992).

To obtain protein phosphatase genes (Pirck et al., 1993) from a *Medicago sativa* somatic embryo cDNA library (Hirt et al., 1991), we found that one of the cDNA inserts (*AnnMs*, see Table I) potentially encoded a protein with similarity to different animal annexins. A homology search at the NCBI with the BLAST net service showed 30 to 37% identity with *AnnMs* to various animal annexins. Considerable similarity (74%) was obtained between *AnnMs* and an unpublished *Arabidopsis* cDNA fragment potentially encoding an annexin-like protein (accession no. Z18518).

Comparisons with the published plant partial protein sequences revealed significant similarities between the single annexin repeat units. The tomato 28-kD fragment of p35.5 (Smallwood et al., 1990), for example, has 51% identity with repeat II of AnnMs. In contrast, comparison of the four AnnMs internal repeat sequences showed only approximately 20% identity. Similar results were obtained when various animal annexins were analyzed.

Table I. *Characteristics of the AnnMs cDNA from M. sativa*

Organism:
Medicago sativa line RA3.
Clone Type; Designation:
cDNA; *AnnMs*.
Source:
cDNA library prepared from suspension-cultured cells that were induced by auxin treatment to form somatic embryos. The library was constructed using the λZap kit from Stratagene.
Method of Isolation:
DNA hybridization screening of 300,000 recombinant phages with PCR-generated fragments of PP1Ms and PP2AMs. Positive clones were sequenced on both strands using the Pharmacia double-strand sequencing kit.
Features of cDNA:
The *AnnMs* clone is 1282 nucleotides in length with a 206-nucleotide 3′ untranslated region and a poly(A) tail.
Features of Deduced Amino Acid Sequence:
The 308-amino acid-long polypeptide potentially encoded by the *AnnMs* open reading frame shows the highest sequence similarity to annexins containing four annexin repeats.
Expression Characteristics:
The *AnnMs* transcript is present in every plant tissue examined, at varying levels.
Antibodies:
Antibodies against AnnMs have not been generated.

Taken together, it appears that, although the plant and animal annexins differ considerably in amino acid sequence, the overall organization into repeated domains is evolutionarily conserved and suggests that these genes are derived from a common ancestor.

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