Plant Gene Register

Genomic Sequence of a Calnexin Homolog from Arabidopsis thaliana¹

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Calnexin is a membrane-bound protein of the ER in animal cells (Wada et al., 1991). It shows considerable similarity to the major calcium-sequestering protein of the ER lumen, calreticulin, with two calcium-binding regions—a high-affinity, low-capacity region in the ER lumen and a low-affinity, high-capacity region in the cytoplasm. The protein is postulated to act as a calcium-regulated chaperone during protein maturation (Ou et al., 1993). We have isolated a genomic sequence showing significant homology to the animal gene over the predicted coding sequence (Table I).

A partial cDNA from Zea mays was isolated from an expression library made from 6-d coleoptiles (Clontech, Palo Alto, CA). The library was screened using a monoclonal antibody raised against a small number of microsomal proteins resulting from a partial purification of plasma membrane Ca²⁺ ATPase (Briars et al., 1988). The partial cDNA showed sequence homology to the calcium-binding region common to calreticulin and calnexin. The fragment was used to screen a genomic library constructed from Arabidopsis thaliana (cv Larasbonerecta), and a 15-kb fragment was isolated and subcloned and the relevant subfragments were sequenced.

The coding region contains five introns, two in the N-terminal region and three in the C-terminal region. The predicted amino acid sequence shows a high level of homology with the animal calnexin, although the terminal highly acidic calcium-binding region is shorter. A cDNA for a putative homolog of calnexin was isolated from A. thaliana (cv Columbia) by Huang et al. (1993); our coding sequence shows 85% identity and 92% similarity determined by FASTA (Wisconsin Genetics Computer Group package); however, the differences are greater than would be expected between cultivars of the same species. A Southern blot probed with DNA from the central calcium-binding region shows multiple

Table I. Characteristics of the sequence

Organism:

Arabidopsis thaliana cv Landsberg erecta.

Source:

Genomic library of partial SauIIIA digest of total DNA in λ Dash II (Stratagene).

Method of Identification:

Heterologous screening with fragment from Zea mays showing homology with calcium-binding regions of calnexin/calreticulin.

Map Position:

Not determined.

Method of Sequencing:

Double-strand sequencing using overlapping nested deletion and synthetic oligos. Chain-terminating method using Sequenase II (United States Biochemical).

Structural Features of DNA:

Five introns: 87, 233, 84, 80, and 90 bp in length.

bands. This, combined with the sequence heterogeneity, suggests that calnexin belongs to a family of related genes.

Received May 17, 1994; accepted June 23, 1994.

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The GenBank accession number for sequence reported in this article is U08315.

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¹ This work was supported by a grant from the Agricultural and Food Research Council.

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