

Plant Gene Register

Structure of a Calmodulin-Binding Protein Kinase Gene from Apple¹

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Reversible phosphorylation of specific proteins is now widely recognized as a major mechanism for controlling a variety of cellular functions in plants as in other eukaryotes (Hanks et al., 1988; Colbran et al., 1989; Poovaiah and Reddy, 1993). The presence of various protein kinase activities in plant cells has been well documented for two decades and, in recent years, several of these protein kinases have been characterized by molecular cloning (Lawton et al., 1989; Biermann et al., 1990; Elliott and Brennan, 1990; Harper et al., 1991). Among these, multifunctional calcium-dependent protein kinases deserved special attention because of their possible involvement in many signal transduction processes implicating cytoplasmic calcium ions as a second messenger (Trewavas and Gilroy, 1991; Roberts and Harmon, 1992).

In addition to these calcium-dependent protein kinases, a messenger encoding a calmodulin-binding protein kinase, homologous to the mammalian type II calcium/calmodulin-dependent protein kinase, has also been found to be expressed in a plant species (Watillon et al., 1993). Characterization of the sequence and structure of the corresponding gene might help to ascertain the biological significance, if any, of its product. Characterization of the promoter region of this gene also constitutes a first step toward the identification of factors controlling its transcriptional activity. Here we describe the sequence of the gene corresponding to the previously reported (Watillon et al., 1993) calmodulin-binding protein kinase cDNA from apple (Table I).

The 3074-bp sequence presented covers the complete coding region interrupted by four introns, as well as 742 bp of DNA located upstream of the ATG and 361 bp of the 3' untranslated region. The intron/exon borders were determined by comparison with the cDNA sequence, and they all conform to the canonical GT/AG 5' and 3' splice sites. A few mismatches were observed between cDNA and genomic sequences, both in coding (nucleotide positions 808, 2054, and 2222 in the genomic sequence) and noncod-

Table I. Characteristics of the calmodulin-binding protein kinase gene from apple

Organism:

Malus domestica (L.) Borkh cv McIntosh Wijcik.

Source:

λGEM11 genomic library.

Cloning Techniques:

Library was screened using the 5' end of the calmodulin-binding protein kinase cDNA as a probe. A 6-kb *Hind*III fragment (identical in size with the *Hind*III fragment detected when Wijcik apple genomic DNA was analyzed by Southern blotting using the same probe) was subcloned into pBluescript and further fragmented in overlapping clones for sequencing using available restriction sites. Both strands were sequenced (by the dideoxy method) using both standard and gene-specific sequencing primers. Because the 3' region of the gene appeared to be missing in this *Hind*III fragment, the genomic library was rescreened using a fragment corresponding to the 3' end of the cDNA. Two *Eco*RI fragments (0.9 and 2 kb in length) turned out to hybridize with the probe and were consequently subcloned. The 3' region of the gene (extending toward the site of polyadenylation in the previously cloned cDNA) was sequenced on both strands using gene-specific primers.

Features of Gene Structure:

3074 nucleotides, including 742 bp upstream and 361 bp downstream of the initiation and termination codons, respectively. The open reading frame is interrupted by four AT-rich introns of 109, 105, 183, and 326 bp in length.

Confirmation:

Identified by comparison with the cDNA sequence.

Characteristics of Deduced Amino Acid Sequence:

Putative polypeptide 415 amino acids long. Mol wt 46,489.

Chromosomal Localization:

Unknown. According to data obtained by partial sequencing of other clones isolated from the genomic library and consistent with the results of genomic Southern analysis, a second copy of this gene is present in the Wijcik apple genome.

Expression Characteristics:

Very low levels of a transcript about 2 kb in length detected by northern blot analysis of poly(A)⁺ RNA isolated from in vitro grown plantlets (leaves and stems). Comparable or undetectable levels (by northern blotting) observed in total or poly(A)⁺ RNA from various organs (including petals, stamens, carpels, leaves, and stems) of orchard-grown plants.

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ing (positions 708, 730, 2724, 2725, and 2981) regions. In this respect, it should be remembered that the previously characterized cDNA fragments were mostly obtained by the Rapid Amplification of cDNA Ends strategy. However, none of these mismatches affects the predicted amino acid sequence of the putatively encoded protein.

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The EMBL accession number for the sequence reported in this article is Z38126.

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