Plant Physiol. (1993) 101: 1399–1400

Plant Gene Register

p34^{cdc2} Protein Kinase Homolog from Mothbean (*Vigna aconitifolia*)[1]

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The molecular machinery that controls cell division in eukaryotes is conserved (Nurse, 1990). The key component of this machinery is a complex of cyclins and p34^{cdc2} protein kinases that is involved in the control of both G_1 to S phase and G_2 to M phase transitions in the cell cycle (Lewin, 1990). Homologs of p34^{cdc2} protein kinase genes have been cloned from a number of eukaryotic organisms (Lewin, 1990) including plants (Doonan, 1991; Martinez et al., 1992; Miao et al., 1993). The function of p34^{cdc2} protein kinase genes from plants has been determined by functional complementation of yeast *cdc2/CDC28* mutations by expression of the plant sequences (Hirt et al., 1991; Miao et al., 1992). Although the expression of p34^{cdc2} protein kinase genes can be detected in differentiated tissues of a plant, the highest level of the expression has been found in meristematic tissues. The expression of these genes has been shown to be responsive to treatment with external mitotic factors such as phytohormones (Hirt et al., 1991; Martinez et al., 1992; Miao et al., 1993) and *Rhizobium* infection (Miao et al., 1993). As a step toward dissection of the *Rhizobium*-mediated signal transduction pathway(s) leading to the initiation of cortical cell division in legume roots, we have isolated a cDNA clone encoding a p34^{cdc2} protein kinase from a mothbean (*Vigna aconitifolia*) nodule cDNA library (Table I).

Table I. *Characteristics of a p34^{cdc2} protein kinase cDNA from mothbean*

Organism:
Mothbean (*Vigna aconitifolia*); root nodules.
Location of Gene:
Nuclear genome, chromosome location not known.
Function:
Encodes p34^{cdc2} protein kinase.
Techniques:
Nodule cDNA library in pcDNA II (Invitrogen, San Diego, CA), dideoxy nucleotide sequencing of both strands.
Method of Isolation and Identification:
Reverse transcription of mothbean nodule total RNA followed by polymerase chain reaction using oligonucleotides 5′-GG(GT)GA(AG)GG(ATC)AC(AG)TACGG-3′ and 5′-ATC(AT)ATCAA(TC)AA(AG)TT(TC)TG-3′, corresponding to the conserved regions of known p34^{cdc2} homologs. A fragment of 378 bp was amplified, confirmed by sequencing, and used to screen a nodule cDNA library.
(G+C) Content:
42.2%.

The mothbean p34^{cdc2} protein kinase cDNA sequence is 1339 bp in length with an open reading frame encoding a peptide of 294 amino acids. Amino acid sequences in the highly conserved regions, including the ATP-binding region, catalytic domain for protein kinases, and the PSTAIR motif, are identical among the p34^{cdc2} genes from yeast (Lorincz and Reed, 1984), mothbean, and soybean (Miao et al., 1993) (Fig. 1). Furthermore, all but 1 of the 17 amino acid residues that have been identified to be essential for p34^{cdc2} protein kinase function in *Schizosaccharomyces pombe* (MacNeill et al., 1991) are conserved in these sequences. The exception is a substitution of a Gly at position 192 in yeast by a Pro residue in the mothbean sequence. The same substitution has also been reported for other plant homologs (Hirt et al., 1991; Miao et al., 1993). Of the two soybean p34^{cdc2} protein kinases (Miao et al., 1993), cdc2-S5 has been shown to be more active in roots and root nodules, whereas cdc2-S6 is highly expressed in shoot meristem, leaves, and flowers. Sequence comparison revealed that the mothbean p34^{cdc2} sequence is more homologous to soybean cdc2-S5 than to cdc2-S6. The difference between the mothbean sequence and soybean cdc2-S5 is only five amino acid residues, whereas, by contrast, it differs in 22 residues from cdc2-S6. Expression of soybean cdc2-S5 is preferentially enhanced by inoculation with *Rhizobium* and treatment with auxin (Miao et al., 1993). The high structural homology of the mothbean p34^{cdc2} protein kinase to soybean cdc2-S5 implies that this gene may be more active in roots and nodules and may play a role in nodule organogenesis.

Mothbean was used as plant material in this study because it is a diploid tropical legume plant with high competency for genetic manipulation, such as easy transformation via *Agrobacterium* infection and regeneration from transgenic callus (our unpublished observation). Suppression of this gene by the antisense RNA approach and isolation of its promoter will allow us to determine the role of this gene in the induction of meristematic activity in root cortical cells following *Rhizobium* infection.

[1] This work was supported by National Science Foundation grants DCB 8904101 and DCB 8819399.

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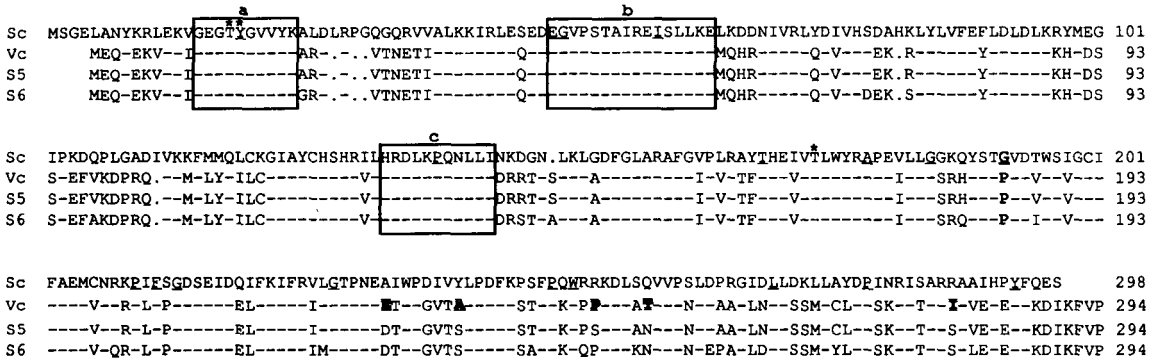

Figure 1. Amino acid sequence comparison of mothbean (*V. aconitifolia*) p34^{cdc2} protein kinase (Vc) with *Saccharomyces cerevisiae CDC28* protein kinase (Sc) (Lorincz and Reed, 1984) and two soybean p34^{cdc2} protein kinases (Miao et al., 1992), cdc2-S5 (S5) and cdc2-S6 (S6). The hyphen (-) indicates an identical amino acid residue, and gaps (.) are generated to align the sequences. In boxes are three highly conserved regions: the ATP-binding domain (a), PSTAIR motif (b), and catalytic domain for protein kinases (c). The conserved phosphorylation sites are indicated by *. The amino acid residues essential for p34^{cdc2} protein kinase activity are underlined; substitution of any of these causes a temperature-sensitive mutant phenotype in yeast. The only exception is at position 192, marked by bold letters, where a Gly in yeast is substituted by a Pro residue (position 184) in the plant sequences. Five amino acid residues that differ between mothbean p34^{cdc2} and soybean cdc2-S5 are indicated by shadowed letters.

Received September 24, 1992; accepted October 29, 1992.
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The GenBank accession number for the sequence reported in this article is M99497.

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