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Plant Gene Register

Cloning and Sequencing of a Full-Length cDNA from *Thlaspi arvense* L. That Encodes a Cytochrome P-450

Michael K. Udvardi[1]*, James D. Metzger, Viji Krishnapillai, W. Jim Peacock, and Elizabeth S. Dennis

Cooperative Research Centre for Plant Science, GPO Box 475, Canberra, Australian Capital Territory 2601, Australia (M.K.U.); Commonwealth Scientific and Industrial Research Organization, Division of Plant Industry, GPO Box 1600, Canberra, Australian Capital Territory 2601, Australia (M.K.U., W.J.P., E.S.D.); United States Department of Agriculture Biosciences Research Laboratory, P.O. Box 5874, State University Station, Fargo, North Dakota 58105 (J.D.M.); and Department of Genetics and Developmental Biology, Monash University, Clayton, Victoria 3168, Australia (V.K.)

Plant Cyts P-450 are membrane-bound hemoproteins that are involved in a variety of oxidative reactions. They participate in the biosynthesis of GAs, membrane sterols, fatty acids, terpenoids, lignin phenolics, flavonoids, and phytoalexins (Donaldson and Luster, 1991). We are interested in the regulation of GA biosynthesis and its possible role in cold-induced stem elongation and flowering in the crucifer, *Thlaspi arvense* L. (Hazebroek et al., 1993). Cyts P-450 appear to catalyze four consecutive steps early in the GA biosynthetic pathway: *ent*-kaurene to *ent*-kaurenol to *ent*-kaurenal to *ent*-kaurenoic acid to *ent*-7α-hydroxykaurenoic acid (Graebe, 1987). We are attempting to clone the genes encoding these Cyts P-450, using the strategy described below.

Cyts P-450 share a number of conserved domains in their amino acid sequences (Bozak et al., 1990). We have taken advantage of this and used a PCR strategy to isolate a novel plant Cyt P-450 from *Thlaspi arvense* L. (Table I). The cDNA is 1648 nucleotides long and encodes a protein with 496 amino acid residues and a mol wt of 56,387. The deduced protein exhibits the characteristic Cyt P-450 Cys heme-iron ligand signature. The deduced protein also shares significant amino acid sequence identity with other plant Cyt P-450: 42% identity with avocado CYP71 (Bozak et al., 1990); 32% with Jerusalem artichoke cinnamate 4-hydroxylase, CYP73 (Teutsch et al., 1993); 31% with petunia flavonoid 3′,5′-hydroxylase, CYP75 (Holton et al., 1993); 21% with periwinkle CYP72 (Vetter et al., 1992); and 20% with flax allene oxide synthase, CYP74 (Song et al., 1993). The gene is represented in the *Thlaspi* cold-induced shoot tip cDNA library at a frequency of 0.02%.

The protein exhibits the characteristic reduced minus CO difference spectrum of a Cyt P-450 when synthesized in yeast transformed with the cDNA (results not shown). However, the function of the protein remains obscure. Interestingly, the protein also contains a pyruvate kinase active site signature, but the significance of this is unknown.

Table I. *Characteristics of a Cyt P-450 cDNA from Thlaspi arvense L.*

Organism:
Thlaspi arvense L. (field pennycress).
Gene:
cDNA encoding Cyt P-450.
Function:
Unknown.
Source:
λZAP cDNA library constructed from vernalized shoot-tip poly(A)$^+$ RNA.
Method of Isolation and Identification:
Total RNA from vernalized shoot tips was reverse transcribed, and the resulting cDNA was used as template in a PCR using degenerate, inosine-containing oligonucleotide primers: (a) 5′-TTGGATCCIGA(AG)IIITT(CT)IIICCIGA(AG)IGITT-3′ and (b) 5′-AAGGATCCIII(GA)CAIIIIC(TG)IIIICCIIIICC(GA)AA-3′, corresponding to conserved domains C and D of Cyt P-450, respectively (Bozak et al., 1990). A 110-bp fragment was amplified, cloned into the *Bam*HI site of pBluescript SK$^-$ (Stratagene), and sequenced. The sequence between primers a and b was used to generate a gene-specific primer (c). A fourth oligonucleotide (d), complementary to the *Eco*RI-*Not*I adapter used in cDNA library construction (Pharmacia), was also synthesized. PCR was then performed using first-strand cDNA from above as template and oligonucleotide primers: (c) 5′-AAAGGATCCTTGAACTTTGAGTTGTTGCCG-3′ and (d) 5′-AAAGGATCCCGCGGCCGCTTTTTT-3′. A 450-bp fragment was amplified, cloned, sequenced, and used to screen the vernalized shoot-tip cDNA library. A full-length cDNA clone was identified by sequencing. Sequence homology to known Cyt P-450 identified the clone as a putative Cyt P-450. The full-length cDNA was expressed in yeast, which then and only then exhibited the reduced minus CO difference spectrum characteristic of Cyt P-450.
Features of the cDNA Structure:
Total length, 1648 nucleotides. Deduced translation start site at nucleotide 23 and stop site at nucleotide 1511.
Features of the Protein Sequence:
Deduced amino acid sequence of 496 residues with predicted mol wt of 56,387. Exhibits characteristic Cyt P-450 Cys heme-iron ligand signature. Also contains a pyruvate kinase active site signature.

[1] Present address: Division of Biochemistry and Molecular Biology, Faculty of Science, The Australian National University, Canberra ACT 0200, Australia.

* Corresponding author; fax 61–6–249–0313.

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