Plant Physiol. (1994) 104: 1091–1092

*Plant Gene Register*

# Cloning and Sequencing of a Full-Length cDNA Coding for Phenylalanine Ammonia-Lyase from Tobacco Cell Culture[1]

**Nobuo Nagai, Fumiya Kitauchi, Makoto Shimosaka, and Mitsuo Okazaki***

Department of Applied Biology, Faculty of Textile Science and Technology, Shinshu University, Ueda, Nagano 386, Japan

PAL (EC 4.3.1.5) plays a key role in linking primary metabolism to phenylpropanoid metabolism by catalyzing the deamination of L-Phe to produce *trans*-cinnamic acid. This reaction is considered a key step in phenylpropanoid metabolism. In bean (Edwards et al., 1985) and parsley (Lois et al., 1989), PAL genes are transcriptionally activated by elicitor treatment, UV light, and wounding. We reported that the addition of kinetin to tobacco (*Nicotiana tabacum* L. Bright Yellow T-13) cell cultures increased PAL activity (about 2-fold) and resulted in high-level accumulations of scopoletin and scopolin (Hino et al., 1982). The increase in PAL activity was shown to result from a stimulation of de novo synthesis of the PAL enzyme, based on immunochemical studies (Nagai et al., 1988). However, the regulatory mechanism is not clearly understood. To clarify the mechanism of this kinetin-mediated PAL induction, we have cloned a full-length PAL cDNA that corresponds to the PAL induced by kinetin.

Total cellular RNA was isolated from kinetin-treated cells (final concentration of 1 μg kinetin/mL) by a modification of the method of Chirgwin et al. (1979). Poly(A)$^+$ RNA was isolated by adsorption and elution from an oligo(dT)-cellulose column and used for the construction of the cDNA library. Double-stranded cDNAs were synthesized and cloned into the *Eco*RI site of λgt11. The cDNA library was screened with a $^{32}$P-labeled PAL cDNA isolated from bean (a 1520-bp open reading frame and a 223-bp 3′ noncoding region) (Edwards et al., 1985).

In this paper, we present the sequence of the full-length cDNA encoding tobacco PAL. It contains a 2136-bp open reading frame capable of coding for a polypeptide with 712 amino acids, a 204-bp 5′ noncoding region, and a 206-bp 3′ noncoding region containing a 19-bp poly(A) (Table I). The molecular mass of the polypeptide was calculated to be 77,400 D from the deduced amino acid sequence.

We compared the nucleotide sequence of the coding region and the deduced amino acid sequence of tobacco PAL cDNA with the partial sequences of PAL cDNA from the other plants. The homology between the nucleotide sequences was 74 to 83% for potato (Joos and Hahlbrock, 1992), tomato (Lee et al., 1992), sweet potato (Tanaka et al., 1989), parsley (Lois et al., 1989), poplar (Subramaniam et al., 1993), soybean

**Table I.** *Characteristics of a PAL cDNA from tobacco cell culture*

Organism:
Tobacco (*Nicotiana tabacum* L. Bright Yellow T-13 callus).

Function:
Encodes a subunit of the tetrameric enzyme PAL (EC 4.3.1.5), which catalyzes the conversion of L-Phe to *trans*-cinnamate and $NH_3$.

Source:
cDNA library constructed on a template of poly(A)$^+$ RNA from a kinetin (1 μg/mL)-treated tobacco cell culture.

Techniques:
A cDNA library constructed in λgt11 was screened with the $^{32}$P-labeled PAL cDNA isolated from bean (Edwards et al., 1985). Restriction fragments of the cDNA insert were subcloned into the bacteriophage M13 mp18 or mp19. The nucleotide sequence of both strands was determined by the dideoxy chain-termination method.

Features of cDNA Structure:
Contains 2546 nucleotides: 204 nucleotides of 5′ untranslated region, an open reading frame of 2136 nucleotides, and 206 nucleotides of 3′ untranslated region containing 19 nucleotides of poly(A). Comparison with other PAL sequences (coding region) shows 83.1% homology with the potato PAL (Joos and Hahlbrock, 1992), 82.3% with that of tomato (Lee et al., 1992), 78.9% with that of sweet potato (Tanaka et al., 1989), 77.9% with that of parsley (Lois et al., 1989), 76.7% with that of poplar (Subramaniam et al., 1993), 75.7% with that of soybean (Frank and Vodkin, 1991), and 74.5% with that of bean (Edwards et al., 1985).

Feature of the Deduced Protein:
Open reading frame of 712 amino acids; predicted molecular mass 77,400 D. Comparison with other PAL sequences shows 88.3% homology with the partial potato PAL (Joos and Hahlbrock, 1992), 88.0% with that of tomato (Lee et al., 1992), 86.3% with that of sweet potato (Tanaka et al., 1989), 84.5% with that of parsley (Lois et al., 1989), 83.5% with that of poplar (Subramaniam et al., 1993), 83.3% with that of soybean (Frank and Vodkin, 1991), and 84.4% with that of bean (Edwards et al., 1985).

Antibodies:
Polyclonal antibodies against PAL from tobacco cell culture are available.

[1] This work was supported by the Asahi Glass Foundation.

* Corresponding author; fax 81–268–22–4079.

Abbreviation: PAL, phenylalanine ammonia-lyase.

(Frank and Vodkin, 1991), and bean (Edwards et al., 1985), and the homology between the deduced amino acid sequences was about 83 to 88% (Table I).

## ACKNOWLEDGMENT

We thank Dr. C.J. Lamb for a gift of bean PAL cDNA.

Received October 1, 1993; accepted November 10, 1993.
Copyright Clearance Center: 0032-0889/94/104/1091/02.
The EMBL accession number for the sequence reported in this article is D17467.

## LITERATURE CITED

**Chirgwin JM, Przybyla AE, Macdonald RJ, Rutter WJ** (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18:** 5294–5299

**Edwards K, Cramer CL, Bolwell GP, Dixon RA, Schuch W, Lamb CJ** (1985) Rapid transient induction of phenylalanine ammonia-lyase mRNA in elicitor-treated bean cells. Proc Natl Acad Sci USA **82:** 6731–6735

**Frank RL, Vodkin LD** (1991) Sequence and structure of a phenylalanine ammonia-lyase gene from *Glycine max*. DNA Seq **1:** 335–346

**Hino F, Okazaki M, Miura Y** (1982) Effects of kinetin on formation of scopoletin and scopolin in tobacco tissue cultures. Agric Biol Chem **46:** 2195–2202

**Joos HJ, Hahlbrock K** (1992) Phenylalanine ammonia-lyase in potato (*Solanum tuberosum* L.): genomic complexity, structural comparison of two selected genes and modes of expression. Eur J Biochem **204:** 621–629

**Lee SW, Robb J, Nazar RN** (1992) Truncated phenylalanine ammonia-lyase expression in tomato (*Lycopersion esculentum*). J Biol Chem **267:** 11824–11830

**Lois R, Dietrich A, Hahlbrock K, Schulz W** (1989) A phenylalanine ammonia-lyase gene from parsley: structure, regulation and identification of elicitor and light responsive *cis*-acting elements. EMBO J **8:** 1641–1648

**Nagai N, Kojima Y, Shimosaka M, Okazaki M** (1988) Effects of kinetin on L-phenylalanine ammonia-lyase activity in tobacco cell culture. Agric Biol Chem **52:** 2617–2619

**Subramaniam R, Reinold S, Molitor EK, Douglas CJ** (1993) Structure, inheritance, and expression of hybrid poplar (*Populus trichocarpa* × *Populus deltoides*) phenylalanine ammonia-lyase genes. Plant Physiol **102:** 71–83

**Tanaka Y, Matsuoka M, Yamamoto N, Ohashi Y, Kano-Murakami Y, Ozeki Y** (1989) Structure and characterization of a cDNA clone for phenylalanine ammonia-lyase from cut-injured roots of sweet potato. Plant Physiol **90:** 1403–1407