

Plant Gene Register

Isolation of a Full-Length cDNA Encoding Cytosolic Enolase from *Ricinus communis*

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In glycolysis and gluconeogenesis, enolase (2-phospho-D-glycerate hydrolase, EC 4.2.1.11) is a ubiquitous enzyme that catalyzes the conversion of 2-phosphoglycerate to PEP. Enolase has been purified and/or cloned from several nonplant sources, e.g. *Bacillus subtilis* (Verma, 1989), *Xenopus* (Segil et al., 1988), and yeast (Chin et al., 1981). In yeast, both *cis*- and *trans*-acting factors have been identified that govern the regulation and expression of the two structural genes, *ENO1* and *ENO2* (Cohen et al., 1986; Holland et al., 1987; Brindle et al., 1990). There are three different isozymes of enolase in higher vertebrates designated α , β , and γ , the genes for which are expressed in a tissue- and development-specific manner (Forss-Petter et al., 1986).

In contrast, relatively little is known about plant enolase. Biochemical analysis has demonstrated the presence of both plastid and cytosolic isozymes of enolase in developing castor seeds (Dennis and Miernyk, 1982). During endosperm development in these seeds, the plastid form represents 30% of total cellular activity during the period of maximum fatty acid biosynthesis. In other castor tissues, however, the ratio of plastid to cytosolic enolase has been shown to vary considerably, and there is no detectable enolase in the chloroplasts from mature leaves (Miernyk and Dennis, 1992). It has also been reported that *Arabidopsis* chloroplasts lack enolase activity (Van der Straeten et al., 1991).

Cytosolic enolase has been cloned from maize (Lal et al., 1991), and in tobacco and *Arabidopsis* the structures of the genes for this isozyme have been determined (Van der Straeten et al., 1991). Plastid enolase has not yet been cloned. A developing castor endosperm cDNA library was screened in an attempt to isolate cDNAs for both plastid and cytosolic enolase. We report here the sequence of a cDNA for the cytosolic isozyme of enolase from this tissue (Table I).

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The GenBank accession number for the sequence reported in this article is Z28386.

Table I. Characteristics of the cDNA encoding cytosolic enolase from *R. communis*

Organism:	<i>Ricinus communis</i> L. cv Baker 296.
Gene Product; Pathway:	Enolase (EC 4.2.1.11). Glycolysis and gluconeogenesis, interconversion of 2-phosphoglycerate and PEP. <i>R. communis</i> cDNA library was constructed in bacteriophage λ gt 11 using mRNA isolated from developing castor endosperm tissue. Clones were identified using antisera raised against overexpressed maize cytosolic enolase (Lal et al., 1991). Restriction fragments from positive signals were subcloned into pUC118 and both strands were sequenced completely using the Sanger dideoxy chain termination procedure.
Gene Copy Number:	Southern blot analysis of genomic DNA cleaved with various restriction endonucleases indicated the presence of a small gene family consisting of two or three genes.
Features of Gene Sequence:	Open reading frame of 1335 bp. There are two putative polyadenylation sequences in the 3' untranslated region.
Features of the Protein Sequence:	The 1335-bp open reading frame encodes a polypeptide of 445 amino acids with a predicted <i>M_r</i> of 47,912.
Subcellular Localization:	Cytosol. Alignment of deduced amino acid sequence with enolase from other sources indicates absence of NH ₂ -terminal extension (transit peptide).

LITERATURE CITED

- Brindle PK, Holland JP, Willett CE, Innes MA, Holland MJ** (1990) Multiple factors bind the upstream activation sites of the yeast enolase *ENO1* and *ENO2*: ABF1 protein, like repressor activation protein RAP1, binds *cis*-acting sequences which modulate repression or activation of transcription. *Mol Cell Biol* **10**: 4872–4885
- Chin CCO, Brewer JM, Wold F** (1981) The amino acid sequence of yeast enolase. *J Biol Chem* **256**: 1377–1384
- Cohen R, Holland JP, Yokoi T, Holland MJ** (1986) Identification of a regulatory region that mediates glucose-dependent induction of the *Saccharomyces cerevisiae* enolase gene *ENO2*. *Mol Cell Biol* **6**: 2287–2297
- Dennis DT, Miernyk JA** (1982) Compartmentation of non-photosynthetic carbohydrate metabolism. *Annu Rev Plant Physiol* **33**: 27–50

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- Forss-Petter S, Danielson P, Sutcliffe JG** (1986) Neuron-specific enolase: complete structure of rat mRNA, multiple transcriptional start sites, and evidence suggesting post-transcriptional control. *J Neurosci Res* **16**: 141–156
- Holland MJ, Yokoi Y, Holland JP, Myambo K, Innes MA** (1987) The GCR1 gene encodes a positive transcriptional regulator of the enolase and glyceraldehyde-3-phosphate dehydrogenase gene families in *Saccharomyces cerevisiae*. *Mol Cell Biol* **7**: 813–820
- Lal SK, Johnson S, Conway T, Kelley PM** (1991) Characterisation of a maize cDNA that complements an enolase deficient mutant of *Escherichia coli*. *Plant Mol Biol* **16**: 787–795
- Miernyk JA, Dennis DT** (1992) A developmental analysis of the enolase isozymes from *Ricinus communis*. *Plant Physiol* **99**: 749–750
- Segil N, Shrutkowski A, Dworkin MB, Dworkin-Rasti E** (1988) Enolase isoenzymes in adult and developing *Xenopus laevis* and characterisation of a cloned enolase sequence. *Biochem J* **251**: 31–39
- Van der Straeten D, Rodrigues-Pousada RA, Goodman HM, Van Montague M** (1991) Plant enolase: gene structure, expression and evolution. *Plant Cell* **3**: 719–735
- Verma M** (1989) Primary structure of *Bacillus subtilis* enolase gene deduced from cDNA sequence. *Biochem Int* **18**: 667–672