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Nucleotide sequence of a cDNA encoding UMP synthase from *Nicotiana tabacum* (GenBank [U22260](#))

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UMP synthase is a bifunctional enzyme that catalyzes the last two steps of the pyrimidine biosynthetic pathway in higher eukaryotes. UMP synthase contains both orotate phosphoribosyl transferase (OPRTase) and OMP decarboxylase (ODCase) activities. OPRTase converts orotic acid to OMP and ODCase converts OMP to UMP (Caroline, 1969).

We have previously prepared a series of mutants which are affected in their ability to metabolize 5-fluoroorotic acid (Santoso and Thornburg, 1992; Santoso and Thornburg, in preparation). To investigate the molecular nature of these mutants we have set out to prepare the molecular tools necessary to characterize these mutants in detail. We report here the isolation of the UMP synthase cDNA from *Nicotiana tabacum*.

A cDNA library constructed in lambda ZapII using poly(A)⁺ RNA isolated from *Nicotiana tabacum* var. SR1 leaves (Dr. Steven Rodermel, Iowa State University) was screened with a UMP synthase cDNA sequence from *Arabidopsis thaliana* (Dr. Michele Minet, Gif-sur-Yvette, France). Eleven positive plaques were identified by plaque hybridization. After co-infection with helper phage R408, the inserts of the 11 clones were excised into plasmid form in the vector Bluescript SK⁺. Sizes of the inserts were determined by restriction analysis and the 5 largest inserts were sequenced via the dideoxy chain termination method. Three of the five clones sequenced contained cDNA sequences homologous to other eukaryotic UMP synthases or URA3 or URA5 sequences.

The largest clone, pRT327, contained an insert of 1261 bp, while the other 2 clones, pRT332 and pRT333, contained inserts of size 974 bp. When the three clones were sequenced and analyzed, regions of overlap were recognized and a large open reading frame corresponding to a 461 amino acid polypeptide was determined. The overlapping regions of the three clones were identical. This open reading frame lacks approximately 10 amino acids at the amino terminus.

Clone pRT327 was used as a probe in southern blot analysis to determine gene copy number on genomic DNA from both *N. tabacum* and *N. plumbaginifolia*. Results of this analysis show that UMP synthase exists as a single copy in *N. plumbaginifolia* and as two copies in *N. tabacum*. Portions of this probe were also used to detect mRNA from *N. plumbaginifolia* callus by northern blot analysis. (Santoso and Thornburg, manuscript in preparation).

Table 1: Characteristics of the UMP synthase cDNA from *Nicotiana tabacum*

Organism:

Nicotiana tabacum var. SR1

Gene Product:

Predicted UMP synthase, pyr5-6 (OPRTase EC [2.4.2.10](#), ODCase EC [4.1.1.23](#)) catalyzes the two step conversion of orotic acid into UMP.

Techniques:

cDNA screening; dideoxy chain termination sequencing; southern blot analysis of *N. tabacum* and *N. plumbaginifolia*

Characteristics of the cDNA:

A 1444 bp sequence lacking the 5' terminus, encodes a sequence homologous to UMP synthases from *Dictyostelium discoideum*, *Homo sapiens*, and *Arabidopsis thaliana*

Characteristics of the deduced amino acid sequence:

The overall protein sequence shows 77.7% identity with the UMP synthase from *Arabidopsis thaliana* and 48.7% identity with the UMP synthase from *Dictyostelium discoideum* and 57.0% identity with the UMP synthase from *Homo sapiens*. Amino acids 1 to 188 of the coding region show 28.3% identity with the *S. cerevisiae ura5* gene and amino acids 189 to 461 show 50.6% identity to the *S. cerevisiae ura3* gene.

Antibodies:

Antiserum prepared against purified squash UMP synthase recognize UMP synthases from a variety of sources including Solanaceae, Brassicaceae, Cucurbitaceae, Graminaceae, Leguminosae, and Umbelliferae (Santoso and Thornburg, unpublished).

Literature Cited

Caroline, DF (1969) Pyrimidine biosynthesis in *Neurospora crassa*: Gene-enzyme relationships. *J. Bacteriol* **100**: 1371-1377 [[Medline](#)]

Santoso D, Thornburg RW (in preparation) Isolation and analysis of rpy2: A lesion affecting normal response to pyrimidine starvation in *Nicotiana plumbaginifolia*.

Santoso D, Thornburg RW (1992) Isolation and characterization of UMP synthase mutants from haploid cell suspensions of *Nicotiana tabacum*. *Plant Physiol* **99**: 1216-1225 [[Agricola](#)]



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