Differential Biochemical Regulation of the URA7- and URA8-encoded CTP Synthetases from Saccharomyces cerevisiae*

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The URA7- and URA8-encoded CTP synthetases (EC 6.3.4.2, UTP:ammonia ligase (ADP-forming)) are functionally overlapping enzymes responsible for the biosynthesis of CTP in the yeast Saccharomyces cerevisiae. URA8-encoded CTP synthetase was purified to apparent homogeneity by ammonium sulfate fractionation of the cytosolic fraction followed by chromatography with Q-Sepharose, Affi-Gel Blue, Mono Q, and Superose 6. The subunit molecular mass (67 kDa) of purified URA8-encoded CTP synthetase was in good agreement with the predicted size of the URA8 gene product. Antibodies raised against a fusion protein constructed from the amino acid sequences of the open reading frames of the URA7 and URA8 genes were functionally overlapping and expressed in Escherichia coli reacted with purified URA8-encoded CTP synthetase. Native URA8-encoded CTP synthetase existed as a dimer which oligomerized to a tetramer in the presence of its substrates UTP and ATP. Maximum URA8-encoded CTP synthetase activity was dependent on Mg2+ ions (Km = 2.4 mM) and 2-mercaptoethanol at the pH optimum of 7.5. The enzyme followed saturation kinetics toward UTP (Km = 74 μM), ATP (Km = 22 μM), and glutamine (Km = 0.14 mM). GTP stimulated (Km = 26 μM) URA8-encoded CTP synthetase activity 12-fold. CTP potently inhibited (IC50 = 85 μM) URA8-encoded CTP synthetase activity and, in addition, caused the dependence of activity toward UTP to become cooperative. The URA8-encoded CTP synthetase and the previously purified URA7-encoded CTP synthetase differed significantly with respect to several biochemical properties including turnover number, pH optimum, substrate dependences, and sensitivity to inhibition by CTP. The URA7-encoded CTP synthetase mRNA was 2-fold more abundant when compared with URA8-encoded CTP synthetase mRNA. Both CTP synthetase isoforms were maximally expressed in the exponential phase of growth.

CTP synthetase (EC 6.3.4.2, UTP:ammonia ligase (ADP-forming)) is the enzyme responsible for the biosynthesis of the nucleotide CTP (1, 2). CTP is a precursor for the synthesis of RNA, DNA, and membrane phospholipids (3, 4). Thus, CTP synthetase activity plays a major role in the growth and metabolism of all organisms. Regulation of CTP synthetase activity is critical to normal cell growth in mammalian cells. Mutant mammalian cell lines lacking normal regulation of CTP synthetase activity exhibit abnormally high intracellular levels of CTP and dCTP (5, 6), resistance to nucleotide analog drugs used in cancer chemotherapy (7–10), and an increased rate of spontaneous mutations (8, 10, 11). Moreover, elevated levels of CTP synthetase activity are characteristic of rapidly growing tumors of liver (12), colon (13), and lung (14).

We are using the yeast Saccharomyces cerevisiae as a model eucaryote to study the regulation of CTP synthetase activity. CTP synthetase is encoded by the URA7 and URA8 genes in S. cerevisiae (15, 16). Comparison of the nucleotide and deduced amino acid sequences of the open reading frames of the URA7 and URA8 genes show 70 and 78% identity, respectively (15, 16). Although there is a high degree of identity between the two CTP synthetases, they are not functionally identical. The CTP concentration in cells possessing only a functional URA7 gene is 78% of that found in wild-type cells whereas the CTP concentration in cells with only a functional URA8 gene is 36% of that found in wild-type cells (16). Moreover, the reduced CTP concentration found in cells possessing only one of the CTP synthetase genes correlates with a reduced rate of cell growth (16). Simultaneous presence of null alleles for both CTP synthetase genes is lethal (16). Thus, the CTP synthetases encoded by the URA7 and URA8 genes are functionally overlapping and play an essential role in the growth of yeast cells.

The URA7-encoded CTP synthetase has been purified to homogeneity and characterized with respect to its physicochemical and kinetic properties (17). URA7-encoded CTP synthetase activity exhibits complex cooperative kinetics characteristic of allosteric enzymes and is allosterically regulated by CTP product inhibition (17). In this study, we questioned whether the activities of the CTP synthetases encoded by the URA7 and URA8 genes were regulated differentially through their biochemical properties. The URA8-encoded CTP synthetase was purified to homogeneity so its biochemical properties could be studied under well-defined conditions. URA8-encoded CTP synthetase differed from URA7-encoded CTP synthetase with respect to several enzymological properties and sensitivity to inhibition by CTP. Thus, these CTP synthetase isoforms were regulated differentially on a biochemical level. In addition, we showed that the URA7-encoded CTP synthetase mRNA was 2-fold greater than the URA8-encoded CTP synthetase mRNA. The differential regulation of the two CTP synthetases may account for the relative contribution of each enzyme for CTP synthesis.
EXPERIMENTAL PROCEDURES

Materials

All chemicals were reagent grade. Growth medium supplies were purchased from Difco Laboratories. Restriction endonucleases, restriction endonuclease recognition sequences, NEB, restriction enzyme manipulation kit, and the protein purification kit used to make maltose-binding protein-CTP synthetase fusion protein were purchased from New England Biolabs. Radiochemicals and GeneScreen were purchased from DuPont NEN. Nucleotides, L-glutamine, phenylmethylsulfonyl fluoride, benzamide, aprotinin, leupeptin, pepstatin, molecular mass standards for gel filtration chromatography, and bovine serum albumin were purchased from Sigma. Centric-10 concentration filters were purchased from Amicon. Protein assay reagent, Affi-Gel Blue, DEAE-Affi Gel Blue, molecular mass standards for SDS-polyacrylamide gel electrophoresis, electrophoresis reagents, and immunoreagents were purchased from Bio-Rad. Q-Sepharose, Mono Q, and Superose 6 were purchased from Pharmacia Biotech Inc.

Methods

Strains and Growth Conditions

Wild-type strain FL100 (MATa, American Type Culture Collection 28383) was used to examine CTP synthetase mRNA abundance. CTP synthetase was purified from strain OK8 (MATa leu2 trp1 ura3 -AURA8) previously isolated in the multicopy plasmid pOK10.1 (16). Strain OK8 has mutations in both the URA7 and URA8 genes (15, 16). Cells were grown in complete synthetic medium (18) without uracil to the exponential phase of growth (1–2 × 10^7 cells/ml) at 30 °C. Cell numbers were determined by microscopic examination with a hemacytometer.

Purification of URA8-encoded CTP Synthetase

All steps were performed at 5 °C.

Step 1: Preparation of Cytosol—Cells were disrupted with glass beads with a Bead-Beater (Biospec Products) in Buffer A (50 mM Tris-HCl, pH 7.5, 1 mM Na2EDTA, 20 mM L-glutamine, 0.3 mM sucrose, 10 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 5 μg/ml aprotinin, 5 μg/ml leupeptin, and 5 μg/ml pepstatin) as described previously (16). Unbroken cells and glass beads were removed by centrifugation at 1,500 × g for 5 min. The cytosolic fraction was obtained by centrifugation at 100,000 × g for 1.5 h.

Step 2: Ammonium Sulfate Fractionation—The cytosolic fraction was diluted to a protein concentration of 5 mg/ml with Buffer A. Enzyme grade ammonium sulfate was added to the cytosol to 45% saturation with slow stirring. After stirring for 2 h, precipitated protein was removed by centrifugation at 12,000 × g for 20 min and dissolved in a minimum volume of Buffer B (50 mM Tris-HCl, pH 7.5, 4 mM L-glutamine, 1 mM Na2EDTA, 10 mM 2-mercaptoethanol, and 10% glycerol). The enzyme preparation was then desalted by dialysis against Buffer B.

Step 3: Q-Sepharose Chromatography—A Q-Sepharose column (1.5 × 12 cm) was equilibrated with Buffer B. The ammonium sulfate fraction was applied to the column at a flow rate of 30 ml/h. The column was washed with Buffer B until all of the unbound protein had been removed from the column. CTP synthetase was then eluted from the column in 3-ml fractions with 10 column volumes of a linear NaCl gradient (0–1.0 M NaCl) in Buffer B. The peak of CTP synthetase activity was eluted from the column as a single peak. Fractions containing activity were pooled and the glycerol concentration in the enzyme preparation was increased to 30%. Purified CTP synthetase was stable at −20 °C for at least 3 months.

Preparation of Anti-URA8-encoded CTP Synthetase Antibodies

An inducible pMAL-c-UR8 plasmid was constructed for the production of a maltose-binding protein-CTP synthetase fusion protein. The fusion protein was used for the generation of anti-URA8-encoded CTP synthetase antibodies. A 0.7 kb fragment was isolated from the plasmid pOK10.1 (16) by Hpal and HindIII digestion by standard recombinant DNA techniques (20). This fragment contained the URA8 open reading frame from codon 245 to 477 (16). The pMAL-c plasmid was digested with Stul and HindIII. The 0.7-kb UR8 fragment was ligated into the pMal-c plasmid at these sites, placing URA8 in frame with the malE gene, to create the inducible pMal-c-URA8 plasmid.

Maltose-binding protein-URA8-encoded CTP synthetase fusion protein was produced in Escherichia coli strain DH5α by induction of its expression from the pMal-c-URA8 plasmid and purified as described previously (17). Antibodies to the maltose-binding protein-URA8-encoded CTP synthetase fusion protein were raised in New Zealand White rabbits by standard procedures (21) at the Pocono Rabbit Farm (Canadensis, PA). IgG antibodies were isolated as described previously (17).

Electrophoresis and Immunoblotting

SDS-polyacrylamide gel electrophoresis (22) was performed with 10% slab gels. Proteins on polyacrylamide gels were visualized with Coomassie Blue. Immunoblot analyses of cytosol and pure CTP synthetase were performed as described previously (23, 24). Immunoblot signals were optimized by analyzing a number of antigen and antibody concentrations and were in the linear range of detectability. The density of the CTP synthetase bands on immunoblots was quantified by scanning densitometry.

Enzyme Assay and Protein Determination

CTP synthetase activity was determined by measuring the conversion of UTP to CTP (molar extinction coefficients of 182 and 1520 M -1 cm -1 , respectively) by following the increase in absorbance at 291 nm (2). The standard reaction mixture contained 50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 1 mM 2-mercaptoethanol, 2 mM L-glutamine, 0.1 mM GTP, 2 mM ATP, 2 mM UTP, and an appropriate dilution of enzyme in a total volume of 0.2 ml. Enzyme assays were performed in triplicate with an average standard deviation of ±3%. All assays were linear with time and protein concentration. A unit of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of CTP/min under the assay conditions described above. Specific activity was defined as units/milligram of protein. Protein was determined by the method of Bradford (25) using bovine serum albumin as the standard. Protein was monitored from column chromatography fractions by measuring absorbance at 280 nm.

Analysis of Kinetic Data

Kinetic data were analyzed according to the Michaelis-Menten and Hill equations using the EZ-FIT Enzyme Kinetic Model Fitting Program (26). EZ-FIT uses the Nelder-Mead Simplex and Marquardt/Nash nonlinear regression algorithms sequentially and tests for the best fit of the data among different kinetic models.

Preparation of RNA and Northern Blot Analysis

Total RNA was extracted from cells using hot phenol as described by Schmitt et al. (27). The RNA was separated by electrophoresis under denaturing conditions using 1% agarose formaldehyde gels (28). Following electrophoresis, RNA was transferred to GeneScreen and probed with a radiolabeled fragment of the URA7 and URA8 genes. URA7 probe was a 1.6-kb fragment isolated from YEplp5-URA7 (17) by EcoRI and HindIII digestion. The URA8 probe was a 1-kb fragment generated by the polymerase chain reaction (29) using primers pokO10.1 (16) as a

1 The abbreviation used is: kb, kilobase(s).

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template and the primers 5'-GGATCCGATATGATTGCCTG-3' and 5'-GGATCCACCTTCGATGTA-3'. The probes were labeled with \(_{32}P\)dCTP by the random priming reaction using a NEBlot kit. Prehybridization and hybridization of blots were carried out at 60 °C in modified Church buffer (30) as recommended by U. S. Biochemical Corp. Ribosomal subunit L32 mRNA (31) was used as a constitutive standard and loading control. The density of the CTP synthetase mRNA bands on Northern blots was quantified by scanning densitometry.

RESULTS AND DISCUSSION

Purification of URA8-encoded CTP Synthetase—The purification of the URA8-encoded CTP synthetase was facilitated by the overexpression of the URA8 gene on a multicopy plasmid in a strain which lacked the URA7-encoded CTP synthetase. A summary of the purification of the URA8-encoded CTP synthetase is presented in Table I. The purification scheme included ammonium sulfate fractionation of the cytosolic fraction followed by chromatography with Q-Sepharose, Affi-Gel Blue, Mono Q, and Superose 6 (Fig. 1). The ammonium sulfate and Mono Q steps resulted in yields of activity over 100%. The reason for this was unclear. However, the removal of an inhibitor or protease could account for the increased yields of activity in these preparations. Overall, CTP synthetase was purified 1263-fold over the cytosolic fraction with an activity yield of 43.7%. Thus, under optimum assay conditions URA8-encoded CTP synthetase existed as a tetramer. A summary of these properties and those for the URA7-encoded CTP synthetase is presented in Table II.

Kinetic Properties of URA8-encoded CTP Synthetase—CTP synthetase catalyzes an ATP-dependent transfer of the amide nitrogen from glutamine to the C-4 position of UTP to form CTP (2, 33). GTP activates the reaction by accelerating the formation of a covalent glutaminyl enzyme catalytic intermediate (2, 33). We first examined the dependence of CTP synthetase activity on UTP and ATP using saturating concentrations of glutamine, GTP, and magnesium ions. Under these conditions all three nucleotides in the reaction mixtures existed as magnesium-nucleotide complexes (32). The kinetics of the enzyme with respect to the UTP concentration at various set concentrations of ATP was complex (Fig. 4A). CTP synthetase exhibited saturation kinetics when the UTP concentration varied (\(K_{m} = 74 \mu M\)) and ATP was held constant at a saturating concentration (0.8 mM). On the other hand, when ATP was held constant at subsaturating concentrations, the enzyme showed positive cooperative kinetics with respect to UTP. For example, the Hill number with respect to UTP at 0.1 mM ATP was 2.2. This complex kinetic behavior was also observed when CTP synthetase activity was measured with respect to ATP at various fixed concentrations of UTP (Fig. 4B). At a saturating concentration of UTP (0.6 mM), CTP synthetase showed saturation kinetics toward ATP (\(K_{m} = 22 \mu M\)). However, the dependence of CTP synthetase activity on ATP at subsaturating concentrations of UTP was cooperative. For example, the Hill number with respect to ATP at 75 \(\mu M\) UTP was 1.6.

The dependence of CTP synthetase activity on glutamine and GTP was examined using saturating concentrations of UTP, A

| Purification step | Total units | Protein | Specific activity | Yield | Purification |
|------------------|-------------|---------|------------------|-------|--------------|
|                  | \(\mu\)mol/ min | mg | units/ mg | % | fold |
| 1. Cytosol       | 5.23        | 1236   | 0.0038         | 100  | 1            |
| 2. Ammonium sulfate | 9.65      | 232    | 0.0416      | 184.5| 10.9         |
| 3. Q-Sepharose   | 8.63        | 72     | 0.1198    | 165  | 31.5         |
| 4. Affi-Gel Blue | 4.77        | 5.35   | 0.8915    | 91.2 | 234.6        |
| 5. Mono Q        | 5.2         | 2.44   | 2.13       | 99.4 | 560.5        |
| 6. Superose 6    | 2.3         | 0.48   | 4.8        | 43.7 | 1263.1       |

CTP Synthetases from S. cerevisiae

Enzymological Properties of URA8-encoded CTP Synthetase—CTP synthetase activity was measured with a Tris-maleate-glycine buffer at pH values ranging from 5.5 to 9.5 (Fig. 3A). Optimum CTP synthetase activity was obtained at pH 7.5. The dependence of CTP synthetase activity on the concentration of magnesium ions is shown in Fig. 3B. Maximum CTP synthetase activity was dependent on 6 mM magnesium ions. In these experiments, activity was measured at saturating concentrations of ATP (2 mM), UTP (2 mM), and GTP (0.1 mM) (see below). At 6 mM magnesium ions, all of the nucleotides present in the enzyme assay would be present as magnesium-nucleotide complexes (32). The dependence of activity on magnesium ions was cooperative. Analysis of the data according to the Hill equation yielded a \(K_{m}\) value for magnesium ions of 2.4 mM and a Hill number of 3. The cooperative dependence of activity on magnesium ions was likely due to the formation of magnesium-nucleotide complexes and subsequent cooperative binding to CTP synthetase (17). Manganese ions could not substitute for the magnesium ion requirement. CTP synthetase activity was totally inhibited by 0.8 mM p-chloromercuriphenylsulfonic acid and 0.8 mM N-ethylmaleimide. The addition of 10 mM 2-mercaptoethanol to the assay system prevented the inhibition of activity by these thiolreactive compounds. When 10 mM 2-mercaptoethanol was added to the assay system by itself, it stimulated CTP synthetase activity by 52%.

CTP synthetase activity eluted from the Superose 6 chromatography column at a position consistent with a molecular mass of 150 kDa (Fig. 1D). This indicated that native URA8-encoded CTP synthetase was a dimer. A characteristic of the URA7-encoded CTP synthetase is nucleotide-dependent oligomerization (17). This property was examined for URA8-encoded CTP synthetase by subjecting the enzyme to Superose 6 chromatography in the presence of 2 mM UTP and 2 mM ATP as described previously (17). Indeed, the enzyme eluted from the column at a position consistent with a molecular mass of 300 kDa (data not shown). Thus, under optimum assay conditions URA8-encoded CTP synthetase existed as a tetramer. A summary of these properties and those for the URA7-encoded CTP synthetase is presented in Table II.

Kinetic Properties of URA8-encoded CTP Synthetase—CTP synthetase catalyzes an ATP-dependent transfer of the amide nitrogen from glutamine to the C-4 position of UTP to form CTP (2, 33). GTP activates the reaction by accelerating the formation of a covalent glutaminyl enzyme catalytic intermediate (2, 33). We first examined the dependence of CTP synthetase activity on UTP and ATP using saturating concentrations of glutamine, GTP, and magnesium ions. Under these conditions all three nucleotides in the reaction mixtures existed as magnesium-nucleotide complexes (32). The kinetics of the enzyme with respect to the UTP concentration at various set concentrations of ATP was complex (Fig. 4A). CTP synthetase exhibited saturation kinetics when the UTP concentration varied (\(K_{m} = 74 \mu M\)) and ATP was held constant at a saturating concentration (0.8 mM). On the other hand, when ATP was held constant at subsaturating concentrations, the enzyme showed positive cooperative kinetics with respect to UTP. For example, the Hill number with respect to UTP at 0.1 mM ATP was 2.2. This complex kinetic behavior was also observed when CTP synthetase activity was measured with respect to ATP at various fixed concentrations of UTP (Fig. 4B). At a saturating concentration of UTP (0.6 mM), CTP synthetase showed saturation kinetics toward ATP (\(K_{m} = 22 \mu M\)). However, the dependence of CTP synthetase activity on ATP at subsaturating concentrations of UTP was cooperative. For example, the Hill number with respect to ATP at 75 \(\mu M\) UTP was 1.6.

The dependence of CTP synthetase activity on glutamine and GTP was examined using saturating concentrations of UTP, A
ATP, and magnesium ions. CTP synthetase showed saturation kinetics with respect to glutamine at saturating and subsaturating concentrations of GTP (Fig. 5A). The enzyme also showed saturation kinetics with respect to GTP at saturating and subsaturating concentrations of glutamine (Fig. 5B). GTP did not affect the enzyme’s affinity for glutamine (Kₘ = 0.14 mM) but did cause an increase in maximum velocity (Fig. 5A). GTP was not an absolute requirement for CTP synthetase activity (Fig. 5B). However, at a saturating concentration of glutamine, GTP stimulated (Kₐ = 26 μM) CTP synthetase activity 12-fold. The activation constant for GTP was not affected by glutamine.

The URA7- and URA8-encoded CTP synthetases exhibited significant differences in their kinetic properties. In contrast to the URA8-encoded CTP synthetase, the URA7-encoded enzyme showed positive cooperative kinetics toward UTP and ATP and negative cooperative kinetics toward glutamine and GTP even when kinetic experiments are performed with saturating substrate concentrations (17). Differences in the kinetic properties of the URA7- and URA8-encoded enzymes were also reflected...
The cellular concentrations of UTP (0.75 mM) and ATP (2.3 mM) are saturating for both the URA7- and URA8-encoded CTP synthetase activities (15, 17). Thus, the URA8-encoded CTP synthetase would be expected to exhibit saturation kinetics toward UTP when ATP was set at a saturating concentration. However, in the presence of 0.1 mM CTP, the enzyme exhibited positive cooperative kinetics (Fig. 6B). An important characteristic of the URA7-encoded CTP synthetase is product inhibition of its activity by CTP (17). The inhibition of CTP synthetase activity by CTP regulates the cellular concentration of CTP in growing cells (17). Strikingly, the inhibitor constant for CTP (85 μM) for URA8-encoded CTP synthetase was 3.5-fold lower when compared with the inhibitor constant (0.3 mM) for the URA7-encoded enzyme (Table II). Furthermore, the inhibitor constant for the URA8-encoded enzyme was 8-fold lower than the cellular concentration (0.77 mM) of CTP (34). These results indicated that in vivo, the URA8-encoded enzyme would be more sensitive to product inhibition by CTP when compared with the URA7-encoded enzyme.

Abundance of the URA7- and URA8-encoded CTP synthetase mRNAs—The abundance of the URA7- and URA8-encoded CTP synthetase mRNAs in wild-type cells was examined by Northern blot analysis. Under the high stringency hybridization conditions used in these studies, the URA7 probe did not hybridize to URA8-encoded CTP synthetase mRNA, and the URA8 probe did not hybridize to the URA7-encoded CTP synthetase mRNA. The level of the URA7-encoded CTP synthetase mRNA was 2-fold more abundant than the level of the URA8-encoded CTP synthetase mRNA (Fig. 7). Ribosomal subunit
L32 mRNA was used as an internal standard in these experiments. Thus, the difference in the abundance of these mRNA levels was attributed to differential expression of these genes as opposed to RNA degradation or to differences in the amount of RNA loaded on the gel.

Based on codon bias values, the URA7 gene product would be expected to be expressed more than the URA8 gene product (16). This notion was supported by the fact that the URA8-encoded enzyme required a greater fold purification to obtain pure enzyme when compared with the URA7-encoded enzyme (Table II). We were unable to determine differential expression of the URA7 and URA8 gene products by immunoblot analysis. Anti-URA7-encoded CTP synthetase antibodies reacted with pure URA8-encoded CTP synthetase, and anti-URA7-encoded CTP synthetase antibodies (17) reacted with pure URA8-encoded enzyme (data not shown). In addition, the subunit molecular masses of the two CTP synthetases were too similar to differentiate on immunoblots.

Effect of Growth Phase on CTP Synthetases—Anti-URA7-encoded CTP synthetase antibodies were used to examine the expression of the CTP synthetases during the exponential and stationary phases of growth in wild-type cells. Maximum expression of the CTP synthetases was found in the early exponential phase of growth (Fig. 8). As cells entered the stationary phase of growth, the expression of the CTP synthetases decreased 5-fold (Fig. 8). We also examined the activity of CTP synthetase during the exponential and stationary phases of growth. The specific activity of the enzyme was 4.3-fold higher in early exponential phase cells when compared with stationary phase cells. The regulation of CTP synthetase expression with growth phase was consistent with the essential role CTP plays in the synthesis of nucleic acids and membrane phospholipids during active cell growth (16).

Concluding Discussion—The URA7- and URA8-encoded CTP synthetases are functionally overlapping enzymes responsible for the synthesis of CTP in S. cerevisiae (16). Studies performed with mutants defective in the URA7 or URA8 genes indicated that the URA7-encoded CTP synthetase is responsible for the majority (78%) of the CTP synthesized in vivo (16). We questioned whether the relative contribution of the URA7- and URA8-encoded CTP synthetases for the synthesis of CTP was influenced by differential biochemical regulation of these enzymes. To address this question under well-defined conditions, we previously purified and characterized the URA7-encoded CTP synthetase (17) and in this work, we purified and characterized the URA8-encoded enzyme. The URA7- and URA8-encoded CTP synthetases had striking differences with respect to several enzymological and kinetic properties including turnover number, pH optimum, substrate dependences, and sensitivity to inhibition by CTP. Importantly, the differential kinetic properties of the enzymes with respect to UTP and ATP and inhibition by CTP were seen in vitro at concentrations within the range of concentrations which occur in vivo. In addition, the products of the URA7 and URA8 genes were differentially expressed. The increased sensitivity of the URA8-encoded enzyme activity to product inhibition by CTP coupled to the lower expression of the URA8-encoded CTP synthetase was consistent with the URA7-encoded CTP synthetase being responsible for the majority of the CTP synthesized in vivo.

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