Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7412–7416, December 1984
Biochemistry

Molecular cloning and nucleotide sequence of cDNA for rat ornithine carbamoyltransferase precursor

(mitochondrial enzyme precursor/transport and processing/presequence/recombinant DNA/sequence homology)

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Communicated by Philip P. Cohen, August 15, 1984

ABSTRACT Messenger RNA of rat ornithine carbamoyltransferase (EC 2.1.3.3), a mitochondrial matrix enzyme, was enriched by immunoprecipitation of rat liver free polysomes, and recombinant plasmids were prepared from the enriched mRNA by a vector–primer method. The cDNA clones for ornithine carbamoyltransferase were identified by hybrid-arrested translation and hybrid-selected translation. One of the clones, designated pOTC-1, contained a 1.6-kilobase insert and hybridized to a mRNA of ≈1.8 kilobases in rat liver. The cDNA clone was subjected to nucleotide sequence analysis. The deduced amino acid sequence indicates that the ornithine carbamoyltransferase precursor consists of the mature enzyme of 322 amino acid residues and an NH_2-terminal peptide extension (presequence) of 32 amino acid residues. The presequence contains 8 basic amino acid residues, no acidic residues, and no hydrophobic amino acid stretch. The amino acid sequence of the rat ornithine carbamoyltransferase was compared with the recently reported sequence of the human enzyme [Horwich, A. L., Fenton, W. A., Williams, K. R., Kalousek, F., Kraus, J. P., Doolittle, R. F., Konigsberg, W. & Rosenberg, L. E. (1984) *Science* 224, 1068–1074]. The sequences of the mature enzyme portion are 93% identical, whereas those of the presequences are 69% identical. There are two highly conserved segments in the presequences of the rat and human enzymes. One of the two conserved segments is significantly similar to a segment of the presequence of yeast mitochondrial elongation factor EF-Tu. These results suggest that the homologous segments are important for the proteins that are synthesized in the cytosol to be transported into the mitochondrial matrix.

Ornithine carbamoyltransferase (EC 2.1.3.3) is a mitochondrial matrix enzyme found in the liver of ureotelic animals, where it catalyzes the second step of urea synthesis. The enzyme has been purified to homogeneity from bovine (1), rat (2, 3), and human livers (4, 5). Current interest in this enzyme protein arises from several distinguishing characteristics. First, ornithine carbamoyltransferase is a good model for analysis of biosynthesis of mitochondrial proteins and their transport into the organelle in higher eukaryotes (6). The enzyme is initially synthesized as a larger precursor in the cytosol and is then transported into the mitochondrial matrix, where it is processed to the mature enzyme concomitantly with the transport (7–9). Experiments with the *in vitro* reconstituted system, in which the ornithine carbamoyltransferase precursor synthesized *in vitro* can be taken up and processed to the mature enzyme by isolated mitochondria, have revealed some of the mechanisms of the transport and processing of the precursor molecule (10–14). Second, deficiency of the carbamoyltransferase activity in humans is associated with an X-chromosome-linked form of hyperammonemia (15). Third, expression of this enzyme is tissue and species specific; the enzyme is expressed almost exclusively in hepatocytes of ureotelic animals. Molecular cloning of cDNA sequence for the enzyme should facilitate further studies on biosynthesis, intracellular transport, and processing of the enzyme and on its deficiency. Horwich *et al.* most recently described the isolation of cloned cDNAs encoding a portion of the rat enzyme (16) and nearly full-length cDNAs for the human enzyme (17), and they determined the nucleotide sequence of the human enzyme cDNA (17). We now report the complete amino acid sequence of the rat ornithine carbamoyltransferase precursor predicted from the nucleotide sequence of a cloned cDNA. Sequence similarities between the presequence of this rat enzyme and those of the human enzyme and another mitochondrial matrix protein are presented.

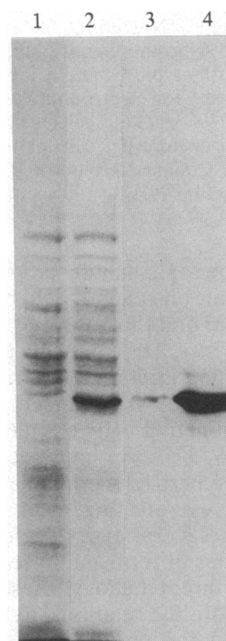

FIG. 1. Enrichment of ornithine carbamoyltransferase mRNA by polysome immunoprecipitation. Total RNA was extracted from free polysomes (lanes 1 and 3) or polysomes immunoprecipitated with anti-ornithine carbamoyltransferase (lanes 2 and 4) by the guanidium thiocyanate/cesium chloride procedure (36), and poly(A)$^+$ RNA was prepared by oligo(dT)-cellulose chromatography (37). Poly(A)$^+$ RNA (1 μg) was translated in a reticulocyte lysate cell-free system (50 μl), and total products (lanes 1 and 2) and immunoprecipitates with anti-ornithine carbamoyltransferase (lanes 3 and 4) were subjected to $NaDodSO_4$/10% polyacrylamide gel electrophoresis and fluorography.

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MATERIALS AND METHODS

Enrichment of Ornithine Carbamoyltransferase mRNA by Polysome Immunoprecipitation. Details of preparation of rat liver free polysomes and polysome immunoprecipitation will be described elsewhere. Briefly, free polysomes were first separated from membrane-bound polysomes by differential centrifugation (18) and then collected by magnesium precipitation (19). Polysomes synthesizing ornithine carbamoyltransferase were precipitated with anti-ornithine carbamoyltransferase IgG and formalin-fixed *Staphylococcus aureus* cells (20). Polysomes remaining in the supernatant of the immunoprecipitation were used for preparation of ornithine carbamoyltransferase mRNA-depleted poly(A)$^+$ RNA.

Construction of Recombinant Plasmids and Transformation. Plasmid cDNA recombinants were constructed essentially according to Okayama and Berg (21), with a modified vector–primer and linker. The vector–primer (pRH401) and linker (pRH20) derived from the pBR322–*Escherichia coli* ρ gene hybrid plasmid (K. Takeuchi, K. Shigesada, and M. Hatanaka, personal communication) were provided by K. Shigesada (Kyoto University, Japan). The plasmid cDNA was prepared using ≈2 μg of immunopurified poly(A)$^+$ RNA and 0.7 pmol (1.6 μg) of the vector–primer DNA. *E. coli* strain DH1 (22) was transformed and selected for ampicillin resistance.

Screening and Identification of Ornithine Carbamoyltransferase cDNA Clones. Transformants were initially screened by differential (23) colony hybridization (24, 25). Duplicate filters were hybridized with ^{32}P-labeled cDNA synthesized from the immunopurified poly(A)$^+$ RNA or the depleted poly(A)$^+$ RNA. Plasmids were isolated from candidate colonies by the alkaline lysis method and contaminating RNA was removed by LiCl precipitation and ribonuclease treatment (26). Ornithine carbamoyltransferase cDNAs were identified by hybrid-arrested translation (27) and hybrid-selected translation (28).

Other Methods. *In vitro* translation in a reticulocyte lysate system (29), immunoprecipitation using fixed *S. aureus* cells (29), $NaDodSO_4$/polyacrylamide gel electrophoresis (30), and fluorography (31) were performed as described. RNA blot analysis was performed as described by Thomas (32). Nick-translation was performed according to the instructions by Amersham. DNA sequencing was carried out by the chemical degradation procedure of Maxam and Gilbert (33) and the dideoxy-termination method of Sanger *et al.* (34) after subcloning of restriction fragments into M13mp9 phage vector (35).

Construction and handling of recombinant plasmids were carried out in accordance with the guidelines for research involving recombinant DNA molecules issued by the Ministry of Education, Science and Culture of Japan.

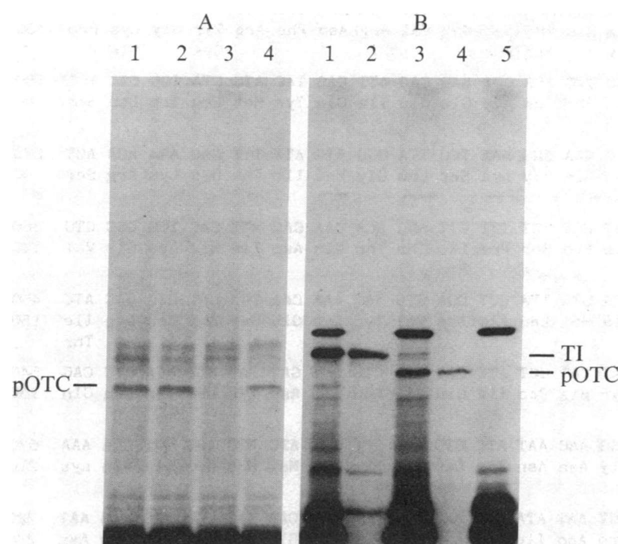

FIG. 2. Identification of a cDNA clone for ornithine carbamoyltransferase by hybrid-arrested translation (*A*) and hybrid-selected translation (*B*). (*A*) *Pvu* II-digested plasmid pOTC-1 (lanes 3 and 4) and a control plasmid (lanes 1 and 2) derived from 3-ml cultures were hybridized with 0.2 μg of poly(A)$^+$ RNA enriched in ornithine carbamoyltransferase mRNA. After hybridization, the mixtures were divided into two equal portions, one of which was retained in hybrid form (lanes 1 and 3), and the other was heated for 60 sec at 100°C and quick-chilled in dry ice/methanol (lanes 2 and 4). RNAs recovered by ethanol precipitation were translated in a reticulocyte lysate system. The cell-free synthesized products were subjected to $NaDodSO_4$/10% polyacrylamide gel electrophoresis and fluorography. pOTC, ornithine carbamoyltransferase precursor. (*B*) Ten micrograms of plasmid DNAs of pOTC-1 (lanes 3 and 4) and pT1-19 (a cDNA clone of rat 3-ketoacyl-CoA thiolase, lanes 1 and 2) immobilized on nitrocellulose filters (3 × 6 mm) were hybridized with free polysomal poly(A)$^+$ RNA (2 μg in 40 μl). The bound mRNAs were eluted and used to program cell-free protein synthesis in a reticulocyte lysate system. Total translation products (lanes 1 and 3) and immunoprecipitates (lanes 2 and 4) were subjected to NaDod-SO_4/polyacrylamide gel electrophoresis and fluorography. Lane 5, total translation products without exogenous mRNA. T1, 3-ketoacyl-CoA thiolase.

RESULTS AND DISCUSSION

Enrichment of Ornithine Carbamoyltransferase mRNA by Polysome Immunoprecipitation. Polysomes synthesizing ornithine carbamoyltransferase were immunoprecipitated from rat liver free polysomes with anti-ornithine carbamoyltransferase and *S. aureus* cells. Aliquots of the immunoselected

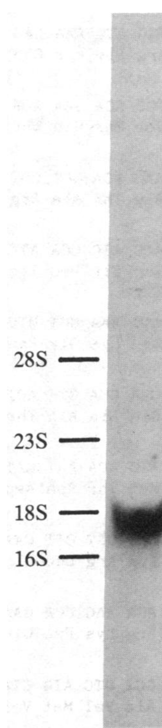

FIG. 3. RNA blot analysis of ornithine carbamoyltransferase mRNA. Four micrograms of hepatic poly(A)$^+$ RNA from a rat fed a 60% casein diet were denatured with glyoxal and separated by electrophoresis in a 1.2% agarose gel. After electrophoresis, the RNA was transferred to a nitrocellulose filter and hybridized with ^{32}P-labeled cDNA probe. Nick-translated insert (4 × 10^8 cpm/μg of DNA) excised from pOTC-1 with *Bam*HI and *Sac* II was used as the hybridization probe. RNA size markers used were rat 28S (5.5 kilobases) and 18S (2.1 kilobases) ribosomal RNA and *E. coli* 23S (3.1 kilobases) and 16S (1.5 kilobases) ribosomal RNA.

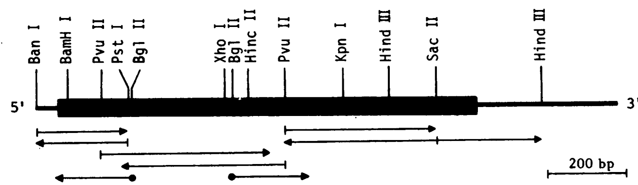

FIG. 4. Restriction map of rat ornithine carbamoyltransferase cDNA (pOTC-1) and the strategy for nucleotide sequence determination. The poly(dA)·poly(dT) and poly(dG)·poly(dC) tails are not shown. Protein coding region is indicated by the thick line. Arrows represent the direction and the length of sequence determined for each independent experiment. Vertical bars at ends of arrows indicate sequences determined by the dideoxy terminator method; solid circles at ends of arrows indicate sequences determined by the chemical degradation procedure. bp, base pairs.

poly(A)$^+$ RNA and free polysomal poly(A)$^+$ RNA were translated in a reticulocyte lysate system, and the total translation products were subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis (Fig. 1). No polypeptide that corresponded to the ornithine carbamoyltransferase precursor was evident among the total translation products programmed with the free polysomal poly(A)$^+$ RNA, whereas a major polypeptide that comigrated with the enzyme precursor was observed among the total products programmed with the immunoselected poly(A)$^+$ RNA. This polypeptide was specifically immunoprecipitated with anti-ornithine carbamoyltransferase. Radioactivity in the carbamoyltransferase precursor accounted for 3.5% of the radioactivity incorporated into the total protein. On the other hand, the enzyme precursor accounted for 0.07% of the radioactivity in the translation products obtained from the free polysomal poly(A)$^+$ RNA. Therefore, polysome immunoprecipitation resulted in a 50-fold purification of ornithine carbamoyltransferase mRNA.

Construction and Identification of Recombinant Plasmids Containing cDNA Sequences Complementary to Ornithine Carbamoyltransferase mRNA. The method of Okayama and Berg (21) was used for cDNA synthesis and cloning because it provides a highly efficient means for obtaining full-length cDNAs. Approximately 6500 transformants were screened for cDNA sequences for ornithine carbamoyltransferase by differential colony hybridization with ^{32}P-labeled cDNAs synthesized from the immunopurified and depleted mRNAs, and 22 positive clones were selected. Among the 22 clones, 11 clones having cDNA inserts >700 base pairs were then analyzed by hybrid-arrested translation. Among the 11 plasmid DNA samples tested, 3 were found to inhibit selectively the synthesis of the carbamoyltransferase precursor (Fig. 2*A*). Furthermore, heat melting of the hybrid samples result-

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-58                 GTGCCTGCCTGCGGAACTCTCTAGACCATAGATTCCTCCTCCACTCTAGCAAGAGAAG  -1

1 ATG CTG TCT AAT TTG AGG ATC CTG CTC AAC AAG GCA GCT CTT AGA AAG GCT CAC ACT TCC ATG GTT CGA AAT TTT CGG TAT GGG AAG CCA   90
1 Met Leu Ser Asn Leu Arg Ile Leu Leu Asn Lys Ala Ala Leu Arg Lys Ala His Thr Ser Met Val Arg Asn Phe Arg Tyr Gly Lys Pro   30
          Phe                             Asn         Phe     Asn Gly     Asn Phe                         Cys     Gln

  GTC CAG AGT CAA GTA CAG CTG AAA GGC CGT GAC CTC CTC ACC CTG AAG AAC TTC ACA GGA GAG GAG ATT CAG TAC ATG CTA TGG CTC TCT  180
  Val Gln Ser Gln Val Gln Leu Lys Gly Arg Asp Leu Leu Thr Leu Lys Asn Phe Thr Gly Glu Glu Ile Gln Tyr Met Leu Trp Leu Ser   60
  Leu    ↑Asn Lys

  GCA GAT CTG AAA TTC AGG ATC AAA CAG AAA GGA GAA TAC TTG CCT TTA TTG CAA GGG AAA TCC TTA GGG ATG ATT TTT GAG AAA AGA AGT  270
  Ala Asp Leu Lys Phe Arg Ile Lys Gln Lys Gly Glu Tyr Leu Pro Leu Leu Gln Gly Lys Ser Leu Gly Met Ile Phe Glu Lys Arg Ser   90

  ACT CGA ACA AGA CTG TCC ACA GAA ACA GGC TTC GCT CTT CTG GGA GGA CAT CCT TCT TTT CTT ACC ACA CAA GAC ATT CAC TTG GGC GTG  360
  Thr Arg Thr Arg Leu Ser Thr Glu Thr Gly Phe Ala Leu Leu Gly Gly His Pro Ser Phe Leu Thr Thr Gln Asp Ile His Leu Gly Val  120
                                                                          Cys     Pro

  AAT GAA AGT CTC ACA GAC ACA GCT CGT GTG TTA TCT AGC ATG ACA GAT GCA GTG TTA GCT CGA GTG TAT AAA CAA TCA GAT CTG GAC ATC  450
  Asn Glu Ser Leu Thr Asp Thr Ala Arg Val Leu Ser Ser Met Thr Asp Ala Val Leu Ala Arg Val Tyr Lys Gln Ser Asp Leu Asp Ile  150
                                                          Ala                                                         Thr

  CTG GCT AAG GAA GCA ACC ATC CCA ATT GTC AAC GGA CTG TCA GAC CTG TAT CAT CCT ATC CAG ATC CTG GCT GAT TAC CTT ACA CTC CAG  540
  Leu Ala Lys Glu Ala Thr Ile Pro Ile Val Asn Gly Leu Ser Asp Leu Tyr His Pro Ile Gln Ile Leu Ala Asp Tyr Leu Thr Leu Gln  180
                      Ser             Ile

  GAA CAC TAT GGC TCT CTC AAA GGT CTC ACC CTC AGC TGG ATA GGA GAT GGG AAC AAT ATC CTG CAC TCC ATC ATG ATG AGT GCT GCA AAA  630
  Glu His Tyr Gly Ser Leu Lys Gly Leu Thr Leu Ser Trp Ile Gly Asp Gly Asn Asn Ile Leu His Ser Ile Met Met Ser Ala Ala Lys  210
              Ser                                 Cys Phe

  TTC GGG ATG CAC CTT CAA GCA GCT ACT CCA AAG GGT TAT GAG CCA GAT CCT AAT ATA GTC AAG CTA GCA GAG CAG TAT GCC AAG GAG AAT  720
  Phe Gly Met His Leu Gln Ala Ala Thr Pro Lys Gly Tyr Glu Pro Asp Pro Asn Ile Val Lys Leu Ala Glu Gln Tyr Ala Lys Glu Asn  240
                                                                  Ala Ser Val Thr

  GGT ACC AGG TTG TCA ATG ACA AAT GAT CCA CTG GAA GCA GCA CGT GGA GGC AAT GTA TTA ATT ACA GAT ACT TGG ATA AGC ATG GGA CAA  810
  Gly Thr Arg Leu Ser Met Thr Asn Asp Pro Leu Glu Ala Ala Arg Gly Gly Asn Val Leu Ile Thr Asp Thr Trp Ile Ser Met Gly Gln  270
          Lys     Leu Leu                                 His                                                         Arg

  GAG GAT GAG AAG AAA AAG CGT CTT CAA GCT TTC CAA GGT TAC CAG GTT ACA ATG AAG ACT GCT AAA GTG GCT GCG TCT GAC TGG ACG TTT  900
  Glu Asp Glu Lys Lys Lys Arg Leu Gln Ala Phe Gln Gly Tyr Gln Val Thr Met Lys Thr Ala Lys Val Ala Ala Ser Asp Trp Thr Phe  300
      Glu

  TTA CAC TGC TTG CCT AGA AAG CCA GAA GAA GTA GAT GAT GAA GTG TTT TAT TCT CCG CGG TCA TTA GTG TTC CCA GAG GCA GAA AAT AGA  990
  Leu His Cys Leu Pro Arg Lys Pro Glu Glu Val Asp Asp Glu Val Phe Tyr Ser Pro Arg Ser Leu Val Phe Pro Glu Ala Glu Asn Arg  330

  AAG TGG ACA ATC ATG GCT GTC ATG GTA TCC CTG CTG ACA GAC TAC TCA CCT GTG CTC CAG AAG CCA AAG TTC TGATGCCTGTCAAGAGGACGAAA 1085
  Lys Trp Thr Ile Met Ala Val Met Val Ser Leu Leu Thr Asp Tyr Ser Pro Val Leu Gln Lys Pro Lys Phe                          354
                                                                      Gln
  AACCCAAAAGACAAAAAAATCTGTTCTTTAGCAGCAGAATAAGTCAGTTTATGTAGAAAAGAGAAGAATTGAAATTGTAAACACATCCCTAGTGCGTGATATAATTATGTAATTGCTTT 1204

  GCTATTGTGAGAATTGCTTAAAGCT                                                                                              1229
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FIG. 5. Nucleotide and corresponding amino acid sequence of plasmid pOTC-1 encoding rat ornithine carbamoyltransferase. Nucleotides are numbered in the 5′ to 3′ direction, beginning with the first residue of the ATG triplet encoding the initiator methionine, and the nucleotides on the 5′ side of residue 1 are indicated by negative numbers. Deduced amino acid sequence is indicated below the nucleotide triplets. Arrowhead indicates the cleavage site and mature polypeptide begins at Ser-33. Predicted amino acids of human ornithine carbamoyltransferase precursor (17) different from those of the rat enzyme precursor are shown below the rat sequence. The 3′ untranslated region sequenced here contains two additional nucleotides inserted into the sequence previously determined (16)—namely, T at position 1072 and A at position 1226. The reason for the discrepancy is not known.

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        ***      **  **          *   *  *** *   *    ** *** ** * *      **      *
        ATG CTG TCT AAT TTG AGG ATC CTG CTC AAC AAG GCA GCT CTT AGA AAG GCT CAC ACT TCC
OTC:    Met Leu Ser Asn Leu Arg Ile Leu Leu Asn Lys Ala Ala Leu Arg Lys Ala His Thr Ser
mEF-Tu: Met Ser Ala Leu Leu Pro Arg Leu Leu Thr Arg Thr Ala Phe Lys Ala Ser Gly Lys Leu
        ATG TCA GCT TTA TTA CCA AGA TTA CTC ACA AGA ACA GCT TTT AAA GCT TCT GGG AAA CTT
        ***      **  **          *   *  *** *   *    ** *** ** * *      **      *

         **     *     *  *         *     * *  *   *
        ATG GTT CGA AAT TTT CGG TAT GGG AAG CCA GTC CAG
        Met Val Arg Asn Phe Arg Tyr Gly Lys Pro Val Gln
        Leu Arg Leu Ser Ser Val Ile Ser Arg Thr Phe Ser ---
        CTG AGG CTC TCT TCA GTA ATT TCT AGG ACC TTT TCT ---
         **     *     *  *         *     * *  *   *
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FIG. 6. Comparison of presequence of rat ornithine carbamoyltransferase precursor (OTC) with that of yeast mitochondrial EF-Tu. Amino acid sequence of ornithine carbamoyltransferase precursor is from Fig. 5 and that of EF-Tu is from Nagata *et al.* (43). Identical amino acids are boxed by solid lines and basic amino acids at the same position are boxed by dashed lines. Identical nucleotides are marked by an asterisk.

ed in full recovery of the enzyme precursor-synthesizing activity. The plasmid with the longest insert (1600 base pairs long), termed pOTC-1, was further analyzed by hybrid-selected translation. The recombinant plasmid pOTC-1 selectively hybridized with a mRNA that directed the synthesis of a polypeptide with a mobility corresponding to the ornithine carbamoyltransferase precursor (Fig. 2*B*, lane 3). This polypeptide was specifically immunoprecipitated by anti-ornithine carbamoyltransferase (lane 4). On the other hand, recombinant plasmid pT1-19 containing a cDNA sequence of rat 3-ketoacyl-CoA thiolase (EC 2.3.1.16) (unpublished observations) selected a mRNA that directed the synthesis of the thiolase (lanes 1 and 2). These results demonstrate that intact ornithine carbamoyltransferase mRNA was specifically selected by pOTC-1.

Size of Ornithine Carbamoyltransferase mRNA. Rat liver poly(A)$^+$ RNA was subjected to agarose gel electrophoresis. The resolved RNAs were transferred to a nitrocellulose filter and hybridized with ^{32}P-labeled cDNA insert excised from the plasmid pOTC-1. The probe hybridized with a major RNA species of $\approx$1.8 kilobases (Fig. 3).

Nucleotide Sequence and Predicted Amino Acid Sequence. Fig. 4 shows the restriction map and the sequence analysis strategy. The sequence was determined on both strands of the cDNA, crossing restriction fragment junctures, except for the region around the COOH terminus, which has already been sequenced by Horwich *et al.* (16). The determined nucleotide sequence and the predicted amino acid sequence are shown in Fig. 5. The translation initiation site was assigned to the methionine codon ATG at nucleotide positions 1–3, because this was the first ATG triplet downstream of the in-frame nonsense codon TAG at positions -12 to -10. The predicted amino acid sequence of serine-33 to methionine-56 is essentially identical to the sequence of the NH_2-terminal region of the mature enzyme, which was determined by sequence analysis of the purified enzyme by Lusty *et al.* (2). The predicted COOH-terminal portion is identical to the COOH-terminal 52 amino acid sequence already confirmed by Horwich *et al.* (16). The COOH-terminal sequence Lys-Phe has been determined for the bovine enzyme (38). The amino acid composition deduced from the nucleotide sequence agrees with the composition determined by amino acid analysis of purified rat liver ornithine carbamoyltransferase (2). The calculated molecular weight is 36,135; this value is in good agreement with the previously estimated values (35,300–39,600) (2, 3, 7, 8). Thus, it is clear that serine-33 to phenylalanine-354 corresponds to mature ornithine carbamoyltransferase. When amino acid and nucleotide sequences of the rat mature enzyme were compared with the human enzyme sequences (17), there was 93% identity in the amino acid sequences (Fig. 5) and 89% identity in the nucleotide sequences.

The present results show that the ornithine carbamoyltransferase precursor contains the NH_2-terminal presequence of 32 amino acids. The possibility that the methionine codon ATG at nucleotide positions 61–63 is the translation initiation site can be excluded, because the NH_2-terminal portion of the presequence was determined to be Met-Leu by Edman degradation of the labeled enzyme precursor synthesized *in vitro* (unpublished results). The calculated molecular weight of the presequence is 3724, this value being close to values estimated by our group (3400) (8) and by Conboy *et al.* (4000) (7). Our postulation that the presequence is basic and not hydrophobic (39) was fully confirmed in the present study. The presequence contains 8 basic amino acid residues and no acidic residues. The presequence has no stretch of hydrophobic amino acids, in sharp contrast to "signal" peptides of secretory proteins (40). The structures of presequences of human ornithine carbamoyltransferase (17) and three other mitochondrial protein precursors have been reported. The latter include ATP synthase proteolipid subunit of *Neurospora* (inner membrane protein) (41), yeast cytochrome *c* peroxidase (intermembrane space protein) (42), and yeast mitochondrial elongation factor EF-Tu (matrix protein) (43). The presequences of the two ornithine carbamoyltransferases and those of the three other proteins share the common features described above, except that the peptide extension of cytochrome *c* peroxidase contains a long apolar segment that is thought to span the inner membrane (42). Comparison of the rat and human presequences shows that there is 69% identity in the amino acid sequences (Fig. 5) and 81% identity in the nucleotide sequences. This indicates that the presequence is much less conserved than is the mature portion of the enzyme. However, there are two highly conserved segments in the presequences. One is the NH_2-terminal segment of 13 amino acids (11 amino acids among the 13 are identical), and the other is the residues methionine-21 to arginine-26. To be noted is that the former segment of the rat presequence has a distinct similarity with the NH_2-terminal portion of yeast mitochondrial EF-Tu presequence, despite the species difference (Fig. 6). These results provide additional support to the concept that the presequences are important for the mitochondrial matrix proteins synthesized in the cytosol to be transported to the proper destination. This proposal must be tested by further studies on the structures of other matrix protein presequences and by experiments including applications of recombinant DNA technology.

We thank Drs. H. Nawa and S. Nakanishi (Kyoto University) for help in cDNA cloning, Dr. H. Hayashi (Gumma University) for determination of the NH_2-terminal sequence of ornithine carbamoyltransferase precursor, Dr. K. Shigesada (Kyoto University) for valuable discussions, and M. Ohara (Kyushu University) for comments on the manuscript. This work was supported in part by Grants-in-Aid 59870009 and 59480441 from the Ministry of Education, Science and Culture of Japan.

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