Mouse ornithine decarboxylase gene: Cloning, structure, and expression

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ABSTRACT We used molecular cloning to isolate a functional gene for mouse ornithine decarboxylase (OrnDCase; L-ornithine carboxy-lyase, EC 4.1.1.17) from a cell line in which that gene had been selectively amplified. The position of the 5' terminus of the mRNA was identified, and the coding sequence was shown to be preceded by a 312- or 313-nucleotide (nt) untranslated leader. The latter is highly G+C rich, particularly in its 5'-most portion. The leader can be anticipated to have extensive and stable secondary structure. The transcription unit of the gene is of relatively small size, ≈6.2 kilobases (kb) from the start site to the proximal site of polyadenylylation. Sequence analysis of DNA near the transcription start position demonstrated the presence of a "TATA" box, but no "CAAT" box. Functional properties of the cloned gene were tested by transfecting it into cultured cells. Expression of the putative full-length gene efficiently conferred ornithine decarboxylase activity on recipient mutant cells deficient in that activity. To assess the function and strength of the OrnDCase promoter region and to delimit its boundaries, we used a transient expression assay. Upstream of a bacterial chloramphenicol acetyltransferase gene was placed a portion of the OrnDCase gene, including the presumed promoter region, spanning a region from ≈3.0 kb 5' of the site of transcription initiation to the first 250 nt of the transcript. When expressed in mouse NIH 3T3 cells, this OrnDCase genomic element was comparable in strength to the Rous sarcoma virus long terminal repeat promoter. A similar construct, truncated so as to retain only 264 base pairs of the OrnDCase gene 5' to the site of transcription start, yielded undiminished levels of expression.

Ornithine decarboxylase (OrnDCase; L-ornithine carboxy-lyase, EC 4.1.1.17) is the initial enzyme in the pathway committed to synthesis of polyamines (1). Because OrnDCase expression is not confined to specific tissues, this enzyme can appropriately be described as encoded by a housekeeping gene. However, unlike most enzymes of that class, the activity of OrnDCase is highly subject to regulation. Intracellular activity can undergo changes of several 100-fold within hours of application of appropriate stimuli (2). One of the best-established determinants of OrnDCase activity is cell growth. Proliferating cells generally have much higher activity than do corresponding nonproliferating cells. In addition, alterations of OrnDCase activity in response to diverse hormones and tumor promoters have been extensively documented.

At least three classes of regulatory phenomena apparently underlie changes of OrnDCase activity: these include changes in the amount of OrnDCase mRNA (3–7), in the efficiency of translation of the mRNA (8–11), and in the intracellular stability of the enzyme itself (11–13). Polyamines negatively regulate the efficiency of translation of OrnDCase mRNA, thereby mediating a form of end-product

inhibition of the initial step of the metabolic pathway leading to their synthesis. This form of regulation has been observed in intact cells (8–11) and in an *in vitro* translation system (14), but its mechanism is unknown. Because the 5' untranslated leader of the OrnDCase mRNA is widely presumed to be involved in this process (15), determination of its structure is of interest.

Cloning and sequencing of mouse (16, 17) and human (18) OrnDCase cDNAs and of yeast (19) and trypanosome (20) OrnDCase genes have been described. The present work constitutes a report of the molecular cloning and characterization of a gene that encodes a mammalian OrnDCase.§

MATERIALS AND METHODS

Genomic Cloning. DNA was purified from D4.1 cells (21), digested with *Eco*RI and *Bam*HI, and fractionated according to size on a sucrose gradient. The fraction enriched in DNA ≈6 kilobases (kb) in length was ligated into the *Eco*RI and *Bam*HI sites of the pUC8 plasmid. *Escherichia coli* strain HB101 transformants were screened using the 707-nucleotide (nt) *Pst* I fragment of mouse OrnDCase cDNA (3) as a hybridization probe and a genomic clone designated pOD1 thus isolated. A similar procedure was used to clone the 5.3-kb *Sst* I fragment of the OrnDCase gene into the *Sst* I site of the pUC18 plasmid, except the 534-nt *Pst* I fragment of the same cDNA clone was used as probe. A plasmid designated pOD100 was thereby obtained. The full-length gene was reassembled from the two inserts by using a *Sal* I site in their region of overlap (Fig. 1).

RNA. RNA was purified from cultured cells as described (22). RNA from the kidneys of C57/Bl6 mice was a gift from G. Watson, University of California, Berkeley.

DNA Sequencing. The methods of chemical cleavage (23) and dideoxy chain termination (24, 25) were used. In addition, the Sequenase sequencing kit (United States Biochemical, Cleveland) was used as directed by the manufacturer.

S1 Mapping. S1 nuclease mapping of RNA·DNA hybrids was done as in ref. 26, except the hybridization temperature was raised from 52°C to 67.5°C. The S1 nuclease digestion was done at 37°C with 1000 units per ml of enzyme for 45 min.

Primer Extension. A synthetic oligonucleotide primer complementary to nt −165 to −146 (the A of the AUG translational initiation codon is designated number 1) was 5' end-labeled as described (23). Hybridization of oligomer (50,000 cpm) to 2 μg of total RNA was done at 45°C for 3 hr

in 5 μl of hybridization buffer (200 mM Tris·HCl, pH 8.5/40 mM MgCl₂/400 mM KCl). After hybridization the buffer was changed to 20 μl of 50 mM Tris·HCl, pH 8.5/10 mM MgCl₂/100 mM KCl/RNasin (1.0 units/μl), 10 mM dithiothreitol containing 0.5 mM each of dNTPs and AMV reverse transcriptase (1.0 units/ml). The extension reaction was done at 42°C for 2 hr.

Cells. NIH 3T3 cells were from M. Verderame (University of California, San Francisco), wild-type Chinese hamster ovary (CHO) cell line 10001 from M. M. Gottesman (National Cancer Institute). C. Steglich (University of South Carolina) provided the ODC⁻ cell line C55.7. This line, derived from a CHO cell line, is auxotrophic for polyamines and will grow rapidly in the same medium as 10001 cells if 0.5 mM putrescine is included (27). D4.1 cells, a derivative of the mouse S49 lymphoma cell line, overproduce OrnDCase and have an amplified OrnDCase gene (3).

Transfections. Cells were transfected by calcium phosphate coprecipitation followed 4 hr later by a 20% (vol/vol) glycerol shock for 2 min (28). For stable transformations, C55.7 cells were cotransfected with 20 μg of pOD12.7 DNA and 1 μg of pSV2neo DNA (29). Ten days later colonies surviving selection in medium containing G418 (450 μg/ml) and 0.5 mM putrescine were analyzed for OrnDCase expression. For transient transfections, NIH 3T3 and CHO 10001 cells were cotransfected with 5 μg of one of several plasmids containing the chloramphenicol acetyltransferase (CAT) gene together with 5 μg of the pRSV-β-galactosidase plasmid DNA (30). Cells were harvested 2 days later, suspended in 25 mM Tris, pH 7.8, at 10⁷ cells per ml and subjected to three freeze–thaw cycles. Microfuged supernatants were assayed for CAT and β-galactosidase activities.

CAT and β-Galactosidase Assays. CAT and β-galactosidase assays were done as in refs. 30 and 31.

Materials. Plasmids pTEI delta S/N, pRSV-CAT, pSV2-CAT, and pRSV-β-gal were gifts from M. D. Walker (University of California, San Francisco). L-[¹⁴C]ornithine and [¹⁴C]chloramphenicol were from Amersham.

RESULTS

Cloning a Functional OrnDCase Gene. We (3, 16) and others (4, 5) have analyzed mouse genomic DNA on Southern blots and concluded that OrnDCase cDNA probes hybridize to multiple loci. We have shown that in the OrnDCase-overproducing mutant mouse cell line D4.1 (32), a derivative of the S49 cell line, one of these genes has become amplified (3). Southern blot analysis indicated that two amplified and overlapping restriction fragments, a 5.7-kb BamHI–EcoRI fragment and a 5.3-kb Sst I fragment, together spanned about 10 kb of contiguous DNA. This 10 kb included all of the cDNA coding sequences and several kilobases upstream of the cDNA 5' end. Each of those two fragments were cloned into plasmid vectors as described to yield pOD1 and pOD100, respectively (Fig. 1). They were subsequently joined at a common Sal I site present in the region of overlap to generate the complete genomic clone pOD12.7. The relationship between the restriction maps of the genomic and cDNA clones was determined by probing Southern blots with radiolabeled cDNA fragments and is diagramed in Fig. 1. From this comparison it is clear that there is a minimum of four introns.

To establish that a functional OrnDCase gene had been isolated, pOD12.7 DNA was used to transform OrnDCase-deficient (and putrescine-dependent) C55.7 CHO cells (27). The cells were cotransfected with the OrnDCase genomic clone and the plasmid pSV2neo (29), expression of which confers resistance to the antibiotic G418. Cultures were subjected to selection in medium containing G418 and putrescine. Among 30 colonies isolated, 10 were OrnDCase⁺ in
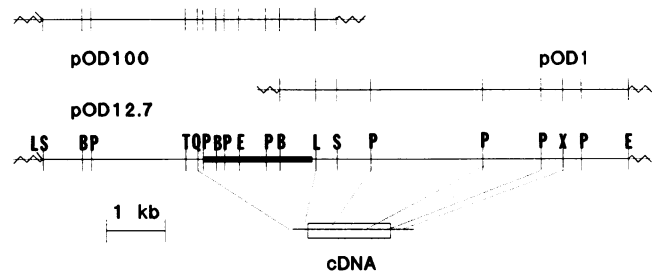

FIG. 1. Physical map of the cloned OrnDCase sequences. The longest horizontal line represents the recombinant plasmid pOD12.7 encompassing the entire OrnDCase gene. This clone was constructed by splicing together two overlapping genomic fragments, contained in plasmids pOD1 and pOD100, at the common Sal I (L) site. The zigzag lines represent vector sequences. The Sal I sites at the insert–vector junctions are pUC polylinker sites used in splicing. Restriction enzyme cleavage sites are indicated as follows: B, BamHI; E, EcoRI; L, Sal I; P, Pst I; S, Sst I; T, Stu I. The transcription start and the first polyadenylylation signal are indicated as Q and X, respectively. Sites common to the cDNA (5) are aligned by dashed lines. The first intron is indicated by a solid box. The coding region of the cDNA is indicated by an open box.

phenotype, as indicated by their ability to proliferate without putrescine. Only those clones able to grow in the absence of putrescine had detectable OrnDCase activity. We have mock-transfected C55.7 CHO cells and have never observed reversion to putrescine independence. Finally, we used S1 analysis to demonstrate that RNA with the authentic 5' terminus of mouse OrnDCase mRNA was present in the transformed cells but was not present in untransformed OrnDCase⁻ CHO cells (Fig. 2), nor in wild-type CHO cells (data not shown).

Mapping the OrnDCase mRNA Transcription Start Site. To map the site of transcription initiation, we used the method of S1 protection. Total RNA extracted from OrnDCase-overproducing D4.1 S49 cells (32), parental wild-type S49 cells, and kidneys of mice, either untreated or treated with androgen to induce OrnDCase mRNA (3–5), were used to protect a 5' end-labeled probe complementary to nt −146 to −446 of the OrnDCase genomic DNA. (Nucleotide positions are enumerated relative to the adenine of the AUG translation initiation codon.) Regardless of which RNA preparation was used to protect the probe, the S1 digestion products were a doublet of about 167 and 168 nt (Fig. 3A), implying
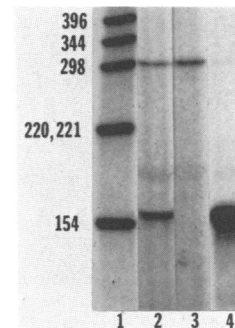

FIG. 2. S1 nuclease analysis of RNA from OrnDCase⁻ hamster cells that have been transformed to the OrnDCase⁺ phenotype by transfection with the murine genomic OrnDCase clone. The 301-nt genomic Sma I–Nco I restriction fragment (nt −446 to −146) was 5' end-labeled at the nucleotide complementary to nt −146 (Fig. 4). This probe was hybridized to the RNAs listed below, S1 digestion was done, and the products were electrophoresed on a sequencing gel. Lanes: 1, End-labeled HinfI restriction fragments of pBR322 as Mᵣ markers; 2, 100 μg of total RNA from pOD12.7-transformed C55.7 cells; 3, 100 μg of total RNA from untransformed C55.7 cells; 4, 15 μg of total RNA from D4.1 S49 cells.

that OrnDCase mRNA has a 5' untranslated leader 145 nt longer, that is, 312–313 nt. The quantity of probe protected from S1 nuclease digestion, seen in Fig. 3A as autoradiographic signal intensity, varies several 100-fold among the various RNA sources. The extent of protection is directly proportional to the amount of OrnDCase mRNA in each RNA source, as assayed previously by RNA blot analysis (3).

To confirm these results by an independent method, the same four RNAs were subjected to primer extension using a synthetic oligonucleotide complementary to the OrnDCase mRNA leader sequence. An oligonucleotide identical to the 5'-most 20 nt of the DNA fragment used as probe in the S1 experiments was synthesized for use as a primer. The largest primer-extension product was a 167- or 168-nt doublet and was produced in quantities proportional to the amount of OrnDCase mRNA present in the reaction (Fig. 3B). There were several shorter extension products, some OrnDCase mRNA concentration dependent and others concentration independent. These are probably incomplete or spurious OrnDCase cDNA transcripts, respectively. No longer primer-extension products were evident; we therefore conclude that the 5' end of the OrnDCase mRNA is uniquely located 167 or 168 nt 5' to the labeled nucleotide used in the S1 mapping and primer-extension reactions. Because the S1 probe and oligonucleotide primer had identical 5' ends, the extension product and S1-protected fragment can be directly

compared on a single gel (Fig. 3C). The doublet fragments produced by each method were identical in mobility and were 167 or 168 nt in size. Hence, the OrnDCase mRNA has a 5' untranslated leader 312 or 313 nt in size.

Nucleotide Sequence of Promoter Region. We sequenced a portion of the cloned OrnDCase gene near the start of transcription to determine the structure of the presumed promoter region and to confirm and extend the sequence of the 5' untranslated leader previously determined from cDNA. Results (Fig. 4) show that the transcription start site is 31 or 32 nt 3' to a TATA box. The DNA sequence of the 5' end of the genomic clone is identical to the cDNA untranslated region from the *Sal* I site 5' to the end of the cDNA, except for the presence of two intervening sequences in the genomic DNA, the first ≈2 kb in size, as diagramed in Fig. 4. Several minor errors in our previously reported cDNA leader sequence have been corrected (see legend for Fig. 4). The 313-nt OrnDCase mRNA untranslated 5' leader sequence is unusual in that the first 199 nt, encoded by the first exon, are 78% G + C bases.

Relative Strength of the OrnDCase Promoter. We compared the OrnDCase genomic expression elements to several viral promoters by examining the ability of each to drive the CAT gene in a transient expression assay. The 5.0-kb region of the OrnDCase gene between the 5'-most *Sst* I site and the *Sal* I restriction site (Fig. 1) includes the entire OrnDCase
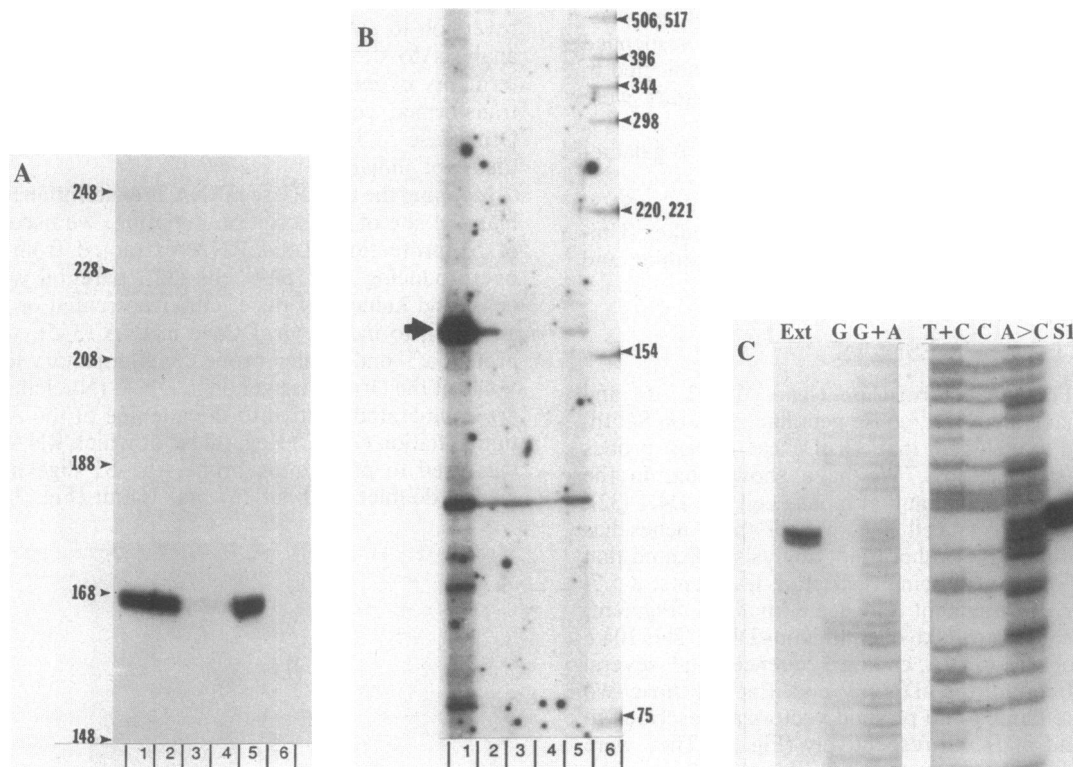

FIG. 3. Analysis of the site of initiation of transcription. (A) S1 nuclease analysis. The 301-bp genomic *Sma* I–*Nco* I restriction fragment (nt −446 to −146), was 5' end-labeled at the nucleotide complementary to nt −146 (Fig. 4). S1 analysis was done as described in Fig. 2. Lanes: 1, 0.5 μg of total RNA from D4.1 S49 cells; 2, 10 μg of total RNA from the kidneys of androgen-induced mice; 3, 10 μg of total RNA from the kidneys of untreated mice; 4, 10 μg of total RNA from wild-type S49 cell; 5, 100 μg of total RNA from wild-type S49 cells; 6, 10 μg of yeast tRNA. Numbers at left represent the size in nucleotides of end-labeled-protected fragments; these were determined by coelectrophoresis of the base-specific chemical cleavage products (24) of the same end-labeled DNA used as S1 protection probe. (B) Primer-extension analysis. A synthetic oligonucleotide complementary to nucleotide positions −146 to −165 (Fig. 4) was 5' end-labeled and hybridized to the RNAs listed below as described. Primer extension was done, and the products were electrophoresed on a sequencing gel. Lanes: 1, 2 μg of total RNA from D4.1 S49 cells; 2, 2 μg of total RNA from androgen-induced mouse kidney; 3, 2 μg of total RNA from untreated mouse kidney; 4, 2 μg of total RNA from wild-type S49 cells; 5, 20 μg of total RNA from wild-type S49 cells; 6, end-labeled *Hin*fI restriction fragments of pBR322 used as size standards. (C) Products of both primer-extension analysis and S1 nuclease analysis of D4.1 RNA (i.e., A and B above) were analyzed by coelectrophoresis with the base-specific chemical cleavage products of the same end-labeled DNA used as S1 protection probe. Because chemical cleavage removes the modified base, size of the S1 and primer-extension products has been augmented by one nucleotide with respect to the sequencing-ladder-size standard. Lanes: Ext = primer extension product; G, G + A, C + T, C, A>C = sequencing ladder; S1 = S1 nuclease digestion product.

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                AGGTACA TGTGCGCATG CACCAGCCGA CTCCCGCCCG
-640
CTGCCCATAG GGCCCTGCGG CATGCTGGCA GCCAGGACTG GTGGTGTGGT

GCGCGTGCGC AGGCCTGCCG CAGGGCGTGT CCGACACGAG GCCCGGCGGG
-540
GAGCGCGGGG CGTATGGGCG GGTGGGTGGG CACGCGCTGC GCCCCGCCCC

ACTGACGCGC CCGGCCCGCG TCCCCGCCTC CGGCGCCGGG ACCCGGGTTG
-440
GCCGCCACGG AGTCCCCGCC CCTCCCCCGC GCCTCCCGCC CGGAACCGAT

CGCGGCTGGT TTGAGCTGGT GCGTCTCCAT GACGACGTGC TCGGCG TATA
-340
AGTAGCGGCG CGTCGCACCG TCGGGCTTTG TCAGTCCCTG CAGCCGCCAC

CGCCGGCCGC CCTCAGCCAG CAGCTCGGCG CCACCTCCGG TCGGCATCTG
-240
CGGCGGGCTC GACGAGGCGG CTGACGGGGC GGCGGCGGCG GGCGGACGGA

CGGACGGACG GGCGCTCCTC GGGGTTTGGC GGCGGCGCCT CCATGGGTCA
-140
GGCCAGCCGG GCCACCGTGC TGTGAG...
```

INTRON 1 (-2KB)
[GTGAGGCGGG ACGCGGGAGG ..-2KB.. CTTGTGTTTC TATTCACTAG]

...TGTT TCCACCACTC CAAGAAGGCA

GCATTCAGAG TTCTTGGCTA AGTCGACCTT GTGAGGAGCT GGTGATAATT
-40
TGATTCCATC TCCAGGTTCC CTGT...

INTRON 2 (89 nt)
[GTAAGTATCG GTTTACTTGT .... TGCAGCTTCC TCTGTTTCAG]
                                             ***
                        ...AAGCAC ATCGAGAACC ATG

FIG. 4. DNA sequence in the region of the mouse OrnDCase gene promoter. The sequence depicted has the same polarity as the mRNA. Nucleotides are numbered with respect to the initial nucleotide of the translation initiation codon. The position and initial and final nucleotides of the first two introns are shown. TATA box is boxed, transcription start site is marked by an arrow, SP1 transcription factor consensus sequence binding site is underlined, and translation initiation codon is marked by asterisks. Comparison of genomic DNA sequence with our previously published cDNA sequence (16) indicated several discrepancies in the G + C-rich leader. The cDNA sequence should be corrected by replacement of a guanine for adenine at position −132, of a cytosine for guanine at position −176, and by insertion of guanines at positions −140, −160, and −163.

promoter, the first intron, and a major portion of the mRNA leader sequence. This fragment was cloned into the Sal I site of plasmid pTE1 delta S/N (30) in place of the herpes simplex virus thymidine kinase (TK) promoter. NIH 3T3 and CHO cells were transformed with CAT-bearing plasmids driven by either OrnDCase, Rous sarcoma virus (RSV), simian virus 40 (SV40) early or TK expression elements. To compensate for possible variations in transfection efficiency, a plasmid carrying the bacterial lacZ gene was included in each transfection (30). CAT activity was then normalized to β-galactosidase activity in each extract.

The relative activities of the TK, SV40 and RSV promoters seen are consistent with earlier reports (30, 31). Most notably, the SV40 construct was more effectively expressed in CHO than in mouse NIH 3T3 cells (Table 1). The OrnDCase expression elements displayed a contrasting preferential utilization; they were relatively weak in CHO cells but stronger in NIH 3T3 cells. These elements showed the following rank order of expression strength: in CHO cells, SV40 > RSV > TK > OrnDCase; in 3T3 cells OrnDCase = RSV > SV40 > TK. The minimal 5′ OrnDCase-flanking sequences necessary for expression were further delimited by deletion. A construct that leaves only 264 base pairs (bp) of DNA upstream of the transcription start site retains full constitutive activity (data not shown).

DISCUSSION

S49 mouse lymphoma D4.1 cells, in which an OrnDCase gene is amplified, were used as a source of DNA for mo-

Table 1. CAT expression directed by DNA containing different 5′ flanking sequences

	Promoter	CAT	β-Gal	CAT/β-Gal × 10³
CHO cells	SV40	12.8	21.3	601
Experiment 1	RSV	9.1	48.9	186
	TK	0.6	8.9	67
	ODC	1.7	44.1	39
CHO cells	SV40	11.5	19.5	590
Experiment 2	RSV	5.2	27.0	193
	TK	0.5	9.7	52
	ODC	1.6	44.4	36
NIH 3T3 cells	SV40	0.9	14.3	63
	RSV	2.3	24.9	92
	TK	0.6	13.4	45
	ODC	1.8	19.8	91

CAT specific activity (CAT) and β-galactosidase specific activity (β-Gal) of each extract were determined. The CAT specific activity of each extract divided by the amount of β-Gal activity present in the same cellular extract is shown to normalize for experimental variation in transfection efficiency.

lecular cloning of that gene. The amplification of the OrnDCase gene, ≈50- to 100-fold (5), and rudimentary information on its restriction sites, obtained from Southern blotting, allowed us to form a small library of size-fractionated DNA in a plasmid vector and to isolate the entire gene as two overlapping clones. The functional integrity of the reassembled cloned gene was demonstrated by transfecting it into OrnDCase-deficient CHO cells. Transfection conferred OrnDCase activity on the cells, and mouse OrnDCase mRNA with authentic 5′ ends was found therein.

The mouse OrnDCase gene is relatively small in size: ≈6.2 kb from transcription start site to the more 5′ of the two sites of polyadenylylation. Comparison of the sequences of cDNA (16) and genomic clones (this paper) shows that two introns, the first of ≈2 kb, interrupt the untranslated leader sequence. Sequencing of the entire gene (P.C. and E. L. Chen, unpublished data) has shown that nine additional introns are present in the coding sequence, making a total of eleven introns within the gene.

The transcription start site lies 31 or 32 bp 3′ to a canonical TATA box. The presence of a TATA box in a housekeeping gene is uncommon but not unprecedented (33). No sequence recognizable as a "CAAT" box was found within several hundred bp 5′ to transcription start. The sequence in the promoter region of the OrnDCase gene is highly G + C rich, a characteristic typical of housekeeping genes (33–45). The GGCGGG hexanucleotide or its inverted complement, shown in some genes to be part of the site of binding of SP1 transcription factor protein (46), was found at four positions 5′ to transcription start and at three positions within the transcribed leader sequence (Fig. 4). Among these seven recurrences of the core GGCGGG motif, there was a single match (GCCCCGCCCC, positions −500 to −491) to the full consensus sequence (46) attributed to authentic sites of SP1 binding.

Primer-extension and S1 protection experiments demonstrate that the OrnDCase message has a 312- or 313-nt 5′ untranslated leader. It is not certain whether the apparent microheterogeneity of the 5′ end of the message is real or an artefact; the fact that two independent techniques yielded identical results suggests the former is more likely. The sequence of the leader is unusual. It is extremely G + C rich, particularly in the 5′ portion: 85 of 102 bases are guanines or cytosines between positions −297 and −196. It would not be surprising if an RNA of such base composition could form one or more stable secondary structures; computer modeling based on energy minimization (47) suggests that a series of alternate secondary structures closely related in energy can

exist (results not shown). The physical reality of such putative secondary structural features has not been directly demonstrated, and their biological function, if any, has not been elucidated. It is imaginable that the leader could play a role in the translational regulation of OrnDCase synthesis in response to polyamines, a form of regulation recently described by us and other laboratories (8–11).

The strength of the OrnDCase genomic expression elements was compared to that of RSV, SV40, and TK promoters by transfecting CHO and NIH 3T3 cells with constructs consisting of each expression unit fused to the CAT gene. In mouse NIH 3T3 cells, the mouse OrnDCase promoter is comparable in strength to the RSV promoter and is somewhat stronger than the SV40 promoter. In CHO cells, the OrnDCase promoter demonstrates less activity than any of the promoters of viral origin. OrnDCase protein is expressed in animal tissue at a low level—even in cells in which it is most highly induced. The contrast between the relatively high expression of CAT driven from the OrnDCase genomic elements and the low protein abundance of endogenous OrnDCase may be accounted for in a variety of ways. The genomic location of the endogenous gene may affect expression. Alternatively, sequences 3' to the *Sal* I site of the OrnDCase gene may be responsible for rapid OrnDCase polypeptide turnover, rapid OrnDCase mRNA turnover, inefficient mRNA processing, or low translational efficiency of the OrnDCase mRNA.

Because we cloned the gene from a mutant cell selected for vast overproduction of OrnDCase, it is important to consider whether the cloned gene may have become altered. The S1 protection experiments yield qualitatively identical results, regardless of whether mRNA from overproducing cells, wild-type cells, or mice provided protection. This result implies that somatic mutations affecting the leader sequence, if any, are trivial. Restriction mapping using Southern blotting with multiple enzymes has shown no discernible difference between the cloned gene and the corresponding gene of wild-type cells in the region 2.5 kb 5' to the site of transcription start (results not shown). We have cloned the same OrnDCase gene using DNA from wild-type cells as a source. Comparative structural analysis will, therefore, answer this question definitively. Because overproduction of OrnDCase in the mutant cells arose in the course of stepwise selection before OrnDCase gene amplification (11, 32, 48), it will be important to compare the genes from wild-type and mutant cells for structure and functional activity of promoter sequences and, when defined, regulatory sequences.

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