Isolation and Expression of a Human Ornithine Decarboxylase Gene*

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We have isolated a human ornithine decarboxylase (ODC) gene from a leukocyte genomic DNA library in order to examine the mechanisms involved in the regulation of ODC gene expression in normal and neoplastic cell growth. Nucleotide sequence analysis shows that the human ODC gene in clone ODC709-A2 consists of 12 exons which encode a protein identical to that inferred from a human ODC cDNA sequence. The 5' end of the gene was determined by S1 nuclease and primer extension mapping. The high G + C content and small open reading frame found in exon 1 may be pertinent to translation regulation of ODC. Conserved sequences and potential promoter elements including a TATA box, a possible CCAAT element, SP1 and AP-2 transcription factor binding sites, and cAMP response elements were identified in the 5'-flanking region. Transfection of mouse tk- (tk-) cells with ODC709-A2 DNA resulted in the production of human ODC mRNA approximately 2.25 kilobases in length. Evidence that the protein synthesized from the human gene is functional is provided by "rescue" transfection of a Chinese hamster ovary mutant cell line, C55.7, which is ODC-deficient. C55.7 cells transfected with ODC709-A2 DNA expressed ODC enzyme activity and proliferated without exogenous putrescine.

The polyamines, putrescine, spermidine, and spermine, are small polycations, which have been shown to be essential for mammalian cell growth and function. Intracellular polyamine concentrations are highly regulated by the enzyme ornithine decarboxylase (ODC), which catalyzes the conversion of ornithine to putrescine, the initial and often rate-limiting step in polyamine biosynthesis. The level of ODC activity in quiescent cells is extremely low but readily induced by a wide variety of growth-promoting agents such as hormones, growth factors, regenerative stimuli, and tumor promoters (reviewed in Williams-Ashman and Canelakis, 1979; Pegg and McCann, 1982; Tabor and Tabor, 1984; and Pegg, 1988). Elevated levels of ODC are sustained in rapidly proliferating cells involved in either normal or neoplastic growth. Furthermore, enzyme-activated inhibitors of ODC, such as difluoromethylornithine, have been used extensively to deplete cellular pools of putrescine and spermidine resulting in growth arrest of normal and neoplastic cells in culture and in animal tumors in vivo (Jänne et al., 1983; Mamont et al., 1984; Sjoerdma and Scheele, 1984; Luk and Baylin, 1986; Luk et al., 1986; Pegg, 1986; Pera et al., 1986; Porter and Sufrit, 1986; McCann et al., 1987).

In view of the strong correlation between cell growth and ODC activity, the question of how normal and neoplastic cells modulate ODC activity is very important to our understanding of cell proliferation. Numerous studies have demonstrated that regulation of ODC at the protein level can occur at multiple steps including efficiency of mRNA translation (Kahana and Nathans, 1985a; Höflia and Pohjanpelto, 1986), rate of protein turnover (Bouvier et al., 1983; Srely and Pegg, 1983), and post-translational interactions and modifications (Heller et al., 1976; Kitani and Fujisawa, 1981; Kahana and Nathans, 1985a; Kahana and Nathans, 1985b; Höflia and Pohjanpelto, 1986). Molecular probes in the form of mouse (Berger et al., 1984; Kahana and Nathans, 1984; Kuntula et al., 1984; McConlogue et al., 1984; Kahana and Nathans, 1985b), rat (van Kranen et al., 1987), and human (Hickok et al., 1987) ODC cDNA clones have been developed to examine the regulation of ODC synthesis at the level of gene transcription. These studies have shown that some growth-stimulating agents enhance ODC synthesis by increasing either the rate of gene transcription or the stabilization of ODC mRNA. Recent reports have described the cloning and characterization of mammalian ODC genes from both mouse (Brabant et al., 1988; Katz and Kahana, 1988) and rat (van Steeg et al., 1988; Wen et al., 1989). However, the intense interest in ODC and polyamine regulation as therapeutic targets in human proliferative diseases such as cancer requires detailed knowledge of the human ODC gene and its products and regulatory elements. We report here the isolation and characterization of a human gene encoding a functional ODC enzyme as the initial phase of our investigation into the basic mechanisms controlling the expression of the human ODC gene.

EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

Structure and Sequence of a Human ODC Gene—In order to isolate a human ODC gene, we screened 500,000 colonies of 12 exons which encode a protein identical to that inferred from a human ODC cDNA sequence. The 5' end of the gene was determined by S1 nuclease and primer extension mapping. The high G + C content and small open reading frame found in exon 1 may be pertinent to translation regulation of ODC. Conserved sequences and potential promoter elements including a TATA box, a possible CCAAT element, SP1 and AP-2 transcription factor binding sites, and cAMP response elements were identified in the 5'-flanking region. Transfection of mouse tk- (tk-) cells with ODC709-A2 DNA resulted in the production of human ODC mRNA approximately 2.25 kilobases in length. Evidence that the protein synthesized from the human gene is functional is provided by "rescue" transfection of a Chinese hamster ovary mutant cell line, C55.7, which is ODC-deficient. C55.7 cells transfected with ODC709-A2 DNA expressed ODC enzyme activity and proliferated without exogenous putrescine.

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EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

Structure and Sequence of a Human ODC Gene—In order to isolate a human ODC gene, we screened 500,000 colonies of...
from a human leukocyte genomic DNA library, designated
10SSL (Lau and Kan, 1983), with a 32P-labeled mouse ODC
cDNA probe. Three recombinant colonies yielded positive
hybridization signals after multiple rounds of screening.
Southern blot analysis of DNA restriction digests indicated
that two of the three clones, ODC705-B1 and ODC717-B1,
were identical to each other but only partially related to the
third clone, ODC709-A2. Restriction fragment mapping with
oligomeric probes derived from the 5' and 3' termini of human
ODC cDNA revealed that DNA from ODC717-B1 and
ODC705-B1 lack at least 2.6 kb of the 3' portion of the ODC
gene sequence. The human DNA insert in ODC709-A2 was
the only clone homologous to ODC cDNA that was not
truncated at either end. The small number of recombinant
clones with homology to ODC supports other reports suggest-
ing that the human ODC gene family is very small (Winquist
et al., 1986; Hickok et al., 1987).

Total genomic DNA from human colon mucosa and DNA
from ODC709-A2 were digested with identical restriction
endonucleases and electrophoresed in the same agarose gel.
The Southern blot of this gel was probed with 32P-labeled
human ODC cDNA pODC2H/10 (Fig. 1). The cDNA probe
detects the same 5-kb HindIII and 10-kb BamHI fragments
in both genomic and ODC709-A2 DNAs. Human genomic
DNA shows four PstI fragments (sizes 8.0, 5.0, 2.6, and 0.68
kb), which hybridize to ODC2H/10, two of which (sizes 5.0
and 2.6 kb) are present in ODC709-A2 DNA. Hickok et al.
(1987) utilized the same ODC cDNA to probe Southern blots
of human genomic DNA and concluded that the two largest
PstI fragments are probably restriction length polymorphs
derived from two ODC gene alleles. The 2.6-kb fragment is
probably common to the two alleles represented by the 8.0-
and 5.0-kb bands. This is supported by densitometric analysis
of the autoradiograph in Fig. 1 showing that the sum of the
relative intensities of the two largest genomic PstI fragments
is the same as that of the 2.6-kb fragment (data not shown).
We conclude from this Southern blot analysis that clone
ODC709-A2 contains an intact ODC gene representing at
least one member of the human ODC gene family.

Furthermore, human ODC sequences have been mapped to
chromosome regions 2pter-p23 and 7cen-qter by hybridizing
a human ODC cDNA probe to a panel of mouse × human
somatic cell hybrid clones containing normal, translocated,
and deleted human chromosomes (Winquist et al., 1986). It
was shown that ODC sequences on human chromosome 2
were located in 9.0- and 2.7-kb PstI restriction fragments and
a 5.8-kb HindIII fragment. Human chromosome 7 contains a
4.4-kb HindIII DNA fragment, which is complementary to
ODC cDNA. The restriction fragments generated from PstI
and HindIII digestion of ODC709-A2 DNA, which hybridize
to ODC cDNA, are similar in size to those fragments derived
from ODC sequences on human chromosome 2 (8.0 and 2.6
kb; and 5.0 kb, respectively). The slight variation in fragment
lengths is probably due to differences in electrophoresis con-
ditions and DNA size markers. We infer from the comparison
of these restriction fragments that the human ODC gene in
ODC709-A2 probably maps to human chromosome 2.

The 10-kb BamHI and 5-kb HindIII fragments of recom-
binant ODC709-A2 were subcloned into Bluescript plasmid
(Stratagene) for sequence analysis. Unidirectional nested
deletions were prepared by limited digestion with exonuclease
III and mung bean nuclease and sequenced by the dideoxy
chain termination method (Sanger et al., 1977). The structure
and nucleotide sequence of the human ODC gene are shown
in Figs. 2 and 3, respectively. Like the mouse (Coffino and
Chen, 1988; Katz and Kahana, 1988) and rat (van Steeg
et al., 1988; Wen et al., 1989) ODC genes, the human ODC gene
is composed of 12 exons, with sequence information sufficient
to produce an ODC mRNA of 2043 nucleotides and 11 introns.
Exons 1, 2, and the first 17 bp of exon 3 represent a 5'-
nontranslated ODC mRNA leader sequence of 335 nucleo-
tides. Exon 12 from the termination codon (TAG) to the
polyadenylation signal (AATAAA) contains a 325 nucleotide
untranslated sequence comprising the 3' end of the ODC
mRNA. The remaining exons encode a protein identical to
the 461-amino acid sequence derived for human ODC from
an ODC cDNA sequence (Hickok et al., 1987). There are only
two differences between the nucleotide sequences of the hu-
mans. ODC genomic and cDNA clones; one difference is located
in the 5'-noncoding region (position 3164), and the other is a
change in the third base of a codon (position 7508), which
does not alter the amino acid sequence. The coding sequences
of the human, mouse, and rat ODC genes are interrupted by
introns at exactly the same nucleotide positions, resulting in
protein-coding exons of identical lengths. All of the human
ODC introns are bordered by GT/AG sequences as required
for proper splicing. All but one of the human introns are
longer than its rodent counterparts. Two of the human introns
each contain a single copy of an Alu family repetitive element.
The first Alu-inserted sequence (position 5250-5637) is 81%
FIG. 3. Nucleotide sequence of the human ODC gene. Numbers on the left indicate a nucleotide number starting at the site of transcription initiation (+1). Underlined sequences represent exons. Asterisks indicate the TATA box (position -31) and the polyadenylation signal (+7947), respectively. Met (+3317) and Val (+7621) denote initial and terminating codons, respectively. Two Alu repetitive elements (positions 5250-5637 and 7102-7388) are overscored by dotted lines. Arrowheads indicate direct repeats bordering the second Alu repeat. Approximately 2 kb of the first intron was not sequenced.
homologous to the Alu consensus sequence (Schmid and Jelinek, 1982) but is truncated at the 5' end by 12 nucleotides and is not bordered by direct repeats. The second Alu element (position 7101-7388) displays 77% homology to the consensus sequence and is bounded by an 11-bp direct repeat. Approximately 2 kb of the first and longest intron was not sequenced. Two approaches were used to determine that this region did not contain additional exons. First, a 2.0-kb HindIII fragment (position 7101-7388) displays 77% homology to the consensus sequence and is bounded by an 11-bp direct repeat. Approximately 2 kb of the first and longest intron was not sequenced. Two approaches were used to determine that this region did not contain additional exons. First, a 2.0-kb HindIII fragment consisting of the entire unsequenced portion of intron 1 failed to hybridize to human ODC mRNA on a Northern blot.
Second, primer extension mapping of the 5' end of the human ODC gene using a synthetic oligonucleotide complementary to sequences 3127–3144 confirmed the exon structure of the ODC gene as shown in Fig. 2.

The 5' End of the ODC Gene—Because the 5' end of the human ODC cDNA is truncated, the transcription start site of the human ODC gene was determined by S1 nuclease mapping. Total RNA isolated from human HCT 116 cells was used to protect a uniformly labeled fragment from ODC709-A2 spanning −51 to +180 nucleotides. Fig. 4A indicates that endogenous human ODC mRNA protects a doublet of 180 and 179 nucleotides, which places the 5' end of exon 1 at the T and G residues at nucleotide positions +1 and +2, respectively. The 5' end of the cloned gene was also determined using total RNA from a cell line designated L709-1, which was derived by transfecting mouse LM(tk−) cells with ODC709-A2 DNA (see below). Fig. 4A shows that L709-1 cells produce human ODC mRNA in abundance and that these transcripts initiate at approximately the same site(s) as the endogenous human ODC transcripts. As expected, total RNA from mouse LM(tk−) cells did not protect this species-specific probe. S1 analysis using an end-labeled fragment from −51 to +72 was carried out to more clearly define the transcription initiation site of the human ODC gene expressed in L709-1 cells. The same fragment was sequenced by chemical cleavage and coelectrophoresed with the S1-protected fragments for direct comparison. Fig. 4B reveals four protected fragments 70–73 nucleotides in size. Most of the human ODC transcripts from ODC709-A2 initiate at residues T (+1) or G (+2). It is not clear whether the multiple protected fragments reflect multiple transcription start sites or S1 mapping artifacts. To confirm these results by an independent method, poly(A') RNA from L709-1 and LM(tk−) cells were subjected to primer extension mapping using a synthetic oligonucleotide complementary to nucleotides +59 to +83. A single primer extension product of 83 nucleotides was detected in L709-1 cells but not LM(tk−) cells (Fig. 4C). This fragment corresponds to transcripts initiated at the same T residue (+1) implicated by S1 analysis. Taken together these mapping studies demonstrate that ODC transcripts originating from both endogenous and cloned human ODC genes initiate at approximately the same site(s) which for the cloned gene is predominantly the T residue at position +1.

The 5' end and the presumed promoter region of the human ODC gene were compared with the corresponding regions of the mouse and rat genes (Fig. 5). The sequences of the first exons of the human and mouse, human and rat, and rat and mouse ODC genes were found to be 68, 60, and 86% homologous, respectively. The nucleotides at the extremities of exon 1 and immediate flanking regions are identical in all three genes. The human ODC exon 1 sequence shares two structural characteristics with both rodent ODC genes which may be

![Fig. 4. Transcription initiation site analysis. A, S1 nuclease analysis with uniformly labeled probe. A 231-bp genomic DNA fragment spanning from −51 to +180 nucleotides was uniformly labeled and hybridized to 20 μg of total RNA from ODC709-A2-transformed L709-1 cells, mouse LM(tk−) control cells, and human HCT 116 colon carcinoma cells. S1 digestion was performed, and the products of one-half of each reaction were fractionated by electrophoresis in a 6% polyacrylamide-urea sequencing gel. Lanes L709-1 and LM(tk−) were exposed for 1.5 h; lane HCT 116 was exposed for 16 h. The sequence ladder (A, G, C, T) generated by sequencing M13mp18 DNA with T7 polymerase (Sequenase) using M13 universal primer was coelectrophoresed as a nucleotide (nt) size marker. B, S1 nuclease analysis with end-labeled probe. A DNA fragment including −51 to +72 nucleotides was end-labeled at the nucleotide complementary to nucleotide +72 and hybridized to 20 μg of total RNA from L709-1. After S1 nuclease digestion, reaction products were electrophoresed on an 8% polyacrylamide-urea sequencing gel. Base-specific chemical cleavage products (T/C, C, G, G/A) of the same end-labeled DNA were coelectrophoresed for size analysis. Chemical cleavage reactions remove the modified base and therefore produce size markers one nucleotide shorter than the corresponding nuclease-protected fragment. C, primer extension analysis. A synthetic oligonucleotide complementary to nucleotides +59 to +83 in exon 1 was end-labeled and hybridized to 5 μg of poly(A') RNA from L709-1 and LM(tk−) cells. Primer extension was performed, and the products were resolved in a 6% polyacrylamide-urea sequencing gel. An M13mp18 sequence ladder (A, G, C, T) generated by T7 polymerase (Sequenase) using M13 universal primer was coelectrophoresed as DNA size markers.](http://www.jbc.org/fig/4.png)
pertinent to translation regulation of ODC synthesis. First, exon 1 and, therefore, the 5' end of the untranslated leader of the ODC mRNA have a G+C content of 81%. As had been proposed for similarly G+C-rich leader sequences in the ODC mRNA, the 5' end of the untranslated leader of the ODC mRNA also shows that there are four GC boxes in human exon 1 (+117, +175 to +207), which encode a peptide sequence Met-Gly-Gln-Ala-Ser-Arg-Ala-Thr-Val-Leu, respectively. The rodent peptide sequences are 70% homologous to the human. It has been shown that the frames of the peptide encoded by the mouse and rat ODC genes contain similar open reading frames. The sequences of the peptide encoded by the mouse and rat open reading frames are identical or similar to consensus sequences for transcription factors. Only one GC box at the beginning of intron 1 (+248) displays significant homology (9 of 10 matched nucleotides) to the full consensus sequence GCCCGCCGCC (Dypan and Tjian, 1985) for SP1 binding sites. Only one GC box at the beginning of intron 1 (+117) displays significant homology (9 of 10 matched nucleotides) to the full consensus sequence GCCCGCCGCC (Dypan and Tjian, 1985) for SP1 binding sites. In addition, the human ODC gene promoter contains one putative binding site for transcription factor AP-2 (-171) and two cAMP response elements (-48, -177). These sequences may be important in the induction of ODC gene expression by phorbol esters and cAMP (Imagawa et al., 1985; Montiminy et al., 1986; Hovis et al., 1986). Fig. 5 also shows that there are four GC boxes in human exon 1 (+31, +38, +88, and +120) and another GC box at the beginning of intron 1 (+248). Interestingly, only the TATA box refers to nucleotide positions within the gene starting at the site (+1) of transcription initiation. Human ODC gene sequences homologous to the following transcription factor DNA-binding sites are underlined: 11 GC boxes, 2 cAMP response elements (CRE), 1 AP-2 site, 1 CCAAT element, and 1 TATA box.
pecific mutations in the ODC gene and its flanking region are necessary to characterize functional promoter and enhancer elements.

Expression of the Human ODC Gene in Clone ODC709-A2—We assayed for the expression of the human ODC gene by DNA-mediated transfer of ODC709-A2 DNA into mouse LM(tk−) cells in culture. Control cells were transfected with DNA from either ODC717-B1, which contains a truncated ODC gene sequence, or CON70, the cosmids vector with little or no human DNA insert. The cosmids vector in each of these DNA inserts includes a thymidine kinase selectable gene marker which was utilized to isolate stably transfected cells grown in HAT media. A total of 34 LM(tk+) colonies was cloned, 24 of which were transfected with ODC709-A2 DNA, 8 colonies from ODC717-B1 DNA and 2 colonies from ODC70 DNA. Polyamine concentrations, ODC enzyme activity, and mRNA levels were determined for approximately one-half of these stably transfected clones and compared with those levels found in mouse LM(tk−) and human colon carcinoma (HCT 116) cell lines. A representative sampling of these results is shown in Table I. ODC activity in cells transfected with ODC709-A2 DNA was enhanced up to 130-fold relative to LM(tk−) cells or cells transfected with either truncated ODC sequences (ODC717-B1) or cosmid vector alone (CON70). The increase in enzyme activity in ODC709-A2-transfected cells corresponds to an increase in the concentration of both ODC mRNA and putrescine (Table I and Fig. 6). Despite the strong induction of ODC activity and mRNA and an increase in putrescine concentration, steady-state levels of spermidine and spermine are apparently unaffected by the incorporation of a human ODC gene into the genome of this mouse cell line. We probed a Northern blot of total RNA from these cells with human ODC cDNA in order to establish that the increase in ODC activity in ODC709-A2 DNA-transfected cells correlated with the expression of the human ODC gene. The results in Fig. 6 demonstrate that the observed increases in ODC mRNA in ODC709-A2-transfected LM cells were linked to the expression of human ODC mRNA. S1 nuclease mapping with human ODC genomic DNA from ODC709-A2 confirmed that the elevated ODC mRNA in transfected L709-1, L709-11, and L709-12 cells was synthesized from the human gene (a representative autoradiograph for L709-1 is shown in Fig. 4). The steady-state levels of human ODC transcripts in ODC-transfected mouse cells was 1.6–67-fold greater (Table I) than that of the endogenous ODC mRNA in the human colon carcinoma cell line HCT 116. Furthermore, the abundant ODC transcripts in the mouse cells were efficiently processed to the 2.25-kb size expected for the human mRNA. Thus the human DNA in ODC709-A2 contains a transcriptional promoter and RNA processing signals capable of directing the synthesis and processing of human ODC transcripts.

Due to the presence of endogenous mouse ODC enzyme in the transfected LM(tk−) cells, it was not clear whether the human ODC mRNA produced from ODC709-A2 DNA was actually responsible for the enhanced levels of ODC activity. The Chinese hamster ovary mutant cell line C55.7, on the other hand, exhibits no endogenous ODC activity or protein and is a putrescine auxotroph, with an absolute requirement for putrescine for proliferation (Steglich and Schettler, 1982; Steglich et al., 1985a, 1985b). We transfected C55.7 cells with DNA from ODC709-A2, or ODC717-B1, or CON70 and selected for those colonies which acquired the capability of growing without exogenous putrescine. We isolated 28 clones, designated C 709-1 through 28, which grew without putrescine from C55.7 cells stably transformed with ODC709-A2 DNA. No colonies grew from cells transfected with cosmid DNA alone or DNA containing truncated ODC sequences or non-transfected cells. A representative number of these "rescued" cells was analyzed for both ODC enzyme activity and polyamine content (Table II). ODC activity was detected in all C55.7 cells rescued by transfection with ODC709-A2 DNA. There was no detectable ODC activity in control C55.7 grown in the presence of putrescine. In addition, the levels of spermidine and spermine in the rescued cells grown without putrescine were comparable to that of C55.7 cells grown in putrescine-supplemented media. The addition of the ODC suicide inhibitor difluoromethylornithine to these transfected cells lowered ODC activity and arrested cell proliferation. These results indicate that clone ODC709-A2 contains a transcriptionally active human ODC gene encoding a functional enzyme. It is also the first demonstration that the DNA-derived amino acid sequence represents a functional ODC enzyme.

Further characterization of the human ODC gene and its mRNA and protein products should begin to clarify the mechanisms which regulate ODC synthesis. Such an understanding of ODC and, thus, polyamine regulation in normal and neoplastic human cells may help realize the therapeutic promise of difluoromethylornithine and other inhibitors of the polyamine biosynthesis pathway.

Acknowledgments.—We are grateful to William Theis and Drs. Rajesh Mehta and Mark Gesell for their expert technical assistance for portions of this study.

Note Added in Proof.—After this paper was accepted, Fitzgerald and Flanagan (Fitzgerald, M. C., and Flanagan, M. A. (1989) DNA (N. Y.) 8, 632-634) and van Steeg et al. (van Steeg, H., van Oostrom C. Th. M., Martens, J. W. M., van Kreyl, C. F., Schepens, J., and Wierenga, B. (1989) Nucleic Acids Res. 17, 8850-8856) reported human ODC gene sequences which were nearly identical to the sequence described here.

REFERENCES

**Human Ornithine Decarboxylase Gene**


**EXPERIMENTAL PROCEDURES**

**Cloning Human ODC** – A human genomic DNA cosmid library, designated 10G5, prepared from lymphocyte DNA (Lau and Ben, 1981) was provided by T. Taggart (Oregon State University). A 1.7-kb EcoRI fragment from cosmid 10G5-22 (pG5-22) obtained from T. Ben (University of South Carolina), was 5'-radiolabeled by random primer extension (Feinberg and Vogt, 1984) and hybridized to replicate filters containing human DNA library 10L50 for 16 h at 65°C in a solution containing 5 X SSC (1 X SSC is 0.15 M sodium chloride/0.015 M NaCl) in buffer 2.5 M sodium acetate, 2.5 mg/ml yeast tRNA, 1.0 X SSC/0.5% SDS at 50°C, and prehybridization was carried out for 2 h at 50°C. Colonies yielding positive hybridization signals were selected for a second round of screening. Three colonies (pG5-1, pG5-2, and pG5-3) were selected for further characterization. Single-stranded (ss) DNA (17, 20, and 25 kb) at 3 h in 9 M formamide/0.1% SDS was transcribed in vitro in both orientations into α-32P labeled DNA (Strategene, La Jolla, CA) for sequence analysis.

**DNA-sequencing** – The sequencing strategy was designed for the positive clones obtained from the screen (Strategene, La Jolla, CA). The nucleotide sequence of the human ODC gene was determined from multiple overlapping cosmids on the same strands or from opposite strands using the Sequenase DNA sequencing kit (United States Biochemical, Cleveland, OH) according to the manufacturer's directions.

**DNA and RNA dot blot hybridization analyses** – Human genomic DNA was obtained from normal colon mucosa as previously described (Silverman et al., 1984). Cosmid and genomic DNAs were digested with restriction enzymes, fractionated by electrophoresis on a 0.7% agarose gel and transferred to a Sare Bio dot hybridization membrane in a Genetron apparatus. Hybridization was performed in a solution containing 5 X SSC and 1% SDS at 50°C for 16 h. Filters were washed with 0.2 X SSC and 0.1% SDS at 55°C for 30 min and 0.1 X SSC and 0.1% SDS at 55°C for 15 min. Filters were hybridized with the ODC cDNA probe at 65°C for 16 h, washed with 0.2 X SSC and 0.1% SDS at 55°C, 30 min; 0.1 X SSC and 0.1% SDS at 55°C, 5 min; and 0.1 X SSC and 0.1% SDS at 55°C, 5 min; and 0.05 X SSC and 0.1% SDS at 55°C, 15 min. Filters were hybridized with the ODC cDNA probe at 65°C for 16 h, washed with 0.2 X SSC and 0.1% SDS at 55°C, 30 min; 0.1 X SSC and 0.1% SDS at 55°C, 5 min; and 0.1 X SSC and 0.1% SDS at 55°C, 5 min; and 0.05 X SSC and 0.1% SDS at 55°C, 15 min.

**Cell culture and DNA isolation** – Cells were grown in monolayers in 24-well tissue culture dishes and harvested by trypsinization. Total cellular DNA was isolated by the method of R. W. Schimke (1987) using a modification of the procedure of F. Sanger et al. (1977) to separate DNA from RNA.

**RNA isolation and dot blot hybridization analysis** – Total RNA was isolated from the cell lines using the method of Chomczynski et al. (1987). Total RNA 

**RESULTS**

**Fig. 3. Structure of the human ODC gene.** The schematic diagram shows the structure of the human ODC gene. Open and solid lines represent non-coding and coding exons, respectively, whereas shaded lines represent introns. The symbols below the boxes indicate the predicted amino acid sequence of the protein. The boxes are drawn to scale, but the sequence of the boxes is not shown. The restriction endonuclease sites displayed are as follows: C, ClaI; E, EcoRI; H, HindIII; P, PstI.
TABLE I

Analysis of Transfected Mouse L(5/12)- Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>ODC mRNA</th>
<th>ODC Activity</th>
<th>NAD</th>
<th>NAA</th>
<th>SPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT 116</td>
<td>1.0</td>
<td>1.08</td>
<td>0.48</td>
<td>2.13</td>
<td>2.72</td>
</tr>
<tr>
<td>L(5/12)</td>
<td>0</td>
<td>2.06</td>
<td>0.31</td>
<td>1.69</td>
<td>0.64</td>
</tr>
<tr>
<td>L/117-81</td>
<td>0</td>
<td>2.18</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>L/109-11</td>
<td>1.6</td>
<td>2.10</td>
<td>0.44</td>
<td>1.75</td>
<td>0.39</td>
</tr>
<tr>
<td>L/109-12</td>
<td>13.0</td>
<td>16.87</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>L/109-3</td>
<td>13.5</td>
<td>16.58</td>
<td>3.0</td>
<td>3.33</td>
<td>0.66</td>
</tr>
<tr>
<td>L/109-1</td>
<td>67.0</td>
<td>263</td>
<td>4.10</td>
<td>2.66</td>
<td>0.65</td>
</tr>
</tbody>
</table>

* Determined as described in "Experimental Procedures"; ND, not determined.

TABLE II

Analysis of Transfected C57 Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>ODC Activity</th>
<th>PEP</th>
<th>SPH</th>
<th>SPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57-1</td>
<td>0.7</td>
<td>2.52</td>
<td>2.01</td>
<td>1.69</td>
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<tr>
<td>C709-4</td>
<td>1.4</td>
<td>0.43</td>
<td>2.74</td>
<td>3.18</td>
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<td>C709-11</td>
<td>0.19</td>
<td>0.58</td>
<td>3.61</td>
<td>2.41</td>
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<tr>
<td>C709-17</td>
<td>0.53</td>
<td>0.25</td>
<td>4.60</td>
<td>2.30</td>
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<tr>
<td>C709-18</td>
<td>0.20</td>
<td>0.60</td>
<td>2.73</td>
<td>3.46</td>
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<tr>
<td>C709-24</td>
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<td>C709-26</td>
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<td>C709-32</td>
<td>2.27</td>
<td>0.48</td>
<td>2.24</td>
<td>2.63</td>
</tr>
</tbody>
</table>

* ODC activity in nanomoles of CO₂ released from [14C]-pyruvaldehyde; PEP, phosphoenolpyruvate; SPH, spermidine; SPM, spermine; concentrations expressed as nanomoles/cell.

Fig. 4. Northern blot of human ODC mRNA in transfected mouse L(5/12)- cells. Total RNA was isolated, electrophoresed in a denaturing gel, and transferred to a nylon filter as described in "Experimental Procedures." 23 ng of ODC mRNA from each lane was probed with human ODC cDNA labeled to a specific activity of 1,000,000 c.p.m. per μg by nick-translating with calf intestinal alkaline phosphatase. The filter was exposed to film for 8 h.
Isolation and expression of a human ornithine decarboxylase gene.
J A Moshier, J D Gilbert, M Skunca, J Dosescu, K M Almodovar and G D Luk


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